Title: Analysis of molecular prognostic factors associated with tumor immune and stromal microenvironment in BEATRICE, an open-label phase 3 trial in early triple-negative breast cancer (eTNBC)

Molinero L, Yu J, Li C, Deurloo R, Dent RA A, Bell R, Brown J, Parmar M, Toi M, Suter T, Steger G, Pivot X, Mackey J, Jackisch C, Hall P, Hegde P, Bais C and Cameron D. Genentech Inc., South San Francisco, CA; F Hoffmann-La Roche Ltd, Basel, Switzerland; National Cancer Center, Singapore, Singapore; Sunnybrook Health Sciences Center, University of Toronto, Toronto, Canada; Deakin University, Geelong, Australia; Clinical Trials Research Unit, University of Leeds, Leeds, United Kingdom; Medical Research Council Clinical Trials Unit, London, United Kingdom; Kyoto University, Kyoto, Japan; Swiss Cardiovascular Center, Bern University Hospital, Bern, Switzerland; Medical University of Vienna, Vienna, Austria; University Hospital Jean Minjoz, Besançon, France; Cross Center Institute, Edmonton, Canada; Klinikum Offenbach, Offenbach, Germany; Leeds Institute of Health Sciences, University of Leeds, Leeds, United Kingdom and University of Edinburgh and Cancer Services, NHS Lothian, Edinburgh, United Kingdom.

Body: Background: TNBC is a mutationaly complex heterogeneous breast cancer subtype. In BEATRICE, adding bevacizumab to standard adjuvant chemotherapy for eTNBC improved neither invasive disease-free survival (IDFS; primary endpoint) nor overall survival (OS) [Cameron 2013; Bell SABCS 2014]. We explored prognostic effects of tumor-associated immune and stromal gene signatures.

Methods: Gene expression (RNA) was assessed in pretreatment archival tumor tissue using an 800-gene nanostring platform. Given the low event rates and lack of bevacizumab effect in BEATRICE, treatment arms were pooled. The biomarker-evaluable population (BEP; all patients with an evaluable biomarker sample and ≥1 postbaseline efficacy assessment) was dichotomized using median gene expression level as the cutoff. Prognostic associations between IDFS/OS and prespecified candidate gene sets/de novo identified clusters were assessed using univariate Cox proportional hazards models.

Results: Baseline characteristics and efficacy were similar in the BEP (988/2591 randomized pts; 38%) and the overall study population. In hierarchical cluster analysis based exclusively on immune gene expression, immune genes were enriched in 33% of samples, intermediate in 38%, and weak in 28%. Further characterization suggested differential prognostic value of distinct immune and stromal cell gene sets (Table). A significant prognostic effect for IDFS and OS was seen for CD8 effector T cell (T_{eff}) and regulatory T cell (T_{reg}) gene signatures, but not for the T_{eff}:T_{reg} ratio. A less pronounced positive prognostic effect was seen for other gene sets representing immune cells, including macrophages, CD4 T cells, and B cells (data not shown). Activated T helper (Th)-1 cell-derived chemokines and negative immune modulators of T cell activity (eg PD-L1) were highly prognostic for IDFS and OS. Both the cytokine IL-8 and ESM1 (target of VEGF-A pathway activation) were associated with worse IDFS and OS. No association was seen between outcome and markers for classic microvasculature (CD31, CD34), cancer-associated fibroblasts (FAP, BGN, DCN), VEGF-A, or VEGF-C.

<table>
<thead>
<tr>
<th>Gene signature</th>
<th>IDFS HR (95% CI)</th>
<th>Interaction p-value</th>
<th>OS HR (95% CI)</th>
<th>Interaction p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{eff}</td>
<td>0.40 (0.28-0.57)</td>
<td>7.2x10^{-7}</td>
<td>0.29 (0.17-0.49)</td>
<td>4.2x10^{-6}</td>
</tr>
<tr>
<td>T_{reg}</td>
<td>0.38 (0.26-0.54)</td>
<td>1.6x10^{-7}</td>
<td>0.23 (0.13-0.40)</td>
<td>2.9x10^{-7}</td>
</tr>
<tr>
<td>T_{eff}:T_{reg} ratio</td>
<td>0.80 (0.58-1.12)</td>
<td>0.2</td>
<td>0.89 (0.57-1.39)</td>
<td>0.6</td>
</tr>
<tr>
<td>Th1</td>
<td>0.45 (0.31-0.64)</td>
<td>8.1x10^{-6}</td>
<td>0.43 (0.27-0.70)</td>
<td>5.8x10^{-4}</td>
</tr>
<tr>
<td>PD-L1</td>
<td>0.42 (0.29-0.60)</td>
<td>1.8x10^{-6}</td>
<td>0.24 (0.14-0.41)</td>
<td>3.4x10^{-7}</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.48 (1.06-2.08)</td>
<td>0.022</td>
<td>1.89 (1.18-3.01)</td>
<td>0.0076</td>
</tr>
<tr>
<td>ESM1</td>
<td>1.73 (1.23-2.43)</td>
<td>0.0017</td>
<td>2.22 (1.38-3.58)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Conclusions: These molecular gene signature analyses in eTNBC confirm that markers of cytotoxic CD8 T cells are associated with good prognosis. This is the first report of a positive prognostic effect of regulatory T cell markers, immune checkpoint modulators, and macrophage-associated markers in the adjuvant TNBC setting. High VEGF-A activity, but not its expression, was associated with worse prognosis. The strong prognostic effect of immune checkpoint modulators suggests equilibrium between cytotoxic T cells and their inhibitors in eTNBC, supporting further exploration of immune checkpoint inhibitors in this therapeutic context.
Title: Lymphocytic infiltration in invasive lobular breast cancer


Body: Background: The presence and prognostic value of tumor infiltrating lymphocytes (TILs) in invasive breast carcinoma has been demonstrated in several studies, especially in the triple-negative and HER2-positive subtypes. So far, TILs have not been investigated with sufficient detail in invasive lobular breast cancer (ILBC). Here we therefore aimed at: first, assessing the distribution of stromal TILs in ILBC; second, correlating the presence of TILs with standard clinical and pathological markers; third, exploring associations of TILs with recurrent genomic alterations; and, fourth, comparing the lymphocytic composition of ER-positive/HER2-negative lobular to ER-positive/HER2-negative ductal tumors.

Material and methods: The percentage of stromal TILs was independently assessed according to Salgado et al. (Ann Oncol 2015) by three pathologists on full-face hematoxylin and eosin slides in a well-annotated retrospective series of 614 primary ILBCs previously characterized at the genomic level. The median value of TILs was used for the analyses. For the association analyses, we focused on the more homogeneous group of ER-positive/HER2-negative ILBC (555/614). Breast cancer-free interval was used as survival endpoint and the analyses were censored at 12 years of follow-up. The comparison of the lymphocytic composition (relative percentage of CD45+ TILs which are CD4+, CD8+ or CD19+) was assessed by FACS in a separate prospective cohort of 51 ER-positive/HER2-negative lobular and 112 ER-positive/HER2-negative ductal tumors.

Results: The intraclass correlation coefficient between the three pathologists was 0.71 (95%CI:0.65-0.76). The median percentage of stromal TILs was 5% and the interquartile range 5-10%, with only 9% of the samples having ≥20%. Greater numbers of TILs were significantly associated with younger age at diagnosis, axillary lymph node involvement, high proliferative tumors as assessed by Ki67, and with the mixed non-classic ILBC subtypes. Greater numbers of TILs were associated with worse prognosis (HR=1.22; 95%CI:1.07-1.38, p=0.003) only in the unadjusted analysis, as it lost significance after adjustment for standard clinical and pathological variables. Greater numbers of TILs were observed in tumors harboring ARID1A, BRCA2, KMT2C and TP53 mutations, as well as chr3p21.31 and chr8q24.23 (PTK2) loss; whereas lower numbers were observed in tumors with ERBB3 mutations as well as chr7p and chr11q14.1 (PAK1) gains. There were no significant differences in the relative proportion of CD4+, CD8+ or CD19+ lymphocytes between ER-positive/HER2-negative lobular and ductal tumors.

Conclusion: In this work, which reports to our knowledge on the largest series of ILBC ever assessed for TILs, we showed that most ILBCs were characterized by low lymphocytic infiltration. Besides the association of TILs with clinical and pathological features of ILBC patients, we found that higher TIL levels were observed in the presence of specific mutations and copy number alterations. Higher numbers of TILs were associated with worse prognosis at the univariate analysis. Finally, based on the assessed markers, we have no evidence of differential lymphocytic composition between ER-positive/HER2-negative lobular and ductal tumors.
Title: Pooled individual patient data analysis of stromal tumor infiltrating lymphocytes in primary triple negative breast cancer treated with anthracycline-based chemotherapy

Loi S, Drubay D, Adams S, Francis PA A, Joensuu H, Dieci MV Vittoria, Badve S, Demaria S, Gray R, Piccart MJ J, Kellokumpa-Lehtinen P-L, Andre F, Dufaure-Gare I, Denkert C, Salgado R and Michiels S. Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia; Gustave Roussy, Villejuif, France; New York University School of Medicine, NY; Helsinki University Central Hospital, Helsinki, Finland; University of Padova, Padova, Italy; Indiana University, Indianapolis, IN; Dana-Farber Cancer Institute, Boston, MA; Institut Jules Bordet, Brussels, Belgium; Tampere University Hospital, Tampere, Finland and Charite Universite Hospital, Berlin, Germany.

Body: Background:
Retrospective analyses from individual clinical trials have suggested that host anti-tumor immunity as measured by stromal tumor infiltrating lymphocytes (TILs) is important for the outcomes of the primary triple negative breast cancer (TNBC) subgroup, but the clinical utility of TILs in day-to-day management of primary TNBC is still limited. Our objective was to conduct a pooled analysis of the clinical trials that have investigated TILs in TNBC patients treated by anthracyclines-based (A) chemotherapy regimens in order to gain a robust understanding of the prognostic value of TILs in this setting.

Material and methods:
Methods were predefined in a protocol. Eligible studies were randomized clinical trials that have evaluated the prognostic associations of TILs (evaluated in the same manner) in patients diagnosed with early stage TNBC treated with A or A plus taxanes (A+T). Cox regression models stratified by trial for invasive disease-free survival (IDFS, primary endpoint) and overall survival (OS), fitting stromal TILs as a continuous variable.

Results:
We collected individual data from 991 TNBC patients included in 6 randomized clinical trials (ECOG2197, ECOG1199, BIG2-98, FinHER, 2 from Gustave Roussy): 62% of patients were treated by A+T and 38% by A alone; 32% of patients had no nodal involvement, 43% of patients had 1-3 nodes and 25% patients more than 3 nodes involved. The average age was 49 years (range 22.6-85 yrs) and the average tumor size 3.0 cm (sd 1.7).

Across the entire data set, the average value of stromal TILs was 20% (sd 17%); 90% of patients had at least 1% stromal TILs. After adjusting for trial, stromal TILs were significantly lower with increasing tumor size (linear model, p<0.0001) but not significantly associated with nodal status categories (p=0.52 and p=0.37) nor age (p=0.25). With a median follow-up of 6.6 years for IDFS and 7.3 years for OS, a total of 363 IDFS events and 273 deaths were observed. Each 10% increase in stromal TILs was associated with a 14% relative reduction in IDFS events (HR=0.86, 95% CI 0.80 to 0.93, p<0.0001) and a 17% relative reduction in deaths (HR=0.83, 95% CI 0.76 to 0.91, p=0.0001). There was no significant evidence for heterogeneity between trials for IDFS (chi2=4.55, p=0.34) nor for OS (chi2=4.45, p=0.34).

In a multivariable analysis adjusted for age, nodal status, tumor size and chemotherapy regimen, stromal TILs added significant independent prognostic information for both IDFS and OS (likelihood chi2=17.9 for IDFS, p<0.0001 and chi2=16.7 for OS, p<0.0001). The adjusted hazard ratio for each 10% increase in stromal TILs was HR=0.86 (0.76-0.92) for IDFS events and HR=0.84 (0.76-0.92) for death.

Conclusion:
This large pooled individual patient data analysis confirms the strong prognostic role of stromal TILS in primary TNBC treated with A or A+T. TILs should now be strongly considered for incorporation as a stratification factor in future clinical trials enrolling TNBC patients. Given the important prognostic role of pre-existing immunity, patients with TNBC are rational candidates for immunotherapy clinical trials.

Funding: Ligue Nationale Contre le Cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: S1-04

Title: Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase Ib JAVELIN solid tumor trial

Dirix LY Y, Takacs I, Nikolinakos P, Jerusalem G, Arkenau H-T, Hamilton EP P, von Heydebreck A, Grote H-J, Chin K and Lippman ME E. Sint Augustinus - University of Antwerp, Antwerp, Belgium; Semmelweis University, Budapest, Hungary; University Cancer & Blood Center, LLC, Athens, GA; CHU Sart Tilman Liege and Liege University, Liege, Belgium; Sarah Cannon Research Institute, London, United Kingdom; Sarah Cannon Research Institute, North Nashville, TN; Merck KGaA, Darmstadt, Germany; EMD Serono, Billerica, MA and University of Miami Miller School of Medicine, Miami, FL.

Body: Background: The programmed death-1 receptor (PD-1) and its ligand (PD-L1) are key therapeutic targets in the reactivation of the immune response against cancer. Avelumab* (MSB0010718C) is a fully human anti-PD-L1 IgG1 antibody being investigated in clinical trials. We report clinical activity of avelumab in a cohort of patients (pts) with locally advanced (LA) or metastatic breast cancer (MBC) refractory to or progressing after standard-of-care therapy (NCT01772004).

Methods: Pts received avelumab at 10 mg/kg Q2W until confirmed progression, unacceptable toxicity, or any criterion for withdrawal occurred. Tumors were assessed every 6 wks (RECIST 1.1). Unconfirmed best overall response was evaluated. Adverse events (AEs) were graded by NCI-CTCAE v4.0. Biopsy or surgical specimens were collected within 90 days prior to 1st dose of avelumab for biomarker analyses. Tumor PD-L1 expression was assessed by immunohistochemistry using various cutoff criteria.

Results: As of 27 Feb 2015, 168 pts (167 female, 1 male) with MBC, including ductal (56.5%), carcinoma NOS (9.5%), lobular (3.6%), or other (30.4%), were treated with avelumab and followed for a median of 10 mo (range 6-15). Median age was 55y (range 31-81), ECOG performance status was 0 (49.4%) or 1 (50.6%), and pts had received a median of 3 prior therapies for LA/M disease (range 0-10; pts must have received prior treatment with taxane and anthracycline, unless contraindicated). Pts were HER2–/ER+ or PR+ (69 [41.1%]), triple negative (TNBC = HER2–/ER–/PR–; 57 [33.9%]), HER2+ (26 [15.5%]), or had unknown biomarker status (16 [9.5%]). Median duration of treatment was 8 wks (range 2-50), and 9 pts (5.4%) remained on avelumab. Any grade treatment-related treatment-emergent AEs (TEAEs) occurred in 120 pts (71.4%); the most common (>10%) were fatigue (33 [19.6%]), nausea (24 [14.3%]), and infusion-related reactions (20 [11.9%]). Treatment-related grade ≥3 TEAEs occurred in 24 pts (14.3%) and included (≥1%) fatigue, anemia, increased GGT, and autoimmune hepatitis (each 3 [1.8%]), and arthralgia (2 [1.2%]). There were 2 treatment-related deaths (acute liver failure, respiratory distress). Unconfirmed objective response rate (ORR) in the entire cohort was 5.4% (9 pts; 95% CI: 2.5, 9.9), with 1 CR and 8 PRs. Five of 9 responses were ongoing at time of cutoff. Stable disease was observed in additional 40 pts (23.8%), for an overall disease control rate of 29.2%. Evidence of tumor reduction by ≥30% was seen in 15 pts (8.9%). There were responders in all biomarker subgroups, including 5 PRs in TNBC (n=57 [8.8%]; 95% CI: 2.9, 19.3). PD-L1 expression was evaluable in 136 pts. Among all pts with PD-L1 expressing immune cells within the tumor, 33.3% (4 of 12) had PRs. In pts with TNBC who had PD-L1+ immune cells within the tumor, 44.4% (4 of 9) had PRs, compared with 2.6% (1 of 39) for TNBC and PD-L1− immune cells.

Conclusions: Avelumab showed an acceptable safety profile and had clinical activity in a subset of pts with MBC. In pts with TNBC, presence of PD-L1 expressing immune cells within the tumor may be associated with clinical responses to avelumab. Further analyses of PD-L1 expression and clinical activity of avelumab in MBC are ongoing. *Proposed INN.
Pituskin E, Mackey JR R, Koshman S, Jassal D, Pitz M, Haykowsky MJ J, Thompson R, Oudit G, Ezekowitz J and Paterson I. University of Alberta, Edmonton, AB, Canada; University of Manitoba, Winnipeg, MB, Canada; Mazankowski Alberta Heart Institute, Edmonton, AB, Canada; Cross Cancer Institute, Edmonton, AB, Canada; Bergen Cardiac Care Centre, Winnipeg, MB, Canada and Cancer Care Manitoba, Winnipeg, MB, Canada.
Title: Abstract Withdrawn

Body:
The miR-424/503 cluster is a breast cancer tumor suppressor with a role in chemoresistance

Silva J, Llobet-Navas D, Rodriguez-Barrueco R, Sanchez-Garcia F and Pe’er D. Mount Sinai School of Medicine, NYC, NY and Columbia University, NYC, NY.

Body: Recently, we have identified the miR-424(322)/503 cluster as an important regulator of mammary epithelial homeostasis. The miR-424(322)/503 cluster was identified as one of the few miRNAs significantly upregulated during involution after pregnancy. By generating a knock-out mouse model, we found that regression of the mammary epithelium after pregnancy was compromised in the absence of miR-424(322)/503. Mechanistically, our studies unveiled that miR-424(322)/503 is induced by the canonical TGF-β-SMAD pathway, and that it orchestrates changes in the mammary epithelium by downregulating the expression of key components of signal transduction (IGF1R) and apoptosis (BCL2) (Llobet et al. Genes & Development 2014).

Remarkably, our new studies have revealed that miR-424(322)/503-/- female mice develop hyperplasia and mammary tumors that are promoted by pregnancy. Thus, we investigated the status of this cluster in human breast cancers. For this we analyzed the METABRIC dataset. These studies revealed that the miR-424(322)/503 cluster was heterozygously deleted in ~16% of breast cancers and that its deletion correlates with lower expression levels of the mature miRNA forms. Importantly, miR-424(322)/503 is located on the X-chromosome and we have confirmed that is monoallelically expressed due to X-chromosome inactivation. Thus, the mutation of the active allele strongly impacts the expression of the miR-cluster. Deletions of the miR-424(322)/503 locus were more frequent in molecular subtypes with aggressive behavior (Luminal B, HER2+ and Basal) and both deletions and low expression of the cluster were associated with poor prognosis and reduced survival.

Some of the miR-424(322)/503 targets that we have previously validated (BCL2 and IGF1R) are involved in resistance to chemotherapy. Thus, we investigated in vivo, utilizing our knock-out mouse model, if loss of miR-424(322)/503 induces resistance to chemotherapeutic drugs. For this we crossed our miR-424(322)/503-/- animals with the HER2+ model FVB/N-Tg(MMTVneu)202Mul/J. Tumors emerging in HER2+/miR-424(322)/503-/- animals presented higher levels of BCL2 and hyperactivation of the IGF1R-AKT signaling compared to HER2+/miR-424(322)/503+/+ counterparts. Furthermore, these tumors were resistant to standard chemotherapy. Importantly, inhibition of BCL2 with ABT-199 and IGF1R with BMS-754807, two compounds currently in clinical trial, completely reverted chemotherapy resistance.

Overall, our data present evidence supporting a tumor suppressor role for miR-424(322)/503 cluster and its implication in resistance to chemotherapy.
Title: A phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathologic residual invasive disease after neoadjuvant chemotherapy (CREATE-X, JBCRG-04)

Toi M, Lee S-J, Lee ES, Ohtani S, Im Y-H, Im S-A, Park B-W, Kim S-B, Yanagita Y, Takao S, Ohno S, Aogi K, Iwata H, Kim A, Sasano H, Yokota I, Ohashi Y and Masuda N. Yeungnam University Hospital, Daegu, Korea; Kyoto University Hospital, Kyoto, Japan; National Cancer Center, Seoul, Korea; Hiroshima City Hospital, Hiroshima City, Hiroshima, Japan; Samsung Medical Center, Seoul, Korea; Seoul National University Hospital, Seoul, Korea; Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; Asan Medical Center, Seoul, Korea; Gunma Prefectural Cancer Center, Ota, Gunma, Japan; Hyogo Cancer Center, Akashi, Hyogo, Japan; National Kyusyu Cancer Center, Fukuoka, Japan; NHO Shikoku Cancer Center, Matsuyama, Ehime, Japan; Aichi Cancer Center, Nagoya, Aichi, Japan; Korea University Guro Hospital, Seoul, Korea; Tohoku University, Sendai, Miyagi, Japan; Kyoto Prefectural University of Medicine, Kyoto, Japan; Chuo University, Tokyo, Japan and NHO Osaka National Hospital, Osaka, Japan.

WITHHELD PENDING PRESS CONFERENCE
Title: High risk premenopausal luminal A breast cancer patients derive no benefit from adjuvant chemotherapy: Results from DBCG77B randomized trial

Nielsen TO O, Jensen M-B, Gao D, Leung S, Burugu S, Liu S, Tykjær Jørgensen CL L, Balslev E and Ejlertsen B. Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; Genetic Pathology Evaluation Centre, Vancouver, BC, Canada; Canadian Immunohistochemistry Quality Control, Vancouver, BC, Canada and University of Ottawa, Ottawa, ON, Canada.

WITHELD PENDING PRESS CONFERENCE
Title: A comparison of the diagnostic performance of 2D synthetic mammography versus digital breast tomosynthesis in 2500 patients

Holt SD D, Sharaiha YM M, Moalla A, Williams HR R, Thomas D and Huws AM M. Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom.

Body: Introduction: A synthetic 2D mammogram (C-view) can be created by combining the individual optimally enhanced 1mm slices of a digital breast tomosynthesis (DBT). Studies show that screening with 2D mammography and DBT increases the cancer detection rate by about 40% and reduces the recall rate by about 25% but it doubles the x-ray exposure to the patient and reading time for the radiologist compared to standard 2D screening. The use of a synthetic 2D instead of a standard 2D film may theoretically overcome these problems by avoiding a second exposure and presenting the detail normally available in DBT in one picture. The hypothesis we wished to test is, "if the synthetic 2D is normal/benign, is there any advantage in also viewing the DBTs?"

Method: We prospectively collected data on 2500 unselected cases presenting symptomatically or at follow up, all of whom underwent DBT on a Hologic® Dimensions machine. From the 3D data sets synthetic 2D mammograms were constructed (Hologic® C-view). One breast radiologist with 13 years experience of interpreting mammograms and 5 years with DBTs was asked to review the 2D synthetic mammograms (CC and MLO) and report them before then reviewing the DBTs and issuing a final report. The mammograms were reported M1 to M5 using the standard BIRADs criteria. The BIRADs scores for each breast were recorded prospectively. Similarly the breast density as assessed by eye was recorded (fatty/average density/dense).

Results: 2500 patients were studied between October 2013 and October 2014. The average age of the women was 58.4 years (range 28-95). Of these some were under follow up after mastectomies so in total there were 4589 individual mammogram sets reported. Table 1 summarises the correlation between the synthetic 2D and DBT reports.

<table>
<thead>
<tr>
<th>BIRAD Classification - Synthetic 2D v DBT</th>
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<tbody>
<tr>
<td>Synthetic 2D</td>
</tr>
<tr>
<td>M1</td>
</tr>
<tr>
<td>M2</td>
</tr>
<tr>
<td>DBT</td>
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<tr>
<td>M4</td>
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<td>M5</td>
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</table>

The correlation is very close, but there were 11 patients in whom the synthetic 2D was reported normal or benign (M1 or M2) but the DBT was reported as M3. Of these 10 were benign on assessment and one malignant. There was one patient reported as M2 on synthetic 2D but M4 on DBT. Assessment confirmed malignancy. Sixteen cases reported as suspicious of malignancy (M3/4) by synthetic 2D were subsequently downgraded to benign after review of the DBT. We estimate that 2D mammography alone would have detected only 68 of the 94 detected by synthetic 2D.

Of the 4589 examinations, 1131 (25%) were assessed as fatty, 1851 (40%) as average density and 1607 (35%) as dense. One cancer was missed in an averagely dense and one in a dense breast.

Conclusion: In a symptomatic and follow up clinic, our study suggests that much radiologist's time and x-ray exposure to the patient could be saved by using synthetic 2D mammograms rather than using 2D/3D combinations. Only if the synthetic 2D is reported M3, 4 or 5 is it necessary to review the DBT. Whilst a similar trial is required to confirm these findings in a screening population, this trial suggests that synthetic 2D mammography could be cost effective compared to combination 2D/3D.
Title: Importance of margin width and re-excision in breast conserving treatment of early breast cancer; a Danish breast cancer cooperative group study of 11,900 women

Bodilsen A, Bjerre K, Offersen BV V, Vahl P, Mele M, Dixon MJ J, Ejlertsen B, Overgaard J and Christiansen P. Aarhus University Hospital, Aarhus, Denmark; Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; Aarhus University Hospital, Aarhus, Denmark; Aarhus University Hospital, Aarhus, Denmark and Breakthrough Research Unit Edinburgh Breast Unit, Western General Hospital, Edinburgh, United Kingdom.

Body: Background: The majority of women with invasive breast cancer are treated surgically by breast conserving surgery (BCS). A significant proportion subsequently undergo re-excision to obtain clear margins. However what constitutes a sufficient negative margin continues to be subject of controversy. The purpose of the study was to investigate the association between margin width and ipsilateral breast tumour recurrence (IBTR) as well as identifying factors associated with residual disease after repeat surgery, and to determine the effect of re-excision on IBTR in a population-based nationwide cohort.

Method: 11,900 patients treated with breast conserving therapy for unilateral invasive cancer in Denmark between 2000 and 2009 were included. All patients received whole breast irradiation and were offered systemic adjuvant treatment according to the guidelines of the Danish Breast Cancer Cooperative Group.

Results: The median follow-up was 4.9 years. The cumulative incidence of IBTR at 5 and 9 year was 2.4% and 5.9%, respectively. No decrease in IBTR with a wider negative margin compared to a narrow but negative margin was seen in adjusted analysis (>0-1 mm vs. 2-4 mm vs. ≥5 mm (reference): HR 1.54 (CI 95% 0.81-2.93) vs 0.95 (CI 95% 0.56-1.62) vs. 1). A final positive margin did however increase the risk of IBTR (HR 2.51; 95% CI 1.02-6.23). Other factors associated with increased IBTR were young age (HR 3.10; 95% CI 1.89-5.10), more than 4 positive lymph nodes (HR 1.80; 95% CI 1.24-2.62), and re-excision (HR 1.53; 95% CI 1.16-2.02). Receiving chemotherapy (HR 0.45; 95% CI 0.33-0.61) or boost (HR 0.43; 95% CI 0.31-0.60) reduced risk of IBTR as did being oestrogen receptor positive treated with (HR 0.35; 95% CI 0.25-0.49) or without (HR 0.43; 95% CI 0.31-0.60) adjuvant endocrine therapy.

Within two months of initial BCS 1342 women (11%) had a re-excision. Residual disease was found in 20% of re-excisions. In adjusted analysis DCIS outside the invasive tumour (OR 2.69; 95% CI 1.99-3.63), positive initial margin (OR 2.26, 95% CI 1.70-2.99, p<0.001), and age <50 years (OR 1.53; 95% CI 1.00-2.31) was associated with increased risk of residual disease. Patients with residual disease after re-excision had in the adjusted analysis an increased risk of ipsilateral breast tumour recurrence (IBTR), regardless of whether residual findings were invasive carcinoma (HR 2.97, CI 95% 1.57-5.62) or DCIS (HR 2.58, CI 95% 1.50-4.45). However no difference was seen for overall survival comparing one procedure with repeat surgery with or without residual disease (p=0.96).

Conclusion: An overall low rate of IBTR was seen. While a final positive margin was associated with a more than two-fold risk of IBTR, no evidence of improved local control was found with wider negative margins compared to narrow. However the finding of residual disease at re-excision was associated with an increased risk of IBTR.
2015 San Antonio Breast Cancer Symposium

**Publication Number:** S2-02

**Title:** The impact of adjuvant denosumab on disease-free survival: Results from 3,425 postmenopausal patients of the ABCSG-18 trial

Gnant M, Pfeiler G, Dubsky PC C, Hubalek M, Greil R, Jakesz R, Wette V, Balic M, Haslbauer F, Melbinger-Zeinitzer E, Bjelic-Radisic V, Artner-Matuschek S, Fitzal F, Marth C, Sevelda P, Mlineritsch B, Steger GG G, Manfreda D, Exner R, Egle D, Bergh J, Kainberger F, Talbot S, Warner D, Fesl C, Singer CF F and On behalf of the Austrian Breast and Colorectal Cancer Study Group. Medical University of Vienna and Comprehensive Cancer Center, Vienna, Austria; Medical University of Vienna and Comprehensive Cancer Center, Vienna, Austria; Medical University Innsbruck, Innsbruck, Austria; Paracelsus Medical University Salzburg and Salzburg Cancer Research Institute, Salzburg, Austria; Breast Center/ Doctor's Office Wette, St. Veit/ Glan, Austria; Medical University Graz, Graz, Austria; Hospital Vöcklabruck, Vöcklabruck, Austria; Hospital Wolfsberg, Wolfsberg, Austria; Medical University Graz, Graz; Breast Center_ Hospital Hanusch-Vienna, Vienna, Austria; Hospital of Sisters of Mercy Linz/ Breast Health Center, Linz, Austria; Hospital Hietzing, Vienna, Austria; Medical University of Vienna, Vienna, Austria; Doctor's Office Manfreda, Klagenfurt, Austria; Karolinska Institute and University Hospital/ Radiumhemmet, Karolinska Oncology, Stockholm, Sweden; Medical University of Vienna, Vienna, Austria; Amgen Ltd, Uxbridge, United Kingdom; Amgen Inc, One Amgen Center Drive, Thousand Oaks, CA and Austrian Breast & Colorectal Cancer Study Group, Vienna, Austria.

WITHHELD PENDING PRESS CONFERENCE
Persistence of circulating tumor cells in high risk early breast cancer patients during follow-up care suggests poor prognosis – Results from the adjuvant SUCCESS A trial

Janni W, Rack B, Fasching P, Haaberle L, Friedl T, Tesch H, Lorenz R, Neugebauer J, Koch J, Jaeger B, Fehm T, Mueller V, Schneeweiß A, Lichtenegger W, Beckmann M, Scholz C, Pantel K and Trapp E. University Hospital Ulm, University of Ulm, Ulm, Germany; University Hospital Ludwig-Maximilians University, Ludwig-Maximilians University, Munich, Germany; University Hospital, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; Medical Center Hamburg-Eppendorf, University Hospital Hamburg-Eppendorf, Hamburg, Germany; University Hospital Heidelberg, Ruprecht-Karls-University, Heidelberg, Germany; University Hospital Nuremberg-Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany; Haemato-Oncology Practice, Bethanien Hospital, Frankfurt, Germany; Gynaecology Practice Lorenz/Hecker, Braunschweig, Germany; Charité University Hospital Campus Virchow, Berlin, Germany and University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Background: Recent data suggest that circulating tumor cells (CTCs) are of prognostic relevance in early as well as metastatic breast cancer (BC). While persisting CTCs immediately after chemotherapy are known to indicate poor prognosis, there is a lack of data regarding the prognostic role of CTCs assessed during long-term follow-up care. Hence the prognostic value of CTCs two years after chemotherapy was analyzed.

Methods: The SUCCESS A trial is a randomized, open-label, 2x2 factorial design Phase III study in high-risk breast cancer patients (N0 or T2–T4 or grade 3 or age ≤ 35 or hormone-receptor negative). Patients were first randomized to adjuvant chemotherapy treatment with 3 cycles of epirubicin-fluorouracil-cyclophosphamide followed by either 3 cycles of docetaxel or 3 cycles of gemcitabine-docetaxel. In addition, patients were randomized to 2 vs. 5 years of zoledronate treatment. Presence of CTCs was assessed using the FDA-approved CellSearch System (Janssen Diagnostics, LLC). CTC positivity was defined as ≥ 1 CTC in 7.5 ml whole blood. To investigate if CTC status 2 years after chemotherapy is of prognostic relevance independent from CTC status before chemotherapy and to evaluate the prognostic relevance of changed CTC status, only patients with data on CTC status before and 2 years after chemotherapy were included. Patient outcomes in terms of overall survival (OS) and disease-free survival (DFS) were analyzed by univariate log-rank tests and multivariate Cox regressions adjusted for age, menopausal status, tumor stage, nodal stage, grading, histological type, hormone receptor status and HER2 status. Survival time was measured beginning with the date of follow-up CTC assessment two years after chemotherapy.

Results: Data on CTC status before and 2 years after chemotherapy were available for 1103 (29.4%) of 3754 randomized patients. The CTC status 2 years after chemotherapy was positive in 204 (18.5%) patients. The median follow-up time was 37 months. Multivariate Cox regressions including CTC status before chemotherapy showed significant independent prognostic role for CTC status 2 years after chemotherapy on OS (hazard ratio (HR) 3.95, 95% confidence interval (CI) 2.13 – 7.32, p < 0.001) and DFS (HR 2.28, 95% CI 1.48 – 3.50, p < 0.001). The prognostic value of CTC status 2 years after chemotherapy was independent from hormone- and HER2-receptor status. Overall, 719 (65.2%) patients were CTC negative before and 2 years after chemotherapy, while 157 (14.2%) had a negative CTC status before and a positive CTC status 2 years after chemotherapy. 180 (16.3%) patients converted from positive to negative CTC status and 47 (4.3%) patients were persistently positive for CTCs. There were significant differences in OS and DFS among the four patient groups (log rank tests, both p < 0.001) and persistently CTC positive patients had the worst outcome in terms of OS and DFS.

Conclusion: The presence of CTCs two years after chemotherapy analyzed during routine breast cancer follow-up care was associated with decreased survival. According to these results, persisting CTCs during long term follow-up independently predict patients’ outcome and may serve as surveillance marker.
2015 San Antonio Breast Cancer Symposium

Publication Number: S2-04

Title: Early survival analysis of the randomized phase II trial investigating the addition of carboplatin to neoadjuvant therapy for triple-negative and HER2-positive early breast cancer (GeparSixto)

von Minckwitz G, Loibl S, Schneeweiss A, Salat CT T, Rezai M, Zahm D-M, Klare P, Blohmer J-U, Tesch H, Khandan F, Fasching PA A, Jakisch C, Nekljudova V and Untch M. German Breast Group, Neu-Isenburg, Germany; University Hospital Heidelberg, Heidelberg, Germany; Hämatothologisch-Onkologische Schwerpunktpraxis Salat/Stoetzer, München, Germany; Luisenkrankenhaus, Düsseldorf, Germany; SRH Wald-Klinikum Gera, Gera, Germany; Praxisklinik Berlin, Berlin, Germany; Charité Breast Centre, Berlin, Germany; Onkologische Gemeinschaftspraxis am Bethanien-Krankenhaus, Frankfurt, Germany; St. Markus Krankenhaus, Frankfurt, Germany; University Hospital Erlangen, Erlangen, Germany; Sana Klinikumk Offenbach, Offenbach, Germany and Helios Klinikum Berlin-Buch, Berlin, Germany.

Body: Introduction
Addition of carboplatin to anthracycline/taxane-based neoadjuvant chemotherapy has shown to improve pathological complete response (pCR; ypT0 ypN0) rates in patients with triple-negative breast cancer in two large phase II studies (GeparSixto: von Minckwitz et al. Lancet Oncol 2014; CALGB 40603: Sikov et al. J Clin Oncol 2015). Participants of the GeparSixto study with triple-negative tumors showed an improvement of pCR rate from 36.9 to 53.2% by the addition of carboplatin (p=0.005); however, no statistically significant difference in pCR rate was observed in the HER2-positive subgroup (36.8 vs 32.8%, respectively). A greater benefit with carboplatin was observed in patients with BRCA mutations or a high homologous recombination deficiency (HRD score) in the tumor (pCR rate of 30% compared to 10% for patients without HRD). So far, it is unknown whether these effects on pCR translate into a survival benefit for the patients. We here report an early survival analysis of the GeparSixto study.

Patients and Methods
In the GeparSixto trial (NCT01426880), patients were treated for 18 weeks with paclitaxel 80mg/m² q1w and non-pegylated-liposomal doxorubicin (NPLD) 20mg/m² q1w. Patients with TNBC (N=315) received concurrently bevacizumab 15mg/kg i.v. q2w until surgery. Patients with HER2+ disease (N=273) received concurrently trastuzumab 6(8)mg/kg q3w and lapatinib 750mg daily. All patients were randomized 1:1 to receive concurrently carboplatin AUC 1.5-2.0 q1w vs no carboplatin, stratified by subtype (HER2+ vs TNBC). Carboplatin dose was reduced from AUC 2.0 to 1.5 by an amendment after 330 patients. Primary objective was pCR rate (ypT0 ypN0). Loco-regional invasive recurrence free survival (LRRFS), distant-disease-free survival (DDFS), invasive disease-free survival (IDFS), and overall survival (OS) were secondary objectives.

Results
595 patients were recruited (8/2011 - 12/2012) in 51 German centers. 296 patients were randomly assigned to receive carboplatin and 299 to no additional carboplatin, of whom 295 and 293 started treatment, respectively. So far, 82 events have been reported after a median of 28 months follow-up. Analysis of updated events by treatment arm in the full study population as well as in the TNBC and HRD subgroups will be presented.

Conclusion
Even if the GeparSixto study was not powered to show carboplatin effects on survival, the expected results will help to assess the overall benefit of carboplatin in TNBC and the power of pCR to predict for DFS and OS.
Title: Event-free and overall survival following neoadjuvant weekly paclitaxel and dose-dense AC +/- carboplatin and/or bevacizumab in triple-negative breast cancer: Outcomes from CALGB 40603 (Alliance)

Sikov WM M, Berry DA A, Perou CM M, Singh B, Cirrincione CT T, Tolaney SM M, Somlo G, Port ER R, Qamar R, Sturtz K, Mamounas E, Golshan M, Bellon JR R, Collyar D, Hahn OM M, Carey LA A, Hudis CA A and Winer EP P. Program in Women's Oncology, Women and Infants Hospital of Rhode Island and Alpert Medical School of Brown University, Providence, RI; MD Anderson Cancer Center, Houston, TX; UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC; New York University Medical Center, New York, NY; Alliance Statistical Center, Durham, NC; Dana Farber Cancer Institute, Boston, MA; City of Hope Comprehensive Cancer Center, Duarte, CA; Mount Sinai Medical Center, New York, NY; Aurora Health Care NCORP, Milwaukee, WI; Colorado Cancer Research Program, Denver, CO; UF Health Cancer Center Orlando, Orlando, FL; Patient Advocates in Research, Danville, CA; University of Chicago Medical Center, Chicago, IL; Memorial Sloan Kettering Cancer Center, New York, NY and Brigham and Women's Hospital, Boston, MA.

WITHELD PENDING PRESS CONFERENCE
2015 San Antonio Breast Cancer Symposium

Publication Number: S2-06

Title: Abstract Withdrawn

Body:
Title: cfDNA analysis from BOLERO-2 plasma samples identifies a high rate of ESR1 mutations: Exploratory analysis for prognostic and predictive correlation of mutations reveals different efficacy outcomes of endocrine therapy–based regimens

Chandarlapaty S, Sung P, Chen D, He W, Samoila A, You D, Bhatt T, Patel P, Voi M, Gnant M, Hortobagyi G, Baselga J and Moynahan ME Ellen. Memorial Sloan Kettering Cancer Center, New York, NY; Novartis Pharmaceuticals Corporation, East Hanover, NJ; Medical University of Vienna, Vienna, Austria and The University of Texas MD Anderson Cancer Center, Houston, TX.

WITHELD PENDING PRESS CONFERENCE
Title: A phase I trial of the safety and immunogenicity of a multiple antigen vaccine (STEMVAC) in HER2 negative advanced stage breast cancer patients

Higgins DM M, Childs JS S, Salazar LG G and Disis ML L. University of Washington-Tumor Vaccine Group, Seattle, WA.

Body: Background: Biologically relevant epithelial to mesenchymal transformation (EMT) associated proteins, a subpopulation of tumor cells with stem like properties, have been identified and are responsible for tumor initiation, metastasis formation and resistance to cancer therapies involved in breast cancer (BC) initiation. It is hypothesized that the acquisition of stem cell properties is driven by EMT induction and that BC stem cells express EMT-associated proteins. A vaccine which would educate the immune system to recognize and eliminate cells that have up-regulated proteins associated with BC stem cells/EMT could eradicate BC at the time of initiation or relapse. We have identified 5 stem cell/EMT proteins that are immunogenic in BC patients and created a vaccine, STEMVAC, composed of extended Th1 epitopes derived from these proteins. STEMVAC is safe and inhibits tumor growth in preclinical murine studies.

Trial design: Phase I dose escalation study evaluating 3 doses of STEMVAC admixed with 100 mcg of GMCSF. Patients enrolled sequentially into 1 of 3 dose arms (10 patients/arm): Arm 1=150 mcg, Arm 2=300 mcg, and Arm 3=600 mcg. 3 patients must complete 3 monthly vaccines and month 4 evaluation with no dose limiting toxicity before further accrual to that arm. Patients may receive 2 boosters, 3 and 9 months after their 3rd vaccine. Toxicity is assessed at baseline through end of study. Serial blood draws for immunologic monitoring is done.

Eligibility criteria: Stage III-IV HER2 negative BC patients treated with standard therapy who: (1) are without evidence of disease or have stable bone-only disease, (2) are 28 days from last chemotherapy, radiotherapy, systemic steroids, (3) have adequate blood counts, (4) have no active autoimmune disease. Endocrine therapy and bisphosphonates are allowed.

Specific aims: (1) Determine the safety of 3 escalating doses of STEMVAC, (2) Determine the most immunogenic dose, (3) Determine whether a STEMVAC Th1 polyepitope plasmid based vaccine elicits persistent T cell memory, and (4) Evaluate if STEMVAC modulates T regulatory and myeloid derived suppressor cells.

Statistical methods: Safety will be determined by laboratory and clinical parameters. Descriptive statistics will be used to summarize changes from baseline. Safety benchmarks to move to the next arm will be grade 3 toxicity rate of ≤ 15% and grade 4 toxicity rate of ≤ 5% in the first 3 vaccinations. Immunogenicity evaluated by generation of antigen specific Th1 immunity via ELISPOT. Immunologic efficacy is defined as achievement of significant increase in Th1 immunity for ≥50% of the immunizing antigens compared to baseline; and a greater proportion of patients developing T-cell immunity to a greater number of the antigens included in the vaccine. Exploratory analysis will be used to assess memory Th1 dominant immune response to all 5 antigens. Treg and MDSC will be defined as present or absent, and the probability of each will be estimated as a simple proportion.

Target accrual: 30 patients-no patients accrued to date.
2015 San Antonio Breast Cancer Symposium

Title: Pilot trial of a type I polarized autologous dendritic cell vaccine incorporating tumor blood vessel antigen-derived peptides in patients with metastatic breast cancer

Baar J, Storkus W, Finke J, Butterfield L, Lazarus H, Reese J, Brufsky A, Downes K, Budd GT and Fu P.  Case Comprehensive Cancer Center - Seidman Cancer Center, Cleveland, OH; Case Comprehensive Cancer Center - Taussig Cancer Center, Cleveland, OH and University of Pittsburgh Medical Center, Pittsburgh, PA.

Body: BACKGROUND. Cancer vaccines based on tumor-associated antigens are rarely curative in advanced cancer. This limitation relates to the heterogeneity of cancer due to defects in antigen presentation and altered immunophenotypes. Therefore, another method to promote anti-tumor immunity is to prime T cells against tumor-associated stromal cells. We have reported that IL-12 gene-therapy of established HLA-A2neg B16 melanomas in HLA-A2 transgenic (Tg) mice resulted in CD8+ T cell-mediated immunity against the host HLA-A2+ stromal cells within the tumor microenvironment (TME). We have also shown that vaccines based on a subset of tumor blood vessel antigen (TBVA)-derived peptides (DLK1310-318, EphA2883-891, HBB31-39, NRP1433-441, RGS55-13 and TEM1691-700) prevented HLA-A2neg MC38 tumor establishment and promoted the regression of melanomas in HLA-A2 Tg mice by CD8+ T cell targeting of HLA-A2+ pericytes and vascular endothelial cells in the TME.

TRIAL DESIGN. Based on this pre-clinical data, we are undertaking a Susan G. Komen-funded (IIR13261822; IND 15722) IRB-approved clinical trial of chemo-immunotherapy using the immunomodulatory drug gemcitabine (GEM) to suppress tumor infiltrating suppressor cells such as myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs) with a dendritic cell (DC) vaccine pulsed with the above six HLA-A2-presented TBVA-derived peptides (DC-TBVA) in 30 HLA-A2+ patients with metastatic breast cancer (MBC). Eligible patients will first undergo leukapheresis for the generation of the DC-TBVA vaccine. Patients will then receive 3 cycles of GEM, 1000 mg/m2 IV on days 1 and 8 of a 21-day cycle for 3 cycles. Patients will then receive the DC-TBVA vaccine administered twice intradermally 7 days apart.

ELIGIBILITY CRITERIA. Patients must be HLA-A2+ and have radiologically measurable MBC, an ECOG performance status of 0-1 and not have any active immune disorders. Prior GEM therapy is acceptable as long as the last dose was ≥ 3 months from registration on this study. Patients may not be on steroids.

SPECIFIC AIMS. The 4 specific aims are to 1) assess the safety of GEM + αDC1-TBVA vaccination, 2) assess the clinical response of MBC to GEM + αDC1-TBVA vaccination, 3) determine the clinical efficacy of GEM + αDC1-TBVA vaccination in generating Tc1 immunity, and 4) correlate changes in MDSC and Tregs with the generation of anti-TBVA Tc1-cell immunity.

STATISTICAL METHODS. Clinical response: if the response rate is less than 10%, then there is probability 0.05 or less of accepting the vaccine therapy; if the response rate is bigger than 32%, then the probability of rejecting the combination is less than 0.2. While the secondary goals of the study are exploratory, there is sufficient statistical power to identify moderate to large effects (i.e., there will be statistical power >.80 to detect changes from baseline in the different immune function parameters that are >0.6 standard deviations of the parameter.)

TARGET ACCRUAL. We will enroll 30 patients over 3 years, with the first patient expected to be enrolled in July 2015.

CONTACT INFORMATION. Joseph Baar, MD, PhD. Seidman Cancer Center. E-mail: joseph.baar@uhhospitals.org.
Title: Adoptive cell transfer (ACT) using tumor infiltrating lymphocytes to target neoantigens in patients with metastatic breast cancer

Goff SL L, Feldman SA A, Somerville R and Rosenberg SA A. Surgery Branch, National Cancer Institute, Bethesda, MD.

Body: Background: Adoptive transfer of tumor infiltrating lymphocytes (TIL) can cure patients with metastatic melanoma, likely based on the recognition of mutated neoantigens (Robbins et al Nature Medicine 2013). Although immunogenic cancer antigens have been found in gastrointestinal cancers (Tran et al Science 2014), this has not been widely studied in patients with breast cancer. The presence of TIL on pathologic examination of triple-negative breast cancers is a positive prognostic marker for disease-free survival and overall survival. This pilot study investigates the ability to grow TIL from breast cancer metastases, to identify personalized non-synonymous mutations and potential neoantigens, and to adoptively transfer TIL into patients with breast cancer.

Trial Design: This is a single-arm, non-randomized pilot study of adoptive immunotherapy in patients with metastatic epithelial cancers with a cohort designated for those patients with breast cancer. Once screened for eligibility, patients undergo metastectomy to obtain tissue for culture of TIL and extensive in vitro studies will be performed to identify TIL cultures reactive to neoantigens. Once robust TIL have been identified, the patient is admitted to the National Institutes of Health Clinical Center for conditioning chemotherapy, TIL infusion and interleukin-2. Treatment and recovery generally entails about three weeks as an inpatient.

Eligibility Criteria: Patients between the ages of 18 and 70 with metastatic breast cancer who have measurable metastatic disease with at least one lesion resectable with minimal morbidity. Patients must be refractory to standard systemic therapy and must have shown progression on at least two lines of chemotherapy prior to infusion of TIL. Patients must be of good performance status (ECOG 0-1) and have three or fewer brain metastases. In addition, patients must meet common hematologic and chemistry lab criteria. Given the nature of immunotherapy and the rigorous treatment, patients are ineligible for the following reasons: dependence on steroids, cardiac dysfunction, active infection, active major medical illness of the respiratory, cardiovascular or immune system.

Specific Aims: The aims are both clinical and research oriented. Of greatest interest is to determine the ability of autologous TIL to mediate tumor regression in patients with metastatic breast cancer. We will also be examining the phenotypic and functional characteristics of TIL derived from breast cancer metastases. We will be attempting to identify non-synonymous immunogenic mutations within resected tumors.

Statistical Methods and Trial Accrual: Twenty-one patients will be initially enrolled in the treatment phase of this cohort to assess toxicity and tumor responses. If two or more of the first 21 patients per groups shows a clinical response (PR or CR), accrual will continue to 41 patients, targeting a 20% goal for objective response. We have currently enrolled three patients on the screening phase of this trial and one patient on the treatment phase.

2015 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-04

Title: A multicenter, phase 1b, first-in-human dose-escalation study of ADXS31-164, a *Listeria monocytogenes*-LLO immunotherapy, in patients with HER2-expressing solid tumors

Tan AR R, Olszanski A, Golan T, Mauro D and Rugo H. Levine Cancer Institute, Charlotte, NC; Fox Chase Cancer Center, Philadelphia, PA; Sheba Medical Center, Tel HaShomer, Ramat Gan, Israel; Advaxis Inc., Princeton, NJ and UCSF, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Background: Wild type *Listeria monocytogenes* (*Lm*) is taken up by antigen-presenting cells (APCs) and has the capability to escape destruction in the phagolysosome and proliferate in the cytosol of the APC. ADXS31-164 is a live attenuated *Lm*-listeriolysin O (LLO) immunotherapy bioengineered to express the intracellular domain 1 and extracellular domains 1 and 2 of chimeric human epidermal growth factor receptor 2 (cHER2) as a fusion protein to a truncated form of the LLO (tLLO) in the cytoplasm of APCs. The resultant immunologic response generates tumor antigen-specific cytotoxic T lymphocytes while also inhibiting regulatory T cells and myeloid-derived suppressor cells in the tumor microenvironment. Preclinical studies have shown ADXS31-164 can delay the progression of tumors in both transplantable and autochthonous HER2-expressing mouse tumor models.

Trial Design: This is an open-label, multicenter Phase 1b trial (NCT02386501). Patients will receive ADXS31-164 every 3 weeks until progression of disease or unacceptable toxicity. Dose escalations will be performed according to a standard 3+3 design starting at $1 \times 10^9$ colony forming units (CFU) to a maximum dose level of $1 \times 10^{10}$ CFU. The maximum tolerated dose (MTD) will be identified as the dose level in which a dose-limiting toxicity is seen in 2 of 6 patients; the previous dose level will be selected as the recommended Phase 2 dose (RP2D). Once the MTD and RP2D have been identified, up to 4 HER2-overexpressing tumor-specific expansion cohorts will be evaluated. Treatment cycles can be repeated at the RP2D (or less) for each patient until a study discontinuation criterion is met or the subject completes 1 cycle of treatment post-observation of complete response. Blood samples will be evaluated for immunologic effects in cycle 1 only. Descriptive statistics will be used to evaluate the safety and tolerability of ADXS31-164.

Objectives: The primary aim of this trial is to evaluate safety and tolerability of ADXS31-164 in patients with solid tumors that express HER2, and to select the RP2D. Secondary objectives include tumor response rates and progression-free survival (measured by Response Evaluation Criteria In Solid Tumors [RECIST] 1.1 and immune-related RECIST criteria). Exploratory analyses will describe and evaluate data from correlative immunologic studies. Key Eligibility Criteria: Patients aged ≥18 years with HER2-positive tumors determined by fluorescence in situ hybridization (FISH) or immunohistochemistry (IHC, at least 1 positive in 1% of the evaluable tumor cells) and an Eastern Cooperative Oncology Group performance status of 0–1 are eligible. Additional criteria include a diagnosis of locally advanced/metastatic solid tumor that has progressed or become intolerant to standard therapy or for which no standard therapy is available, measurable and/or evaluable disease per RECIST 1.1, and a left ventricular ejection fraction within normal limits.
Body: Background: Immune-based strategies involving T-cell activation have recently shown significant activity in multiple tumor types. The presence of immune elements in breast cancers has prognostic and predictive impact. Thus, strategies that optimize the interplay between a breast cancer and the effected individual's immune system may be therapeutic. Interleukin-12 (IL-12), a pro-inflammatory cytokine, reverses immune escape mechanisms induced by myeloid derived suppressor and dendritic cells which, in turn, improves the function of activated CD8+ T cells and promotes tumor stroma collapse. Because tumor neoantigens may be generated in response to chemotherapy, IL12-mediated immune modulation may be optimal in patients with chemotherapy-sensitive metastatic breast cancer. Ad-RTS-hIL-12 (Ad) is a novel gene therapy candidate expressing IL-12 under the control of an orally-administered activator ligand, veledimex (V) through the proprietary RheoSwitch Therapeutic System® (RTS).

Trial Design: Open-label, phase 1b/2, single-arm, single-center study of Ad+V in women with stable or responsive disease after \( \geq 12 \) weeks of 1st or 2nd-line chemotherapy. Eligible patients will be placed on chemotherapy-holiday and enter the immunotherapy phase, consisting of a single cycle of Ad administered intratumorally (Day 1), along with V (80 mg QDx7). HER2-directed antibody therapy may be continued during the immunotherapy phase for women with HER2- disease.

Key Eligibility Criteria: Women \( \geq 18 \) years with histologically-confirmed locally advanced or metastatic breast cancer of any subtype who have achieved a partial response (PR) or stable disease (SD) to 1st or 2nd-line chemotherapy are eligible. Exclusion criteria include use of immunosuppressive drugs, compromised immune function, autoimmune disorder, or brain metastases.

Specific Aims: To evaluate the safety and tolerability of Ad+V immunotherapy in eligible women. Secondary endpoints include 12 week overall response rate, 12 week disease control rate and the impact of treatment on exploratory immune biomarkers.

Statistical Methods: Safety and efficacy will be evaluated separately for HER2-/HER2+ patients. Tumor response will be evaluated by RECIST v1.1 at 6 and 12 weeks. To ensure safety, stopping rules defined by grade 3/4 adverse events and 12-week progression rate were adopted.

Target Accrual: Up to 40 patients, including up to 8 patients (20%) with HER2+ disease.

Summary: Ad+V is a novel gene therapy which controls local expression of IL-12 and may induce tumor stroma collapse and stimulation of an anti-cancer T cell immune response. The ability to regulate the production of IL-12 by modulating V dosing may result in an improved therapeutic index in combination with standard of care. The data from this study will directly inform future studies.

Study Contact (Clinical Trials.gov: NCT02423902).
Title: A phase III randomized trial of atezolizumab in combination with nab-paclitaxel as first line therapy for patients with metastatic triple-negative breast cancer (mTNBC)

Emens L, Adams S, Loi S, Schmid P, Schneeweiss A, Rugo H, Chui S and Winer E. Johns Hopkins University School of Medicine, Baltimore, MD; New York University School of Medicine, NY, NY; Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia; Barts Cancer Institute, Queen Mary University London, London, United Kingdom; Heidelberg University Hospital, Heidelberg, Germany; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; Genentech, Inc., South San Francisco, CA and Dana-Farber Cancer Institute, Boston, MA.

Body: Background: The management of mTNBC is a therapeutic challenge, and chemotherapy remains the mainstay of treatment. Atezolizumab (atezo; MPDL3280A) is a humanized anti-PDL1 antibody that inhibits PD-L1 binding to PD-1 and B7.1 but leaves PD-L2/PD-1 binding intact. mTNBC has high levels of tumor-infiltrating immune cells (IC), increased PD-L1 expression and high mutation rates that may generate immunogenic neoantigens, making it an attractive candidate for PD-L1–targeted therapy with atezo. Accordingly, atezo monotherapy has demonstrated durable responses in mTNBC (Emens et al, AACR 2015). In addition, atezo combined with nab-paclitaxel has shown promising tolerability and activity in mTNBC (Adams et al, SABCS 2015; pending). Nab-paclitaxel has high anti-tumor activity that may favorably alter the immune microenvironment. Based on these preliminary results, a Phase III multicenter, randomized, double-blind, placebo-controlled trial (IMpassion130) was designed to evaluate the efficacy and safety of nab-paclitaxel combined with atezo as first-line therapy for mTNBC.

Methods: Patients are randomized 1:1 to receive atezo (840 mg) or placebo on days 1 and 15 plus nab-paclitaxel (100 mg/m²) on days 1, 8, and 15; all treatments are given on a 28-day cycle. Patients are stratified by the presence of liver metastases, prior taxane therapy and the PD-L1 status of IC (IC0 vs IC1/2/3). PD-L1 expression is centrally evaluated by immunohistochemistry using the SP142 assay. To capture pseudoprogression and delayed responses to atezo, patients with radiographic progression may continue to receive open-label atezo alone or with nab-paclitaxel until unacceptable toxicity or loss of clinical benefit.

Eligibility criteria: This study will enroll patients with histologically documented locally advanced or metastatic TNBC, no prior systemic therapy for advanced TNBC, ECOG PS 0-1 and measurable disease per RECIST v1.1. Patients with significant cardiovascular or CNS disease (except asymptomatic treated CNS metastases), autoimmune disease or prior immune checkpoint blockade therapy are excluded.

Endpoints: The co-primary efficacy endpoints are progression-free survival (PFS) in all patients and in PD-L1–selected patients. Secondary endpoints include overall survival, objective response rate, response duration, safety/tolerability, pharmacokinetics and health-related quality of life. Tumor biopsies are obtained at baseline and at progression to evaluate potential biomarkers associated with therapeutic response and resistance.

Statistical methods/target accrual: PFS will be compared between treatment arms (nab-paclitaxel vs. nab-paclitaxel plus atezo) using the stratified log-rank test. The hazard ratio for disease progression or death will be estimated using a stratified Cox proportional hazards model. Kaplan-Meier methodology will be used to estimate the median PFS for each treatment arm. About 350 patients will be enrolled at ≈ 120 sites globally.

Sponsor: Genentech, Inc. ClinicalTrials.gov identifier NCT02425891.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT1-01-07

Title: nab-paclitaxel (nab-P) plus nivolumab (Nivo) in human epidermal growth factor receptor 2 (HER2)–negative recurrent metastatic breast cancer (MBC)

Waterhouse D, Gutierrez M, Bekaii-Saab T, DeRosa W, Wainberg Z, George B, Duval Fraser C, Ko A, Pierce DW W, Stergiopoulos S and Soliman H.  Oncology Hematology Care Inc, Cincinnati, OH; John Theurer Cancer Center, Hackensack, NJ; Ohio State University Comprehensive Cancer Center, Columbus, OH; Regional Cancer Care Associates LLC, Morristown, NJ; UCLA Hematology/Oncology, Santa Monica, CA; Medical College of Wisconsin, Milwaukee, WI; Celgene Corporation, Summit, NJ; Celgene Corporation, Summit, NJ; Celgene Corporation, Summit, NJ; Celgene Corporation, Summit, NJ and Moffitt Cancer Center, Tampa, FL.

Body: Background: Nivo is an inhibitory antibody against programmed death receptor-1 (PD-1), a regulator of antitumor immunity. Nivo is approved for treatment of unresectable or metastatic melanoma and disease progression (PD) following ipilimumab or, in BRAF V600 mutation–positive melanoma, following a BRAF inhibitor, and for metastatic squamous non-small cell lung cancer (NSCLC) following PD during or after platinum-based chemotherapy. Nivo and other immune checkpoint inhibitors are also being investigated in other tumor-types. nab-P is a novel taxane formulation and does not require prophylaxis with immunosuppressive steroids. It has demonstrated superior efficacy over control regimens in phase III studies of MBC, pancreatic cancer, and NSCLC. This open-label, 6-arm, multicenter phase I trial will evaluate the safety of Nivo with nab-P in 3 cancer types (2 arms/disease): MBC, advanced NSCLC (+ carboplatin), and advanced pancreatic cancer (+ gemcitabine). The study design for the MBC portion is described below.

Methods: Eligibility criteria include histologically/cytologically confirmed HER2-negative MBC; 1 prior chemotherapy for MBC, including an anthracycline unless clinically contraindicated; no relapse < 12 months after taxane adjuvant therapy; measurable disease by RECIST v1.1; ECOG performance status 0-1; adequate organ function; and preexisting peripheral neuropathy grade < 2. Patients (pts) with MBC will be treated in 2 arms: nab-P 100 mg/m2 on days 1, 8, and 15 of each 28-day cycle plus Nivo 3 mg/kg on days 1 and 15 starting at cycle 3 or nab-P 260 mg/m2 on day 1 of each 21-day cycle plus Nivo 5 mg/kg on day 15 starting at cycle 3. Pts will be treated until PD or allowed to continue treatment beyond RECIST v1.1–defined PD if they continue to meet study eligibility; do not have rapid PD or clinical deterioration or unacceptable toxicities; and can benefit from continuation of study treatment in the treating physician's opinion and will not delay an imminent intervention to prevent serious complications of PD. The primary endpoints of the study are the number of pts with dose-limiting toxicities (DLTs) in each treatment arm (part 1) and the percentage of pts with grade 3/4 treatment-emergent adverse events (TEAEs) or treatment discontinuation due to a TEAE (parts 1 and 2). Part 1 of the study will assess whether the starting dose of Nivo is deemed safe (≤ 1 DLT in 6 pts); otherwise, the Nivo dose will be de-escalated and assessed in a new cohort at the next lower dose level. The Nivo dose in combination with nab-P deemed safe in a treatment arm may be further assessed in part 2 of the study, with enrollment expanded to an additional ~ 14 pts/arm (total of 20 Nivo-treated pts/arm). Secondary study endpoints include TEAEs leading to dose reduction, delay, interruption, or treatment discontinuation; progression-free survival; overall survival; disease control rate; overall response rate; and duration of response (per RECIST v1.1). Exploratory endpoints include tumor-associated PD-L1 expression, modulation of immune activation in the tumor and peripheral blood in response to Nivo treatment, Nivo serum levels, and development of anti-globulin antibodies. ClinicalTrials.gov identifier NCT02309177.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT1-02-01

Title: Pilot study of prognostic utility of circulating tumor cells (CTCs) assessed by AdnaGen technology and clinical outcome of patients with stage III breast cancer who completed locoregional and systemic treatment


Body: Background: Detection of high number of CTCs (>5) before initiation of first-line therapy in patients with metastatic breast cancer is associated with shorter progression free survival and overall survival. The most widely used method is CellSearch (Veridex, Raritan, NJ). It relies on immunomagnetic capture of CTCs, using antibodies against the epithelial cell adhesion molecule (EpCAM). Although the US Food and Drug Administration approved CellSearch assay for clinical use. In addition to isolation and enumeration, a promising area of research is genomic CTCs characterization which entails phenotyping and molecular expression profiling of CTC subsets consisting of those of epithelial origin (CTC-Epi), others undergoing epithelial to mesenchymal transition (CTC-EMT), or expressing cancer stem cell-like phenotype (CTC-CSC; CD44+ CD24low, ALDH+), respectively. EMT is a molecular process to acquire the traits needed to execute the multiple steps of metastasis. Through the EMT process, epithelial cells lose cell-cell contacts and cell polarity, downregulate epithelial-associated genes, acquire mesenchymal gene expression and undergo major changes in their cytoskeleton. Currently, a CTC detection kit is available to detect CTCs expressing EMT-associated genes by semiquantitative RT-PCR (Adna EMT2/Stem Cell test). EMT will be detected by measuring EMT-inducing transcription factors such as TWIST1, SNAIL1, SLUG, ZEB1 and FOXC2) by RT-PCR

Objectives. Primary objective: To investigate if activated pathways in CTCs are correlated with clinical outcome of patient with stage III breast cancer. Secondary objective: To prospectively determine if assessment of the pathways profiling in CTCs can be used to stratify NED breast cancer patients

Patients Eligibility: Inclusion: histologically confirmed invasive breast cancer (any subtype), clinical stage III, no evidence of distant metastasis by PET-CT or CT scan of chest and abdomen, and body scan, age 18 years or older, pts must be scheduled to start neoadjuvant/adjuvant therapy, ECOG PS 0-2. Pts must sign a written informed consent. Exclusion: distant metastasis, investigational therapy, prior history of other malignancies within the last 2 years, except non-melanoma skin cancer. This study (PA12-0097) was approved by IRB of UT MD Anderson Cancer Center.

Trial Design. This is a pilot, international, multicenter, prospective, blood sample collection from 200 patients with clinical or pathologic stage III breast cancer.

Statistical Analysis: This study is a 7-year study (84 months). Pts will be classified as to the presence [negative (neg) vs. positive (pos)] of CTC and as to the expression of a biomarker (neg vs. pos). The primary endpoint of the study is breast cancer recurrence. Time to recurrence curves for the four breast cancer patient groups (neg/neg, neg/pos, pos/neg, or pos/pos) will be estimated using the Kaplan-Meier method and differences in the recurrence rates will be evaluated by the log-rank test at the end of the study (84 months). The confidence intervals for the quantiles of the recurrence distribution will be based on the sign test as described by Brookmeyer and Crowley.
Title: The DETECT study program – Personalized treatment in metastatic breast cancer based on circulating tumor cells

Schramm A, Friedl TWP WP, Huober J, Jäger B, Rack B, Trapp E, Fasching PA A, Taran F-A, Hartkopf A, Schneeweiss A, Müller V, Aktas B, Pantel K, Meier-Stiegen F, Wimberger P, Kümmel S, Gebauer G, Müller L, Janni W and Fehm T. University Hospital Ulm, Ulm, Germany; Gynecology and Obstetrics, Klinikum der Ludwig-Maximilians-Universität, Munich, Germany; Gynecology and Obstetrics, University Hospital Erlangen, Erlangen, Germany; Gynecology and Obstetrics, University Hospital Tübingen, Tuebingen, Germany; University Hospital Heidelberg, Heidelberg, Germany; Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Essen, Essen, Germany; University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Essen, Essen, Germany; University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany; Gynecology and Obstetrics, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Gynecology and Obstetrics, University Hospital Dresden, Technische Universität Dresden, Dresden, Germany; Interdisciplinary Breast Center Unit Kliniken-Essen-Mitte, Essen, Germany; Gynecology Marienhospital Hamburg, Hamburg, Germany and Private Practice Onkologie UnterEms, Leer Ostfriesland Leer, Leer, Germany.

Body: Circulating tumor cells (CTCs) are found in patients with primary and metastatic breast cancer (MBC), respectively, and discordance in HER2 and hormone-receptor status between primary tumor, metastases and CTCs is well described. Treatment decisions are still based on the expression profile of solid tumor samples whereas CTCs are thought to cause tumor progression by blood-derived metastases. Nevertheless, targeted therapy based on expression profile of CTCs is not established in clinical routine. Individualized treatment decisions based on presence and phenotype of CTCs will be analyzed within the DETECT study program.

Metastatic breast cancer patients with HER2-negative MBC are screened in DETECT III and IV for presence of CTCs by using the CellSearch System (Janssen Diagnostics) which is FDA approved for enumeration of CTCs. Patients are enrolled into the different cohorts according to HER2-phenotype of CTCs. Since February 2012, women with HER2-negative MBC and HER2-positive CTCs are treated in the multicenter randomized Phase III study DETECT III with standard therapy with or without additional HER2-targeted therapy with Lapatinib. For standard therapy, physicians can choose between exemestane, letrozole and anastrozole for endocrine therapy, or docetaxel, paclitaxel, capecitabine, vinorelbine and non-pegylated liposomal doxorubicin for chemotherapy. Efficacy of CTC-based anti-HER2 treatment is evaluated by analyzing CTC-clearance rate after treatment.

Patients with only HER2-negative CTCs are recruited for the multicenter open-label phase II study DETECT IV. Since December 2013, women with hormone-receptor positive MBC receive endocrine therapy (tamoxifen, exemestane, letrozole or anastrozole) plus everolimus in DETECT IVa. In February 2015, DETECT IV was extended by the eribulin-cohort which offers a cytotoxic treatment with eribulin for women with triple-negative or hormone-receptor positive, chemotherapy demanding MBC (DETECT IVb). Progression free survival is used for assessment of clinical efficacy with overall survival and disease control rate as secondary objectives.

DETECT V, a multicenter open-label phase III study starting in summer 2015, randomizes patients with hormone-receptor positive, HER2-positive MBC to a dual HER2 targeted therapy (Trastuzumab and Pertuzumab) combined with either endocrine therapy or cytotoxic treatment. Quality of life determined by occurrence of adverse events is compared between both treatment arms. For prediction of endocrine treatment response, an “Endocrine Responsiveness Score” is calculated based on expression of estrogen-receptor and HER2 on detected CTCs.

More than 1200 patients are already screened in the DETECT study concept. Thus, it is the worldwide largest study concept with therapy decisions resulting from CTC-testing and CTC-phenotypization. The accompanying translational research programs evaluates further markers for molecular characterization of CTCs and prediction of therapy response.

Conclusion and Contact
The value of CTC phenotypes for making decisions on therapy interventions and predicting treatment responses in patients with MBC is tested in the DETECT study concept. The findings will help to move a step forward towards a more personalized anti-cancer therapy.
**Title:** A phase II study of single-agent PF-03084014 in patients with advanced triple-negative breast cancer with or without activating genomic alterations in NOTCH receptors

**Body:** Background: PF-03084014 is a reversible, noncompetitive, selective gamma-secretase inhibitor that blocks the NOTCH signaling pathway. Pre-clinical studies have demonstrated that PF-03084014 has strong anti-tumor activity in a subset of breast cancer models that harbor NOTCH receptor activating genomic alterations (NA+). Genomic data from the Cancer Genome Atlas have also shown that the NOTCH pathway is altered via multiple mechanisms in about 13% of patients (pts) with triple-negative breast cancer (TNBC). These results, together with the need for more effective therapies for TNBC, support the evaluation of PF-03084014 as single-agent treatment in pts with NA+ TNBC.

Study design: A8641020 is a multi-center, open-label, single-arm phase II study investigating the administration of PF-03084014 as a single-agent for the treatment of pts with NA+ TNBC. In addition, the study includes a subset of pts whose tumors test negative for activating genomic alterations in NOTCH receptors (NA-), to evaluate in pts the antitumor activity of PF-03084014 previously observed in pre-clinical animal models of NA- TNBC. Other eligibility criteria include ECOG PS ≤2, measurable disease, and availability of tumor tissue for central profiling of NOTCH genomic alterations (archival or de-novo specimens). For NA- TNBC pts, at least 1 prior line of therapy for advanced disease is required to enter the study. Pts will be enrolled in 2 parallel cohorts: cohort 1, n = 15 pts with NA+ TNBC and cohort 2, n = 15 pts with NA- TNBC. All pts will receive PF-03084014 at the starting dose of 150 mg BID given orally and continuously in 21-day cycles. Treatment will continue until disease progression, patient refusal, or unacceptable toxicity.

Endpoints: The primary endpoint is objective response (OR) in pts with NA+ TNBC, as assessed using RECIST version 1.1. Secondary endpoints include OR in pts with NA- TNBC, progression-free survival, duration of response, one-year survival, overall survival, and pharmacodynamic effects of PF-03084014 in tumor specimens and peripheral blood.

Statistical methods: The final analysis of the primary endpoint is planned after 15 response-evaluable pts with NA+ TNBC have been treated.

Accrual: Approximately 30 pts will be enrolled across Europe and North America. As of April 2015, 8 pts were accrued. Reference Study ID Numbers: NCT02299635; 2014-002286-30.
Title: Evaluation of the use of oral care to prevent oral mucositis in estrogen receptor positive metastatic breast cancer patients treated with everolimus: Phase III randomized control trial

Niikura N, Ohta Y, Hayashi N, Naito M, Kashiwabara K, Watanabe K, Yamashita T, Mukai H and Umeda M. Tokai University School of Medicine; St. Luke's International Hospital; Nagoya University Graduate School of Medicine; University of Tokyo; Hokkaido Cancer Center; Cancer and Infectious Diseases Center Tokyo Metropolitan Komagome Hospital; National Cancer Center Hospital East and Nagasaki University Graduate School of Biomedical Sciences.

Body: Background:
In patients with estrogen receptor (ER)-positive advanced breast cancer, everolimus plus exemestane prolongs progression-free survival compared to exemestane monotherapy. However, as an adverse event from everolimus, oral mucositis (all grades) has been reported in 58% of all patients and 81% of Asian patients. Although no established prevention method is available, a previous study reported that professional oral care might prevent oral mucositis, and dentists have hypothesized that such care can reduce the occurrence of oral mucositis induced by everolimus. To evaluate this hypothesis, we compare the incidence of oral mucositis with and without professional oral care.

Method:
This is a randomized, multi-center, open-label, phase III study to evaluate the efficacy of professional oral care in preventing oral mucositis induced by everolimus in postmenopausal ER-positive metastatic breast cancer (MBC). Patients will be randomized into professional oral care and control groups (1:1 ratio). All patients will receive everolimus (10 mg daily) with exemestane (25 mg daily) and will continue everolimus until disease progression. Before the initiation of everolimus, instruction on a professional brushing method will be provided to both groups by specialists. In the professional oral care group, patients will receive teeth surface cleaning, scaling, and tongue cleaning before starting everolimus, and will continue to receive professional oral care weekly from oral surgeons throughout the 8 week treatment. In the control group, patients will brush their own teeth and gargle with 0.9% sodium chloride solution or water. The primary endpoint is the incidence of all grades of oral mucositis. The secondary endpoints are the incidence of over grade 2 and over 3 oral mucositis as determined by an oncologist and oral surgeons. The endpoints include onset and duration of oral mucositis. Major eligibility criteria include: 1) Postmenopausal women with ER positive MBC, and 2) No more than one prior chemotherapy treatment for MBC. Target accrual is 200 patients with a two-sided type I error rate of 5% and 80% power to detect 25% risk reduction. This study has just begun, and 5 of a planned 200 patients have been enrolled. (This study was registered with the UMIN 000016109).
Title: FINESSE - An open, 3-cohort, phase II trial testing oral administration of lucitanib in patients with FGFR1-amplified or non-amplified oestrogen receptor positive metastatic breast cancer

Andre F, Daly F, Azim Jr HA A, Agrapart V, Fumagalli D, Gingras I, Guitart M, Lange A, Turner NC C, Pierrat M-J, Loibl S, Poiret C, Curigliano G, Loi S, Pallis A, Piccart M and Cortes J. Institut Gustave Roussy, Villejuif Cedex, France; Frontier Science (Scotland) Ltd, Kincraig, United Kingdom; Breast Data Centre, Institut Jules Bordet, Brussels, Belgium; Laboratoires Servier, Surennes, France; Breast International Group, Brussels, Belgium; The Royal Marsden NHS Foundation Trust, London, United Kingdom; German Breast Group/Sana Klinikum Offenbach, Neu-Isenburg, Germany; Istituto Europeo di Oncologia, Milan, Italy; Translational Breast Cancer Genomics Lab Peter MacCallum Cancer Centre, Melbourne, Australia and Vall d’Hebron University Hospital, Barcelona, Spain.

Body: Background: Lucitanib is a potent, oral inhibitor of the tyrosine kinase activity of Fibroblast Growth Factor Receptors 1-3 (FGFR1-3), Vascular Endothelial Growth Factor Receptors 1-3 (VEGFR1-3) and Platelet-Derived Growth Factor Receptors α/β (PDGFRα/β). FGF aberrancy, as defined by amplification of either FGFR1, or 11q (containing FGF ligands 3, 4, CCND1, and 19), or both, is a hallmark genomic alteration that can be observed in up to 25% of patients with breast cancer. In a phase I clinical trial of lucitanib at daily doses of 5 to 20 mg, heavily pretreated patients with advanced breast cancer patients and FGF aberrancy experienced an objective response rate (ORR) of 50% and a median progression-free survival (PFS) over 9 months (Soria et al, 2014). This compelling clinical activity has led to the initiation of a global clinical development program for lucitanib in breast cancer.

Trial design: this is a phase II trial testing the efficacy of lucitanib at the dose of 15 mg daily in patients with ER+/HER2- metastatic breast cancer who have received at least one first-line systemic anticancer therapy in the metastatic setting. After informed consent, metastatic tissue (fresh biopsy or archival) is centrally evaluated by FISH for FGFR1- and/or 11q- amplification. Based on FISH results, patients are allocated to cohort 1 (FGFR1-amp), cohort 2 (11q-amp) or cohort 3 (neither). Patients with dual amplification are allocated to cohort 1. The primary objective is to evaluate the ORR of single agent lucitanib in the three cohorts. Secondary objectives include clinical benefit rate, PFS, safety and pharmacokinetics in addition to exploratory biomarker analyses. A Simon two-stage design will be used for each of the cohorts to test the null hypothesis that the ORR is 5% or less versus 20% using a one-sided test with 5% level of significance and 90% power. In each cohort separately, an initial 21 patients with measurable disease at baseline will be assessed at the end of stage 1. If at least 2 patients respond per the pre-specified criteria, this cohort will accrue additional 20 patients. The null hypothesis will be rejected if there are at least 5 responders among all 41 patients.

Eligibility Criteria: ER+/HER2- metastatic breast cancer who have received at least a first line of systemic anticancer therapy and no more than 2 line of chemotherapy with or without targeted therapy in the metastatic setting and have ECOG performance status ≤ 2. Patients with uncontrolled hypertension and at risk of developing hypertension related complications are not eligible.

Conclusion: FINESSE is a phase II trial testing lucitanib, a multikinase inhibitor, in three selected populations in order to investigate the ORR in FGFR1 or 11q amplified or non-amplified populations and to explore the role of FGFR1 or 11q amplifications through correlative translational analyses. As of May 21st 2015, 40 patients have been enrolled, 19 of them in the FGFR1-amplified arm.
Title: A phase 2 study of abemaciclib in patients with brain metastases secondary to hormone receptor positive breast cancer

Background:
Abemaciclib, an oral drug administered twice daily on a continuous schedule, is an inhibitor of both CDK4 and CDK6. In study JPBA, abemaciclib demonstrated evidence of single-agent activity in a cohort of patients with heavily pretreated metastatic breast cancer (MBC, median of 7 prior therapies); all responses were observed in women with hormone receptor positive (HR+) disease. Preclinical results demonstrating that abemaciclib crosses the blood-brain barrier coupled with the clinical responses observed in study JPBA support further investigation of abemaciclib in the current phase 2 trial (JPBO) of patients with brain metastases secondary to HR+ breast cancer.

Trial design:
Study JPBO (NCT02308020) is an open-label, phase 2 trial that will evaluate the safety and efficacy of abemaciclib 200 mg administered orally every 12 hours in patients with HR+ MBC and brain metastases. The study will consist of 3 parts; 2 of these parts will each accrue from 23 to 56 patients. These 2 parts will include patients with HER2+ breast cancer (Part A) and HER2- breast cancer (Part B). Part C will include approximately 8 MBC patients with either HER2+ or HER2- disease who have 1 to 3 intracranial lesions and for whom surgical resection is clinically indicated, with the goal of assessing drug concentrations in plasma, CSF, and brain tumor tissue. These patients may resume abemaciclib post-operatively.

Eligibility criteria:
Eligible patients include women with HR+ MBC who have completed local therapy ≥14 days prior to abemaciclib treatment, a life expectancy ≥12 weeks, and a Karnofsky performance status of ≥70. Part A includes MBC with confirmed HER2 overexpression and/or amplification (HER2+) status. Part B includes MBC that does not demonstrate HER2 overexpression and/or amplification (HER2-). For Parts A and B, patients will have ≥1 new or not previously irradiated measurable metastatic brain lesion ≥10 mm in the longest diameter or a progressive previously irradiated metastatic brain lesion identified by gadolinium-enhanced MRI. For Part C (surgical), patients have either HER2+ or HER2- MBC with brain lesion(s) for which surgical resection is clinically indicated and agree to provide post-treatment brain tumor tissue.

Specific aims:
The primary efficacy measure is objective intracranial response rate (complete response + partial response) as defined by Response Assessment in Neuro-Oncology brain metastases response criteria. Secondary intracranial objectives include best overall response, duration of response, disease control rate, and clinical benefit rate. The following overall objectives (intracranial + extracranial) include: overall survival, objective response rate, and progression-free survival. Change in neurologic symptoms will also be assessed.

Statistical methods:
Two separate Simon 2-stage designs will be employed for Part A and Part B. Each design assumes a 1-sided type-I error of 0.05 and 80% power. All tests will be conducted at a 2-sided alpha level of 0.05, unless otherwise stated. All confidence intervals will be given at a 2-sided 95% level.

Target accrual:
Approximately 120 patients.

Contact information:
For further information, please contact 1-877-CTLILLY (1-877-285-4559).
A phase III, randomized, open label, multicenter, controlled trial of niraparib versus physician’s choice in previously-treated, HER2 negative, germline BRCA mutation-positive breast cancer patients. An EORTC-BIG intergroup study (BRAVO study)

Balmana J, Tryfonidis K, Audeh W, Goulioti T, Slaets L, Agarwal S, Lema N, Cameron D and Turner N. Val d’Hebron, University Hospital, Barcelona, Spain; EORTC Headquarters, Brussels, Belgium; Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles; Breast International Group, Brussels, Belgium; TESARO, Inc, Waltham, MA; University of Edinburgh, Edinburgh, United Kingdom and The Royal Marsden NHS Foundation Trust, London, United Kingdom.

**Background**
Germline BRCA mutations (gBRCAmut) amongst breast cancer (BC) patients ranges between 5% - 25% depending on family history of BC or ovarian cancer (OC). Cells treated with PARP inhibitors (PARPi) accumulate defects that lead to their death in the absence of homologous recombination (HR) repair (e.g. gBRCAmut). Niraparib is a PARPi in development. As the TNT study showed better results of 1st line carboplatin vs docetaxel in gBRCAmut patients, a rise of a platinum-exposed population is expected. Due to prior experience in OC where platinum sensitivity of HR deficient cells is linked with PARPi sensitivity, it seems that patients who progress on or soon after platinum should be excluded from PARPi therapy, without sparing from PARPi those who may retain sensitivity. The protocol was amended to include platinum-exposed patients.

**Trial Design**
Bravo is a multicenter (North America, Europe & Israel) EORTC & BIG phase III trial sponsored by TESARO. Patients with gBRCAmut will be randomized (2:1) to niraparib (per os) vs physician’s choice (PC) (eribulin or capecitabine or gemcitabine or vinorelbine)-NCT01905592.

**Eligibility Criteria**
- Patients with deleterious/suspected deleterious gBRCAmut locally or centrally. Central confirmation will be done. If post inclusion, based on a previous test, mutation is not centrally confirmed, they can participate based on their physician/own preference.
- Up to 2 prior chemotherapy lines for metastatic disease (MD).
- Previously untreated for MD are allowed if they relapse during/within 12 months of (neo-) adjuvant chemotherapy.
- Prior therapy must include a taxane and/or anthracycline. Previously received platinum in the MD can be enrolled if they did not progress while on or within 8 weeks from the last day of the platinum administration. Those who received (neo-) adjuvant platinum are eligible, if relapsed 12 or > months after the last platinum dose.

**Specific aims**
Primary endpoint is PFS assessed by blinded central review. Secondary are OS, safety, PFS on local investigator assessment, time to treatment failure, response & duration of response, health-related quality assessments & tests for companion diagnostic test development.

**Statistical methods**
The PFS analysis will be done after 232 PFS events in the population of centrally confirmed gBRCAmut randomized patients. Assuming that median PFS is 3 months for PC & 6 months for niraparib (hazard ratio=0.5), there is 99.6 % power (1-sided alpha=0.025) to detect a difference from 3 - 6 months. Sample size ensure power for an OS comparison. Assuming an increase in OS from 9 -13 months, with a hazard ratio of 0.69, there is 80% power at 1- sided alpha of 0.025 when 265 deaths are observed. Assuming 40% of patients will be randomized based on local test and 15% won't be mutated centrally, an over-enrollment of 18 patients is needed to obtain the 306 in the efficacy population. A futility interim analysis will happen at 40% of PFS events.

**Present accrual and target accrual**
1200 patients will be registered & 306 randomized. Accrual will finish in 2 years.
Title: LORELEI: A phase II randomized, double-blind study of neoadjuvant letrozole plus taselisib (GDC-0032) versus letrozole plus placebo in postmenopausal women with ER-positive/HER2-negative, early-stage breast cancer

Oliveira M, de Azambuja E, Saura C, Dubsky P, Zardavas D, Fesl C, Bardia A, Soberino J, Ciruelos Gil E, Ng V, Fredrickson J, Stout TJ, Singel SM, Hsu JY, Piccart M, Grant M and Baselga J. Vall d’Hebron University Hospital, Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain; SOLTI Breast Cancer Research Group, Barcelona, Spain; Jules Bordet Institute, Brussels, Belgium; Breast Data Centre at the Jules Bordet Institute, Brussels, Belgium; Medical University of Vienna, Vienna, Austria; ABCSG Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria; Breast International Group (BIG aisbl), Brussels, Belgium; Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA; 12 de Octubre University Hospital, Madrid, Spain; Genentech, Inc., South San Francisco, CA and Memorial Sloan-Kettering Cancer Center, NY, NY.

Body: Background: Taselisib is an orally bioavailable, potent, selective inhibitor of Class I PI3-kinase (PI3K) alpha, gamma, and delta isoforms, with 30-fold less inhibition of the PI3K beta isoform relative to the alpha isoform showing enhanced activity against PIK3CA mutant cancer cell lines. Clinical data have demonstrated confirmed partial responses in patients with PIK3CA mutant breast cancer (BC) treated with single-agent taselisib. Enhanced antitumor activity has been noted when taselisib is combined with either letrozole or fulvestrant in preclinical and Phase Ib clinical studies.

Methods: LORELEI is a Phase II, two-arm, randomized, double-blind, multicenter, study of neoadjuvant letrozole and taselisib versus letrozole and placebo in postmenopausal women with newly diagnosed ER+/HER2-, untreated, Stage I-III operable BC. Other eligibility criteria include tumor size ≤2 cm by magnetic resonance imaging (MRI), ECOG PS 0-1, and evaluable tumor tissue for PIK3CA genotyping. Patients treated with anti-diabetic drugs are not eligible. Patients are randomized (1:1) to receive continuous 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. Stratification is based on tumor size and nodal status. The co-primary endpoints are overall objective response rate (ORR) by centrally assessed breast MRI via modified RECIST criteria and pathologic complete response (pCR) rate in breast and axilla at time of surgery in all randomized patients and PIK3CA mutant patients. Secondary endpoints include ORR by centrally-assessed MRI and pCR rate in PIK3CA wild-type patients. The sample size was calculated to detect an absolute percentage increase of 24% in ORR with 80% power and an absolute percentage increase of 18% in pCR rate. An interim safety analysis will be conducted by an Independent Data Monitoring Committee. As of 1st Jun 2015, 54 of the 330 patients have been enrolled, and global enrollment is ongoing (clinicaltrials.gov NCT02273973).

Contact information:
Reference Study ID Numbers: GO28888/BIG-3-13/SOLTI 1205/ABCSG 38
Phone: 888-662-6728 (US Only)
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Body: Background: Breast cancer stem cells (BCSC) have the ability to self renew and generate the full range of cells that make up a bulk tumor. Experimental models and retrospective clinical observations point to BCSC as responsible for tumor recurrence and metastasis. CXCR1, one of the receptors for CXCL8, has been identified on BCSC. Reparixin, an allosteric inhibitor of CXCR1, reduced BCSC in breast cancer xenografts (Ginestier C et al., JCI 2010) both as single agent and in combination with taxane chemotherapy. In a phase Ib trial in women with metastatic HER2-negative BC, the combination of escalating doses (400 to 1200 mg three times per day) of reparixin with weekly paclitaxel resulted in a low incidence and severity of adverse reactions, a sizeable response rate and time-to-progression, with some long-term responders (Schott AF et al., SABC 2014).

Trial Design: In this randomized, double-blind phase 2 trial patients will be randomized (1:1) to paclitaxel 80 mg/m2 on days 1, 8 and 15 of 28-day cycles in combination with reparixin or placebo oral tablets 1200 mg three times daily on days 1-21. Treatment continues until disease progression, unacceptable toxicity or withdrawal of consent. An independent Data Monitoring Committee has been appointed to oversee the trial. An independent Radiology Review will be performed for analysis of primary and secondary endpoints. Disease response will be assessed every 8 weeks. Patients will be followed up to 12 months after last enrolled patient completes treatment.

Eligibility Criteria: Patients must be female aged ≥18 years with untreated metastatic TNBC who have relapsed >12 and >6 months after the end of a taxane- or non taxane-based (neo)adjuvant chemotherapy regimen, respectively. They must have measurable disease, ECOG PS of 0-1, adequate organ function, and no history or evidence of brain metastases (brain CT or MRI required). Tumor tissue must be available from a metastatic site or from primary tumor for confirmation of diagnosis and correlative studies. Key exclusion criteria are pre-existing peripheral neuropathy G>1 and any disease significantly affecting gastrointestinal function.

Specific Aims: Primary: to evaluate progression-free survival (PFS) rate by independent assessment. Secondary: to determine median PFS, overall survival (OS), objective response rates and safety of the combination treatment. Exploratory: to determine median time to new tumor metastasis (TTM), proportion of patients progressing with new metastatic lesions, incidence and severity of peripheral neuropathy, and to evaluate BCSC in metastatic tissue

Statistical Methods: The trial design provides 80% power to detect an increase in 6 month PFS from 30% to 50% with a 2-sided 5% significance level (Chi-square test). Kaplan-Meier curves will be produced for median PFS, OS outcomes and exploratory median TTM. Appropriate descriptive statistics will be provided for safety variables.

Present Accrual and Target Accrual: Target accrual is 190 patients. Patients will be enrolled internationally in US and Europe.

Contact Information: info@dompe.com
2015 San Antonio Breast Cancer Symposium

**Publication Number:** OT1-03-08

**Title:** DESIREE - A multicenter, randomized, double-blind, phase II study to evaluate the tolerability of an induction dose escalation of everolimus in patients with metastatic breast cancer

Loibl S, Furlanetto J, Barinoff J, Bauerschlag D, Herr D, Lübbe K, Maass N, Müller V, Mundhenke C, Schmidt M, Schwedler K, Thill M, Gkantrigas I, Burchardi N and von Minckwitz G. German Breast Group, Neu-Isenburg, Germany; Sana Klinikum Offenbach, Offenbach, Germany; Agaplesion Markus Krankenhaus, Frankfurt am Main, Germany; Universitätsklinikum Schleswig-Holstein, Kiel, Germany; University Hospital Würzburg, Würzburg, Germany; Diakoniekrankenhaus Henriettenstiftung, Hannover, Germany; University Hospital Hamburg-Eppendorf, Hamburg, Germany; University Hospital Schleswig-Holstein, Kiel, Germany; University Hospital Mainz, Mainz, Germany and Kantonspital Luzern, Luzern, Germany.

**Body:**

**Background:** The BOLERO-2 study demonstrated a relevant benefit for patients who received everolimus in addition to exemestane in patients who progressed during/after a non steroidal aromatase inhibitor (NSAI), which led to approval of everolimus in this indication. However, in routine use a high rate of intolerability due to side effects is reported. The most common side effect of everolimus is mucositis with a reported high rate of intolerability especially during the first 12 weeks of treatment. Mucositis is also considered to be the leading cause for treatment discontinuation not related to tumor progression. In the neoadjuvant GeparQuinto study, a dose-escalation schema was successfully used to improve tolerability of everolimus together with cytotoxic agents.

**Methods:** DESIREE (NCT02387099) is a randomized, double-blind, phase II study of everolimus in addition to exemestane in patients who progressed during or after NSAI. Patients will be randomized in a 1:1 ratio to receive either everolimus 10 mg/day (week 1-3: 4x2.5 mg/day, blinded; week 4-24: 10mg/day, open according to label) or an escalating dose of everolimus as follows: week 1: 1x2.5 mg verum + 3x placebo/day; week 2: 2x2.5 mg verum + 2x placebo/day; week 3: 3x2.5 mg verum + 1x placebo/day; week 4-24: 10 mg/day (open according to label).

The primary aim of the study is to evaluate the incidence of the first episode of mucositis grade 2-4 (WHO's oral toxicity scale) any time during a 12 week period after start of everolimus treatment. Secondary objectives are to compare the cumulative rate of mucositis grade 2-4 at 24 weeks after start of treatment, the cumulative rate of mucositis grade 1 and any grade at 12 and 24 weeks after start of treatment, the rate of patients on 10 mg daily at 12 weeks and 24 weeks after start of treatment, the clinical benefit rate after 24 weeks, safety, time to onset of grade ≥2 mucositis, the cumulative everolimus dose at 4 weeks, the relative dose intensity for everolimus, and quality of life using the FACT-B and the QSDQ questionnaire. Biomaterial (whole blood, serum, plasma and optional primary tumor/metastasis tissue) will be collected to evaluate potential biomarkers predicting safety and compliance. Overall, 156 evaluable patients (78 in each arm) are required to detect a clinically relevant difference of 20% in the mucositis rate between treatment arms using a continuity-corrected χ²-test on a significance two sided level alpha of 0.2 and a power of 90%. The rate was estimated to be 40% and 20% in the control arm and the treatment arm, respectively.

**Results:** The study will be conducted in up to 60 German centers. Recruitment will start in June 2015. Enrollment is planned to be completed within 24 months.

**Conclusion:** The combination of everolimus and exemestane has shown to improve the outcome of patients with metastatic breast cancer. In the DESIREE trial a dose-escalating schema will be employed to enhance patient compliance and tolerability necessary to achieve an adequate dose-intensity.
Title: FAIRLANE: A phase II randomized, double-blind, study of the Akt inhibitor ipatasertib (Ipat, GDC-0068) in combination with paclitaxel (Pac) as neoadjuvant treatment for early stage triple-negative breast cancer (TNBC)

Saura C, Isakoff SJ J, Calvo I, Patt D, Andersen J, Gonzalez-Martin A, Fisher J, Ciruelos E, Gil-Gil M, De la Peña L, Choi Y, Jia S, Singel S, Patel PH H, Baselga J and Oliveira M. Vall d'Hebron Institute of Oncology (VHIO); SOLTI Breast Cancer Research Group; Massachusetts General Hospital Cancer Center; Centro Integral Oncológico Clara Campal; Texas Oncology Center; Compass Oncology; MD Anderson Cancer Center; Carolinas Healthcare System; Hospital Universitario 12 de Octubre; ICO L'Hospitalet, Barcelona; Genentech and Memorial Sloan-Kettering Cancer Center.

Body: Background: TNBC often exhibits activation of PI3K/Akt signaling, associated with loss of PTEN expression, low INPP4B expression, and/or increased AKT3 amplification. Inhibition of the PI3K/Akt pathway in diverse cancers leads to radiosensitization and/or chemosensitization. Ipat is an oral, potent ATP-competitive small molecule inhibitor of all three isoforms of Akt. The combination of Ipat with taxanes in preclinical models resulted in enhanced efficacy relative to either Ipat or chemotherapy alone. In a Phase Ib clinical study, the combination of Ipat with diverse chemotherapy regimens was well-tolerated and resulted in RECIST responses, particularly pts with tumors having PI3K/Akt activation.

Methods: FAIRLANE is a randomized, double-blind, placebo controlled, multicenter, neoadjuvant Phase II study designed to estimate the efficacy of Ipat combined with pac versus placebo combined with pac in women with Stage Ia /Ila TNBC. Approximately 150 pts (Pts) will be enrolled, randomized in a 1:1 ratio, and stratified by PTEN status, node involvement, and tumor size. Pts will receive 3 cycles of Ipat 400 mg or placebo orally once daily on Days 1 to 21 of each 28-day cycle, along with pac 80 mg/m2 every 7 days for a total of 12 doses. All pts will undergo pretreatment and Day 8 tumor tissue acquisition to evaluate pathway biomarkers. Following three cycles of treatment, pts will undergo surgery. The primary efficacy endpoint, pCR within the breast and axilla (ypT0/Tis ypN0) in all pts and in pts with PTEN low tumors, will be assessed by local pathology evaluation following completion of neoadjuvant therapy and surgery. Additional endpoints include objective response rate, safety, BCS rate, pharmacokinetics, and pathway biomarkers. Following surgical resection of primary tumor, pts are expected to continue post-operative treatment with a standard adjuvant chemotherapy regimen at physician's discretion. The study is open for accrual. Clinical trial information: NCT02301988.
Title: A phase I followed by a randomized phase II trial of two cycles carboplatin-olaparib followed by olaparib monotherapy versus capecitabine in BRCA-1 or -2 mutated Her2 negative advanced breast cancer as first line treatment (REVIVAL study)

Body: Background
Preclinical studies revealed that the combination of platinum compounds and olaparib is additive and possibly even synergistic in cell models with BRCA1 or -2 mutations. Early clinical trials suggested high benefit of olaparib with induction carboplatin in BRCA1 and -2 mutation carrier enriched populations. However, there is no evidence yet that carboplatin-olaparib has a superior benefit-risk compared to current standard therapy in advanced breast cancer in BRCA1 and -2 mutation carriers.

Trial design
We initiated a phase-I/II study due to an olaparib formulation change from capsule to tablet. During phase-I a traditional 3+3 dose escalation study is performed. Carboplatin will be dose escalated in 1 step from AUC 3 to AUC 4 with a constant olaparib dose of 25 mg BID. Olaparib is then dose escalated in 3 steps to 50, 75 and 100 mg BID until > 1/6 patients develop a DLT, the previous safe dose-level will be determined the MTD. After the MTD is established a randomised phase-II trial will be initiated where patients are randomised between standard capecitabine 1250 mg/m2 BID day 1-14, q day 22 or 2 cycles carboplatin-olaparib followed by olaparib monotherapy 300mg BID. After progression, patients in the experimental arm receive capecitabine, all other patients receive physicians choice of paclitaxel, vinorelbine or eribulin at standard dose. A compassionate use program with olaparib is available for patients in the standard arm after progression on second line treatment.

Eligibility criteria
In phase-II patients with histological or cytological proof of advanced BRCA1 or -2 mutated HER2 negative breast cancer are eligible if they are ≥18 years, have measurable disease according to RECIST 1.1 criteria, a WHO performance status of 0–2, a life expectancy ≥ 3 months and a negative pregnancy test. Pretreatment should contain an anthracycline and/or taxane in the (neo)adjuvant setting, unless not indicated. In the advanced setting only hormonal pre-treatment is allowed. Minimal laboratory values ANC ≥ 1.5 x 10^9 /L, Hb ≥ 6.2 mM (no transfusions in the last 28 days), platelet count ≥ 100 x 10^9 /L, serum bilirubin ≤ 1.5 x ULN, ASAT and ALAT < 2.5 x ULN and a serum creatinine ≤ 1.5 x ULN or creatinine clearance ≥ 50 mL/min.

Aims
In phase-1 we establish the MTD for treatment in phase-II where we study progression free survival on first line treatment(PFS1) compared with standard of care capecitabine.

Statistical methods
Toxicity analysis in phase-I can take place after all patients completed their 28 day DLT period.
A total of 104 events in 110 patients on first line treatment need to be observed in phase-II to detect a clinical meaningful improvement in median PFS1 in the experimental arm from 4 to 7 months, assuming an accrual of 2 years and a follow-up of ≥6 months, providing a power of 80% (two-sided significance level of 5%). An interim analysis for futility and efficacy will be performed when 52 events have been observed.

Accrual
It is expected that 15-20 patients are needed in phase-I, inclusion is due around November 2015. Phase-II will be multicentre and is expected to start accrual December 2015.
**Title:** Phase III randomized, placebo-controlled clinical trial evaluating the use of adjuvant endocrine therapy +/- one year of everolimus in patients with high-risk, hormone receptor (HR) positive and HER2-negative breast cancer (BC): SWOG/NRG/Alliance S1207 (NCT01674140)

Chavez-MacGregor M, Barlow WE E, Pusztai L, Goetz MP P, Rastogi P, Ganz PA A, Mamounas EP P, Paik S, Bandos H, Gralow J, Lew DL L and Hortobagyi GN N. The University of Texas MD Anderson Cancer Center; Cancer Research and Biostatistics, SWOG Statistical Center; Yale Cancer Center, Yale School of Medicine; Mayo Clinic; NRG Oncology/NSABP and UPMC Cancer Center; UCLA Jonsson Comprehensive Cancer Center; NRG Oncology/NSABP and UF Health Cancer Center; NRG Oncology/NSABP, and the Severance BioMedical Science Institute and Yonsei University College of Medicine; NRG Oncology, and the University of Pittsburgh, Graduate School of Public Health; University of Washington/Seattle Cancer Care Alliance and SWOG Statistical Center.

**Body:** Background: Abnormalities of the PI3kinase/AKT/mTOR signaling network are common in BC. This pathway is associated with resistance to endocrine therapies among HR+ tumors. Everolimus, an mTOR-inhibitor, increases the biological activity of endocrine therapy. S1207 evaluates the role of everolimus in combination with endocrine therapy in the adjuvant setting.

Methods: Specific aims/ design: Randomized phase III double-blinded, placebo-controlled trial. Primary objective is to assess whether the addition of everolimus to standard adjuvant endocrine therapy improves invasive disease-free survival (DFS) among patients with high risk, HR+ BC. Secondary objectives include overall survival, distant recurrence-free survival, safety, adherence and QoL. Patients are randomized to receive standard adjuvant endocrine therapy in combination with one year of everolimus (10 mg PO daily) or placebo. Submission of tissue specimens/blood samples is required for translational studies Eligibility criteria: Patients with histologically confirmed HER2-negative and HR+ invasive BC treated with surgery, adjuvant chemotherapy and radiation therapy (if indicated) are eligible if they have: node-negative disease and tumors >2cm and a recurrence score (RS) >25; 1-3 positive nodes and RS >25 or grade 3 in the absence of RS; >4 positive lymph nodes regardless of RS. Patients >1 positive lymph node after completing neoadjuvant chemotherapy are eligible. Statistics/Target accrual:Parallel randomization design with equal allocation to the two treatment groups, the study will randomize 3,500 patients. All analyses are intent-to-treat with the primary analysis conducted 3 years after the last patient is randomized. The study has 90% power (with 2-sided $\alpha$=0.05) to detect an effective hazard ratio of 0.75 for everolimus versus placebo, corresponding to a gain in DFS of approximately 4.3% at 5 years. All patients will be followed for 10 years. Support: NIH/NCI NCTN Grants CA180888, 180819, 180868, 180821, 180822 189867, and in part by Novartis Clinical trial information: NCT01674140.
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

Schmid P, Ferreira M, Dubey S, Zaiss M, Harper-Wynne C, Makris A, Brown V, Kristeleit H, Patel G, Perelló A, Jones A, Mithal N, Ruiz I, Kümmel S, Brunt AM, Guerra JA, Gonzalez Cao M, Saura C, Mousa K, Sarker S-J, Coetzee C, Swann R and Cortes J. Queen Mary University London, London, United Kingdom; Instituto Portugues De Oncologia Porto, Porto, Portugal; Derriford Hospital, Plymouth, United Kingdom; Praxis Fuer Interdisziplinaere Onkologie und Haematologie, Freiburg, Germany; Kent Oncology Centre, Maidstone, United Kingdom; Mount Vernon Cancer Centre, Northwood, United Kingdom; Nottingham City Hospital, Nottingham, United Kingdom; Queen Elizabeth Hospital, Woolwich, United Kingdom; Royal Sussex County Hospital, Brighton, United Kingdom; Hospital Son Espases, Palma, Spain; Royal Free Hospital, London, United Kingdom; Kent and Canterbury Hospital, Canterbury, United Kingdom; Hospital Sant Joan de Reus, Tarragona, Spain; Kliniken Essen-Mitte, Essen, Germany; Royal Stoke University Hospital, Stoke-on-Trent, United Kingdom; Hospital Universitario De Fuenlabrada, Madrid, Spain; Instituto Dexeus, Barcelona, Spain and Hospital Universitari Vall d'Hebron, Barcelona, Spain.

Body: Background: Resistance to endocrine therapy remains a major clinical challenge in ER+ breast cancer. Aberrant PI3K/AKT/mTOR pathway activation frequently occurs in ER+ breast cancer and is associated with resistance to endocrine therapy. However, there is increasing evidence that inhibition of only mTORC1 with rapalogues such as everolimus sets off a negative feedback mechanism that leads to increased AKT signalling and is linked with treatment resistance. AZD2014 is a dual inhibitor of both mTORC1 (rapamycin-sensitive) and mTORC2 (rapamycin insensitive); compared to rapalogues, AZD2014 has a broader range of growth inhibitory activity in preclinical models based on a more profound mTORC1 inhibition and the additional inhibition of mTORC2. AZD2014 is especially effective in ER+ breast cancer models, showing superior activity to everolimus both in hormone-sensitive and resistant models.

Trial objectives: The main aims of this trial are (i) to determine whether dual inhibition of mTORC1 and mTORC2 with AZD2014 will increase the activity of endocrine therapy with fulvestrant in ER+ breast cancer, (ii) whether inhibition of mTORC1 and mTORC2 using AZD2014 will have superior anti-tumour activity compared to inhibition of mTORC1 alone with everolimus, and (iii) to compare the safety and efficacy of two schedules of AZD2014.

Methods: MANTA is an international investigator led, sponsored, open-label, randomised phase II trial. Patients are randomised in a 2:3:3:2 ratio to receive either fulvestrant (500mg) alone, fulvestrant and AZD2014 at a continuous daily schedule (50mg BD), fulvestrant and AZD2014 at an intermittent schedule (2 days on, 5 days off; 125mg BD) or fulvestrant and everolimus (10mg OD). Patients are stratified by disease measurability and response to prior endocrine therapy. Treatment is given until disease progression (RECIST 1.1), intolerable toxicity or elective withdrawal.

Eligibility criteria: This study enrols post-menopausal women with ER+, HER2-negative, advanced or metastatic, hormone refractory breast cancer. Patients must have at least one measurable lesion and no life-threatening visceral disease. Patients with significant pulmonary dysfunction, cardiovascular disease or uncontrolled diabetes are excluded. Patients must not have had previous treatment with fulvestrant, PI3K/Akt or mTOR inhibitors or no more than one line of chemotherapy for metastatic breast cancer.

Endpoints: The primary endpoint is progression-free survival . Secondary endpoints include objective response rate, change in tumour size, clinical benefit rate, overall survival, duration of response, patient reported outcomes and pharmacokinetic parameters of AZD2014 and fulvestrant. Archival tumour tissue must be available to evaluate biomarkers associated with therapeutic response and resistance.

Target accrual: Approximately 316 patients will be enrolled at ~90 sites in the UK, Germany, Spain, Portugal, France, Hungary, Romania, Georgia, and South Korea. Recruitment opened in 2014 and 121 patients have been recruited to date. NCT02216786.
Title: A phase II, double blind, randomised, placebo-controlled study of the AKT Inhibitor AZD5363 in combination with paclitaxel in triple-negative advanced or metastatic breast cancer (TNBC)(NCT02423603)

Schmid P, Wheatley D, Baird R, Chan S, Abraham J, Tutt A, Kristeleit H, Patel G, Bathakur U, Bishop J, Harper-Wynne C, Sims E, Copson E, Perren T, Stein R, Poole C, Cartwright H, Sarker S-J, Mousa K and Turner N. Queen Mary University of London, London, United Kingdom; Royal Cornwall Hospital, Truro, Cornwall, United Kingdom; Addenbrookes Hospital, Cambridge, United Kingdom; Nottingham City Hospital, Nottingham, United Kingdom; Velindre Cancer Centre, Cardiff, United Kingdom; Guy's & St Thomas' Hospital, London, United Kingdom; Queen Elizabeth Hospital, London, United Kingdom; The Royal Sussex County Hospital, Brighton, United Kingdom; Yeovil District Hospital, Somerset, United Kingdom; Glan Clwyd Hospital, Denbighshire, United Kingdom; Maidstone Hospital, Kent, United Kingdom; Queens Hospital, Essex, United Kingdom; Southampton General Hospital, Southampton, United Kingdom; St James' University Hospital, Leeds, United Kingdom; University College London Hospitals, London, United Kingdom; University Hospital Coventry, Coventry, United Kingdom and Royal Marsden Hospitals, London, United Kingdom.

Body: Management of metastatic TNBC remains a challenge. Chemotherapy is the mainstay of treatment but benefits are frequently short-lived with rapid development of resistance. The PI3K/AKT/mTOR pathway has been implicated in many ways in TNBC, making inhibition of AKT an attractive therapeutic target. Based on downstream pathway activation signatures, PI3K pathway activation appears higher in TNBC compared to other molecular subtypes, despite a relatively low percentage of activating PI3K mutations. Alternative means of activating the PI3K pathway have been identified in TNBC, including loss or mutation of PTEN (up to 35%) and INPP4B (up to 30%) and/or amplification of PIK3CA, AKT2 or AKT3, resulting in increased activation of AKT. Induction of AKT by chemotherapy can be an early compensatory mechanism that can be exploited therapeutically to increase the efficacy of chemotherapy. Preclinical TNBC models with activated AKT signalling have been shown to be highly sensitive to AKT inhibitors. AZD5363 is a potent pan-AKT inhibitor with good oral bioavailability. Multiple lines of investigation have demonstrated strong synergistic effects between AKT inhibition and taxane chemotherapy in models of TNBC both in vitro and in vivo, providing rationale for the combination of AZD5363 and paclitaxel in TNBC. PAKT is designed to test the hypothesis that inhibition of AKT will increase the anti-tumour activity of paclitaxel chemotherapy in TNBC. The study will try to characterize those patients who may benefit from this treatment to identify potential predictors of sensitivity.

PAKT is an international investigator led and sponsored, double-blind, placebo controlled, randomised phase II trial. Patients are randomised 1:1 to receive paclitaxel weekly (90mg/m²) on days 1, 8, and 15 plus AZD5363 (400mgBD) or placebo (400mgBD) on days 2-5, 9-12, 15-19 (28 day treatment cycles). Patients are stratified by the number of metastatic sites and the interval from the end of adjuvant chemotherapy. Treatment is given until disease progression (RECIST 1.1), intolerable toxicity or elective withdrawal. Tumour assessments are carried out every 8 weeks. PAKT enrols patients with histologically documented locally advanced/metastatic TNBC (ER≤Allred2, PR≤Allred2, HER2=0,1+or2+), no prior systemic therapy for advanced TNBC, ECOG PS 0-2 and measurable disease per RECIST v1.1. Patients with brain metastases, significant cardiovascular disease, motor polyneuropathy are excluded. The primary endpoint is progression-free survival. Secondary endpoints are objective response rate, change in tumour size, clinical benefit rate, overall survival, duration of response, and patient reported outcomes. Archival tumour tissue must be available to evaluate potential biomarkers associated with therapeutic response and resistance. PFS will be compared between treatment arms by the stratified log-rank test. HR for disease progression/death will be estimated using a stratified Cox proportional hazards model. Kaplan-Meier methodology will be used to estimate the median PFS for each arm. Approximately 140 patients will be enrolled at ≈65 sites in the UK, France, Hungary, Romania, Georgia & South Korea.
Title: SANDPIPER: Phase III study of the PI3-kinase inhibitor taselisib (GDC-0032) plus fulvestrant in patients with estrogen receptor-positive, HER2-negative locally advanced or metastatic breast cancer enriched for patients with PIK3CA-mutant tumors

Baselga J, Cortés J, De Laurentiis M, Diéras V, Harbeck N, Hsu J, Jin H, Schimmoller F, Wilson TR R, Im Y-H, Jacot W, Krop IE E and Verma S. Memorial Sloan-Kettering Cancer Center, Memorial Hospital, NY, NY; Vall d'Hebron University Hospital, Barcelona, Spain; Istituto Nazionale Tumori Fondazione G. Pascale, Naples, Italy; Institut Curie, Paris, France; Brustzentrum der Universität München, Munich, Germany; Genentech Inc., South San Francisco, CA; Samsung Medical Center, Seoul, Korea; Institut du Cancer de Montpellier, Montpellier, France; Dana-Farber Cancer Institute, Boston, MA and Division of Medical Oncology, Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Background: PIK3CA mutations are one of the most frequent genomic alterations in breast cancer (BC), being present in ~40% of estrogen receptor (ER)-positive, HER2-negative breast tumors. PIK3CA mutations promote growth and proliferation of tumors and mediate resistance to endocrine therapies in BC. Taselisib is a potent and selective PI3-kinase (PI3K) inhibitor that displays greater selectivity for mutant PI3Kα than wild-type PI3Kα. Taselisib has enhanced activity against PIK3CA-mutant BC cell lines, and clinical data include confirmed partial responses in patients with PIK3CA-mutant BC treated with taselisib either as a single agent or in combination with fulvestrant.

Trial design: SANDPIPER is a double-blind, placebo-controlled, randomized, phase III study, designed to evaluate efficacy and safety of taselisib plus fulvestrant in patients with ER-positive, HER2-negative locally advanced or metastatic BC. Patients will be randomized 2:1 to receive either taselisib (4 mg daily) or placebo in combination with fulvestrant (500 mg intramuscular on Days 1 and 15 of Cycle 1, and on Day 1 of each subsequent 28-day cycle). Randomization will be stratified by visceral disease, endocrine sensitivity, and geographical region. The study enriches for patients with PIK3CA-mutant tumors who will be randomized separately from patients with non-mutant tumors.

Eligibility: Postmenopausal women with ER-positive, HER2-negative, locally advanced or metastatic BC are eligible if they have disease recurrence or progression during or after aromatase inhibitor treatment. A valid PIK3CA-mutation result via central assessment is required prior to enrollment.

Aims: The primary efficacy endpoint is investigator-assessed progression-free survival (PFS) in patients with PIK3CA-mutant tumors. Other endpoints include overall survival (OS), objective response rate (ORR), clinical benefit rate (CBR), duration of objective response, safety, pharmacokinetics, and patient-reported outcomes. Efficacy in patients without PIK3CA-mutant tumors will be an exploratory endpoint.

Statistical methods: The primary efficacy analysis population will include all randomized patients with PIK3CA-mutant tumors. Patients will be grouped according to treatment arm assigned at randomization. Median PFS and OS will be estimated using Kaplan-Meier methodology in each treatment arm. Cox proportional-hazards models will be used to estimate the hazard ratio with 95% confidence intervals (CIs). ORR, CBR, and their 95% CIs will be estimated by treatment arms. Duration of objective response will be estimated by treatment arms using the Kaplan-Meier methodology. Quality of life will be analyzed and summarized by treatment arms. Safety will be analyzed for all treated patients according to actual treatment received.

Accrual: Target enrollment is 600 pts from ~165 sites and ~23 countries. The study is open for enrollment and 11 patients have been enrolled as of May 31, 2015. Clinicaltrials.gov ID: NCT02340221.

Contact information: For more information or to refer a patient, email global.rochegenentechtrials@roche.com or call 1-888-662-6728 (USA only).
The METRIC trial: A randomized international study of the antibody-drug conjugate glembatumumab vedotin (GV or CDX-011) in patients with metastatic gpNMB-overexpressing triple-negative breast cancer (TNBC)

Melisko M, Yardley DA, Blackwell K, Forero A, Ma C, Monetero A, Daniel BR, Wright G, Fehrenbacher L, Chew H, Ferrario C, Nanda R, Seller Jr M, Guthrie T, Vance K, Ouellette G, He Y, Bagley RG, Zhang J and Vahdat LT. University of California, San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Sarah Cannon Research Institute/Tennessee Oncology, PLLC; Duke University Medical Center; University of Alabama; Washington University; Cleveland Clinic; Chattanooga Oncology Hematology Associates; Florida Cancer Specialists; Kaiser Permanente; University of California Davis Comprehensive Cancer Center; Segal Cancer Center-Jewish General Hospital; University of Chicago; Crescent City Research Consortium, LLC; Baptist Cancer Institute; Alabama Oncology; Celldex Therapeutics, Inc. and Weill Cornell Medical College.

Body: Background
Glycoprotein NMB (gpNMB) is an internalizable transmembrane protein overexpressed in approximately 20% of breast cancer (BC), including approximately 40% of TNBC. gpNMB is a poor prognostic marker in BC (Rose CCR 2010) and preclinically has been implicated in tumor invasion, metastasis, and angiogenesis. GV is a novel antibody-drug conjugate targeting the potent cytotoxin monomethylauristatin E (MMAE) to gpNMB overexpressing cancer cells.

In a Phase I/II study and the Phase II "EMERGE" study, GV demonstrated promising activity with TNBC patients (pts) deriving the greatest benefit and exhibiting the highest degree of gpNMB overexpression. GV was well-tolerated with the most frequent treatment-related toxicities consisting of rash, neutropenia, and neuropathy. In subset analyses of the EMERGE trial, objective response rate (ORR) was 30% (7/23) for GV vs. 9% (1/11) for investigator's choice in tumors with gpNMB overexpression (>25% of tumor epithelium); 18% (5/28) vs. 0% (0/11) in TNBC; and 40% (4/10) vs. 0% (0/6) in gpNMB-overexpressing TNBC for GV and IC respectively, with apparent improvements in progression-free survival (PFS; hazard ratio (HR) = 0.11) and overall survival (OS; HR = 0.14).

Trial design
The METRIC Trial (NCT#01997333) is an international (USA, CA, Aus), two-arm phase II study. Pts are randomized 2:1 to GV (1.88 mg/kg IV q 21 days) or capecitabine, a current standard of care for this population (2,500 mg/m² daily for d1-14, q21 days) until progression or intolerance. Crossover is not permitted.

Eligibility criteria
Key eligibility criteria include: ≥25% of tumor epithelium gpNMB+ by central immunohistochemistry (IHC) screening of archival tissue; estrogen receptor and progesterone receptor <10% and HER2 negative [0-1+ IHC, or ISH copy number <4.0/ratio <2.0] by local assessment; ECOG 0-1; taxane resistance; anthracycline exposure (if indicated); ≤2 chemotherapy regimens for advanced BC; measurable disease; no persistent Grade ≥2 toxicity.

Specific aims
The primary endpoint is PFS per independent, blinded central review committee according to RECIST 1.1. Secondary endpoints are ORR, duration of response, OS, safety, pharmacokinetics and pharmacodynamics. Exploratory endpoints are quality of life and/or cancer-related pain.

Statistical methods and target accrual
The trial has 85% power to detect a PFS HR of 0.64 with two sided α = 0.05. The hypothesized median PFS is 4.0 months for capecitabine and 6.25 months for GV. Target accrual is open for 300 pts.
EMBRACA: A phase 3, open-label, randomized, parallel, 2-arm international study of the oral PARP inhibitor talazoparib (BMN 673) versus physician's choice in BRCA mutation subjects with locally advanced and/or metastatic breast cancer

Litton JK K, Blum JL L, Im Y-H, Martin M, Mina L, Roché H, Visco F, Yang X, Lokker NA A, Lounsbury DL L and Eiermann W. The University of Texas MD Anderson Cancer Center, Houston, TX; Baylor Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX; Samsung Medical Center, Seoul, Republic of Korea; Hospital General Universitario Gregorio Marañón, Madrid, Spain; Indiana University School of Medicine, Indianapolis, IN; Institut Universitaire du Cancer Toulouse, Toulouse, France; National Breast Cancer Coalition, Washington, DC; BioMarin Pharmaceutical Inc., Novato, CA and Interdisziplinäres Onkologisches Zentrum München, München, Germany.

Background: Cancer cells with deleterious mutations in breast cancer susceptibility genes 1 and 2 (BRCA1/2) are deficient in the DNA double-strand break repair mechanism, rendering them highly dependent on the single-strand break repair pathway, which is initiated by poly-(ADP-ribose) polymerase (PARP) [1-3]. In cells with deleterious BRCA1/2 mutations, PARP inhibition is synthetically lethal because of accumulation of irreparable DNA damage [1-3]. Talazoparib (BMN 673) exhibits a novel two-pronged approach in treating BRCA1/2-mutant tumors: 1) potent catalytic inhibition of the PARP enzyme; and 2) trapping of PARP at sites of DNA damage [4-7]. The capacity to trap PARP-DNA complexes varies widely across PARP inhibitors and is not correlated with catalytic inhibition potency [4-7]. In preclinical models, trapping PARP on DNA is more potent at inducing cancer cell death than enzymatic inhibition of PARP alone [4,7]. Talazoparib is the most potent clinical-stage PARP inhibitor tested to date with the highest efficacy at trapping PARP-DNA complexes [7]. Talazoparib has shown single-agent antitumor efficacy in several solid tumor types and was generally well tolerated in a phase 1/2 clinical study [8].

Methods: This open-label, randomized, parallel, 2-arm, phase 3 international trial (EMBRACA) compares the safety and efficacy of talazoparib with physician's choice treatment (capecitabine, eribulin, gemcitabine, or vinorelbine) in patients with advanced breast cancer. The primary objective is progression free survival (PFS). Secondary objectives include objective response rate (ORR), overall survival (OS), and safety. Exploratory objectives include duration of response (DOR) for objective responders and health-related quality of life measurements. Subject eligibility includes age ≥ 18 years with histologically/cytologically confirmed breast carcinoma, locally advanced and/or metastatic disease, germline BRCA1/2 mutations, ≤ 2 prior chemotherapy-inclusive regimens for locally advanced and/or metastatic disease, prior treatment with a taxane and/or anthracycline, and Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. Subjects (n=429) will be randomized 2:1 to receive either talazoparib oral capsules (1 mg/day, 21-day cycles) or physician's choice treatment. This trial is currently enrolling patients from the United States, Asia/Pacific, Europe, Israel, and South America (NCT01945775).

This study is funded by BioMarin Pharmaceutical Inc.

References:
**Title:** ABRAZO: An international phase 2 (2-stage, 2-cohort) study of the oral PARP inhibitor talazoparib (BMN 673) in BRCA mutation subjects with locally advanced and/or metastatic breast cancer

**Body:**

Background: Cancer cells with deleterious mutations in breast cancer susceptibility genes 1 and 2 (BRCA1/2) are deficient in the DNA double-strand break repair mechanism, rendering them highly dependent on the single-strand break repair pathway, which is initiated by poly-(ADP-ribose) polymerase (PARP) [1-3]. In cells with deleterious BRCA1/2 mutations, PARP inhibition is synthetically lethal because of accumulation of irreparable DNA damage [1-3]. Talazoparib (BMN 673) exhibits a novel two-pronged approach in treating BRCA1/2-mutant tumors: 1) potent catalytic inhibition of the PARP enzyme; and 2) trapping of PARP at sites of DNA damage [4-7]. The capacity to trap PARP-DNA complexes varies widely across PARP inhibitors and is not correlated with catalytic inhibition potency [4-7]. In preclinical models, trapping PARP on DNA is more potent at inducing cancer cell death than enzymatic inhibition of PARP alone [4,7]. Talazoparib is the most potent clinical-stage PARP inhibitor tested to date with the highest efficacy at trapping PARP-DNA complexes [7]. Talazoparib has shown single-agent antitumor efficacy in several solid tumor types and was generally well tolerated in a phase 1/2 clinical study [8].

Methods: This 2-stage, 2-cohort, phase 2 international study (ABRAZO) evaluates the safety and efficacy of talazoparib in patients with a deleterious germline BRCA1 or BRCA2 mutation with locally advanced and/or metastatic breast cancer. Eligible subjects will be assigned to one of two cohorts based on prior chemotherapy for metastatic disease. Cohort 1 (n=70) includes patients with a complete response (CR) or partial response (PR) to platinum-containing regimens for metastatic disease. Cohort 2 (n=70) includes patients who have received >2 prior chemotherapy regimens in the metastatic setting but have not had prior platinum therapy for locally advanced or metastatic disease (prior adjuvant or neoadjuvant therapy with a platinum is allowed). The primary objective is objective response rate (ORR). Secondary objectives include clinical benefit response (CBR) rate, duration of response (DOR), progression-free survival (PFS), and overall survival (OS). Health-related quality of life (QoL) assessments are an exploratory objective. Eligible subjects will receive oral talazoparib (1 mg/day, 21-day cycles) until disease progression or unacceptable toxicity. This trial is currently enrolling patients from the United States and Europe (NCT02034916). This study is funded by BioMarin Pharmaceutical Inc.

References:

2015 San Antonio Breast Cancer Symposium

Publication Number: OT1-03-18

Title: COLET: A multistage, phase 2 study evaluating the safety and efficacy of cobimetinib in combination with paclitaxel as first-line treatment for patients with metastatic triple-negative breast cancer

Kim S-B, Miles D, Rhee J, Yan Y, Hsu J and Brufsky A. Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Mount Vernon Cancer Centre, London, United Kingdom; Genentech, Inc., South San Francisco, CA and University of Pittsburgh, Pittsburgh, PA.

Body: Background: Cobimetinib (cobi) is a potent, highly selective inhibitor of MEK that has shown benefit when administered in combination with the BRAF inhibitor vemurafenib in BRAFV600-mutated metastatic melanoma. Preclinical data suggest that up-regulation of the MAPK pathway is a potential resistance mechanism against taxane chemotherapy. Clinically, the combination of MEK inhibition and taxane chemotherapy in non–small cell lung cancer patients (pts) has improved response rate (RR) and progression-free survival (PFS). Because most triple-negative breast cancer (TNBC) pts develop resistance to taxane chemotherapy and because genetic alterations (including mutations and gene amplifications) in the MAPK pathway are present in many TNBC tumors, the combination of taxane chemotherapy and MEK inhibition could be an effective treatment option.

Study design: COLET (WO29497) is a multistage study designed to evaluate the safety of and to estimate the efficacy of cobi /paclitaxel in pts with metastatic or locally advanced TNBC who have not previously received systemic therapy for metastatic disease. The study will be conducted in 2 stages: an initial safety run-in stage of approximately 12 pts, followed by a randomized stage in which approximately 100 pts will be randomly assigned in a 1:1 ratio to receive either cobi + paclitaxel or placebo + paclitaxel. Pts will receive paclitaxel 80 mg/m2 on Days 1, 8, and 15 of each 28-day cycle and cobi/placebo 60 mg/day on Days 3-23 of each 28-day cycle until progression of disease or toxicity occurs.

Key eligibility criteria:

• Metastatic or locally advanced (not amenable to curative resection) TNBC
• No prior systemic therapy for metastatic or unresectable locally advanced TNBC
• Neoadjuvant or adjuvant chemotherapy or radiation therapy is allowed if completed >6 months before the start of study treatment
• Measureable disease using Response Evaluation Criteria In Solid Tumors, version 1.1 (RECIST v1.1)
• History of or active untreated or unstable brain metastases or requiring corticosteroids for brain metastases precludes eligibility
• Left ventricular ejection fraction (LVEF) greater than the institutional lower limit of normal or above 50%

Specific aims of the safety run-in stage: Determine the safety and tolerability of cobi when administered in combination with paclitaxel.

Specific aims of the randomized stage: Investigator-assessed PFS (primary end point); safety; pharmacokinetics; the effect of intrinsic subtypes and genetic alterations in PFS; mechanisms of resistance; and health-related quality of life.


Statistical methods: In the randomized stage, pts will be followed up until a total of 60 PFS events occur across the 2 arms. This provides 77% power to detect a hazard ratio of 0.5 at a two-sided significance level of 0.05.

Contact information: Registered with ClinicalTrials.gov, identifier NCT02322814. For more information, please contact Roche/Genentech trials, 888-662-6728 (US only) or reference study ID WO29479 at www.roche.com/about_roche/roche_worldwide.htm.
Title: Design of a phase 1b/2 study to evaluate the efficacy and safety of eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer


Body: Eribulin mesylate, a microtubule inhibitor, is indicated for treatment of patients (pts) with metastatic breast cancer (MBC) with ≥2 prior chemotherapies for metastatic disease, including an anthracycline or taxane. Pembrolizumab is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for treatment of pts with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAFV600-mutation positive, a BRAF inhibitor. Pembrolizumab is also being evaluated for treatment of metastatic triple-negative breast cancer (mTNBC) and multiple tumor types. We report the design of an open-label, single-arm, multicenter, phase 1b/2 study to evaluate safety and efficacy of the eribulin and pembrolizumab combination in pts with mTNBC previously treated with 0 to 2 lines of therapy in the metastatic setting.

From August 2015 to February 2017, approximately 95 pts (aged ≥18 yrs) will be enrolled (n=12 phase 1b; n=83 phase 2) to reach 80 evaluable pts. Accrual has not yet commenced. Pts with measurable disease of ≥1 lesion >10 mm in long-axis diameter (nonlymph nodes) or >15 mm in short-axis diameter (lymph nodes) and an ECOG status of 0 or 1 will be included. Exclusion criteria include pts who received adjuvant chemotherapy within the past 6 months, chemotherapy/biological therapy within the past 3 wks, or radiation or small molecule targeted therapy within the past 2 wks. Pts will also be excluded if they have an autoimmune disease requiring immunosuppression or were previously treated with eribulin or any anti-PD-1, PD-ligand (L) 1, or PD-L2 agent.

Phase 1b includes a safety run-in cohort in which ≥6 pts will receive intravenous (IV) eribulin 1.4 mg/m2 (equivalent to 1.23 mg/m2 eribulin [expressed as free base]) on day (d) 1 and d8 and IV pembrolizumab 200 mg on d1 of a 21d cycle. Dose-limiting toxicity (DLT) will be assessed in the first cycle. This dose will be selected as the recommended phase 2 dose (RP2D) if ≤1 pt has a DLT or, if necessary, alternative doses may be explored. Pts will be stratified by prior chemotherapy for MBC (0, ∼70%; 1–2, ∼30%) and will receive RP2D combination treatment. Pts can remain on 1 or both study drugs with clinical benefit until intercurrent illness, unacceptable toxicity, or disease progression. Bayesian predictive probability of response rate, based on the goal of claiming combination superiority at study end, will be used to monitor response rate after postbaseline tumor assessments for ≥38 pts. The study can be stopped early for efficacy or futility.

Primary objectives include determination of safety and tolerability (phase 1b) and evaluation of objective response rate of the drug combination; secondary endpoints include evaluation of progression-free survival (PFS), overall survival (OS), and duration of response (DOR). PFS, OS, and DOR will be analyzed using Kaplan–Meier product-limit estimates. Median PFS, OS, and cumulative probability of PFS, OS, and DOR at 6 and 12 months will be presented with 2-sided 95% confidence intervals. Relationship between PD-L1 tumor status and efficacy endpoints will also be evaluated.

For further information please contact Erhan Berrak (erhan_berrak@eisai.com).
Title: A phase 2 study of pembrolizumab (MK-3475) monotherapy for metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086


Body: Background: TNBC is defined by a lack of ER and PR expression and the absence of HER2 overexpression. As such, the only approved systemic treatment option approved for mTNBC is chemotherapy, which is associated with median survival of <1 year. The programmed death receptor 1 (PD-1) pathway is frequently altered in cancer and used by tumors to evade an immune response. Pembrolizumab, an anti–PD-1 monoclonal antibody that prevents PD-1 from binding to its ligands, PD-L1 and PD-L2, has shown durable antitumor activity and a manageable toxicity profile in many advanced cancers, including mTNBC. In the phase 1b KEYNOTE-012 study, pembrolizumab 10 mg/kg given every 2 weeks (Q2W) provided an ORR of 18.5% and a 6-month PFS rate of 24.4% (RECIST v1.1, central review) in a cohort of 32 heavily pretreated patients with PD-L1–positive mTNBC.

Trial Design: KEYNOTE-086 is a 2-part, multicohort, nonrandomized, phase 2 trial of pembrolizumab monotherapy for women and men with mTNBC (ClinicalTrials.gov, NCT02447003). Key eligibility for all cohorts include age ≥18 years, centrally determined mTNBC, ECOG PS 0 or 1, measurable disease per RECIST v1.1 by central review, and provision of a tumor sample for assessment of ER, PR, HER2, and PD-L1 status at a central laboratory. PD-L1 expression will be assessed by immunohistochemistry using the 22C3 antibody (Merck), with positivity defined as PD-L1 expression in the stroma or in ≥1% of tumor cells. Part 1 includes 2 cohorts that will enroll simultaneously. In cohort A, up to 160 pts with any PD-L1 status who have received ≥1 systemic treatment for metastatic breast cancer, were treated with an anthracycline and a taxane in the (neo)adjuvant and/or metastatic setting, and had documented disease progression on their most recent therapy will be enrolled. In cohort B, up to 40 pts with PD-L1–positive tumors who have received no prior systemic therapy for metastatic breast cancer will be enrolled. Part 2 is an expansion cohort of cohort A that will enroll up to 45 pts with tumors strongly positive for PD-L1 expression; part 2 will be initiated only if ≥1 response is observed in the cohort A PD-L1-strong-positive population. The definition of strongly positive PD-L1 expression will be determined in part 1. In all cohorts, pts will receive pembrolizumab 200 mg Q3W for 24 mo or until disease progression, intolerable toxicity, or patient or investigator decision. Clinically stable pts may continue pembrolizumab beyond initial RECIST-defined progression. Response will be assessed per RECIST v1.1 by central review every 9 wk for the first 12 mo and every 12 wk thereafter. AEs will be monitored throughout treatment and for 30 days thereafter (90 days for serious AEs and events of clinical interest). Primary end point is ORR. Secondary end points include duration of response (DOR), disease control rate, PFS, and OS. Efficacy and safety will be evaluated in all patients who receive ≥1 pembrolizumab dose. The Kaplan-Meier method will be used to estimate DOR, PFS, and OS.

Current Status: Enrollment in KEYNOTE-086 will begin in June 2015 and continue until up to 245 patients are accrued. For additional information on KEYNOTE-086, contact MerckClinTrialfSupport@merck.com.
Title: PALLAS: PAlbociclib Collaborative Adjuvant Study: A randomized phase 3 trial of palbociclib with adjuvant endocrine therapy versus endocrine therapy alone for HR+/HER2- early breast cancer

Mayer E, DeMichele A, Dubsky P, Barry W, Metzger O, Symmans WF, Burstein H, Miller K, Wolff A, Rastogi P, Loibl S, von Minckwitz G, Goulioti T, Zardavas D, Fesl C, Koehler M, Huang Bartlett C, Chen L, Piccart M, Winer E and Gnant M. Dana-Farber Cancer Institute, Boston, MA; Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; Alliance for Clinical Trials in Oncology Foundation, Chicago, IL; PrECOG, Philadelphia, PA; Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria; MD Anderson Cancer Center, Houston, TX; Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD; Simon Cancer Center, Indiana University, Indianapolis, IN; University of Pittsburgh Medical Center, Pittsburgh, PA; NSABP Foundation, Pittsburgh, PA; German Breast Group, Neu-Isenburg, Germany; Pfizer, Inc, NY, NY; Breast International Group, Brussels, Belgium and Institute Jules Bordet, Brussels, Belgium.

Body: Background:
Cell cycle inhibition is a target of interest for novel cancer therapeutics. Palbociclib (P) is an orally active inhibitor of CDK4/6 which arrests the cell cycle at the G1-S transition. P has demonstrated efficacy in phase II and III randomized trials for first-line and pre-treated hormone receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancer (MBC), with hazard ratios 0.42-0.49 (Finn et al, Lancet Oncol 2015, Turner et al, NEJM 2015), and is approved in combination with letrozole as first-line therapy for HR+/HER2- MBC. Given confirmed benefits of P and endocrine therapy for MBC, the PALLAS study was designed to determine if the addition of P to adjuvant endocrine therapy (ET) improves outcomes over ET alone in HR+/HER2- early breast cancer.

Trial Design: PALLAS is an international open label phase III trial randomizing patients 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 comparing iDFS excluding cancer of non-breast origin, DRFS, LRRFS, OS, as well as safety. The principal translational science objective is to determine the predictive or prognostic utility of defined genomic subgroups with respect to iDFS and OS. Additional translation objectives include evaluation of tissue and blood biomarkers predictive of benefit or resistance, cfDNA, pharmacogenomics, adherence, BMI, and patient-reported QOL. Eligible patients (pts) may be pre- or post-menopausal, have stage II-III breast cancer, HR+/HER2- by ASCO CAP guidelines, and have recovered from prior therapies. Pts may have already initiated ET, but randomization must occur within 12 months of diagnosis and 6 months of initiation of ET. An FFPE block must be received at the central sample repository for eligibility. Total planned accrual to the trial is 4600 pts, providing 85% power to detect a 25% risk reduction in iDFS from ET alone using a stratified logrank test with an overall one-sided alpha = 0.025. Pts will be randomized 1:1 stratified by stage, receipt of prior chemotherapy, age, and geographic location. Interim analyses for safety, futility/efficacy and sample size re-estimation are planned. PALLAS will open in 9/2015; current accrual will be updated at time of presentation.
Title: Analytical validation of a standardized scoring protocol for Ki67: Phase-3 of an international multicenter collaboration

Body: Aims: (i) To determine if between-pathologist agreement for Ki67 is adequate for clinical application, following a standardised scoring protocol. (ii) To compare between-pathologist agreement of scoring hot-spots vs a global method averaging Ki67 across each section.

Background: The nuclear proliferation biomarker Ki67 has multiple potential roles in breast cancer, including aiding decisions based on prognosis, but has unacceptable between-laboratory variability. The International Ki67 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 the most important source of variability. Phase 2 showed that significant improvements in agreement could be achieved when scoring the same tumors on tissue microarrays by following clearly defined scoring methods. We now assess whether acceptable performance can be achieved on core-cut biopsies using a standardised method.

Methods: Three adjacent sections from each of 30 primary ER+ breast cancers were centrally stained for Ki67 to assemble three sets of 30 stained tumor sections, circulated around 22 laboratories in 11 countries. Ki67 was scored by 2 methods by all labs: (a) global: 4 fields of 100 cells each were selected to represent any heterogeneity (b) hotspot: the field with highest Ki67 staining percentage was selected and 500 cells scored. Ki67 scores were log2-transformed for statistical analyses and back-transformed for presentation. The primary objective was to assess if either method could achieve an intraclass correlation coefficient (ICC) significantly greater than 0.8, considered substantial to almost-perfect agreement. Secondary objectives were to assess which method had highest observed ICC and to assess whether pathologists identified the same “hotspots”.

Results: The ICC for the global method was 0.88 (95%CI: 0.81-0.93) and therefore met the prespecified success criterion. The ICC for the hotspot method was 0.84 (95%CI: 0.77-0.92) and therefore had a CI which extended below the success criterion. Across the 22 labs, geometric mean value of the 30 scores ranged from 14.4 to 27.9 for the global method and from 17.4 to 40.2 for the hotspot method. The overall mean (95% CI) of these values was 19.8 (18.5-21.3) and 26.4 (24.6-28.3), respectively. Visually, there was moderately strong agreement in location of selected hotspot in the core-cuts across laboratories. The impact of variability of the Ki67 scores for estimating prognosis using the integrated IHC4 + clinical treatment score will be assessed. After selection of the areas to score, the median times for cell counting were 3 and 4 minutes for the global and hotspot methods, respectively.

Conclusions: The global method met the prespecified criterion of success; it should now be evaluated for clinical validity in appropriate cohorts of samples. The hotspot method showed slightly less agreement between labs. The time taken for scoring is practical using counting software we are making publicly available. Establishment of external quality assessment schemes is
likely to improve the agreement between labs further.
(Supported by a grant from the Breast Cancer Research Foundation).
Title: Ki-67 proliferation index supported by digital quantitation in breast cancer: A comparative study


Body: Introduction: In cases of breast cancer, in addition to hormone receptor and Her2 status, proliferation markers (mitotic index, Ki-67 proliferation index = KIPI) also have therapeutic implications. The 2013 St. Gallen consensus guideline includes 14% cut-off point (20% by many experts) for KIPI to distinguish luminal A-like and luminal B-like subtypes, that might be associated with remarkable intra-/interobserver variability applied in daily pathological routine utilizing semiquantitative (SQ) "eye-balling" method.

Objective: The comparison of conventional SQ method and digital image-analysis (DIA) processes for the detection of KIPI.

Methods: Three hundred and forty-seven breast cancer patients' samples with 99.24 months median follow-up data were included in our study (ethical approval: IKEB #7/2008 and 7-1/2008). Tissue microarrays (TMA) were prepared from the representative paraffin-embedded tumor blocks. After performing Ki-67 (MIB1 clone, #0505 by iOT on Ventana Benchmark XT autostainer by Roche) immunoreaction, conventional evaluation of KIPI was performed by 3 breast pathologists independently (SQ1-3). Digital image analysis was supported by PatternQuant (Pannoramic Viewer v15.3 and QuantCenter 2.0, 3DHistech Ltd.) applying a fully automatic tumor tissue recognition module with KIPI detection (DIA-1), and an adjustable module (DIA-2) with the possibility of manual corrections to exclude false detections. Interobserver variability was estimated with intra-class correlation coefficient (ICC). Digital pathological methods were compared to the - currently gold standard - SQ determination of KIPI using SPSS 22 statistical program.

Results: The three pathologists' SQ evaluations demonstrated a remarkable concordance (ICC=0.889; 95% CI= 0.834-0.922). A reference KIPI value (KIPI-RV) was derived from mean values of SQ2 and SQ3, since no significant difference was found between them (p=0.617). KIPI-RV and DIA-2 showed no significant difference (p=0.754), and excellent concordance (ICC=0.979; 95% CI=0.975-0.982). Significant difference has occurred between KIPI-RV and results of DIA-1 (p=0.001). Upon dichotomizing KIPI value at 14%, no significant difference was found between KIPI-RV and DIA-2 (p=0.262), while KIPI-RV and DIA-1 differed (p=0.006). For prognosis prediction, all three methods were able to perform statistically significant division of our patients into 2 cohorts with distinct DFS at 14% (p<0.017-0.038). At 20% threshold of KIPI, DIA-1 failed (p=0.053), while KIPI-RV and DIA-2 were able to separate good and unfavorable prognosis patients' cohorts (p=0.01; p=0.004).

Conclusion: The DIA processes are objective methods in the evaluation of KIPI. The fully automated DIA-1 method differed most from SQ results. Digital image analysis adjusted by a pathologist (our DIA-2 method) reached high concordance with results of SQ. Further refinement and validation are needed to verify applicability of automatic tumor pattern recognition software in diagnostic practice. Our results confirm that SQ evaluation of KIPI is reliable.

This study was supported by the research grant from Hungarian Society of Medical Oncology 2014 and research grant from Doctoral School of Ph.D. Studies, Semmelweis University 2014.
Title: Variability of Ki67 labeling index depending on different measurements in luminal type breast cancers

Kim S, Moon B-I and Sung SH. Ewha Womans University School of Medicine, Seoul, Korea; Ewha Womans University School of Medicine, Seoul and Ewha Womans University School of Medicine, Seoul, Korea.

Body: Background: Hormone receptor-positive and HER2-negative breast cancers are divided into luminal A (LA) and luminal B (LB) types using a cutoff of 14% Ki67 labeling index (LI). LB type breast cancer is associated with poor prognosis compared with LA, and considered as an indication of adjuvant chemotherapy. The main obstacles in classification of luminal type breast cancers are non-standardized measurement and Ki67 heterogeneity. We aimed to assess Ki67 variability depending on different methods of measurement and spot selection, and to assess Ki67 heterogeneity in luminal type breast cancers.

Design: We retrospectively retrieved 174 Ki67 stained slides of surgically excised invasive breast cancers with ER+ and/or PR+, HER2-, and Ki67 LI of 10-20% range. On each slide, 3 spots of low, intermediate, and high Ki67 labeling densities were captured via camera snapshot. And then, Ki67 LI was measured by digital image analysis (DIA) and manual count (MC) on 3 captured spots. Each case was classified into LA and LB types based on Ki67 LI measured by 4 methods; hot spot index by DIA; average index by DIA; hot spot index by MC; average index by MC. Ki67 heterogeneity was defined by the tumor with Ki67 KI <5% in low labeled spot, and ≥14% in high labeled spot by DIA. Correlation between the Ki67 heterogeneity and pathological parameters were analyzed.

Result: Ki67 LI measured by DIA was higher than by MC in 3 spots (low: 4.95 vs. 3.39, p<0.001; intermediate: 10.07 vs.9.26, p=0.058; high: 14.88 vs. 14.03, p=0.025) with the biggest difference in low labeled spot. The 4 methods showed poor agreement on classification of LA/LB types. The proportion of LB type ranged from 3.4% to 60.9% depending on methods.

Classification of luminal A and luminal B types by different methods

<table>
<thead>
<tr>
<th></th>
<th>Luminal A</th>
<th>Luminal B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot spot index-DIA</td>
<td>68 (39.1%)</td>
<td>106 (60.9%)</td>
</tr>
<tr>
<td>Average index-DIA</td>
<td>157 (90.2%)</td>
<td>17 (9.8%)</td>
</tr>
<tr>
<td>Hot spot index-MC</td>
<td>82 (47.1%)</td>
<td>92 (52.9%)</td>
</tr>
<tr>
<td>Average index-MC</td>
<td>168 (96.6%)</td>
<td>6 (3.4%)</td>
</tr>
</tbody>
</table>

Forty two of 174 cases (24.1%) showed Ki67 heterogeneity. Ki67 heterogeneity was most frequent in T2 (p=0.001) and histologic grade 3 (p=0.023) tumors.

Comparison of pathological features according to Ki67 heterogeneity

<table>
<thead>
<tr>
<th>Ki67 heterogeneity</th>
<th>Absent</th>
<th>Present</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>132(75.9%)</td>
<td>42 (24.1%)</td>
<td></td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>95(84.1%)</td>
<td>18(15.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>T2</td>
<td>32(58.2%)</td>
<td>23(41.8%)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>5(83.3%)</td>
<td>1(16.7%)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>30(90.9%)</td>
<td>3(9.1%)</td>
<td>0.023</td>
</tr>
<tr>
<td>2</td>
<td>93(74.4%)</td>
<td>32(25.6%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9(56.2%)</td>
<td>7(43.8%)</td>
<td></td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma, NOS</td>
<td>114(74%)</td>
<td>40(26%)</td>
<td>0.475</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>8(88.9%)</td>
<td>1(11.1%)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion: Ki67 LI is markedly variable depending on different measurements and spot selections. Ki67 heterogeneity exists in 24.1% of luminal breast cancers in this series. Therefore, whole section slides should be preferred for Ki67 LI measurement, rather than tissue microarray or biopsy specimen which may not entirely reflect the proliferation index of the tumor.

<table>
<thead>
<tr>
<th></th>
<th>Mucinous carcinoma</th>
<th>Others (invasive micropapillary, tubular, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4(80%)</td>
<td>1(20%)</td>
</tr>
<tr>
<td></td>
<td>6(100%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>
Title: Early stage breast cancer prognostication using whole tumor or Ki67 heterogeneity-based digital imaging

Barnes M, Srinivas C, Xu C, Dean S, Singh S, Henricksen LA, Wik N, Morris D, Magliocco A and LaFleur B. Roche Diagnostics, Mountain View, CA; Tom Baker Cancer Centre, Calgary, AB, Canada and Moffitt Cancer Center, Tampa, FL.

Body: Introduction:
Accurate prognosis of hormone-positive early stage breast cancer patients offers the opportunity to make more informed follow-up choices, for example the addition of adjuvant chemotherapy. More recently patient prognostication based on immunohistochemistry-scored protein expression (ER, PR, Her2 and Ki67) in the ATAC trial has been described as IHC4 (C-index of 0.78). However, IHC4 clinical translation has not occurred and may be hindered by the need for a clinically-validated standardized assay as well as pathologist scoring reproducibility. To address this idea, we employed a standardized assay system and automated scoring using digital image analysis to assess either whole tumor (WT) IHC expression values or Ki67 heterogeneity (Ki67H) quantification. The goals were to 1) establish a prognostic model based on and potentially improving the IHC4 concept and 2) improve pathologist scoring reproducibility.

Material and Methods:
A paraffin-embedded whole tissue cohort consisting of 120 cases of hormone-positive, HER2-negative, stage I and II, breast cancer patient samples were re-stained with standardized ER, PR, HER2, and Ki67 IHC assays. Three pathologists independently scored conventional glass slides microscopically (CM) and annotated WT on H&E and Ki67 heterogeneous regions on whole slide scanned images (WSI) for each case separately. The annotations were separately registered across serial stained slides and also scored via the digital pathology algorithm.

Results:
The mean patient age at the time of diagnosis is 63 years with a maximum follow-up of 18 years. Patients with regional and/or distal recurrence compose 26% of the cohort with a median recurrence free survival of 8.5 years. Clinical variables (CV) plus WT (C-index 0.74, r² 0.38) or Ki67H (C-index 0.77, r² 0.39) models improved on patient prognostication each as compared to the IHC4 plus CV (C-index 0.70, r² 0.19) in this cohort. High inter-pathologist reproducibility for the IHC score, as measured by concordance correlation coefficient was noted for Ki67H (0.90).

Conclusions:
Novel algorithmic scoring methodologies such as WT and Ki67H may improve on the IHC4 concept with high inter-pathology reproducibility. We are currently validating in a larger 600 patient cohort.
Agreement rates of pathologist-derived manual and digital read and image analysis quantitation for membrane and nuclear-based immunohistochemistry biomarkers in breast cancer clinical studies


Body: Introduction: Over the past 18 years, pathologists have had an increasing responsibility of quantitating immunohistochemistry (IHC) markers with the expectation of high intra- and inter-reader reproducibility. These markers are related to treatment prediction or patient prognosis and are predominately represented by estrogen receptor (ER), progesterone receptor (PR), human epithelial growth factor receptor 2 (HER2), and Ki-67. Many agencies have instituted quality programs to increase these metrics with dramatic effect; however, improvement is still needed. Digital imaging-based quantitation of IHC offers the potential for increasing intra- and inter-reader reproducibility. In this study, we culminate digital and imaging analysis data across multiple regulatory submissions to understand intra- and inter-reproducibility metrics in the context of five breast cancer biomarkers.

Materials and Methods: For each IHC marker (ER, PR, HER2, Ki-67, and p53), 120 invasive whole tissue breast cancer cases were included from retrospective clinical archives and restained. Cases were represented by binning into three IHC staining categories (ER, PR, Ki-67, and p53: 0 to 0.99%, 1 to <10%, and >=10%; HER2: 0/1+, 2+, 3+) representing 33% of samples for each binned category. Three pathologists (9 total) scored each case per marker as positive/negative around a cut-point (ER and PR: 1%; Ki-67 and p53: 10%, and HER2: 2+) utilizing three scoring modes (manual read [MR], digital read [DR], and imaging analysis [IA]) with a 1-2 week washout. Primary end-point of DR and IA was non-inferiority to MR. Statistical analyses were performed using pair-wise analyses of MR vs DR or IA.

Results: IA inter-reader reproducibility overall percent agreements (OPA) were at least similar to manual read OPAs for ER, PR, HER2, and Ki-67. HER2 IA OPA showed an improvement over MR. DR inter-reader reproducibility OPAs were similar to manual reads for ER, PR, HER2, and p53. Ki-67 DR inter-reader reproducibility underperformed MR (Table 1). Intra-reader reproducibility OPAs across scoring modalities with respect to any single scorer were similar for ER, PR, HER2 and Ki-67 (>88% 95%CI 81.3-99.1%), while p53 was lower (82.9% 95%CI 75.1-91.6%).

<table>
<thead>
<tr>
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<th>MR (95% CI)</th>
<th>DR (95% CI)</th>
<th>IA (95% CI)</th>
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<tbody>
<tr>
<td>ER</td>
<td>94.9% (91.4-97.8%)</td>
<td>92.0% (87.8-95.8%)</td>
<td>95.3% (92.0-98.2%)</td>
</tr>
<tr>
<td>PR</td>
<td>94.4% (90.9-97.2%)</td>
<td>94.0% (90.2-97.1%)</td>
<td>94.1% (90.3-97.2%)</td>
</tr>
<tr>
<td>HER2</td>
<td>85% (77-90%)</td>
<td>92% (86-96%)</td>
<td>95% (89-98%)</td>
</tr>
<tr>
<td>Ki-67</td>
<td>85.6% (80.4-90.4%)</td>
<td>76.6% (70.9-82.2%)</td>
<td>86.8% (82.1-91.4%)</td>
</tr>
<tr>
<td>p53</td>
<td>78.8% (72.2-83.3%)</td>
<td>80.6% (75.0-86.0%)</td>
<td>81.7% (76.4-86.8%)</td>
</tr>
</tbody>
</table>

Table 1: Inter-reader reproducibility OPAs across MR, DR, and IA for each breast cancer marker

Conclusions: Overall, DR and IA IHC-based quantitation across these biomarkers showed at least similar results to MR. Some markers over or underperform from this MR baseline most likely related to analysis approach and/or marker heterogeneity.
Title: Digital image analysis outperforms manual scoring for breast cancer subclassification and prognostication

Stålhammar G, Rosin G, Kis L, Lippert M, Moelholm I, Grunkin M, Bergh J and Hartman J. Karolinska Institutet, Stockholm, Sweden; St. Erik Eye Hospital, Stockholm, Sweden; Karolinska University Hospital, Stockholm, Sweden; Visiopharm A/S, Hoersholm, Denmark; Cancer Center Karolinska, Stockholm, Sweden and Karolinska University Hospital, Stockholm, Sweden.

Body: Introduction
Categorization according to the four gene expression-based 'intrinsic' subtypes "Luminal A", "Luminal B", "HER2-enriched" and "Basal-like" is the method of choice for practical prognostic and predictive value in the heterogeneous spectrum of Breast Cancers. Gene expression tests are however not yet universally available, which has created an opportunity for routine immunohistochemical stains to act as surrogate markers (biomarkers) for the gene expression-based subtypes. As recommended by international expert consensus, the expressions of Estrogen receptor α (ER), Progesterone receptor (PR), Human Epidermal Growth factor Receptor 2 (HER2) and the proliferation-associated protein Ki67 are scored during the routine pathological work-up of breast cancer specimens. Thus, congruence of these biomarker tests to the gene expression tests are of utmost importance as discrepancies in classification induces dissimilar treatment decisions. In this study, we compare a novel system for Digital Image Analysis (DIA) with the manual scoring of biomarkers used in current clinicopathological routine and suggest methods to improve their congruence to gene expression assays, provide more robust prognoses for survival as well as reduce time consumption for pathologists.

Methods
1 tissue micro array (TMA) cohort and 2 cohorts of primary breast cancer specimens (total n = 436) with >20 years survival data, were sectioned into physical glass slides and digitally scanned at ×20 and then reviewed for manual vs. DIA test congruence to PAM50 gene expression assays in terms of classification into the four intrinsic subtypes, and their prognostic power. This included the evaluation of 6 different methods for DIA biomarker testing. The DIA system used was the Visiopharm Integrator System (VIS) by Visiopharm A/S, Hoersholm, Denmark. In short, this system facilitates ER, PR and Ki67-testing by a 'sandwich' technology, in which each biomarker slide is aligned with an adjacent 3 µm slide stained with a pancytokeratin marker. Thus, non-tumor tissue is to a large extent automatically excluded from analysis and only cells that express cytokeratin are eligible for automatic detection of positivity or negativity of the respective biomarker.

Results
60.8 % of the cases in the TMA cohort was classified in concordance with PAM50 (κ = 0.46) using DIA. Classification with regard to HER2-enriched, Basal-like and Luminal tumors without dichotomization of A and B subtype (thereby avoiding the impact of Ki67-scoring) was 80.9 % (κ = 0.63). In the whole slide cohorts, DIA performed with 95 % of the cases classified in concordance with PAM50 (κ = 0.90), compared to 67 % (κ = 0.59) for the manual method recommended by international consensus. In addition to this DIA produced better prognostication vs. the manual method in terms of hazard of all-cause death for each subtype.

Conclusion
The system for DIA evaluated here outperforms manual scoring in both predictive and prognostic value. It also has the potential to reduce time consumption for pathologists, as many of the steps in the workflow is either automatic or feasible to manage without pathological expertise. Based on the findings in this study however, TMA cannot be recommended for DIA scoring of Ki67.
Title: Distribution pattern of Ki67 immunoreactivity in ductal intraepithelial neoplasia (DIN): Correlation with lesion grade and potential utility

Ozerdem U and Tavassoli FA A. Yale University School of Medicine, New Haven, CT.

Body: INTRODUCTION: Ki67 labeling index has been proposed as an independent predictive and prognostic factor in patients with ductal intraepithelial neoplasia (DIN). Ki-67 labeling index of 14% has been suggested as a useful cut-off for stratifying DIN patients for adjuvant radiotherapy and hormonal therapy. No data is available regarding either the distribution pattern of Ki67 immunoreactivity within the ducts involved by DIN or potential correlation of these patterns with lesion grade. In this study, the pattern of distribution of the nuclei immunoreactive with Ki67 was examined in DIN1C (DCIS, grade 1), DIN2 (DCIS, grade 2), and DIN3 (DCIS, grade 3) to determine if distinctive patterns could be identified and if these patterns would correlate with lesion grade.

METHODS: 47 consecutive DIN cases were retrieved from our departmental files. Of these, 5 qualified as DIN1C, 28 as DIN2 and 14 as DIN3. H&E and Ki67 immunostains were evaluated on each case to elucidate the distribution of Ki67 positive proliferating epithelial cells within the ducts. The DIN cases were evaluated and the patterns of distribution recorded for each case as either basal or haphazard within the epithelial proliferation. Statistical analysis was performed using Chi-square test with Graphpad PRISM statistical analysis software.

RESULTS: There was a statistically significant difference between the 3 groups in terms of basal vs haphazard Ki67 immunostaining (Chi –square test, P=0.0001). Basal staining pattern was dominant (100%) among the DIN1c cases, while haphazard staining pattern was the dominant (100%) distribution among the DIN3 cases. One half of the DIN2 cases showed basal staining pattern, while the other half showed a haphazard staining pattern. This feature could be useful in separating DIN lesions into low grade and high grade eliminating grade 2. We also quantified necrosis on a scale of 0 to 2; 0 indicating absence of necrosis and 2 reflecting comedo type necrosis. Necrosis was more common in the ducts with haphazard Ki67 distribution. The extent of necrosis varied significantly between DIN1c, DIN2 and DIN3 (Chi –square test, P<0.0001) CONCLUSIONS: Two distinct patterns of Ki67 immunoreactivity are seen in DIN lesions; the basal pattern is characteristic of DIN1 (low grade DIN), whereas a haphazard pattern is dominant in DIN3 (high grade DIN). These patterns could be used to divide grade 2 DIN into low grade and high grade. This approach is easier and potentially more reproducible than counting the percentage of Ki67 positive cells. The information could be useful from a prognostic standpoint and may well be predictive of potential response to radiation, hormonal and targeted therapies.
Title: An impending avalanche-breast cancer among women ≥ 90 years of age

Ozerdem U and Tavassoli FA A. Yale University School of Medicine, New Haven, CT.

Body: INTRODUCTION: Pubmed database shows no study on pathological features of breast carcinoma in extremely old women (≥90 years). The data post 1980s is limited due to the definition of such age limit as "older than 75". This study redefines age extreme in breast pathology to better correlate with current patient demographics, and provides characteristics of breast cancer in this population. Little information exists about the breast cancer in extremely old women. This investigation elucidates the growing number of breast cancer diagnoses in extremely old women (≥90 years) in a single institution in 2000s.

MATERIALS AND METHODS: The database of Yale University Department of Pathology was searched for the terms: "breast carcinoma," "age ≥90" for the past 15 years. Clinicopathologic features of the cases that fit the criteria were studied.

RESULTS: A total of 135 patients (134 female; 1 male) aged ≥90 years were identified with a diagnosis of infiltrating carcinoma. A surge in breast cancer diagnoses among elderly patients was noted in 2000s compared to the earlier decade. Only 10 cases were diagnosed between 1990 and 1999 (one case/year) compared to 125 cases diagnosed between 2000 and 2015 (8.3 cases/year). Of these 135 patients, 117 had infiltrating ductal carcinoma (IDC); 16 had infiltrating lobular carcinoma (ILC),1 had pure squamous cell carcinoma. One patient had IDC and ILC ipsilaterally, while another had bilateral IDC. The median age was 92 (range: 90-107), median tumor size was 2.0 cm (range: 0.2-13.0), and the median modified Bloom & Richardson score was 6 (range: 3-9). Among the 117 IDCs, 11 had mucinous, 6 had apocrine, 1 had medullary, 1 had cribriform differentiation. Ductal intraepithelial neoplasia (DCIS) was present in 46% of the cases, while lobular intraepithelial neoplasia (LCIS) was identified in 8% of the cases.

CONCLUSIONS: Our data shows an increasing number of breast carcinomas diagnosed among patients ≥90 years of age with a morphological distribution similar to other age groups. This increased frequency is particularly notable in the last 15 years compared to 1990s. While increased life expectancy is a factor, better delivery of screening to elderly patients and patient awareness are important additional contributors to this increase. Extremely old women particularly those in good health may potentially benefit from breast cancer screening. As the number of substantially older patients with breast cancer increases, we have to be prepared to manage the disease in this population. Further studies are warranted to elucidate the frequency of breast cancer in extremely old women and the optimal management that would probably have to be individualized to the patients' health status.
Title: Ductal carcinoma in situ: A comparative study between histopathological characteristics and imaging findings

Tang X, Yamashita T, Kumaki N, Tokuda Y and Masuda S. Nihon-University School of Medicine, Tokyo, Japan; Tokai University, School of Medicine, Isehara, Kanagawa, Japan; Tokai University School of Medicine, Isehara, Kanagawa, Japan and Tokai University School of Medicine, Isehara, Kanagawa, Japan.

Body: Background: The treatment policy for ductal cancer in situ (DCIS) of the breast greatly depends on the spreading diagnosis. However, a problem is that we cannot compare imaging findings with the histopathology of DCIS. The purpose of this study was to investigate the histopathological characteristics of DCIS and the association with imaging findings.

Methods: Subjects were 128 patients from Tokai University Hospital, diagnosed with DCIS. A positive finding on ultrasonography (USG) was defined as Breast Imaging Reporting and Data System (BI-RADS) of US category 3 or above, in mammography (MMG) it was Japan Breast Cancer Society category 2 or above, and in MRI it was BI-RADS-MRI category 3 or above. Histopathologically, we re-classified DCIS into 3 subtypes.

Table. Histopathological classification of the 3 DCIS subtypes

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>Architectures of DCIS</th>
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<tbody>
<tr>
<td>Type 1</td>
<td>Flat and/or micropapillary</td>
</tr>
<tr>
<td>Type 2</td>
<td>Cribriform and/or papillary</td>
</tr>
<tr>
<td>Type 3</td>
<td>Solid and/or comedo, solid or comedo with any other architecture patterns, e.g. solid and cribiform or papillary, etc.</td>
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The microscopic examination items included the nuclear grade, necrosis and calcification, stromal reactions surrounding DCIS, distribution of DCIS, and with or without adenosis or other benign changes in the background breast. The automated image analysis using figures captured from virtual system are planned to evaluate concentration of DCIS distribution.

Results: 1) The clinical characteristics and association between imaging findings and histopathological classification of the 3 subtypes of DCIS are summarized as: a) Histopathologically, in type 3, there was a higher frequency of necrosis and calcification in the ducts of DCIS ($\chi^2, p<0.001$), the number of dilated peri-ductal capillaries was greater than in type 1 ($p=0.023$), and the distribution of DCIS was concentrated in type 3 ($p=0.020$); b) In imaging findings, type 3 was easier to detect than type 1 on USG ($p=0.008$), but there were no significant differences in MMG and MRI. 2) The 14 DCIS cases that could not be detected by USG, showed slight edematous or myxoid change in the stroma histopathologically ($p<0.001$), and were less likely to be detected by MRI ($p=0.004$). 3) The 6 MRI un-detected cases were less likely to be detected by USG ($p=0.004$), and the occurrence of adenosis or other benign changes in the background breast interfered with MRI ($p=0.010$). Peri-ductal capillaries seemed to be an important factor for MRI detection ($p=0.007$). 4) The results of automated image analysis will be presented.

Conclusion: USG imaging reflected the histopathological subtypes of DCIS, myxoid changes of the stroma, and the concentration of DCIS ducts. MRI was correlated with the peri-ductal capillaries of DCIS and the changes in the background breast, while MMG can make up for the shortcomings of USG and MRI. It is important for us to keep the histopathological type in mind and interpret the imaging findings comprehensively, when we do a spreading diagnosis of DCIS.
Title: Pleomorphic lobular carcinoma in situ (PLCIS)-presentation, associated lesions and outcome

Shaaban AM M, Smith S, Bradley S, McMahon M and Sharma N. Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom and St James's University Hospital, Leeds, United Kingdom.

Body: Pleomorphic lobular carcinoma in situ (PLCIS) is an uncommon lesion characterised by dyscohesive lobular cells showing high grade nuclei. It is commonly associated with comedo necrosis and luminal calcifications and hence diagnosed on mammographic screening. Data on the presentation, focality, associated lesions, optimal treatment and outcome of PLCIS is patchy.

Methods
Cases diagnosed as PLCIS between 2005 and 2015 were identified from the imaging and pathology databases of two UK large breast screening units. Cases diagnosed on diagnostic core biopsy/VAB or surgical excisions were included. Comprehensive data was collected on age, mode of presentation (screening vs symptomatic), imaging (mammography, ultrasound and MRI), surgical management, histological features on core biopsy and excision including type, grade and immunohistochemical profile of associated ductal carcinoma in situ (DCIS) and invasive carcinoma.

Results
86 cases with the diagnosis of PLCIS (confirmed by review and e-cadherin negativity) were identified. The mean patient age at diagnosis was 61.04 years, range: 39-84 years. 32 cases were treated with wide local excision with/without axillary procedure. A total of 38 patients were screen detected & 36 cases were diagnosed in the symptomatic setting. Others presented as incidental calcifications on family history screening, incidental histological findings in breast reductions and risk reducing mastectomy.

On mammography, 6 patients presented with an asymmetrical density, with or without calcifications, 25 with calcifications, 44 as a mass and 2 as stromal deformity. No mammographic abnormality was found in 9 cases. PLCIS was multifocal in 19.7% of cases, diffuse in 9.9%, focal in 69% and multi-centric in 1.4% on imaging.

Histologically, PLCIS was the most advanced lesion on core biopsy without associated DCIS or invasive disease (pure PLCIS) in 23 patients. Of these, surgical excision revealed an invasive carcinoma in 7 cases (upgrade rate = 30.4%). Six more patients presented as DCIS and PLCIS on core biopsy; three of whom (50%) had invasive disease on excision.

Classical LCIS was associated with PLCIS in 27/86 cases (31.3%). The most common type of associated invasive carcinoma on surgical excision was invasive classical lobular carcinoma (ILC, 40 cases), followed by invasive pleomorphic lobular carcinoma (IPLC, 27 cases). Ductal no special type carcinoma, solid papillary and tubulo-lobular carcinoma were also identified. The size of PLCIS on excision ranged from 1-80mm. DCIS was associated in 26.7% of cases. The majority of invasive cancers were of grade 2 (53.5%) and 3 (19%). The tumors were ER positive (53 cases), PR positive (43 cases) and HER2 negative (52 cases).

Conclusion
PLCIS is an uncommon in situ carcinoma presenting via mammographic and also in the symptomatic setting. Unlike classical LCIS, PLCIS is a disease of postmenopausal women. It is multifocal in approximately one fifth of the cases.

PLCIS is commonly associated with classical LCIS and both ILC and IPLC. When identified in core biopsy, the upgrade rate in this series was 30.4% which increased to 50% if the lesion co-existed with DCIS. The associated cancers are often ER positive, HER2 negative. These findings support managing those lesions surgically as per DCIS.
Title: Nuclear immunohistochemical IKK-ε expression in flat epithelial atypia (FEA) of the breast: A predictor of ipsilateral ADH, in-situ or invasive malignancy?

Williams PA A, Parra-Herran CE E, Ayroud Y, Islam S, Gravel DH H, Robertson SJ J and Pratt C. University of Ottawa, Ottawa, ON, Canada and EORLA, Ottawa, ON, Canada.

Body: Background: Flat Epithelial Atypia of the breast (FEA) is associated with in situ and invasive low grade neoplasia. However, the role of excision after FEA on biopsy is controversial as rates of upgrading to atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS) or invasive carcinoma in subsequent excision are relatively low. Problems include difficulties in inter-observer reproducibility and lack of morphologic or immunohistochemistry (IHC) tools that better identify cases at risk for concurrent ADH/Carcinoma. Nuclear image analysis may be useful, but is not widely available. IKK-ε, part of the NF-κB activating pathway, is absent in normal breast epithelium and non atypical (usual) ductal hyperplasia, but is over-expressed in >30% of breast cancers. In addition, in our experience ADH/DCIS shows IKK-ε staining, mostly cytoplasmic. Of note, in prostate cancer, nuclear accumulation of IKK-ε has been described in hormone sensitive prostate disease while cytoplasmic accumulation is associated with metastatic progression. No previous studies of IKK-ε levels in FEA are reported. Here we report IKK-ε status in FEA and correlation with ipsilateral, synchronous ADH, DCIS or invasive carcinoma.

Method: Resection specimens from 61 patients with diagnosis of FEA were retrieved. Presence of ADH/carcinoma and laterality (ipsi or contralateral) was recorded. Synchronous neoplasia was defined as ADH, DCIS or invasive carcinoma diagnosed within 6 months of the diagnosis of FEA. Presence of FEA was confirmed by three observers using strict morphologic criteria. IHC for IKK-ε was performed using ABCAM, rabbit anti-IKK-ε (ab7891) and pH 6 citrate buffer heat-induced epitope retrieval for 20 minutes. IHC slides were scanned and FEA regions captured for blind scoring of nuclear and cytoplasmic staining. Cut off for positive nuclear staining was 10% and cytoplasmic staining was graded as negative, weak, moderate or strong positive.

Results: 40 patients had ipsilateral synchronous ADH/carcinoma, and 21 did not. Within these groups, 6 patients had contralateral ADH/carcinoma (2 with and 4 without ipsilateral neoplasia). While cytoplasmic staining showed no difference between the groups, nuclear positivity was more frequent in cases with ipsilateral synchronous ADH/carcinoma, \( \chi^2(1, N = 61) = 5.1, p = .025 \) (Table 1). In contrast, there was no correlation between IKK-ε staining and ADH/carcinoma in the opposite breast (p=.25).

Table 1

<table>
<thead>
<tr>
<th>Nuclear IKK-ε</th>
<th>Synchronous Ipsilateral ADH/DCIS/Carcinoma</th>
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<tbody>
<tr>
<td></td>
<td>Negative (%)</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (48)</td>
</tr>
<tr>
<td>Positive</td>
<td>10 (25)</td>
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</table>

Conclusion: Nuclear IKK-ε staining may prove useful in predicting synchronous ipsilateral ADH or malignancy in cases of FEA in biopsy material. Given its more frequent association with ipsilateral synchronous ADH/carcinoma, IKK-ε nuclear expression in FEA may represent a step in continuous local oncogenesis rather than a general marker of risk. Given the pleiotropic role of IKK-ε in growth and survival, the significance of the shift from nuclear staining in FEA to cytoplasmic staining in ADH/DCIS may reflect different signaling pathways and requires further investigation. Further validation of our findings in larger cohorts is necessary.
**Title:** Comparison of local clinical subtyping to central molecular classification using microarray-based expression test in breast cancer patients


**Body:**

**Background:**
Measurement of estrogen receptor (ER), progesteron receptor (PR) and human epidermal growth factor receptor 2 (HER2) status in early breast cancer is critical for informing treatment recommendations. Targetprint®, a commercially available microarray-based test, measures mRNA levels of ER, PR and HER2 genes. The aim of this study was to investigate the concordance and accuracy of local clinical subtyping by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) with TargetPrint®.

**Material and Methods:**
We collected data retrospectively from 109 early breast cancer patients from 17/5/2012 to 17/4/2015. All of them underwent surgery. ER, PR and HER2 status were assessed by IHC in tumor samples. For HER2 IHC 2+ cases, additional FISH was used. These results were compared with microarray mRNA quantifications. Microarrays were all performed in formalin-fixed paraffin-embedded (FFPE) tumor samples. Accuracy of TargetPrint® was evaluated with positive (PPV) and negative (NPV) predictive value considering IHC as "Gold Standard". Concordance between techniques was evaluated with percentage of concordance and Cohen's κ coefficient. The interpretation of κ Coefficient is done by correlating its value with a qualitative scale (Landis and Koch, 1977): 0 is considered poor; 0,01-0.20 is slight; 0,21-0.40 is fair; 0,41-0.60 is moderate; 0,61-0.80 is substantial; 0.81-1 is almost perfect.

**Results:**
100% of tumor samples were RE positive for both IHC and TargetPrint®. All 109 patients resulted HER2 IHC negative, 3 of them were HER2 TargetPrint® positive. Regarding RP, 80% of tumor samples were IHC and TargetPrint® positive, 11% IHC and TargetPrint® negative, 5% negative by IHC and positive by TargetPrint® and 4% positive by IHC and negative by TargetPrint®. For ER, concordance was 100%, κ=1. For PR, concordance was 90.83%, κ=0.65 (95%CI 0.45-0.85). In the case of HER2, percentage of concordance was 97.25%, κ=0 (kappa paradox). TargetPrint had PPV 1 and NPV 0 assessing ER. For PR, TargetPrint PPV is 0.93 and NPV is 0.75. For HER2, TargetPrint PPV is 0 and NPV is 0.97.

**Conclusions:**
To the best of our knowledge, this is the first study exploring concordance between IHC/FISH and Targetprint® in FFPE tumor samples. According to previous data in fresh tissue, almost perfect concordance for ER and HER2 as well as substantial concordance for PR were seen. We suggest lack of accuracy in IHC technique, in microarray test or intra-tumor heterogeneity as possible reasons for the less consistent accordance in PR. It would be interesting to further design a prospective study to address this question.
Title: Does HER2 immunohistochemistry-guided targeted FISH analysis help identify intratumoral heterogeneity in breast cancer?

Gulbahce HE, Factor RE E, Geiersbach KB B and Downs-Kelly E. University of Utah School of Medicine, Salt Lake City, UT and ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

Body: Introduction: The updated 2013 ASCO/CAP HER2 guidelines proposed revised scoring with the aim of capturing all patients eligible for targeted therapy. The current guidelines also address intratumoral heterogeneity using immunohistochemistry (IHC) as a platform to identify and target heterogeneously staining areas for FISH analysis. We wanted to determine if identifying the most intense areas of staining in IHC equivocal / 2+ cases would help identify genetic heterogeneity and potentially more FISH amplified patients.

Materials and Methods: Our lab offers HER2 immunohistochemistry by HercepTest™ (Dako) or 4B5 (Ventana), and dual probe FISH (Abbott Molecular or Dako IQ). We offer HER2 testing as IHC or FISH only, and also as a reflex FISH following equivocal (2+) IHC. HER2 tests performed and interpreted after 10/2013, following the implementation of the ASCO/CAP 2013 guidelines, to 5/2015 were included in this study. Both IHC and FISH were manually read following ASCO/CAP 2013 guidelines by one and two observers respectively. Equivocal (2+) cases with heterogeneous staining showing areas with \( \geq 10\% \) weak or moderate circumferential membrane staining were circled by a pathologist and reflexed to HER2 FISH. In those cases where samples were received for FISH testing only, the pathologist circled the entire area of invasive tumor on H&E slides. All circled tumor areas were evaluated before signal enumeration of the FISH probes.

Results: 1805 HER2 FISH test requests received in our laboratory during the study period had interpretable FISH results. 1210 of these cases did not have prior HER2 IHC testing performed in our laboratory (“FISH only”), 595 had reflex FISH testing following equivocal (2+) IHC (“IHC2+/reflex FISH”). Amplification rates between the “IHC2+/reflex FISH” group of patients with targeted areas for FISH and the “FISH only” group where the entire invasive carcinoma was marked on H&E slide and scored were similar (16.3% and 20% respectively). 10/595 (1.7%) “IHC2+/reflex FISH”, and 15/1210 (1.4%) “FISH only” groups showed genetic heterogeneity by FISH with discrete population of amplified tumor cells respectively (p=0.4).

<table>
<thead>
<tr>
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<th>FISH Testing Utilizing Entire Tumor Area * n=1210</th>
<th>FISH Testing Utilizing Most Intense Staining Areas (following equivocal IHC) n=595</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Heterogeneity</td>
<td>15 (1.4%)</td>
<td>10 (1.7%)</td>
</tr>
<tr>
<td>Amplified</td>
<td>242 (20.0%)</td>
<td>97 (16.3%)</td>
</tr>
</tbody>
</table>

*includes equivocal IHC, and FISH as first line of testing

Conclusion: The updated HER2 ASCO/CAP guidelines recommend that intratumoral heterogeneity for HER2 may be easier to detect with IHC which can be used to guide FISH enumeration. In our study, identifying areas of more intense staining on IHC slides for targeting FISH analysis did not result in a difference in identification of genetic heterogeneity.
Do estrogen receptor negative and progesterone receptor positive breast tumors really exist? Attitude for not making them real

Molly D, Bertaut A, Blanchet C, Beltjens F, Charon-Barra C, Loustalot C, Desmoulins I and Arnould L. GF Leclerc Cancer Center, Dijon, France.

Body: Background: Breast tumors with negative estrogen receptor (ER-) and positive progesterone receptor (PR+) are rare (from 0 to 3.4 % according to the studies), and their existence is contested. These markers determine cancer molecular subtypes which play a determinant role for both the management and the prognosis of breast cancers. It is then essential to document the real existence of ER-/PR+ tumors. The present study aimed at determining if ER-/PR+ tumors share more basal or luminal characteristics.

Methods: Between 2000 and 2015, 50 patients with ER-/PR+ breast tumors, representing 0.6 % of all breast cancers diagnosed in our institution, were included in this study. Their clinical (age, node status), morphological (size, histological type, Elston and Ellis (EE) grade, necrosis, inflammation, pushing margins, central scar, mitotic index) and immunohistochemical characteristics (ER, PR, HER2, CK5/6 and EGFR status) were compared with those of 50 luminal and 50 triple negative (TN) tumors randomly selected in our lab database. At the time this abstract is written, the Ki67 index determination is still in progress. Five of these ER-/PR+ tumors were also given a molecular test (Nanostring). Qualitative variables were compared using Chi2 or Fisher test, quantitative variables were compared using Student or Mann & Whitney tests. To take into account for multiple comparisons, p-values less than 0.025 were considered as significant.

Results: The results are summarized in table 1. For almost all the analyzed criteria, ER-/PR+ tumors present statistical difference with luminal ones. On the contrary, they share most of TN tumors characteristics. The 5 molecular analyzes performed on ER-/PR+ samples showed the following phenotypes: 3 basal, 1 HER2 enriched and 1 luminal. For this last one, new immunohistological analyzes reveal in fact an ER+ staining.

Conclusion: This study tends to support that ER-/PR+ tumors may not exist and are likely to be TN cancers or less frequently false negative ER+ tumors. In a practical point of view, we think that 1) when a tumor shows ER-/PR+ and TN characteristics, it is probably a false positive PR staining, and the tumor has to be considered as a TN one, 2) when an ER-/PR+ tumor don't fit the triple negative tumors characteristics, ER must be retested in order to exclude a true luminal tumor.

Table 1: Study results

<table>
<thead>
<tr>
<th></th>
<th>ER-/PR+ tumors</th>
<th>ER+ tumors</th>
<th>TN tumors</th>
<th>p ER-/PR+ vs ER+</th>
<th>p ER-/PR+ vs TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (mm)</td>
<td>23.1</td>
<td>25</td>
<td>25.2</td>
<td>0.0044</td>
<td>0.4822</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>&lt;10^-4</td>
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<td>HER2 +</td>
<td>29.8</td>
<td>7.8</td>
<td>0</td>
<td>0.0051</td>
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</table>

Results are given in %, excepting mitotic index and size.
Publication Number: P1-01-15

Title: Breast cancer biomarkers: Global tumor biomarker quality assurance study of intratumor heterogeneity

Dabbs DJ J and Clark B. Magee-Women's Hospital of UPMC, Pittsburgh, PA.

Body: Introduction
Biomarkers (BM) are often performed on core biopsies (CB) of breast tumors for treatment purposes, yet comprehensive information on BM heterogeneity is lacking. This quality assurance study is to survey BM intra-tumor heterogeneity (ITH) by comparing BM from CB and the examination of entire breast tumors from surgical excisions (SE). This interim report supplies data on 45/100 collected cases to date.

Methods:
Whole tumor cases, 3.0 cm or less were collected in years 2013-2014, excluding neoadjuvant cases and were examined with CB by two pathologists. All tissues were processed using 2013 ASCO/CAP guidelines. Immunohistochemistry (IHC) for ER/PR/Her2/Ki67 was performed on breast core biopsies and each block of a tumor surgical excision (1-13 blocks per case). IHC was performed using the Ultra Ventana-Roche device with FDA IVD clones ER (SP1), PR (1E2) Her2 (4B5) Ki67 (30-9). Whole slides were semi-quantitated for ER/PR using HScore (0-300), Her2 was interpreted with 2013 ASCO/CAP guidelines and Ki67 proliferation index (PI) as estimated whole slide percent of tumor cell nuclear stain. Mean HScores and PI were calculated for all BM on SE, and CB deviations from the SE mean were calculated for ER/PR/Ki67. ITH of BM was assessed by comparing results among all tissue blocks for each case. Her2 results were descriptive. Eight cases lacked CB Ki67 PI.

Results
There were no cases in which categorical hormone receptors results (positive/negative) differed between CB and SE. 2/45 (4%) cases were Her2 positive, and 13/45 (28%) were equivocal by IHC with one case amplified by in situ hybridization. Comparing ER between CB/SE, 3/45 differed in HScores by 30% or more. 10/45 SE ER had ITH of 25% or more among different tissue blocks. 14/45 cases had CB/SE PR difference in HScore of 30% or more. 18/45 cases had SE PR with ITH HScore of 25% or more between tissue blocks.

Ki67 PI differed between CB/SE by more than 25% in 18/37 cases, with ITH among tissue blocks of more than 25% in 23/37 cases. The variability of CB-SE results and ITH among tissue blocks are summarized in table 1.

Table 1 Summary of Results

<table>
<thead>
<tr>
<th>CB-SE ER</th>
<th>ITH ER</th>
<th>CB-SE PR</th>
<th>ITH PR</th>
<th>CB- SE PI</th>
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<tr>
<td>&gt;30%</td>
<td>&gt;25%</td>
<td>&gt;30%</td>
<td>&gt;25%</td>
<td>&gt;25%</td>
<td>&gt;25%</td>
</tr>
<tr>
<td>3/45</td>
<td>10/45 (22%)</td>
<td>14/45 (31%)</td>
<td>18/45 (40%)</td>
<td>18/37(49%)</td>
<td>23/37(62%)</td>
</tr>
</tbody>
</table>

CB-SE: core biopsy vs surgical excision; ER PR as H Score

Conclusions
In this interim report of 45/100 cases, (1) ITH of 25% or more occurred for ER content in 22 % of cases (2) similar ITH exists for PR content in 40% of cases. (3) Ki67 PI tissue block ITH was expressed with variation of 25% or more in 23/37 cases. (4) While this degree of variability of BM may not affect treatment decisions, the tissues show sufficient BM variability that should stimulate concern for accuracy of results of prognostic gene expression profiles and genomic testing of breast tumors.
Body: Background: The expression of estrogen receptor alpha (ERα) and progesterone receptor (PR) in breast carcinomas is a strong predictor of the efficacy of hormonal therapy for breast cancer patients as well as providing a degree of prognostic information. Anti-ERα (clone EP1) and anti-PR (clone PgR 1294) configured as FLEX ready-to-use antibodies have been tested on the Dako Omnis automated staining platform. These products are in performance evaluation and are not commercially available. A series of concordance studies were performed to evaluate the performance characteristics of these monoclonal antibodies on breast cancer tissue specimens: anti-ERα clone EP1/Dako Omnis was compared to (a) anti-ERα clone EP1/Autostainer Link 48 (238 specimens) and to (b) anti-ERα clone SP1/Autostainer (116 specimens), and anti-PR clone PgR 1294/Dako Omnis was compared to (a) anti-PR clone PgR 636/Autostainer Link 48 (289 specimens) and to (b) anti-PR clone 16 (Leica Biosystems, Newcastle, UK) (144 specimens). In addition, the specificity of the ER and PR antibodies for Dako Omnis was evaluated on a set of normal tissue specimens.

Methods: Formalin-fixed, paraffin-embedded (FFPE) human breast carcinoma specimens and normal tissues were obtained from commercial providers or local hospitals. The specimens had no associated personal information and were not traceable back to the tissue donors. Tissue pretreatment and immunohistochemical staining were performed using the recommended protocol for each antibody and staining platform. The stained slides were evaluated for nuclear ER or PR expression according to ASCO/CAP guidelines (≥1% cut-off for positive) by pathologists who were blinded from the staining method and specimen ID. The concordance studies included breast cancer specimens covering the clinical range of ER or PR expression with approximately half the specimens in the negative (<1%) category, and at least 10% of the specimens in the weakly positive (≥1 ≤10%) category in each study. Two-sided Wilson Score 95% Confidence Intervals were calculated using JMP software (SAS Institute, USA). For the analytical specificity studies the presence or absence of specific staining in the various normal tissue types was recorded.

Results: High concordance rates were observed with both anti-ERα clone EP1/Dako Omnis and anti-PR clone PgR 1294/Dako Omnis compared to the other ER/PR antibodies, with overall agreement rates exceeding 95% in all of the comparative studies. On a set of normal tissues, specific positive nuclear staining was observed only in tissue types known to express ERα or PR.

Conclusions: Monoclonal antibodies anti-ERα clone EP1 and anti-PR clone PgR 1294 configured as FLEX ready-to-use on Dako Omnis are sensitive and specific assays for detecting estrogen receptor and progesterone receptor in FFPE tissues. In comparison testing for assessment of hormonal receptor status on breast carcinoma specimens, anti-ERα clone EP1/Dako Omnis and anti-PR clone PgR 1294/Dako Omnis were highly concordant with commercially-available ER or PR antibodies.
Title: Integrative pathology: Analysis of cellular multiplex technology to detect proteomic, genomic and DNA data from fine needle aspiration biopsy specimens

Mittendorf EA A, Dogan B, Morgan R, Chargin A, Wu Y, Cornett-Risher S and Shults K. The University of Texas MD Anderson Cancer Center, Houston, TX and Penfold-Patterson Research Institute, Frankfort, MI.

Body: Background: An integrative system capable of detecting proteomic, genomic and DNA content from cell isolates obtained by fine needle aspiration (FNA) biopsy may offer distinct advantages in diagnosing breast cancer and monitoring response to therapy. Cellular Multiplex™ is such a system. An initial pilot study evaluating this technology established a series of variables that could separate normal from cancerous elements using cells obtained from an FNA performed on excised tumors and reduction mammoplasty specimens. In order for the technology to be clinically relevant, it must perform robustly on intact tumors. The current study was therefore undertaken to validate Cellular Multiplex™ on cells obtained by FNA performed on intact tumor at the time of diagnosis.

Methods: Patients undergoing lumpectomy requiring either needle or 125I seed localization were identified. FNA was performed on intact tumor (A samples) at the time of radiographic localization prior to lumpectomy and repeated on the excised tumor (B samples). Cells obtained by FNA were placed in a proprietary fixative then hybridized and stained to detect multiple mRNA and protein targets along with DNA content. Estrogen receptor, progesterone receptor and HER2 were included in the panel of targets and compared to the routine clinical pathology report. Cell morphology was assessed by mean corpuscular volume. Samples were analyzed using an EC800 (Sony Biotechnology, San Jose, CA) and the results from matched A and B samples were compared using the Mann-Whitney Wilcoxon Rank Sum test. The study is designed to enroll 50 patients. Here we report an analysis of the first 9 cases.

Results: The cell number obtained from the excised tumors were 3-4 times greater (median to median) than obtained from the intact tumor. There was no statistical difference in the expression of the 2 mRNA targets, 8 protein targets, DNA content and cell morphology between the A and B samples. The parameters derived from Cellular Multiplex matched the standard pathologic features reported on the clinical pathology report in 8 of 9 cases. In the one discrepant case, Cellular Multiplexing detected ER positive cells in a case where standard pathologic evaluation with immunohistochemistry reported the tumor to be estrogen receptor negative.

Conclusions: This interim analysis demonstrates that the Cellular Multiplex technology is working well using cells obtained by FNA performed on intact tumors with readouts matching those obtained from excised specimens. If confirmed in the remaining patients, these data suggest that this technology will be applicable for the evaluation of intact tumors thereby making it relevant for multiple clinical indications including diagnosis and monitoring response to neoadjuvant therapy.
**Title:** Impact of routine subspecialty pathology review on the care of women age $\leq 65$ years with lymph node negative breast cancer

Lupichuk S, Yang H, Ibnshamsah F and Roldan Urgoiti G. Tom Baker Cancer Centre, Alberta Health Services, Calgary, AB, Canada; Calgary Laboratory Services, Alberta Health Services, Calgary, AB, Canada and King Fahad Specialist Hospital, Dammam.

**Body:** **Purpose:** Changes in pathological features of lymph node negative breast cancer (BC) can have a profound impact on local-regional and systemic therapy recommendations. The impact of routine subspecialty pathology review on the care of women with node negative BC has not been well described.

**Methods:** All women diagnosed with node negative BC from January 1, 2012 through December 31, 2012 who had been referred to the Tom Baker Cancer Centre were identified. As per routine institutional practice, patients $\leq 65$ years at the time of referral whose pathology had originally been reported by a general pathologist, had their pathology automatically sent for subspecialty review by a breast pathologist. For the cohort who had their pathology sent for subspecialty review, medical records were examined to determine if original pathology was changed and if treatment recommendations were altered.

**Results:** Of 468 patients diagnosed with node negative BC, 187 patients had their pathology initially reported by a general pathologist and hence their pathology was sent for routine subspecialty review. Of these 187 patients, 82 had at least one change on pathology. The most common pathology change type was an increase in tumour grade (35), followed by increase in tumour size (18), and documentation of LVI (14). Lymph node involvement, was identified in only 3 cases. There was at least one change in treatment recommendation for 21 of the 187 patients (11.2%). The most common change in treatment recommendation was inclusion of adjuvant chemotherapy (10). Had Oncotype DX funding been in place during the study period, 14 cases would have become eligible for testing and 8 would have become ineligible for testing according to our current institutional testing guideline (ER-positive, HER2-negative, grade 2 or 3, and node negative). Nine of 26 treatment recommendation changes occurred in Oncotype DX eligible patients.

<table>
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<th>Type of change</th>
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<th>Positive to negative</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>(increase) 18</td>
<td>(decrease) 9</td>
<td>27</td>
</tr>
<tr>
<td>Nodal status</td>
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<td>3</td>
</tr>
<tr>
<td>Margins</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Grade</td>
<td>(increase) 35</td>
<td>(decrease) 3</td>
<td>38</td>
</tr>
<tr>
<td>LVI</td>
<td>14</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>ER</td>
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<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>HER2</td>
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<tr>
<td>Other*</td>
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<td>-</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>75 changes</td>
<td>17 changes</td>
<td>113 changes in 82 patients</td>
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</tbody>
</table>

*Additions to report (size, sentinel node), histologic subtype, multifocal, perineural invasion, margin distance, isolated tumour cells in node.

Impact of pathology changes found with routine subspecialty review on treatment recommendation
<table>
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<tr>
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<td>Trastuzumab</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>26</strong></td>
<td><strong>13</strong></td>
<td><strong>26</strong></td>
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</table>

**Conclusions**: Routine subspecialty pathology review of lymph node negative BC yielded substantial changes in pathological features and subsequent alterations in treatment recommendations. Optimal practice with the availability of gene signature testing requires further investigation.
Title: Genomic analysis of single cells isolated by a pulse laser retrieval system

Lee H-B, Kim S, Lee K-M, Jung Y, Lee AC, Kim J, Bae S, Ryu HS, Yoo T-K, Moon H-G, Noh D-Y, Kwon S and Han W. Seoul National University College of Medicine, Seoul, Republic of Korea; Interdisciplinary Program of Bioengineering, Seoul National University, Seoul, Republic of Korea; Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; Department of Electrical Engineering and Computer Science, Seoul, Republic of Korea; Seoul National University College of Medicine, Seoul, Republic of Korea and Institutes of Entrepreneurial BioConvergence, Seoul National University, Seoul, Republic of Korea.

Body: Background: Isolating tumor cells of interest and harvesting histologically pure samples is important for genomic studies. Laser capture microdissection (LCM) is an established method to obtain such purified cell populations for various applications including DNA, gene expression, and single cell analyses. However, LCM possesses problems such as limited optical resolution, cell fragmentation from dissection, and adherence of adjacent tissue to the cells which interrupts with single cell isolation from tissue sections. To overcome these obstacles, we developed a high-throughput pulse laser retrieval system which uses a wavelength that minimizes damage to the cellular content and is processed with a sacrificial layer that provides applicable optical resolution. The aim of this study was to evaluate the performance of the pulse laser retrieval system to provide appropriate samples for genomic analysis using breast cancer tissue.

Methods: An indium tin oxide (ITO) coated glass slide was prepared using fresh frozen breast cancer tissue sections of 4 thickness and stained by hematoxylin and eosin. The slide was mounted on the cell isolation machine and imaging was performed with a charge-coupled device camera using a 20× lens. Following identification of the target cells by a pathologist, nano-second pulsed laser (wavelength= 1064nm) was irradiated on the target. Isolated cells were collected in a polymerase chain reaction tube and whole genome amplification (WGA) was carried out using Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare Life Sciences, Pittsburgh, PA, USA). Amplified genomic DNA was fragmented and Illumina sequencing libraries were constructed. Sequencing was carried out to generate data with 0.1–0.2× depth throughout the whole genome for each sample. Copy number variation (CNV) was analyzed by the Variable binning algorithm.

Results: Whole genome amplification was performed using bulk tissue and 10 captured single cells from the same specimen. No difference in amplification coverage was observed between the two samples. A CNV analysis of captured single cells revealed similar CNV profiles with those in a matched bulk tumor. Whole exome sequencing (WES) of captured single cells yielded a variant frequency of 15% at a read depth of 15× and 50M base coverage, compared to 0% at 100× and 50M for WES using bulk tumor and 0.5% at 1200× and 100K for targeted sequencing using bulk tumor. Laser capture was performed for DCIS and stromal cells from the same slide. CNV analysis of the two samples showed minimal CNV in normal stromal cells in contrast to DCIS where multiple CNVs were observed.

Conclusions: Newly developed pulse laser retrieval system is suitable for capturing single cells for genomic analysis of breast cancer. WGA, WES, and CNV analysis was successfully carried out using the captured single cells and showed no difference in profile compared to those performed with bulk tissue. This method may have the potential to replace LCM for certain applications such as single cell analyses.
Title: A review of 66 consecutive patients investigated for mammographic abnormalities by digital tomosynthesis guided vacuum assisted breast biopsy

Munir A, Moalla A, Williams HR R, Thomas D, Huws AM M and Holt SD D. Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom.

Body: OBJECTIVE: Vacuum-assisted breast biopsy (VABB) has replaced surgical biopsy for the assessment of mammographic abnormalities that are not evident clinically and or on ultrasound examination. The aim of this study was to determine the indications for, and accuracy of, vacuum-assisted breast biopsy (VABB) performed using digital breast tomosynthesis (DBT) guidance. (Hologic® Dimensions, Affirm guidance and Eviva handsets).

MATERIALS AND METHODS:
Design: Retrospective medical record and histopathologic review.

Patients and method: We introduced DBT guided VABB in June 2014 having previously investigated such patients using the prone table technique. This is a review of the first 66 consecutive patients investigated using this technique up to April 2105. The following information was reviewed: Indication for VABB, (mammographic classification M1-5, type of abnormality – calcifications/mass/distortion), complications of the procedure itself, (failure to complete, infection, haematoma), the result of the multidisciplinary team (MDT) review of imaging/pathologic correlation and the outcome for the patient.

RESULTS: In one case it proved impossible to locate the lesion and this patient has been excluded from further analysis. The mean age of the patients was 57 years (30-80years). VABB was proposed for patients with lesions initially reported as highly suspicious (M5) 4 patients (6%), suspicious (M4) in 18 patients (28%), intermediate (M3) in 37 patients (57%) or benign (M2) in 6 patients (9%). Mean size of the lesion was 13mm (range 3-100mm). Forty-four patients (68%) presented with micro calcifications, 14 (21%) with distortions in and 7 (11%) with masses.

There were no complications (infection or haematoma) that required further management following the procedure. Review by the MDT agreed that all biopsies were adequate and removed representative tissue from the lesion (No B1s). Review showed that the histology was benign and consistent in 30 (46%) patients all of whom were discharged to routine screening. 19 (29%) cases were reported as B3 (ADH, flat atypia, LCIS or ALH) in whom all the calcifications had been removed in 13 (20%) and the patients discharged and 6 (9%) went to open biopsy for residual calcifications all of whom were benign on final analysis. There was one (1.5%) radial scar reported as B4 that went to open excision and proved benign. 15 (23%) proved malignant (B5a, B5b) and went on to definitive treatment (with one patient entered into the LORIS low risk DCIS trial).

The procedure is quicker, more accurate (related to the higher resolution and larger window of the receptor plate) and involves less radiation exposure (often involving only one DBT exposure) when compared to performing the same procedure on the Hologic Platinum prone table.

CONCLUSION: DBT-guided VABB is an accurate, convenient and safe procedure.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-02-03

Title: Retrospective analysis of the accuracy of ultrasound-guided core needle biopsy in the diagnosis of breast invasive ductal carcinoma: Experience in Chinese population

Wang W, Guo Y, Xing H, Wang K and Zhai X. China-Japan Union Hospital, Jilin University, Changchun, China and China-Japan Union Hospital, Jilin University, Changchun, China.

Body: Background: Percutaneous imaging-guided core needle biopsy is a reliable alternative to surgical biopsy for a histological diagnosis. Nowadays, core needle biopsy is considered to be the standard technique for histological diagnosis of breast lesions. However, the accuracy of ultrasound-guided core needle biopsy in predicting tumor grade, which is scored according to mitotic index, tubular differentiation, and nuclear atypia, is not well established. The aim of this study is to evaluate the concordance of histological diagnosis between ultrasound-guided core needle biopsies and subsequent excision specimens of breast invasive ductal carcinomas.

Methods: We retrospectively reviewed the medical records of 875 consecutive female patients diagnosed with surgically proven breast invasive ductal carcinomas those were biopsied under sonographic guidance preoperatively, using 14-gauge core needles exclusively, from June 2011 to May 2015. A minimum of four cores were taken per lesion. Tumor grade was assigned using the standard modified Scarff-Bloom-Richardson system. Core biopsy pathological diagnosis and grades were compared with final surgical excision specimens. The diagnostic coincidence rate and the agreement rate were expressed in percentages and in kappa statistics; the rates of overestimation and underestimation were also assessed. The correlation among tumor size, diagnostic coincidence rate, and agreement rate was also evaluated.

Results: Compared with the postoperative pathological diagnosis, the diagnostic coincidence rate of ultrasound-guided core needle biopsy was 95.54% (836/875). 39 cases were diagnosed with ductal carcinoma in situ or intraductal carcinoma by core needle biopsy. The overall agreement rate between core needle biopsy and surgical pathology grade was 69.26%. Agreement rate by biopsy grade was 78.40% (225/287) for grade 3, 74.76% (308/412) for grade 2, and 53.28% (73/137) for grade 1. Core needle biopsy underestimated 22.51% (197/875) and overestimated 8.23% (72/875) of the lesions. Small tumors were inclined to be more easily misdiagnosed as ductal carcinoma in situ or intraductal carcinoma, and large tumors were more likely to show underestimation rather than overestimation when discordant (p=0.002). For mass lesions with a diameter less than 10 mm, the agreement rate (62.33%) was lower (p=0.003).

Conclusion: Ultrasound-guided core needle biopsy accurately predicts high-grade breast tumors but is moderately accurate for lower-grade lesions. Large tumor size negatively impacts the accuracy of tumor grade found on biopsy and is associated with underestimation. Our finding indicates that ultrasound-guided core needle biopsy has proven to be a reliable technique for performing a biopsy for breast invasive ductal carcinoma, and has important significance in estimating breast carcinoma grade.
Lipoproteins regulate the effects of macrophages and mesenchymal stem cells on radiation response of inflammatory breast cancer cells

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Body: Inflammatory breast cancer (IBC) represents the most aggressive manifestation of breast cancer and results in up to 10% of all breast cancer-related deaths. Lack of IBC-specific targetable drivers suggests involvement of other cell types besides breast tumor epithelial cells. The multidisciplinary treatment of IBC consists of pre-operative neoadjuvant chemotherapy (with targeted agents for Her2- and ER-positive cases), radical mastectomy, followed by radiotherapy. Given that: 1) radiation therapy is a main component in treatment of IBC, 2) we have previously shown that high-density lipoproteins (HDL) mediates outcomes after radiation for IBC, and 3) the possible involvement of tumor micro-environment, it is critically important to understand the role of microenvironment such as tumor-associated macrophages and mesenchymal stem cells (MSC) on radiation response of IBC cells and how this stroma-epithelial crosstalk is regulated by lipoproteins. Here, we used in vitro co-culture system and 2D clonogenic assays of radiation resistance to examine the impact of both polarized human THP1 macrophages and MSCs on radiation response of human IBC cell lines. Further, we determined the effect of MSC-educated THP1 and THP1-educated MSCs, when co-cultured with IBC cells, on radiation response. We also determined the effect of HDL on radiation response of IBC cells co-cultured with M2-polarized (TLR3) MSCs. Our findings demonstrate that while LPS-treated, M1-polarized MSC (TLR4) co-cultured with IBC cell lines SUM149 and IBC3 leads to radio-sensitization, co-culture of IBC cells with Poly(I:C)-treated, M2-polarized MSC (TLR3) leads to radio-resistance of IBC cells. In a similar manner, co-culture of IBC cells with THP1 macrophages polarized to either M1 phenotype (LPS and IFN-γ treated) or M2 phenotype (IL4 and IL13 treated), mediate radio-sensitivity and radio-resistance, respectively, of SUM149 IBC cells. In order to provide a more comprehensive model of macrophage-MSC-IBC crosstalk, we co-cultured IBC cells with THP1 macrophages that have been previously co-cultured (i.e. "educated") with MSC and compared the effect of MSC-educated THP1 versus non-educated on radiation response of IBC cells. Our data show that MSC-education of THP1 enhances radioresistance of IBC SUM149 and KPL4 cells co-cultured with THP1 cells. In a recent publication, we showed that HDL radiosensitize IBC cells and decrease their self-renewal potential. To expand our recently published findings, here we tested whether HDL co-treatment has any effect on MSC-M2 (TLR3)-mediated radioresistance of IBC cells. Our current findings, show that co-treatment of HDL inhibits MSC-M2-mediated radioresistance of SUM149 IBC cells. In sum, these data suggest that cells within tumor micro-environment such as macrophages and MSCs regulate radiation response of IBC cells and this can be altered by lipoproteins.
Title: Estrogens contribute to cytokine upregulation and cancer stem cell recruitment upon breast cancer contact with mature human mammary adipocytes: Effects of estrogen type and adipocyte donor weight


Body: Consequences of the obesity epidemic on cancer morbidity and mortality are not fully appreciated. While obesity confers increased cancer risk and worse outcome, mechanisms thereof are not fully known. We show prolonged co-culture of fat cells (human adipocyte stem cells, differentiated adipocytes or mature adipocytes) from breast tissue together with breast cancer lines or cultured primary dissociated human breast tumor cells increases secretion of six different pro-inflammatory cytokines, each of which contributes to tumor progression through cancer stem cell recruitment. Prolonged exposure to fat cells or to each cytokine increases the proportion of cells that form mammosphere and express ALDH1 activity in vitro and that can initiate primary orthotopic tumors and metastasis in vivo. Adipocyte and cytokine exposures activate Src, and Src family kinase activity leads to induction of embryonic transcription factors that upregulate miR302b. miR302b induction is Sox2-dependent, promotes cytokine-driven sphere formation, and in turn, stimulates cMYC and SOX2 expression. Src is not only activated by adipocyte or cytokine exposures, it is also required to sustain cytokine induction, since Src inhibitors decrease cytokine production after co-culture. Cytokine upregulation was much greater after co-culture of ER+ breast cancer cells with mature, aromatase positive, adipocytes than with adipocyte stem cells. Cytokine induction was estrogen regulated. The mechanisms of cytokine induction, ER-coactivation and effects of different estrogenic ligands will be presented. Present data illuminate the increased risk of breast cancer after menopause, particularly in obese women and the increased breast cancer mortality with obesity: cancer cell invasion into local fat, in the presence of high local aromatase and intracellular estrogen would establish feed-forward loops to activate Src, maintain pro-inflammatory cytokine production and increase tumor initiating cell abundance, tumor growth and metastasis. These data link obesity related pro-inflammatory cytokines to Src activation and cancer initiating cell abundance, and provide a novel rationale for Src inhibitors together with endocrine therapy for breast cancer.
Title: Obesity increases the lipid mediator, sphingosine-1-phosphate in the tumor and tumor microenvironment, and promotes tumor progression

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Body: INTRODUCTION: While obesity is an established independent prognostic factor for breast cancer patients, the underlying mechanisms how obesity promotes breast cancer progression are not well understood. Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid mediator produced by sphingosine kinases (SphKs) that plays critical roles in inflammation and cancer progression. Our hypothesis is that obesity increases levels of S1P in both tumor and its microenvironment, which promotes obesity-induced inflammation and breast cancer progression. The aim of this study is to test the hypothesis.

METHODS: A high fat diet (HFD) was fed C57Bl/6 mice, which is the most commonly used murine diet-induced obesity model. E0771 cells derived from C57Bl/6 mice were implanted into mammary fat pads of mice that were fed with HFD or normal diet (ND) for 12 weeks prior to the implantation. Western blot, immunofluorescent staining, RT-qPCR and LC-ESI-MS/MS assays were used.

RESULTS: Mice fed with HFD for 20 weeks developed severe obesity with an almost 2 fold increase of body weight compared with ND fed mice. E0771 mouse breast cancer cells were implanted into mammary fat pad of C57Bl/6 mice, and the mice fed with HFD developed significantly larger tumors within 30 days than those fed with ND. Expression of pro-inflammatory cytokines, IL-6 and TNF-α, was increased in the tumors of HFD fed mice, compared to those fed with ND. Furthermore, immunofluorescent analysis with anti-F4/80 antibody showed that tumors from HFD fed animals recruited significantly more tumor associated macrophages than those from ND fed animals. Expression of SphK1 and S1PR1, but not SphK2, was increased in the tumors from mice fed HFD. Mass spectrometry analysis revealed that while S1P levels in the normal breast mammary fat pad were increased with HFD feeding, S1P levels were even higher in breast tumors. Consistent with increased SphK1 and S1P in tumors, S1P was also significantly increased in the tumor interstitial fluid, which is a component of the tumor microenvironment and bathes cancer cells in the tumor. S1P levels were also increased in the serum of the tumor-implanted animals fed with HFD compared with those fed with ND, while minimal changes in S1P were evident in the serum of non-tumor bearing mice fed with HFD. Furthermore, S1P levels in the lungs of tumor-implanted animals fed with HFD were significantly higher than those fed with ND.

CONCLUSION: S1P is increased not only in tumor, but also in tumor microenvironment such as tumor interstitial fluid by obesity. Our results suggest that S1P has a role in obesity-induced inflammation and the cancer progression. This work was supported by the Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research Grant Number 15H05676 and 15K15471 for M.N and 15H04927 for W.T. M.N. is supported by the Uehara Memorial Foundation, Nakayama Cancer Research Institute, and Tsukada Medical Foundation. K.T. is supported by NIH/NCI grant R01CA160688 and Susan G. Komen Investigator Initiated Research Grant IIR12222224.
Response to cyclooxygenase-2 inhibition is regulated by collagen dense stroma

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Body: Women with dense breast have over four-fold risk for breast cancer and high breast density is correlated to increased collagen. In our previously characterized mouse model of MMTV-PyVT x Col1a1tm1jae, increased collagen levels in mammary tumors lead to enhanced tumor formation and progression. Additionally, high collagen density in vitro elevated expression of PTGS2, the gene for cyclooxygenase-2 (COX-2), by over four-fold. Because COX-2 over-expression is observed in 40% of invasive breast carcinoma cases and correlates with poor prognosis, we hypothesized that inhibition of COX-2 may be an effective target in the context of mammary tumors arising in dense tissue. Col1a1tm1jae (HD) or wild-type (wt) tumor mice were randomly assigned at 11 weeks of age to daily treatment with vehicle or celecoxib for 21 days. Tumors in HD mice were larger (p < 0.05) and expressed higher levels of COX-2 (p < 0.05) and PGE2 (p < 0.01). COX-2 and PGE2 expression levels were both decreased with COX-2 inhibition (wt and HD = p < 0.0001, for both markers). Cell proliferation decreased in both wt and HD tumors when COX-2 was inhibited (Epithelium wt and HD p < 0.0001; stroma wt = p < 0.05 and HD = p < 0.01). Notably, several cytokines, immune and stromal cell counts were elevated in HD tumors alone, and COX-2 inhibition reversed this effect. These findings indicate that COX-2 has a direct role in modulating tumor progression in dense matrices and it may be an effective therapeutic target for women with dense breast tissue.
Title: SPARC expression in primary metastatic breast cancer

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Body: Aim: To evaluate the prognostic value of the expression of secreted protein acidic and rich in cysteine (SPARC) in primary metastatic breast cancer (PMBC).

Patients and Methods: Fifty-two patients with PMBC diagnosed between 2005 and 2012 at x German centers were retrospectively analyzed for expression of SPARC in tumor cells using an immunoreactive score (IRS) integrating staining intensity and percentage of positive cells (IRS 0-12), and in stroma based on immunohistochemical (IHC) staining intensity only (IHC 0-3+). Association between SPARC expression, tumor characteristics and progression-free survival (PFS) and overall survival (OS) was analyzed.

Results: Only Her2 expression was associated with expression of SPARC in stroma (p 0.028, OR 13.9 95% 1.3-145.5) but not in tumor cells. SPARC expression in stroma was associated with shorter PFS (hazard ratio (HR) 2.6; 95% confidence interval (CI) 1.2-5.4; p 0.014), but not in tumor cells and shorter OS (HR 4.1; 95% CI 1.04-16; p 0.041) for SPARC expression in stroma of breast tumor. No clear association between expression of SPARC in tumor cells and outcome could be detected.

Conclusion: Only SPARC expression in stroma might be associated with shorter PFS and OS in patients with PMBC. This finding is in line with the known key role of expression of SPARC in the metastastatic process. Confirmation in prospective clinical trials is warranted.
Leptin as a mediator of tumor-stromal interactions promotes breast cancer stem cell activity

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Body: Breast cancer stem cells (BCSCs) play crucial roles in tumor initiation, metastasis and resistance to anticancer therapies. These cells rely for their properties on complex interactions with the tumor microenvironment through networks of cytokines and growth factors. In this study, we investigated how leptin, as a mediator of tumor-stromal interactions, may affect BCSC activity using breast cancer cell lines and patient-derived samples. We found that conditioned media (CM) from cancer associated fibroblasts and breast adipocytes significantly increase mammosphere formation in breast cancer cells. Depletion of leptin from stromal cell-CM as well as inhibition of leptin signaling by using a full leptin receptor antagonist peptide LDFI completely abrogated this effect. Accordingly, mammosphere cultures exhibited increased leptin receptor expression and leptin exposure enhanced mammosphere formation. Microarray analyses revealed a similar expression profile of genes involved in stem cell biology in mammosphere cells treated with stromal cell-CM and leptin. Interestingly, leptin is able to increase the mammosphere formation in metastatic breast cancer cells isolated from patients (n = 10) and this can be blocked by using peptide LDFI. In addition, leptin receptor (OBR) mRNA expression, analyzed in cells from metastatic fluids, directly correlated with mammosphere formation activity \( r=0.68, \ p=0.05; \ n=8 \). Finally, Kaplan–Meier survival curves indicated that OBR expression correlated with reduced overall survival in breast carcinomas \( HR=1.9, \ p=0.022 \). Together, our results suggest that leptin/leptin receptor may represent a potential therapeutic target that can block the stromal-tumor interactions that drive BCSC-mediated disease progression.
The "panta rhei" of breast cancer: Gene expression timeline analysis during progression of microinvasive breast cancer microenvironment


Body:

Background. Tumors develop by progression through a series of stages. It is now widely accepted that cancer is attributed to the accumulation of genetic alterations in cells. Every cell of the tumor microenvironment is constantly changing in the flow of the cancer progression. A number of genes have been identified as having functions in various stages of progression in promoting cancer progression in experimental models. However, the association between gene expression alterations and resulting phenotypic alterations with respect to the aggressiveness and migration potential of cancer cells is not fully understood. Therefore, elucidation of genotype–phenotype correlation will be required to further understand the complex process of progression and invasion. All tumors require at least some stroma to meet their needs of nutrition, waste removal, and structure. It has become clear in recent years that stroma is essential for tumor maintenance and growth and has potential as a therapeutic target. Here, we aimed to give a chronological order of gene expression changes given in the dynamical framework of microinvasive breast cancer microenvironment.

Materials and Methods. RNA-seq (Ion Proton technology) was performed on three microinvasive breast cancers, applying new modifications to the usual protocol. For each of them we microdissected 7 different portions of the tumor (around 200 cells), 4 related to the breast epithelium and 3 to the stroma. The regions were selected on the basis of their grade of progression. Breast epithelium was chronologically subdivided in normal breast epithelium (NBE), carcinoma in situ (CIS), emerging invasive fingers (EIF) and invasive breast cancer (IBC). For each of the breast epithelium subdivisions we collected the adjacent stroma (S) except for the in situ portion: S-NBE, S-EIF and S-IBC.

Results: Whole transcriptome analysis performed on each microdissected regions reveals a series of gene expression changes occurring during cancer progression in the breast epithelium along with the adjacent stroma. The dendogram analysis, based on the whole gene expression data of each patient revealed a perfect group organization of the various microdissected portions of stroma and mammary epithelium. Within the dendogram, the organization of Normal, In Situ, EIF and Invasive tissue respected perfectly the biological assumptions.

Conclusions: More thorough analyses are needed to give a clear view of the flow of molecular events starting from the normal breast epithelium to the microinvasive stage, as well as to give a better understanding of the stroma-epithelium molecular means of communication. The analysis of all the molecular changes occurring in the breast epithelium and in the stroma of microinvasive cancer could lead to the development of new therapeutic targets.
Tartrate-resistant acid phosphatase (TRAcP) dependent polarization of breast cancer-promoting macrophages

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Background: Breast cancer associated macrophages often promote tumor growth and metastasis by conditioning the tumor microenvironment and suppressing adaptive immune responses. Tartrate-resistant acid phosphatase (TRACP) is a novel serum biomarker associated with systemic macrophage burden in a variety of chronic inflammatory diseases and is strongly expressed by tumor-associated macrophages. We tested the hypothesis that TRACP is required for M2 macrophage polarization.

Methods: Macrophage cell lines were polarized in vitro and tested for TRACP, iNOS and Arg-1 expression and their ability to promote or repress breast cancer cell growth and invasion in vitro and in vivo. A novel TRACP deficient, Acp5 mutant mouse was used as a macrophage source and as a breast cancer host to confirm a role for TRACP for macrophages to support cancer growth.

Results: TRACP was up-regulated in concert with M2 polarization and down-regulated in M1 polarized cells. TRACP expression correlated with macrophage promotion of tumor growth and invasion in vitro. Although TRACP-deficient macrophages could be induced to express Arg-1 when stimulated with IL-4 plus IL-13 (M2 phenotype), the TRACP deficient macrophages behaved like M1 cells suppressing tumor growth and invasion compared to than WT cells. Tumor xenografts grew slower in primary subcutaneous grafts and metastasized less extensively in intravenous grafts in TRACP deficient mice compared to WT. Furthermore, the tumor metastatic patterns could be reversed in WT animals by co-grafting TRACP-deficient macrophages and in TRACP deficient hosts by co-grafting WT macrophages.

Conclusions: TRACP expression is normally a phenotypic marker for alternatively activated macrophages, but not necessary for expression of Arg-1. In a host environment of TRACP deficiency, Arg-1 positive macrophages can be generated by cytokine stimulation in vitro and by tumor in vivo, however, TRACP deficiency still conveyed a tumor suppressive phenotype in cell based studies and in intact animals. TRACP plays a role in functional polarization of M2 macrophages and their ability to promote breast cancer growth and metastasis.
Title: Pegylated recombinant human hyaluronidase PH20 (PEGPH20) enhances efficacy of eribulin mesylate (HALAVEN®) in triple negative breast cancer xenografts


Body: Hyaluronan (hyaluronic acid, HA), a glycosaminoglycan found in tissue throughout the body, overaccumulates in the tumor microenvironment (TME) of many non-hematologic malignancies, including breast cancer. HA overaccumulation in breast cancer patients correlates with tumor progression and decreased survival (Tammi 2008). Pegylated recombinant human hyaluronidase PH20 (PEGPH20), an investigational therapeutic agent entering Phase 3 clinical development in pancreatic cancer, enzymatically removes HA from the TME. In preclinical animal models, PEGPH20-mediated HA degradation is associated with remodeling of the tumor stroma, reduction of tumor pressure, expansion of tumor blood vessels and facilitated delivery of chemotherapy (Thompson 2010, Provenzano 2012, Jacobetz 2013). Accordingly, preclinical studies investigated the combination of PEGPH20 with eribulin mesylate (ERI, HALAVEN®), a microtubule dynamics inhibitor with a novel mechanism of action (Towle 2001, Jordan 2005), currently approved for treatment of certain patients with advanced breast cancer. NCr nu/nu mice were inoculated subcutaneously with human triple-negative breast cancer (TNBC) HCC1806 or HCC1806/HAS3 cells; the latter subline was engineered to accumulate high HA levels, confirmed by immunohistochemistry, via overexpression of hyaluronan synthase 3 (HAS3). When tumors reached 350 mm³, animals were randomly assigned to four treatments groups: vehicle, ERI (0.7 mg/kg, IV, QW), PEGPH20 (37.5 µg/kg, IV, BIW), or ERI plus PEGPH20. In the parental HCC1806 model, addition of PEGPH20 did not significantly change the antitumor effects of ERI. In contrast, combining PEGPH20 with ERI in the HCC1806/HAS3 model increased the antitumor effects of ERI by 27% (94.5% vs. 119.7% TGI, ERI alone vs. ERI+PEGPH20, respectively; p=0.05) and resulted in 6 of 7 complete tumor regressions.

In a complementary study in HCC1806/HAS3 tumors evaluating ERI pharmacokinetics with and without PEGPH20, mice were assigned to three treatments groups: ERI (0.5 mg/kg, IV), simultaneous ERI plus PEGPH20 (37.5 µg/kg, IV); or ERI plus PEGPH20 predosed 24 h prior to ERI. Animals were sacrificed at 0.5, 1, 4, 24, 48, 72 and 96 h post ERI dose, and ERI levels in tumor, muscle, plasma and liver were subsequently analyzed by liquid/liquid extraction and LC-MS/MS chromatography. Simultaneous administration of ERI and PEGPH20 increased ERI maximum tumor concentration (Cmax) slightly and approximately doubled ERI tumor exposure (AUC); whereas the 24 h pretreatment with PEGPH20 approximately doubled ERI Cmax and increased ERI AUC more than two-fold. No significant differences in plasma ERI levels were observed between groups, and no significant differences in ERI levels in liver or muscle tissue were observed between groups. Taken together, these data suggest that PEGPH20-mediated HA removal significantly increases both ERI tumor concentrations and antitumor effectiveness in an HA-high TNBC model. A clinical phase 1b/2 clinical trial is planned to evaluate PEGPH20 plus ERI in first-line HER2-negative metastatic breast cancer.
Title: Breast milk exosomes promote breast cancer progression

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Body: Background: The stimulation of extant neoplastic cells vs. their inhibition during breast involution is a key concept in whether pregnancy and lactation decrease or increase breast cancer risk. The time of weaning, a period of breast involution and remodeling, appears critical to future breast cancer risk, as during involution the breast microenvironment becomes tumor promotional. Both matrix metalloproteinases (MMPs) and transforming growth factor (TGF)β isoforms have been implicated in this process. We previously reported that TGFβ2 (but not TGFβ1) expression was significantly higher in milk collected from the cancer containing (vs. matched clinically normal) breast of women diagnosed with cancer during lactation.

Hypothesis: TGFβ2 in milk exosomes from healthy lactating women modulates the development and progression of breast cancer.

Methods: Matched (early, mature and wean-early involution) milk samples were collected from 13 lactating women, exosomes isolated and the levels of five (MMP2, 3, 9; TGFβ1, 2) proteins measured. Based on the results, additional wean samples were analyzed for TGFβ2 expression and samples divided based on their TGFβ2 expression. The highest and lowest TGFβ2 expressing milk exosome samples were co-cultured with MCF-7 breast cancer cells grown in exosome-depleted media. Cell proliferation was measured after 24h. Confocal microscopy was performed after 72h to evaluate epithelial to mesenchymal transition (EMT) in the cells. A TGFβ2 neutralizing blocking antibody was applied after 72 h co-culture.

Results: The greatest increase in expression (four-fold) at the time of involution was in TGFβ2. Confocal microscopy performed 24h after co-culture confirmed the uptake of the exosomes (both those expressing hi and lo TGFβ2) in the MCF-7 cells. There was a significant increase in cell proliferation compared to control (p<0.001) in cells treated with hi TGFβ2 milk exosomes. 72h after co-culture, bright field microscopy demonstrated that MCF-7 cells treated with hi TGFβ2 underwent EMT including the formation of filopodia, whereas those treated with low TGFβ2 did not. A TGFβ2 blocking antibody reversed the EMT phenotype and filopodia formation.

Impact: Pregnancy has a lasting influence on breast cancer risk, and pregnancy associated breast cancers (PABCs) diagnosed after delivery are generally aggressive, with a poor prognosis. Part of the reason for the development of PABCs, and the aggressive nature of PABCs may be the influence of high-expressing TGFβ2 exosomes in the milk on the breast ductal epithelium.
The epithelial to mesenchymal transition: Identifying a signature of recurrence in ductal carcinoma in situ

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Background: The epithelial to mesenchymal transition (EMT) plays a critical role in the progression from non-invasive to invasive breast carcinomas (IBC). It is characterized by alterations in gene expression, changes in cellular polarity, the disruption of tight junctions; production of metalloproteinases, transforming growth factor-β (TGFβ) induction, expression of cancer stem cell markers, hypoxia, decrease in e-cadherin expression, along with other molecular biological events. Several transcription factors including ZEB1/2, TWIST1, SNAIL1/2, FOX family, GATA4/6 are involved in the process. There is a need to identify the molecular events driving the progression of ductal carcinoma in situ (DCIS); and to derive a signature that differentiates DCIS lesions that have the potential to recur as a subsequent DCIS, an IBC, or to not recur. To catalog the changes associated with EMT that may reveal a clinically relevant signature of progression from DCIS to DCIS or IBC recurrences using a panel of 200 genes related to EMT.

Methodology: RNA was extracted from formalin-fixed paraffin embedded (FFPE) sections of pure primary DCIS lesions representing three categories of outcome: those that did not recur; those that recurred with a subsequent DCIS; and those that recurred with invasive cancer. RNA abundance profiling was performed using Nanostring platform and data processing using an R statistical environment. Levels of mRNA abundance were modelled as a function of recurrence status. Coefficients were fit to terms representing the effect and the standard errors of the coefficient were adjusted with an empirical Bayes moderation. Model-based t-tests were then used to test if the coefficients were significantly different from zero.

Results: Using a technical control sample, pairwise comparisons across three replicates showed high correlation (ρ=0.99, P<2.2x10-16 for all 3 comparisons), suggesting the robustness of the assay. In our preliminary survey of 45 patients across the three groups, we have identified a number of genes that showed differential mRNA abundance levels between patients who recurred (either DCIS or invasive recurrence) vs. those who did not recur. Using Random Forest analysis in a leave-one-out cross-validation approach, we were able to obtain a classifier with a sensitivity of 82% and specificity of 58%. Based on these initial findings, an additional 200 samples have been processed to support these initial findings.

Conclusion: The current literature provided increasing evidence that transcriptomic patterns reflecting the EMT may reveal novel biomarkers and elucidate molecular mechanisms leading to improved prognosis. Among breast carcinomas, differential expression of the EMT genes has been associated with a worse outcome, among estrogen receptor-negative and basal-like carcinomas. However, the understanding of the role of EMT genes in DCIS is limited; therefore, to elucidate whether the EMT plays a role in the progression of DCIS, we have designed an EMT gene panel that also includes genes that are significant prognosticators for IBC, including ER, PgR, Ki67 and HER2. In an exploratory analysis of cases trained based on clinical outcome, the sensitivity for predicting recurrence (whether DCIS or invasive) was 82%.
Title: The N-terminus of Twist1 is responsible for interacting with transcriptional repressors to promote EMT and metastasis of breast cancer cells

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Body: Twist1, a basic helix-loop-helix transcription factor, plays a key role to mediate epithelial-mesenchymal transition (EMT) and promote breast cancer metastasis. However, knowledge about Twist1 structure-function relationships to cancer-related phenotypes is limited. Therefore, we studied the requirement of Twist1 N-terminus in Twist1-dependent breast cancer metastasis. We showed that the amino-terminus of Twist1 was the dominant negative mutant of Twist1. Overexpression of Twist1 N-terminus exhibited different cell morphology and motility in vitro. Inoculation of Twist1 N-terminus overexpression cells into SCID mice showed delayed tumor formation and reduced lung metastasis. Furthermore, Twist1 N-terminus overexpression induced expression change of EMT markers, including E-cadherin, β-catenin, vimentin and Twist1 both in vivo and in vitro. Co-immunoprecipitation and mass spectrometry revealed that Twist1 N-terminus interacted with several members of the Mi2/nucleosome remodeling and deacetylase (Mi2/NuRD) complex, HDAC2, 3 and 7, MTA1 and 2, RbAp46/48, and many corepressors including NCoR1 and 2, which released them from the proximal region of E-cadherin promoter for transcriptional activation. These data suggest that Twist1 N-terminus is required for Twist1-mediated transcriptional programs and breast cancer metastasis.
Title: Predictive value of de novo and induced epithelial-mesenchymal transition in locally advanced breast cancer treated with neoadjuvant chemotherapy

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Body: The dynamic transformation of an adherent proliferative epithelial cell to a migratory and invasive mesenchymal state that can drive tumour metastasis has been widely acknowledged in in vitro models as epithelial-mesenchymal transition (EMT). We have characterized EMT status in tissues from 35 locally advanced breast cancer (LABC) patients before and after receiving anthracycline and taxane-based neoadjuvant chemotherapy (NAC). Routine analyses for ER, PR, HER2, lymphovascular invasion (LVI) and tumour staging parameters were available for all patients and five year recurrence and survival data was available for 34. Six patients (17%) had a pathological complete response (pCR), five of whom were hormone receptor (HR) negative and one HR positive. 11 patients (43%) had had disease recurrence and 10 (40%) had died from breast cancer at five years follow up.

Core biopsy tissue specimens were available prior to NAC from all 35 patients. Resected tissue following NAC was available from 17 cases with residual disease. Tissue sections were stained for the epithelial marker cytokeratin 19 (CK19) and the mesenchymal marker vimentin (VIM). Fluorescent, multi-channel microscopy identified co-localization of CK19 and VIM within tumour cells, indicating the presence of EMT.

Evidence of EMT prior to NAC was seen in 14/35 (40%) of LABC cases. There was no association between EMT status pre-NAC and pCR which was observed in 2/14 EMT positive and 4/21 EMT negative patients. However, in patients with detectable EMT pre-NAC there was significantly improved five year disease-free survival (86 vs. 52%, p=0.04) and a trend to improved five year overall survival (86 vs. 62%, p=0.12) compared to cases that were EMT negative pre-NAC.

Of the 17 cases without a pCR with tissue available for assessment of pre- and post-NAC EMT status, seven had disease recurrence and six died by five years. Four cases that were EMT negative pre-NAC developed EMT positive tumour cells following NAC, and all have subsequently developed metastatic disease and died from breast cancer. Two cases lost detectable EMT after chemotherapy, both of whom remain alive. In contrast to pre-NAC EMT, induction of EMT following NAC was associated with trends to worse five year disease-free and overall survival (45 v 75%, p=0.20) and (56 v 75%, p=0.40). Additionally, when events past five years are included in analysis, detectable EMT in the post-NAC tissue sample (induced and retained) correlated with a trend to increased recurrence (p=0.09) and to a statistically significant increase in overall mortality (p=0.04).

This is the first study to explore EMT induction and loss during NAC in the clinical setting. Although patient numbers are few, the data show EMT induction during chemotherapy in a moderate proportion of cases. Observations of significantly superior five year disease free survival in patients without detectable EMT pre-NAC and significantly inferior overall survival in those with visible EMT post-NAC need to be interpreted with caution. Larger studies are needed to further examine this potential prognostic differential between EMT detectable either before or after NAC, and to explore how this may guide therapy.
Title: A novel mechanism of epithelial-mesenchymal transition in breast cancer metastasis: Involvement of prostanoid receptor

Kwong A, Siu MT T, Cheuk I, Ho JC C, Chen J and Shin VY Y. The University of Hong Kong, Hong Kong; Hong Kong Sanatorium & Hospital, Hong Kong; Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong and Stanford University School of Medicine.

Body: Background: Triple-negative breast cancer is associated with higher metastatic rate and poor prognosis than other subtypes of breast cancer due to lack of targeted therapy. Epithelial-mesenchymal transition (EMT) is linked with metastasis with phenotypic conversion of epithelial cells. However, the regulation of EMT in breast cancer metastasis remains largely unstudied. Recent attention has focused on targeting the downstream of COX-2 pathway, understanding the role of prostanoid receptors in breast cancer metastasis may help the development of effective therapeutic interventions for patients with metastasis.

Methods: A stable EP2-expression cell line (MB-231-EP2) was used to study tumorigenesis and distant metastasis in human breast cancer metastatic model. Localization of EP2 and EMT markers were examined by immunostaining and immunofluorescence. Profiles of drug transporters genes were compared between siEP2 and siControl cells. Functional role of EP2 on cell proliferation, invasion and apoptosis were assessed. Alteration of EMT markers were examined by real-time PCR and Western blot analysis.

Results: Expression of EP2 receptor were higher in human primary tumors than non-tumor tissues. EP2 receptor was predominantly expressed in metastatic tumors than primary tumors in human breast cancer metastatic mice model. The metastatic tumors showed a higher Ki67 (cell proliferation) and CD31 (angiogenesis) than primary tumors in the xenograft tissues. Larger tumors and poor survival were seen in MD-231-EP2 bearing mice when compared with control. Silencing of EP2 by siRNA markedly reduced cell proliferation and invasion, but increased apoptosis and expression of solute carrier family 19 member A3 (SLC19A3) gene. Interestingly, SLC19A3 had a lower expression in primary tumors and was inversely correlated with EP2 expression. Ectopic expression of SLC19A3 suppressed cell proliferation and invasion through the restoration of E-cadherin and other EMT markers (Twist, Zeb1 and Snai2). Immunofluorescence staining showed that the localization of Twist and E-cadherin were altered in siEP2 cells.

Conclusion: Our results showed that EP2 promoted EMT and breast cancer metastasis through the downregulation of SLC19A3 expression. Taken together, targeting EP2/SLC19A3 signaling pathway maybe a potential treatment for metastasis and adjuvant chemotherapy to reduce the metastatic risk.
Title: Inflammatory breast cancer cells show a particular pattern of canonical and non-canonical TGFβ signalling, possibly affecting cancer cell motility


Body: Introduction
Inflammatory breast cancer (IBC) is an aggressive form of breast cancer with an elevated metastatic potential. Recent evidence suggests that TGFβ signalling may be an important driver of the disease. Here, we describe data from patient samples and preclinical models that corroborate this hypothesis.

Materials and Methods
The xCELLigence system was used to profile a series of 3 IBC and 3 subtype-matched non-IBC cell lines for the cell motility inducing capacity of a panel of chemokines: TGFα, TGFβ, EGF, FGF, HGF, IGF, PDGF, CCL2, CCL5, CCL12 and CXCL21. Significant results were confirmed using classical wound healing assays (WHA). A series of 79 IBC and 133 non-IBC patient samples was evaluated for nuclear SMAD2, -3 and -4 protein expression using immunohistochemistry (IHC). In a subseries of 14 IBC and 21 non-IBC patient samples, protein expression and Affymetrix gene expression data were integrated and Expression2Kinases was used to identify key components of TGFβ signalling in IBC.

Results
Whereas TGFβ-induced cell motility in all non-IBC cells, we noted a 18-fold reduction of cell motility in IBC cells. Classical WHA showed a near complete wound closure (90% reduction of the wound area) after 24hrs of TGFβ treatment in non-IBC cells. Under similar conditions in IBC cells, the reduction of the wound area was less than 10%. IHC on patient samples revealed increased nuclear SMAD2 protein expression in combination with attenuated nuclear SMAD3 protein expression in IBC, independent of classical clinicopathological variables. Integration of protein and gene expression data demonstrated that nuclear SMAD2 expression in IBC is mediated through the canonical TGFβ signalling pathway, whereas the absence of nuclear SMAD3 expression is due to impaired non-canonical, p38MAPK/ATF2-dependent TGFβ signalling. Of note, ATF2 expression is specifically attenuated in IBC.

Discussion
This study provides the basis for continued research into the role of TGFβ in IBC. We show that, unlike non-IBC cells, IBC cells do not induce cell motility in response to TGFβ stimulation. This observation can be explained by impaired non-canonical p38MAPK/ATF2-dependent TGFβ signalling in IBC, which is essential for SMAD3-driven epithelial to mesenchymal transition (EMT). SMAD2 on the other hand is a proven driver of EMT-independent modes of cell motility. Our results strengthen the vision that EMT is not required for IBC cell invasion.
Title: Essential role of notch-4/STAT3 signaling in epithelial-mesenchymal transition of tamoxifen-resistant human breast cancer

Bui QT Thu and Kang KW. College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Korea.

Body: Epithelial-mesenchymal transition (EMT) is process in which epithelial cells undergo unique morphologic changes characterized by a transition from epithelial cobblestone phenotype to elongated fibroblastic phenotype (mesenchymal phenotype) leading to increased motility and invasion. Our previous study demonstrated that tamoxifen (TAM)-resistant human breast cancer (TAMR-MCF-7) cells showed the increased expression of mesenchymal marker proteins compared to the parent MCF-7 cells. Notch plays a crucial role in the promotion of EMT during both development and tumor progression. Especially, Notch-1 and Notch-4 were reported as prognostic markers in human breast cancer. Here, we found that the basal expression and activity of Notch-4 were significantly increased in TAMR-MCF-7 cells compared to control MCF-7 cells. Suppression of Notch-4 by either Notch inhibitors or Notch-4 siRNA significantly attenuated the EMT signaling. Interestingly, long-term treatment with DAPT, a Notch inhibitor, eventually led to partial reversal of EMT by up-regulating E-cadherin expression. Activated or tyrosine-phosphorylated STAT3 (pYSTAT3) protein is supposed as a critical signaling molecule in the regulation of tumorigenesis and metastasis of cancer cells. We further found that TAMR-MCF-7 cells exhibited constitutive STAT3 phosphorylation. Suppression of Notch-4 activation attenuated the activated STAT3 elevation in TAMR-MCF-7 cells. We hypothesized that Notch-4 regulated EMT in TAMR-MCF-7 cells via STAT3 signaling. Intrasplicenic injection model of liver metastases was performed using TAMR-MCF-7 cells. Mice were received subcutaneous injections daily with DAPT (10 mg/kg) formed smaller tumor size in spleens and showed less micrometastatic tumor burden in livers compared to group treated with vehicle. In conclusion, inhibition of Notch signaling may have efficacy in the treatment of breast cancer metastasis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-06-01

Title: Abstract Withdrawn

Body:
Title: A force-sensitive organoid assay to quantify regenerative potential of single primary human mammary cells

Scheel CH H, Linnemann JR R, Miura H, Meixner LK K, Irmler M, Kloos UJ J, Hirschi B, Bartsch HS S, Sass S, Beckers J, Theis FJ J, Gabka C and Sotlar K. Institute of Stem Cell Research, Helmholtz Center Munich, Neuherberg, Germany; Institute of Experimental Genetics, Helmholtz Center Munich, Neuherberg, Germany; Institute of Pathology, Medical School, Ludwig Maximilian University Munich, Munich, Germany; Institute of Computational Biology, Helmholtz Center Munich, Neuherberg, Germany; Technical University Munich, Freising, Germany; Technical University Munich, Garching, Germany and Nymphenburg Clinic for Plastic and Aesthetic Surgery, Munich, Germany.

Body: We have developed an organoid regeneration assay to quantify the ability of freshly isolated, single human mammary epithelial cells to generate complex branched ductal structures with basal and luminal features. For this purpose, cells are cultured in adherent or floating collagen gels, corresponding to a rigid or compliant matrix. In both conditions, single luminal progenitors form spheres, whereas basal cells generate branched ductal structures. In compliant but not rigid collagen gels, branching ducts in multicellular structures generated by basal cells develop a lumen and alveoli at their tips. Importantly, branched structures generated by single basal cells express basal and luminal markers at correct positions, thereby demonstrating bi-potential. Functionally, basal cells in branched structures display cellular contractility, which we reveal to be required for alveologenesis. In conclusion, branched structures generated by single basal cells in compliant collagen gels resemble terminal ductal-lobular units (TDLU), the functional units of the mammary gland.

To prospectively isolate basal cells with regenerative potential, we added the membrane metallo-endopeptidase CD10 as a cell surface marker to existing sorting protocols, thereby enriching for TDLU-formation and enabling rigorous quantification of regenerative potential by extreme limiting dilution assay (ELDA). Moreover, the use of CD10 as a cell surface marker reveals the presence of stromal cells with endothelial qualities within the CD49fhi/EpCAM– population, previously labeled basal. In summary, we describe a defined in vitro assay system to quantify primary human mammary epithelial cells with regenerative potential and systematically investigate their interaction with the physical environment at distinct steps of morphogenesis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-06-03

Title: Epithelial to mesenchymal transition (EMT) regulates the spontaneous generation of GD2+ breast cancer stem-like cells through NFκB activation

Battula VL, Sun J, Nguyen K, Hortobagyi G and Andreeff M. UT MD Anderson Cancer Center, Houston, TX.

Body: Breast cancer recurrence may be a consequence of persistent breast cancer stem-like cells (BCSCs) that survive chemotherapeutic or hormonal therapy. Therefore, targeting BCSCs could complement standard chemotherapy. We discovered that the ganglioside GD2 is expressed on and defines BCSCs (Battula et al., JCI, 2012), as consequence of activation of the enzyme GD3 synthase (GD3S). Inhibition of GD3S expression inhibited breast cancer metastasis to lung. We also observed that GD2- breast cancer cells spontaneously generate GD2+ cells in vitro. As induction of EMT generates a stem cell–like phenotype, we hypothesized that EMT regulates the generation of GD2+ breast cancer cells. To test this hypothesis, MDA-MB-231 and SUM159 cells were cultured in vitro and the percentage of GD2+ cells was measured over time. Interestingly, the percentage and absolute number of GD2+ cells increased in a time-dependent manner, suggesting the spontaneous generation of GD2+ cells. Concomitantly, mesenchymal-related markers including vimentin, N-cadherin, and twist increased 3 to 6-fold. To further investigate whether this process is operational in vivo, GFP+ MDA-MB-231 cells were transplanted into mammary fat pads of NOD/SCID mice. Each week, a group of mice was sacrificed, tumors were extracted and the number of GFP+GD2+ cells was determined by flow cytometry. In line with our in vitro results, we observed significant increases in GD2+ BCSCs with increasing tumor volume from 15.1%±4.6% to 37%±8.7% over a 6 week period, suggesting that breast cancer cells spontaneously undergo EMT during tumor progression and generate GD2+ BCSCs.

To identify possible targets to inhibit EMT in breast cancer cells, proteomic analysis using Kinexus® antibody arrays revealed activation of NFκB and focal adhesion kinase (FAK) signaling in GD2+ breast cancer cells. The activation of NFκB (phospho p65) in GD2+ cells was validated by CyTOF mass cytometry using metal tagged antibodies. These data suggest that inhibition of NFκB signaling may inhibit GD2+ BCSC growth. Indeed, the IKK inhibitor BMS345541 reduced GD2+ cells by >95% and inhibited GD3S expression (determined by qRT-PCR) in a dose- and time-dependent fashion. In contrast, treatment with doxorubicin increased the percentage of GD2+ cells, from 13.5±2.5% to 21±2.6% in MDA-MB-231 cells, suggesting that GD2+ cells are resistant to doxorubicin. In addition, treatment with BMS345541 inhibited the ability of breast cancer cells to form mammospheres by >90% in vitro. In-vivo tumorigenesis assay demonstrated that BMS345541 induced a significant decrease (p <0.01) in tumor volume, and increased survival of tumor bearing mice: median survival was 78 days for BMS345541-treated mice vs. 58 days for controls (p<0.002).

Conclusion: GD2+ BCSCs are spontaneously produced during tumor progression by EMT and NFκB and FAK mediated signaling might regulate this process. Inhibition of NFκB and FAK signaling pathways may inhibit the spread of BCSCs and reduce breast cancer metastases.
Title: 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.
The incidence of/mortality from the most aggressive triple negative breast cancer (TNBC) is higher in African American-(AA) than Caucasian (CA)-women. In contrast, breast cancer in Hispanic-women is generally less aggressive. There is an ongoing debate as to whether AA-women have an increased incidence of TNBCs that have poor outcome or there are unique biological factors in AA-women that promote aggressive biology. Through unique approaches, we provide evidence for distinct biology in the normal breasts of CA-women, AA-women, and Hispanic-women. Using resources from the Komen Normal Tissue Bank and a primary cell culturing system that enabled propagation of normal epithelial cells of different lineages including mature-luminal, luminal-progenitor, and stem cells from different ethnic groups, we have identified a subpopulation of CD44high/CD24- cells that are unique to AA-women (p=0.0001). This AA-women-specific subpopulation expressed higher levels of Collagen 3A1, Collagen 5A2, CTNNB1 (β-Catenin of Wnt), FOXC2, and ZEB1 compared to common CD44+/CD24+ subpopulation in every breast. Gene expression pattern in the AA-specific CD44high/CD24- population showed marked similarity to gene expression pattern in the recently described PROCR+ multi-potent mammary stem cells. Indeed, the breast epithelial cells of AA-women were enriched for PROCR-positive stem cells compared to CA-women or Hispanic-women (p=0.015). In contrast to cells from CA- and AA-women, cells isolated from the healthy breast of Hispanic-women displayed mostly differentiated features as they were enriched for CD49f-/EpCAM+ and CD271-/EpCAM+ mature cells. Cells from CA-women, AA-women and Hispanic-women are currently being immortalized to determine the cell types that are preferentially immortalized in each of these ethnic groups. These results suggest ethnicity-dependent differences in Wnt, extracellular matrix, and epithelial to mesenchymal transition (EMT) signaling in normal breast epithelial cells and differences in the proportion of cells that are susceptible to immortalization/transformation.

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Title: Mammary stem cell modulation of wildtype and Trp53 null stem cells by CAPE (caffeic acid phenethyl ester), a potential therapeutic agent

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Body: CAPE is the major active component of propolis, a widely available, non-toxic, honeybee natural product with anti-inflammatory, antioxidant, and antitumor properties. We have previously shown that CAPE inhibits growth of breast cancer cells and the tumorigenic potential of breast cancer stem cells. We have identified inhibition of histone deacetylase (HDAC) as one mechanism of action, which suggests that it mediates its effects through epigenetic modifications. We postulated that CAPE may be useful in chemoprevention for women at high risk for triple-negative breast cancers since the cell-of-origin hypothesis states that these cancers likely arise from transformation of mammary stem or progenitor cells, whose self-renewal is maintained via epigenetic states.

We tested the effect of CAPE on wildtype (WT) and Trp53 null mammary stem cell (MaSC) self-renewal from BALB/c mice cultured as mammospheres (MMS). Primary mammary epithelial cells were cultured as MMS for 7 days, dissociated into single cells, re-cultured in the presence of CAPE for 7 days and passaged in secondary and tertiary passages without CAPE. MMS frequency and differentiation potential was analyzed using immunofluorescence detection of luminal marker, cytokeratin 18, basal marker, cytokeratin 14, and progesterone receptor (PR). Chromatin states were identified using ATAC-seq and open chromatin areas unique to CAPE treated murine MMS were used for pathway analysis performed by Ingenuity Pathway Analysis, Gene Set Enrichment Analysis and confirmed by Integrative Genome Viewer.

CAPE treatment resulted in a dose dependent decrease in both WT and p53 null mammosphere forming efficiency that persisted in secondary and tertiary passages, suggesting reduced self-renewal. CAPE treatment also shifted differentiation from predominantly basal K14 to luminal K18-positive in both WT and p53 null MMS and increased PR expression in WT MMS. ATAC-seq of CAPE treated WT MMS showed significant pathway enrichment for p53 signaling, SOX2 signaling, and enrichment of open chromatin for several genes including the SMARCA4 gene, which regulates transcription of genes involved in stem cell renewal. ATAC-seq of CAPE treated Trp53 null MMS showed that genes defining early and late response to estrogen were particularly important. Significant canonical pathways included Aryl hydrocarbon receptor signaling, whose upregulation results in inhibition of self renewal and has been targeted as a potential drug target for estrogen receptor negative breast cancer. The integrin signaling pathway was also highly enriched.

These data suggest that CAPE both inhibits MaSC self-renewal and shifts the lineage commitment to a luminal, ER + lineage. ATAC-seq demonstrated genomic effects that are important in differentiation, SC renewal and adhesion. These data suggest that CAPE may have an effect on lineage commitment in support of our chemoprevention strategy to reduce triple-negative breast cancer.
Title: Msi1 in maintaining breast cancer stem cell involves the AKT/PI3K pathway

Nahas GR R, Sinha GA A, Sherman LS S, Walker ND D and Rameshwar P. Rutgers University-Graduate School of Biomedical Sciences, Newark, NJ.

Body: Musashi1 (Msi1) was originally described in neural stem cells in a role influencing neural differentiation in the Numb/Notch pathway. Due to its role in neural stem cells, there has been much interest in the role of Msi1 in the breast cancer (BC) stem cell population. In this vein, with we have demonstrated a possible feedback loop between the stem cell marker OCT4 and Msi1, in addition to other stem cell-associated genes. Flow cytometry analyses demonstrated that the subset of BC cells (BCCs) that we previously identified as those with high Oct4 was also enriched for Msi1. Msi1 knockdown BCCs showed decreased doubling time and with limited ability to be passaged, indicating the loss of the self-renewal subset needed for cell passaging. These in vitro findings were consistent with the inability of the Msi1 knockdown BCCs to undergo serial passages in vivo. We therefore examined the Msi1 knockdown BCCs for intracellular proteins that could explain the reduced cell growth and the reduced initiating cells. We selected the AKT/PI3K pathway due to its recent connection to the maintenance of BC stem cells. Msi1 knockdown repressed several molecules within the AKT/PI3K pathway: PTEN, AKT, and PI3K. There were no significant differences found however, in the apoptotic factors, BCL-2 and Caspase-3. Upon further investigation, we observed increases in molecules that are linked to decreased cell proliferation and senescence, p16, p53 and p21. Since Msi1 is an RNA binding protein, it is possible that its loss could leave RNAs for binding to miRNAs and this might be partly responsible for the decrease in key intracellular molecules needed for the survival and proliferation of the Msi1 knockdown BCCs. Further studies are needed to investigate how miRNAs and Msi1 interact to maintain the survival of BCCs. Finally, Msi1 KD positively affects the expression of the immune checkpoint inhibitor PD-1L, suggesting increased PD-1L expression in cells that are not of the CSC phenotype. The studies may identify Msi1 or its associated molecules as a potential therapeutic intervention for BC.
Title: Stem cell markers CD44+CD24- and ALDH1 in primary breast cancers and metastatic sentinel and non-sentinel lymph nodes

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Body: Background and Aim
Studies of Aldehyde dehydrogenase 1 (ALDH-1) and CD44+/CD24- have suggested them as cancer stem cell (CSC) markers in breast cancers and the clinicopathologic and prognostic significance of these CSC markers have been widely investigated. However, expressions of CSC in metastatic axillary lymph nodes (ALN) of breast cancers have not yet been the center of interest. Therefore, the object of this study is to explore breast CSC markers CD44+/CD24- and ALDH-1 in the primary breast tumor and metastatic ALNs by retrospectively analyzing the CSC expressions on metastatic tumor cells within ALN.

Method
180 surgically resected breast cancers were selected and among them 165 cases had undergone sentinel lymph node (SLN) with or without non-sentinel lymph node (NSLN) dissections. 50 SLNs (30.3%) and 43 NSLNs (30.9%) had metastatic tumor cells and total of 74 cases were involved with axillary lymph node metastasis. Double immunohistochemistry of CD44/CD24 and single immunohistochemical staining of ALDH-1 were applied on paraffin embedded breast tissue specimens and lymph node specimens to evaluate the CSC phenotypes of primary tumor and metastatic lymph nodes.

Results
The prevalence of CD44+/CD24- and ALDH-1(+) tumor cells in primary breast cancer was 76.7% and 45.0%. In triple negative breast cancers (TNBC) contained significantly higher percentage (49.2%) of ALDH-1 positive tumor cells (p=0.001). CD44+/CD24- phenotype was present in 65.2% and 40.0% in metastatic SLNs and NSLNs, respectively. The analysis of metastatic sentinel lymph nodes and breast cancers with CSCs indicated that there are significant relationships with ALDH-1(+) (p<0.001) and CD44+/CD24- (p<0.001) phenotypes. CSC expression in metastatic NSLNs and breast tumors also showed significant association (p=0.001). However, CSCs in metastatic SLNs and NSLNs did not show any significant relationships to the intrinsic molecular subtypes.

Conclusions
This study investigated the impact of the stem/progenitor phenotype defined by CD44 positivity/CD24 negativity and ALDH-1 positivity in sentinel lymph nodes (SLN) on non-sentinel lymph node (NSLN) metastases. CSC phenotypes expressed in metastatic SLNs and NSLNs, independently showed significant association with the primary breast tumors. However, in future, the authors recommend that the exploration of a much larger study including a vast amount of pool will provide a more reliable result.
Title: Proto-oncogene PELP1 signaling regulates breast cancer stem cells via G9a/EHMT2

Viswanadhapalli S, Mann M, Sareddy GR R, Xaionan L, Vankayalapati H, Brann D and Vadlamudi RK K. UT Health Sciences Center at San Antonio, San Antonio, TX; Oncolexis Therapeutics, Inc., Dallas, TX and Medical College of Georgia, Augusta, GA.

BACKGROUND: Evolving evidence suggests that cancer stem cells (CSCs) evade hormonal therapy and therapy resistance occurs due to regrowth of tumor cells from cancer stem cells that escaped hormonal therapy or remained in the body after tumor resection. Recent studies suggest that estrogen stimulates breast cancer stem-cells and G9a/EHMT2 plays a critical role in stem cell maintenance. Proline, glutamic acid, and leucine rich protein (PELP1) is a proto-oncogene that functions as a critical coregulator of several nuclear receptors and other transcription factors. PELP1 is commonly overexpressed in hormone-related cancers, and is prognostically linked to shorter breast cancer survival. Recent studies from our lab discovered PELP1 interacts with G9a/EHMT2. However, it remains unknown whether PELP1-G9a signaling plays a role in breast cancer stem cell proliferation. The objective of this study is to develop small molecular inhibitors that block G9a/EHMT2 interactions and to test their utility.

METHODS: We isolated CD44high/CD24low CSCs from three breast cancer cell lines (ZR75, MCF7, T47D) using FACS. To test the effect of PELP1 inhibitors on CSCs, we cultured CSCs in SFM in the presence or absence of PELP1 inhibitors for a period of 7-10 days. Cells were analyzed for spheroid formation, morphological changes, immunofluorescence for differentiation markers, protein (Western) and RNA (RT-qPCR) analysis. Expression of differentiation markers K19 and K14 and stem cell markers CD133, CD44, Id1, Nestin, Musashi-1, SOX2, Notch2, and OCT1 was determined.

RESULTS: Using mapping studies, we identified a small peptide inhibitor (PIP1) that interferes PELP1 interaction with G9/EHMT2. Utilizing Hit-Ligand interaction site with the PELP1 hot spot residues based on 3D alignment and shape, we have identified 61 potential hits from Ligand-Based screening using a 10,000 Diverse Set. Screening of these 61 potential hits using MTT based cell viability assays identified three small organic molecule inhibitors (peptidomimetics) as leads. All three peptidomimetics (#20, #29, #34) showed activity similar to PELP1 peptide inhibitor 1 (PIP1) in assays using three different breast cancer cell lines. Further, PELP1 targeting peptidomimetic disrupted PELP1 interaction with G9a/EHMT2. Peptidomimetic treatment inhibited the proliferation of tamoxifen therapy resistant cells. In mechanistic studies, we found that knockdown of PELP1 inhibited stem cell maintenance. In FACS analysis of ZR75, ZR75-PELP1 and ZR75-PELP1KD cells, the percentage of CD44high/CD24low cells correlated with PELP1 status. Accordingly, in mammosphere formation assays, PELP1 targeting peptidomimetic significantly inhibited the formation of mammospheres and the size of the mammospheres was also substantially decreased. Further, in self-renewal assays, peptidomimetic-treated cells had decreased self-renewal capacity.

CONCLUSIONS: Collectively, our studies have discovered an essential role for PELP1 in breast cancer stem cell maintenance and identified the PELP1- G9a/EHMT2 axis as a potential therapeutic target for reducing stemness. Further, the novel small molecule inhibitors of PELP1 could be used for therapeutic targeting of breast cancer stem cells and therapy resistance.
Characteristics, treatment and outcomes of breast cancer diagnosed during pregnancy and the year after delivery


Body: Background:
Pregnancy associated breast cancer (PABC) is defined as breast cancer (BC) diagnosed during pregnancy or during the year after delivery. Whether PABC is associated with inferior outcomes compared to non-PABC is uncertain. Data suggests characteristics and outcomes of BC diagnosed during pregnancy and BC diagnosed within the year after delivery may differ. However, most previous research has not separated BC cases diagnosed during pregnancy from those diagnosed within the year after delivery.

Methods:
We performed a single institution retrospective cohort study of women diagnosed at the Johns Hopkins Hospital with PABC between 1985-2014 and matched controls. Women with BC diagnosed during pregnancy and BC diagnosed during the year after delivery formed two separate case groups. Controls were matched 2:1 to each of the cases by time period of diagnosis, age (+/- 5 years) and extent of disease at diagnosis. Clinicopathologic features, treatment and outcomes were compared between each case group and its respective controls. Univariate Cox modeling stratified by matching set was used to compare time to relapse between cases and their matched controls.

Results:
Of 140 PABC cases identified, BC was diagnosed during pregnancy in 65 and during the year after delivery in 75. 135 controls were matched to the cases diagnosed during pregnancy and 145 controls were matched to the cases diagnosed during the year after delivery. Compared to their controls, cases diagnosed after delivery were more likely to have grade 3 tumors (81% versus 60%) and less likely to be hormone receptor (HR)-positive (62% versus 82%). Similarly, compared to their controls, cases who were pregnant at diagnosis were more likely to have grade 3 tumors (77% versus 57%) and less likely to be HR-positive (54% versus 75%). The frequency of HER2-positivity between cases diagnosed during pregnancy or during the year after delivery was similar to their respective control groups. A higher proportion of cases diagnosed during pregnancy underwent mastectomy than their controls, but this was not statistically significant (74% versus 67%). Most patients in both case groups and both control groups received chemotherapy. The proportions of patients in both case groups compared to their controls who received radiation were similar. Rates of relapse were high in the entire study population. There was a non-significant increased risk of relapse for both the cases diagnosed during pregnancy compared to their controls (HR 1.77, 95% CI 0.844-3.73, p 0.13) and for the cases diagnosed after delivery compared to their controls (HR 1.51, 95% CI 0.70-3.24, p 0.30).

Conclusions:
In our study population, women diagnosed with BC during pregnancy or within the year after delivery were more likely to have high grade and HR-negative disease than controls matched for age, extent of disease and time period of diagnosis. Rates of recurrence were high among our young study population. Findings must be interpreted with caution due to small sample size, but suggest that rates of relapse were not significantly higher among the cases diagnosed during pregnancy or within the year after delivery compared to their controls.
Title: 5-year overall survival of early breast cancer during pregnancy: A multicenter French case control study

Vanlemmens L, Ploquin A, Delaloge S, Rouzier R, Lesur A, Frenel J-S, Loustalot C, Bachelot T, Provansal M, Ferrero J-M, Coussy F, Debled M, Kerbrat P, Vinceneux A, Djelila A, Baron M, Jebert S, Decoupigny E, Tresch E and Bonneterre J. Centre Oscar Lambret, Lille, France; Institut Gustave Roussy, Villejuif, France; Institut Curie, Paris, France; Institut de Cancérologie de Lorraine, Vandoeuvre les Nancy, France; Institut de Cancérologie de l'Ouest - Centre René Gauducheau, St Herblain, France; Centre Georges Francois Leclerc, Dijon, France; Centre Léon Bérard, Lyon, France; Institut Paoli Calmettes, Marseille, France; Centre Antoine Lacassagne, Nice, France; Hopital Saint Louis, Paris, France; Institut Bergonié, Bordeaux, France; Centre Eugene Marquis, Rennes, France; Hopital Universitaire Bretonneau de Tours, Tours, France; Centre Francois Baclesse, Caen, France and Centre Henri Becquerel, Rouen, France.

Body: Background: Breast cancer (BC) during pregnancy (BCP) is a rare situation that requires collaboration between oncologists, surgeons and obstetricians. The main objectives of this study were to compare the overall survival (OS) and disease free survival (DFS) of a multicenter cohort of pregnant patients (pts) with those of matched control pts.

Methods: Patients from 27 centers and diagnosed between 2000 and 2006 with histological confirmed M0 invasive BC were included in this retrospective study. For the cohort of BCP, pts whose pregnancy was interrupted were not eligible. Controls were matched to BCP pts on 5 criteria: clinical T (of TNM), hormonal receptor (HR) status, HER2 status, administration of neo-adjuvant chemotherapy and pathological nodal status in the absence of neo-adjuvant chemotherapy. Survival times were estimated from the date of diagnosis using Kaplan-Meier method. OS was calculated until death from every cause, DFS was calculated until relapse or death from every cause; patients alive were censored at the date of last news.

Results: 100 BCP pts were identified. Their clinical and pathological characteristics were described on a previous presentation (SABCS 2013 P6-06-07). Matched controls could not be found for 12 BCP pts. 88 BCP pts were matched with 204 controls. The only differences between the 2 populations in terms of characteristics or treatment were more radical mastectomy (p=0.036) and fewer taxane administrations in the BCP group (p=0.06). The median duration of follow-up was 8.2 years for cases and 7.7 years for controls. There were no differences between BCP pts and controls in 5-year OS: 83.4%, IC 95% (73.5-89.8) vs 83.8%, IC 95% (77.9-88.3) nor 7-year OS: 76.5% (65.5-84.4) vs 78.1% (71.5-83.3) (p=0.52). The 5-year DFS was 58.6% IC 95% (47.3-68.3) vs 67.2% IC 95% (60.2-73.2) (p= 0.16). However, 5-year DFS was lower in HR+ BCP pts subgroup than in HR+ control group (56.7% IC 95% (40.7-69.8) vs 70.9% IC 95% (61.4-78.5) (p=0.023).

Conclusion: This multicenter French large study confirmed that there are no differences on OS and DFS between pregnant and no pregnant pts, though this might not be true for HR subgroup.
Reproductive factors and subtype specific breast cancer risk

Anderson WF F, Pfeiffer RM M, Wohlfahrt J, Ejlertsen B, Jensen M-B and Kroman NT T. National Cancer Institute, Bethesda, MD; Statens Serum Institut, Copenhagen; Danish Breast Cancer Group, Copenhagen and Righopitalet, Copenhagen, Denmark.

Introduction: Reproductive history and breast cancer risk reportedly differ by the estrogen receptor (ER±) and by the joint expression of ER and the human epidermal growth factor-2 receptor (ER±/HER2±). However, large sample sizes are needed to identify risk factor associations for the relatively less common ER- subtypes.

Material and Methods: We, therefore, linked two large-scale and population-based Danish registries to assess the associations for parity, number of live births, and age at first live birth (AFLB) with receptor-specific breast cancer risk. Relative risks (RRs) and 95% confidence intervals (CIs) for associations were estimated with Poisson regression models.

Results: With nearly 31 million women-years of follow-up, there were 45786 Danish women between the ages 20-84 years who developed an invasive breast cancer during the study period 1992-2011. Parity significantly reduced risk for ER+ and ER+/HER2- subtypes (RR for ER+/HER2- = 0.92; 0.87, 0.98) and suggestively increased risk for ER- and ER-/HER2- subtypes (RR for ER-/HER2- = 1.16; 0.99, 1.36). RRs increased with advancing AFLB for ER+ cancers, especially among premenopausal women; and were elevated for ER- cancers among age groups 12-19 years and 30-34 years compared to the reference age group 20-24 years.

Conclusion: Associations of breast cancer risk and reproductive history varied among Danish women by ER± and by ER±/HER2±, consistent with receptor-specific etiological heterogeneity. Risk estimates for ER+ and ER+/HER2- cancers were similar to the well-established associations for breast cancer overall, whereas relative risks for ER- and ER/HER2- cancers tended to be null or the inverse of ER+ associations.
Introduction: The clinical classification of breast cancer into at least four intrinsic subtypes has advanced targeted therapy and improved prognosis. However, for etiological classification, it has been proposed that breast cancer is comprised of just two main subtypes: basal-like and non-basal-like. Evidence for these two etiologic subtypes emerges strongly from bimodal age frequency distributions at diagnosis. In the absence of RNA-based intrinsic subtyping, estrogen receptor (ER) expression is a useful surrogate for these two distinct etiologic classes. Using data from the population-based Carolina Breast Cancer Study (CBCS), we examined evidence for a two-component (ER-positive vs. ER-negative) mixture model for breast cancer biologic/etiologic heterogeneity.

Methods: Automated digital scoring of ER expression was performed on immunohistochemistry-stained tissue microarrays comprising 1,920 invasive breast cancer cases from CBCS. Clinical classification of ER status has changed over time as new data have emerged regarding optimal treatment-relevant thresholds, but optimal etiologic thresholds have not been established. Therefore, we considered ER status as a quantitative, categorical variable with cut points of <1% (ER-negative) vs. ≥1% (ER-positive), with ER-positive cases further categorized as highly positive (≥80-100%), intermediate (≥40-<80%), low (≥10-<40%) or borderline (≥1-<10%). Smoothed age frequency distributions at diagnosis (i.e., density plots) were constructed and logistic regression adjusted for age and race was conducted to assess associations between patient and tumor characteristics and level of ER positivity.

Results: As expected for etiologically-distinct entities, ER-negative and highly ER-positive tumors showed predominantly unimodal early-onset and late-onset age distributions at diagnosis with peak frequencies near ages 50 and 70 years, respectively. However, tumors with low and intermediate positivity showed bimodal patterns, consistent with a mixture of two main subtypes. Consistent with these age distribution patterns, young age (<40 years) at diagnosis was associated with an elevated odds ratio (OR) for low positive (OR 1.8; 95% CI 1.1-2.9), borderline (OR 1.9; 95% CI 1.1-3.3) and ER-negative disease (OR 2.2; 95% CI 1.5-3.2). Relative to highly ER-positive tumors, low ER-positive tumors were more likely to be node-positive (OR 1.4; 95% CI 1.0-1.9), higher grade (combined grade III; OR 1.9; 95% CI 1.3-2.9), and were more likely to harbor a p53 mutation (OR 2.0; 95% CI 1.2-3.5).

Conclusions: While etiologic differences between dichotomized ER-negative and ER-positive breast cancer categories have been well described in many epidemiologic studies, differences in breast cancer etiology across quantitative levels of ER expression have not been so well-characterized. In this study, we report that ER-positive tumors with low positivity share etiologic features of ER-negative tumors, including young age at diagnosis and aggressive tumor characteristics. These data provide additional support for a two-component breast cancer mixture model, with quantitative level of ER positivity reflecting the relative distributions of ER-positive and ER-negative tumor populations.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-07-05

Title: Weight change across the life-course and breast cancer risk among pre and postmenopausal women

Colditz G, Eliassen H, Toriola A, Hankinson S, Willett W and Rosner B. Brigham and Womens Hospital, Boston, MA; Washington University School of Medicine, St Louis, MO and University of Massachusetts, Amherst, MA.

Body: Obesity is well established as a cause of postmenopausal breast cancer incidence and mortality. In contrast, adiposity in early life is inversely related to breast cancer incidence. To better integrate understanding of these relations, we assess adiposity in childhood, in late adolescence, and in adult years, as well as change in weight in relation to total invasive breast cancer and subtype defined by receptor status.

The Nurses' Health Study cohort was established in 1976 when 121,701 female US registered nurses ages 30-55 responded to a mailed questionnaire about risk factors for breast cancer including reproductive factors, hormone use, anthropometric variables, benign breast disease (BBD), and family history of breast cancer. The risk factors have been updated by repeat questionnaires every 2 years. We followed a cohort of 77,232 women from 1980 to 2006 (1,408,188 person-years), with routinely updated risk factor information, documenting 4,254 incident cases of invasive breast cancer. ER and PR status were obtained from pathology reports and medical records. A total of 2,065 ER+/PR+ tumors, 604 ER-/PR- tumors, 520 ER+/PR- tumors were identified among women with complete information on breast cancer risk factors.

Weight at age 18 was inversely related to incidence of pre and postmenopausal breast cancer. The relative risk (RR) per 50lb weight difference at age 18 was 0.80 (95% CI = 0.73, 0.88). However, the inverse association is completely explained by weight at age 10. Weight at age 10 was more strongly inversely related to ER-PR- breast cancer, RR per 50lb difference in weight at age 10 = 0.48; 95% CI 0.32 – 0.74. Weight gain from 10 to 18 was not related to risk. After controlling for weight at age 18, weight loss of 10lb or more was significantly related to lower risk of breast cancer (RR=0.80; 0.66, 0.95) overall. The association was stronger for premenopausal breast cancer (RR=0.51; 0.30, 0.88). Long-term weight change was positively related to total incident breast cancer risk and most clearly to postmenopausal cancer (RR per 50lb weight gain since age 18 =1.26; 1.19-1.34). Weight gain during premenopausal years (weight at menopause minus weight at age 18), and after menopause, were both directly related to increased risk. Weight gain of 30 or more pounds during premenopause increased risk of postmenopausal breast cancer compared to less than 10lb weight change (RR=1.25; 1.14-1.38) and weight gain of 30 or more pounds after menopause carried similar increase in relative risk (RR=1.24; 1.10-1.41). These results were unchanged after control for weight at age 10, and were stronger for ER+PR+ breast cancer. The associations for long-term weight gain and postmenopausal breast cancer are stronger for never vs. ever users of hormone therapy, but are significantly positively associated in both groups.

In conclusion, adiposity in childhood has a protective lifelong relation to breast cancer risk most clearly seen for ER-PR- disease. There are deleterious effects of long-term weight gain both pre- and post-menopause. Weight loss in premenopausal years significantly reduces risk of breast cancer. Weight change can importantly modify breast cancer risk.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-07-06

Title: Weight gain during pre- and postmenopausal years results in earlier onset of breast cancer. The Tromsø cohort study

Lofterød T, Frydenberg H, Flote VG G, Risberg T, Eggen AE E, McTiernan A, Mortensen E, Wist EA A, Akslen LA A, Reitan JB B, Wilsgaard T and Thune I. Oslo University Hospital, Oslo, Norway; Tromsø University Hospital of Northern Norway, Tromsø, Norway; The Arctic University of Norway, UiT, Tromsø, Norway; Fred Hutchinson Cancer Research Center, Seattle, WA and Haukeland University Hospital, Bergen, Norway.

Body: Background: Obesity is both an independent risk factor, and a prognostic factor of postmenopausal breast cancer. In contrast, the association between premenopausal obesity/leanness, and subsequent weight gain and breast cancer outcomes, is still unclear. Furthermore, the association between adult weight gain, weight and age at diagnosis, and tumor characteristics is less studied.

Methods: During 1979-2007, a total of 18 990 women, aged 18-87 years, answered questionnaires and underwent clinical examination at a total of five repeated health surveys (attendance rate 68-82%). Height and weight were measured at each survey, and before surgery, among those women diagnosed with breast cancer during follow-up. Careful review of the respective medical records, including histopathological workup, was performed. Multivariate Cox Proportional Hazard models were used to study the importance of Body Mass Index (BMI kg/m^2) and weight change on breast cancer risk, and to evaluate variation in breast tumor characteristics.

Results: During a median follow-up of 23.3 years, 579 women with invasive breast cancer were identified, and the cases were histologically verified. These breast cancer cases had a mean age at diagnosis of 56.3 years, and mean BMI at diagnosis of 25.3 kg/m^2. Most (67 %) of the breast cancer patients had estrogen receptor (ER) positive tumors, 48 % had progesterone receptor (PgR) positive tumors, and 41 % had lymph node positive disease. We divided all participating women in three groups of weight change (< 5kg, 5-15 kg, >15 kg). When we compared women with less than 5 kg weight gain, to women with weight gain 5-15 kg, and to women with weight gain above >15kg, we observed a RR of 1.43 (95% CI 1.07-1.90) and a RR of 1.86 (95% CI 1.30-2.68), respectively, for postmenopausal breast cancer. We divided women by quartiles of BMI (kg/m^2) at entry, and observed that women in the lowest quartile of BMI (≤ 21.45 kg/m^2), who had a subsequent weight gain >15 kg, had a RR of 2.40 (95% CI 1.07-5.38) for postmenopausal breast cancer compared to women with the same BMI at entry, but who remained stable in weight. We observed a 6 year difference in age at diagnosis for women diagnosed with breast cancer, who at study entry were in the same BMI group (< 25kg/m^2), but subsequently either experienced a large weight gain (>15 kg), or remained stable in weight (59.5 years vs. 64.4 years, p=0.007). Furthermore, we observed a 15 year difference in age at diagnosis for women diagnosed with breast cancer, who at study entry were in the same BMI group (≥ 25kg m^2), but subsequently either experienced a large weight gain (> 15 kg), or remained stable in weight (60.3 years vs. 74.9 years, p=0.007).

Conclusion: Avoiding large weight gain during pre- and postmenopausal years may both protect against, and delay onset of postmenopausal breast cancer. Our findings support the importance of weight gain as a modifiable lifestyle factor for early onset of breast cancer.
Title: The incidence of and survival after breast cancer recurrence


Body: Background: Patients who complete definitive therapy for primary, stage I-III breast cancer may develop recurrent disease. However, little is known about the outcomes patients experience after recurrence when treated outside of a clinical trial because population-based datasets usually do not capture recurrence status. We describe the overall survival of patients after developing recurrent breast cancer, identify factors independently associated with improved survival, and compare survival for patients with recurrent versus de novo stage IV metastatic disease.

Methods: The cancer registries from two Kaiser Permanente (KP) sites participating in the Cancer Research Network, KP Colorado and KP Northwest, provided data on adult women diagnosed 2000-2011 with primary breast cancer and followed through death, disenrollment or study end (12/31/2012). Among patients with stage I-III disease who completed definitive therapy, recurrence was captured via manual chart abstraction. Survival time was calculated from the date of recurrence or the date of de novo stage IV disease. Multivariable modeling identified factors independently associated with restricted mean survival time (RMST) through 7 years, controlling for age, race, income, co-morbidity, year of recurrence, time from primary diagnosis to recurrence, type of recurrence (local vs. regional/distant), use of chemotherapy or radiation at recurrence, and characteristics of the primary cancer that pre-dated recurrence (i.e., primary stage, grade, hormone-receptor status, and use of chemotherapy or radiation therapy for the primary diagnosis). We compared overall survival after developing recurrent versus de novo stage IV disease after matching for age, race, income, co-morbidity and year.

Results: From 7,216 breast cancer diagnoses we identified 506 cases of recurrent disease and 219 cases of de novo stage IV disease (7% and 3%, respectively). Most recurrences were regional or distant (81%). From the time of recurrence, median survival was 21 months and 2-year survival was 47%. Factors significantly associated with inferior RMST included regional/distant vs. local recurrence (-35.6 months; P<.01), primary stage III vs. stage I disease (-13.6 months; P<.01), and chemotherapy for the primary diagnosis (-9.6 months; P=.02), but not race, income, grade, or primary cancer hormone-receptor status. Patients for whom the interval from diagnosis to recurrence was >4 years vs. <1 year had a longer RMST (+18.9 months; P<.01). Receipt of chemotherapy at the time of recurrence was associated with inferior RMST; the magnitude of this association was higher among patients with local (-18.5 months) versus regional/distant disease (-3.2 months). Women with regional/distant recurrence had significantly worse RMST than those with de novo stage IV disease (-10.3 months; P<.01).

Conclusions: Recurrent breast cancer is at least two-fold more common than de novo stage IV disease. Among patients who develop recurrence, characteristics of the primary cancer and its treatment are associated with survival after recurrence. Survival differences between patients with recurrent and de novo stage IV disease suggest that prognostic estimates and treatment paradigms should be tailored.
**Title:** Time trends in incidence rates and survival for women with de novo metastatic lobular vs. ductal carcinoma, a population-based study

Di Meglio A, Freedman RA A, Lin NU U, Barry WT T, Metzger-Filho O, Keating NL L, Winer EP P and Vaz-Luis I. Dana Farber Cancer Institute, Boston, MA; IRCCS San Martino University Hospital - IST National Cancer Research Institute, Genova, Italy; Harvard Medical School, Boston, MA and Brigham and Women's Hospital, Boston, MA.

**Body:** Background: Survival for metastatic breast cancer (MBC) patients (pts) has modestly improved over time. Until the early 2000’s, incidence rates for invasive lobular carcinoma (ILC) had steadily risen, in contrast to the stable rates observed for invasive ductal carcinoma (IDC). Historically, ILC was deemed to have a more favorable prognosis than IDC. Nevertheless, data on recent time trends in incidence and survival of lobular vs. ductal histology among newly diagnosed MBC pts are limited.

Pts and Methods: Using the Surveillance, Epidemiology, and End Results (SEER) 9 registries, we included 10,767 pts diagnosed with de novo lobular or ductal MBC from 1990-2011, and followed through 2012. Time trends in annual age-adjusted incidence rates were analyzed, stratified by histology. Multivariable Cox regression models were fit to investigate the association of year of diagnosis and overall survival (OS) by stratum, adjusting for features presented in Table 1. We examined interactions between year of diagnosis and histology. In sensitivity analyses, we modeled year of diagnosis as categorical, and restricted the cohort to hormone-receptor positive pts.

Results: 9,376 (87%) pts had IDC and 1,391 (13%) had ILC. Overall, we found a 1.4 fold increase in incidence rates for de novo MBC over the study period, (with a 1.3- and 2.6-fold increase for IDC and ILC, respectively). OS improved over the study period for the overall cohort (Hazard ratio (HR) of death=0.99; 95% confidence interval (CI)=0.98-0.99; 1% decrease/year; 5% decrease/5 years; p=.0059 for the interaction year of diagnosis-histology on OS). ILC pts had better outcomes than IDC pts (median OS=28 vs. 21 months; adjusted HR of death= 0.93; 95%CI=0.87-0.99). For IDC pts, we found a statistically significant improvement in OS over time (HR of death=0.98; 95%CI=0.98-0.99; 2% decrease/year; 6% decrease/5 years). However, we observed no significant change in survival outcomes for ILC pts (HR of death=1.01; 95%CI=0.99-1.02) (Table 1). Results from sensitivity analyses were similar.

Conclusions: From 1990-2011, incidence rates for de novo MBC increased. In this cohort, ILC pts had a better prognosis than IDC pts. Nevertheless, although we found an expected overall improvement in OS for MBC pts, this effect was restricted to IDC pts, with no significant improvement among ILC pts. Dedicated studies are warranted to understand whether our results can be confirmed in other datasets and to investigate the reasons driving this discrepancy, such as the impact of patterns of care, new drug approvals, and tumor molecular subtype.

<table>
<thead>
<tr>
<th>Cohort characteristics</th>
<th>IDC N= 9,376 (87%)</th>
<th>ILC N= 1,391 (13%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>40-49</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>50-59</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>60-69</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>≥70</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>75</td>
<td>85</td>
</tr>
<tr>
<td>Black</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Other/Unknown</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Grade</td>
<td>I</td>
<td>4</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>III/IV</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>17</td>
</tr>
<tr>
<td>Hormone Receptor</td>
<td>+</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td><strong>Incidence</strong></td>
<td>Rates (per 100,000/year)</td>
<td></td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td>1990</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>1.54</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>2.17</td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td>HR of death (95% CI)*</td>
<td></td>
</tr>
<tr>
<td>Year of diagnosis</td>
<td>1-year trend</td>
<td>0.98(0.98-0.99)</td>
</tr>
<tr>
<td></td>
<td>5-years trend</td>
<td>0.94(0.92-0.96)</td>
</tr>
</tbody>
</table>

p<.01 for differences between groups

*Adjusted for: cohort characteristics, SEER registry, and marital status
Body: Background
Young women (<40 yrs) with breast cancer (YWBC) account for 7-12% of BC diagnoses. BC is the leading cause of cancer death in this group (G). Age-specific data on outcome and appropriate treatment (Rx) are lacking. YWBC appear to have more biologically aggressive subtypes and a higher risk of relapse and death. We studied the clinico-pathological (ClinPath) characteristics in YWBC, examining how outcomes/Rx have evolved.

Methods
YWBC were identified from pathology databases at 2 tertiary centers. Pts were divided into 2 cohorts: BC diagnoses from 2000-2007 (G1) and 2008-2015 (G2). ClinPath and Rx data were retrieved from clinical, radiology and histology databases. Statistical analysis was performed using SPSS.

Results
We identified 347 pts. Tumor features are shown in Table I. Median age is 36 (23-39). By histology, 90.8% (n=315) had invasive ductal carcinoma, 53.1% (n=181) had Grade III BC and 56.3% (n=171) had lymphovascular invasion. Pregnancy-associated BC occurred in 10.7% (n=34). Mastectomy (MX) was performed in 53% (n=176) and axillary lymph node clearance (ALNC) in 63.8% (n=192 [G1: 84.3% vs. G2: 48.6%, p<0.001]).

Table 1

<table>
<thead>
<tr>
<th>Tumor features</th>
<th>Group 1 (n=149)</th>
<th>Group 2 (n=198)</th>
<th>Total (n=347)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median tumor size (mm)</td>
<td>25 (60.3%)</td>
<td>22 (51.5%)</td>
<td>22.5</td>
<td>p=0.115</td>
</tr>
<tr>
<td>Node positivity</td>
<td>88 (60.3%)</td>
<td>100 (51.5%)</td>
<td>188 (55.3%)</td>
<td>p=0.109</td>
</tr>
<tr>
<td>Median node count</td>
<td>4 (1-44)</td>
<td>1 (1-30)</td>
<td>2 (1-44)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>148 (43.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
<td>70 (20.5%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>23 (7.3%)</td>
<td></td>
</tr>
<tr>
<td>Biomarker status*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/HER2-</td>
<td>76 (53.1%)</td>
<td>120 (60.6%)</td>
<td>196 (56%)</td>
<td>p=0.086</td>
</tr>
<tr>
<td>HER2+</td>
<td>41 (28.7%)</td>
<td>45 (22.8%)</td>
<td>86 (27%)</td>
<td>p=0.031</td>
</tr>
<tr>
<td>Triple negative (TN)</td>
<td>26 (18.2%)</td>
<td>33 (16.8%)</td>
<td>59 (17%)</td>
<td>p=0.291</td>
</tr>
</tbody>
</table>

* Missing data n=6

Rx characteristics are shown in Table 2. 85 pts received neo-adjuvant therapy (NAT); 48.3% (n=41) ER+/HER2-, 27% (n=23) HER2+ and 24.7% (n=21) TNBC. Pts receiving NAT in G2 trended towards improved pCR rate (G2: 24.6% vs G1: 8.3%, p=0.057). Endocrine Rx alone was received by 9.8% (n=22); 13.6% (n=18) in G2 vs 4.3% (n=4) in G1. OncotypeDx(ODx) was used in 23 pts (14.9%) (median score 17), 1 had a DR (ODx Score = 18).

Table 2
<table>
<thead>
<tr>
<th>Tx characteristics</th>
<th>n=347</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>Total 300 (86.4%)</td>
</tr>
<tr>
<td></td>
<td>NAT 85 (28.3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathological Complete Response (pCR)*</th>
<th>pCR (n=16, 19.8%)</th>
<th>No pCR (n=65, 80.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/HER2-</td>
<td>18.8% (n=3)</td>
<td>53.9% (n=35)</td>
</tr>
<tr>
<td>HER2+/ER+</td>
<td>18.8% (n=3)</td>
<td>13.8% (n=9)</td>
</tr>
<tr>
<td>HER2+/ER-</td>
<td>31.2% (n=5)</td>
<td>9.2% (n=6)</td>
</tr>
<tr>
<td>TNBC</td>
<td>31.2% (n=5)</td>
<td>23.1% (n=15)</td>
</tr>
</tbody>
</table>

| Local relapse | 1 (6.2%) | 1 (1.5%) |
| Distant relapse (DR) | 0 | 22 (33.8%) |

*Data incomplete n=4

DR occurred in 50 pts (16%), including 13 (20.3%) HER2+ pts. Of note, 92.3% (n=12) of these were in G1. Relapse rates (RR) in TN and ER+/HER- pts were 19.6% (n=11) and 13.7% (n=26) respectively. There was a higher RR in G1 (34.8% vs 11.4%, p<0.001). Overall survival in pts with stage IV dx was 32 mos in G1 and 48 mos in G2.

Conclusion
In line with existing data, locally advanced dx is more prevalent in YWBC. MX and ALNC rates were high and most received multimodal Rx. The extent of axillary surgery declined. Pts in G2 had lower volume BC at diagnosis suggesting increasing awareness. TN and HER2+ subtypes accounted for a slightly higher proportion of BC cases. Pts with PCR had better outcomes. Only 16% relapsed with metastatic dx. The impact of HER2 Rx is highlighted by reduced RR in HER2+ G2 pts. Outcomes were unchanged in pts with ER+/HER2- and TNBC. These remain a priority for future research.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P1-07-10

**Title:** Increasing population rates of in-situ breast cancer [DCIS] are associated with reduced breast cancer (BrCa) mortality. A case for screening mammography and "overdiagnosis" linked to outcome benefits

Ragaz J, Qian H, Shakeraneh S, Fox J, Wilson KS S., Simpson JS S., Yoon J-Y and Wong H. School of Population and Public Health [SPPH], University of British Columbia [BC], Vancouver, BC, Canada; Centre for Health Evaluation and Outcome Sciences, Providence Health Care Research Institute, St. Paul’s Hospital, University of B.C., Vancouver, BC, Canada; BC Cancer Agency [BCCA], Victoria, BC, Canada; UBC Medical School, Vancouver, BC, Canada and St. Michael Hospital, University of Toronto, Toronto, ON, Canada.

**Body: INTRODUCTION:**
Following the first phase of this project [Ref 1], we correlate here the rates of DCIS with BrCaMOR, in two regions of Canada - British Columbia [BC] and Atlantic Provinces [Atl.P].

We previously reported higher compliance in screening mammography [ScreenMam] and therapeutic [TH*] guidelines [GUIDELINES] for both DCIS and invasive BrCa in BC compared with Atl.P [Ref. 2].

**METHODS:** Annual age-specific rates [cases / 100,000 population] of DCIS, and BrCaMOR between BC vs Atl.P, were obtained for 17 age groups of 5 years (years 0-4 to 85+) and averaged each 5-year period from 1975-1979 up to 2005-2009. To compare age distribution, DCIS rates and BrCaMOR between the two regions, we selected four birth cohorts, age 30-34, 35-39, 40-44 and 45-49 in 1975-1979. From those, we tabulated the DCIS incidence and BrCaMOR for each birth cohort when they reached ages 50-54 and 60-64. We assumed that the rates of DCIS reflect annual ScreenMam practices.

Data were obtained from the Public Health Agency of Canada based on the Canadian Cancer Registry database at Statistics Canada.

<table>
<thead>
<tr>
<th><strong>RESULTS [N/100,000 population]</strong></th>
<th>British Columbia</th>
<th>Atlantic Provinces</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age / years</strong></td>
<td>DCIS</td>
<td>BrCaMOR</td>
</tr>
<tr>
<td><strong>Age 50 â– 54</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980-84</td>
<td>13.1</td>
<td>52.2</td>
</tr>
<tr>
<td>1985-89</td>
<td>21.2</td>
<td>46.6</td>
</tr>
<tr>
<td>1990â– 94</td>
<td>29.2</td>
<td>44.4</td>
</tr>
<tr>
<td>1995â– 99</td>
<td>45.5</td>
<td>43.8</td>
</tr>
<tr>
<td><strong>Age 60 â– 64</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990-94</td>
<td>28.0</td>
<td>72.1</td>
</tr>
<tr>
<td>1995-99</td>
<td>49.3</td>
<td>61.9</td>
</tr>
<tr>
<td>2000-04</td>
<td>49.3</td>
<td>65.8</td>
</tr>
<tr>
<td>2005â– 09</td>
<td>51.4</td>
<td>53.1</td>
</tr>
</tbody>
</table>

**CONCLUSIONS:**
1. Our study shows across all age cohorts higher rates of DCIS and lower BrCaMOR in BC compared to Atl.P - results consistent with higher ScreenMam rates in BC than Atl.P.
2. These data are compatible with the concept that a higher diagnostic rate for early lesions such as DCIS [i.e. frequently designated as "Overdiagnosis"] by ScreenMam, and subsequent earlier Guideline TH*, contributes to lower BrCaMOR.

*TH Guidelines: surgery, radiation, Tamoxifen for DCIS; and the same + chemotherapy for early invasive disease.
References:
1. J. Ragaz, H. Wong, H. Qian, J. Fox, K. Wilson, A. Coldman: Cancer Research, May 1, 2015 75; P3-07-28
Title: Improved long term survival from breast cancer over three decades: Persistent disparities with age, but not socioeconomic status, in a socialized health care system

Malin A, Ashfield AM M, Purdie CA A, Jordan LB B and Thompson AM M. Ninewells Hospital and Medical School, Dundee, Tayside, United Kingdom and MD Anderson Cancer Center, Houston, TX.

Body: Background
Socioeconomic status and patient age have been associated with disparities in survival for breast cancer. We previously reported breast cancer diagnosed in a geographically defined population treated in a single cancer center in a socialized health care system (free health care) with a 5 year survival of 70% (95% CI 67-74%) (reported in 1998) and, in the same cohort, a 10 year survival of 52.7% (95% CI 50.03-55.1%) (reported in 2001). Since that time, multidisciplinary team discussion of every patient at diagnosis, before and after surgery, together with improved drug therapy and radiotherapy techniques have been implemented using national guideline, evidence-based protocols. This study examined survival for the population based cohort of women from the same geographical region treated over 5 years from 2000 to seek evidence of improved outcomes and persistent disparities compared with historical practice.

Methods
Between 2000-2004, a cohort of 1851 women were treated at a single cancer center, representing 98% of the incident breast cancer patients in the geographic region. Prospective electronic clinical data collection was retrospectively manually cross checked with patient records and death certification. While breast screening practice changed little over that time, multidisciplinary team discussion of every patient, second and third generation chemotherapy regimens and hypofractionation of radiotherapy were introduced when compared with pre-2000.

Results
At a median 8.8 years follow-up, amongst 1851 women there were 768 deaths, 342 from breast cancer, and 405 women diagnosed with recurrent disease. The 5 year breast cancer specific survival was 82% and disease free survival 80% (87% and 84% respectively for operable disease) and at 10 years 76% breast cancer specific survival and 75% disease free survival (82% and 78% respectively for operable disease). There were the expected associations between tumor size, grade, node status, estrogen receptor, progesterone receptor, HER2 and disease free or overall survival. Breast cancer specific mortality was significantly better in the <50 years (HR 0.60; 95% CI 0.42-0.85) and 50-69 years (HR 0.69; 95% CI 0.51-0.95) age groups compared with women >70 years. While deprivation was an independent risk factor for all-cause mortality, after adjustment for age, tumor size, grade and node status, breast cancer specific survival was not significantly associated with socioeconomic status.

Conclusions
Over a 30 year period, survival from breast cancer has dramatically improved, likely due to multidisciplinary evidence based practice, including new drugs and enhanced delivery of radiotherapy. While disparities by socioeconomic status have declined, women >70 years age continue to have a worse breast cancer specific survival.
Title: Prognosis of clinico-pathological breast cancer subtypes in routine clinical care

Hennigs A, Heil J, Gondos A, Riedel F, Marme F, Sinn H-P, Schirmacher P, Kauczor H-U, Debus J, Golatta M, Schtz F, Sohn C and Schneeweiss A. Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany; Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany; Department of Pathology, University of Heidelberg, Heidelberg; Department of Radiology, University of Heidelberg, Heidelberg, Germany; Department of Radio-Oncology, University of Heidelberg, Heidelberg, Germany and National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany.

Body: Background / Aim: To analyze oncological outcome of breast cancer (BC) subtypes in routine clinical care in specialized breast care units (BCU).

Patients and methods: A prospectively followed cohort of 4110 female cases with primary, non-bilateral, non-metastatic BC treated between 01.01.2003 and 31.12.2012 has been analyzed for the whole cohort and separately for the five routinely used clinico-pathological subtypes (i.e. Luminal A, Luminal B (=Her2 neg.), Luminal B (=Her2 pos.), HER-2, triple negative). The median follow-up of the cohort was 51 month. We calculated estimates for local control rate (LCR), disease-free survival (DFS), distant disease-free survival (DDFS), overall survival (OS) and relative overall survival (ROS).

5 year outcome results referred to 5 different endpoints (using Kaplan-Meier method) of all patients with primary, non-metastatic, non-bilateral breast cancer treated at Heidelberg Breast Care Unit between 01.01.2003 and 31.12.2012

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>All patients (including in-situ)</th>
<th>Patients with invasive cancer (excluding in-situ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=4102 (including 499 in-situ cases)</td>
<td>n = 3603</td>
</tr>
<tr>
<td>LCR [%] (95% CI)</td>
<td>96.1 (95.6 ; 96.6)</td>
<td>96.1 (95.7 ; 96.5)</td>
</tr>
<tr>
<td>DFS [%] (95% CI)</td>
<td>85.0 (84.2 ; 85.8)</td>
<td>83.7 (82.8 ; 84.6)</td>
</tr>
<tr>
<td>DDFS [%] (95% CI)</td>
<td>86.9 (86.1 ; 87.7)</td>
<td>85.7 (84.8 ; 86.6)</td>
</tr>
<tr>
<td>OS [%] (95% CI)</td>
<td>91.3 (90.5 ; 92.2)</td>
<td>90.5 (89.6 ; 91.4)</td>
</tr>
<tr>
<td>ROS [%] (95% CI)</td>
<td>95.5 (94.3 ; 96.7)</td>
<td>94.7 (93.4 ; 96.0)</td>
</tr>
</tbody>
</table>

CI, confidence interval; LCR, local recurrence rate; DFS, disease-free survival; DDFS, distant disease-free survival; OS, observed overall survival; ROS, relative (age adjusted) overall survival

Outcome results referred to 5 different endpoints (using Kaplan-Meier method) according to clinico-pathological tumor subtype or in-situ tumor. Results in percent at 5 years (95% CI).

<table>
<thead>
<tr>
<th>Tumor Subtype</th>
<th>All patients (n = 499)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lum A-like</td>
<td>99.1 (98.7 ; 99.5)</td>
</tr>
<tr>
<td>Lum B1-like</td>
<td>95.2 (-)**</td>
</tr>
<tr>
<td>Lum B2-like</td>
<td>95.0 (86.3 ; 100)</td>
</tr>
<tr>
<td>HER2+</td>
<td>90.5 (-)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>89.6 (87.1 ; 92.1)</td>
</tr>
<tr>
<td>CIS (n = 499)</td>
<td>96.2 (94.3 ; 98.1)</td>
</tr>
<tr>
<td>DFS [%] (95% CI)</td>
<td>92.2 (90.9 ; 93.5)</td>
</tr>
<tr>
<td>DDFS [%] (95% CI)</td>
<td>82.2 (80.5 ; 83.9)</td>
</tr>
<tr>
<td>OS [%] (95% CI)</td>
<td>95.1 (94.1 ; 96.1)</td>
</tr>
<tr>
<td>ROS [%] (95% CI)</td>
<td>100.0 (98.5 ; 99.8)</td>
</tr>
</tbody>
</table>
Results: LCR, DFS, DDFS, OS and ROS over 5 years for the whole cohort of invasive cases were 96.1%, 83.7%, 85.7%, 90.5% and 94.7%, respectively. Luminal A tumors were the most frequent (44.7%) and showed the best outcome with LCR, OS and ROS over 5 years at 99.1%, 95.1% and 100.0%, respectively; while triple negative tumors presented the poorest outcome with LCR, OS and ROS over 5 years at 89.6%, 78.5% and 80.1%, respectively.

Conclusions: This outcome analysis of a large cohort of patients with primary BC diagnosed, treated and prospectively followed on a routine basis at a specialized BCU in Germany confirmed general and detailed clinico-pathological subtype outcome data of clinical trials.
Title: Abstract Withdrawn
Title: A statewide, population-based study of molecular subtypes of female breast cancer: Treatment and associated factors

Loch MM M, Zhang L, Hsieh M-C, Wu X-C and Chen VW W. Louisiana State University Health Sciences Center, New Orleans, LA.

Body: Background: Breast cancer is now recognized as a heterogeneous disease with distinct biological molecular subtypes which have different prognoses and treatment options. We conducted the first statewide, population-based study to examine systemic treatment among invasive breast cancer (IBC) patients by subtype and determined their associated factors with treatment.

Methods: We analyzed data from the Louisiana Tumor Registry (LTR) and a Centers of Disease Control and Prevention (CDC)-funded special project of Enhancing Cancer Registry Data for Comparative Effectiveness Research (CER), which included Louisiana women diagnosed with microscopically-confirmed invasive breast cancer in 2011. Patient's socio-demographics, tumor characteristics and detailed information on the treatment, including chemotherapy, hormone, radiation and targeted therapy received within 12 months of diagnosis were collected from hospitals, radiation facilities, and medical oncology clinics. Systemic treatments received by each subtype were examined. Univariate and multivariate logistic regression analyses were used to identify factors associated with not receiving systemic treatment. Analyses were carried out using SAS version 9.4.

Results: About 70% of patients were hormone receptor (HR)+/ human epidermal growth factor receptor 2 (HER2)-, 15% triple negative (HR-/HER2-), 10 % HR+/HER2+ and only 5% HR-/HER2+. Among IBC patients with known HR and HER2 status, 72-78% with HR+ had hormonal therapy, 61-75% with HER2+ had Herceptin. About 0.5-6.1% of patients were given therapies which are contradictory to HR/HER2 status and 11-23% of IBC patients with known molecular subtypes did not receive any systemic treatment. Factors associated with not receiving systemic therapy include triple negative subtype and age ≥65 years. Patients younger than 50 years and with more advanced stages were more likely to receive systemic treatment. Race/ethnicity, grade, lymph node involvement, and comorbidity were not associated with receiving systemic treatment, adjusting for other covariates.

Conclusions: About 12-29% of breast cancer patients in our data set were not receiving treatment consistent with their HR/HER2 status or treatment guidelines. Some possible explanations may be advancing age, death prior to therapy and refusal by patients and/or family. Race, lymph node status and comorbidity were not associated with receiving systemic therapy after adjusting for other covariates. Further studies are needed to explore reasons why patients are not receiving therapy that is concordant with the guidelines and the access to care.
Title: The outcomes for super elderly patients over 80 years old after breast cancer surgery

Nakamura R, Matsuzaki H, Sakamoto M, Suda K, Hayama S and Sangai T. Chiba Cancer Center Hospital, Chiba, Japan; Funabashi Municipal Medical Center; Kameda Medical Center; Juntendou University Urayasu Hospital and Chiba University.

Body: (Purpose)
Considering the dramatic increase in average life expectancy throughout the world, the management of super-elderly patients over 80 years old (SEP) with breast cancer has become a global issue. However, there have been few clinical trials for SEP until now. The reasons for this were a small population, unpredictable prognosis, a large number of non-cancer-related deaths and a lower function of multiple organs in SEP. Surgical treatment or post-operative treatment based on evidence of clinical trials for SEP has also not been unclear. We hypothesized that the outcome of SEP with breast cancer compared with other ages were similarly depended on the breast cancer subtypes.

The aim of this study was to clarify the breast cancer related survival (BRS) rate and overall survival (OS) rate at 5 years for SEP according to breast cancer subtype.

(Methods)
We retrospectively analyzed 407 patients over 80 years old at initial operation between April, 1994 and April 2015 from 4 institutions of Chiba Youth Breast Oncology Research Group. Overall, 366 patients with stage I to Stage IIIc were included. 41 patients with Stage 0 or IV were excluded in this study.

We compared the clinical characteristics, OS and BRS rates among the breast cancer subtype: such as ER positive HER2 negative (ER group), ER negative HER2 negative (TN group), ER negative HER2 positive (HER2 group) and ER positive HER2 positive (ER/HER2 group).

Univariate and multivariate analyses were performed to identify the factors of Tumor size, Lymph node, Ly, ER, HER2 and characteristics, associated with the OS and BRS.

(Results)
The median age of the 366 patients was 83 years (range 80-96 years). The median follow-up duration was 32 months (range, 2-120).

During the follow-up period, 25 (9.4%) patients in the ER group, 19 (27.5%) in TN group, 4 (22.2%) in HER2 group and 2 (20.0%) patients in ER-HER2 group died.

The 5 year OS and BRS rates were 89.2%, 97.1% in ER group, 64.6%, 81.2% in TN group, 61.5%, 33.3% in HER2 group and 83.3%, 100% in ER-HER2 group, respectively.

Univariate and multivariate analyses revealed that ER was one prognostic factor to OS and BRS.

ER positive patients treatment with Aromatase inhibitor had significantly longer survival rates than treatment with Tamoxifen or no treatment (p=0.05).

There were no significant differences in OS or BRS of TN patients according to the use of chemotherapy (n=7) versus non treatment (n=61).

(Conclusions)
The prognosis and clinical course of super elderly patients with breast cancer depended on subtype. Adjuvant therapy for ER group was one prognostic factor to OS and BRS.
Title: Triple-negative breast cancer: A single-centre retrospective cohort study of 408 TNBC cases with a focus on elderly patients


Body: Background: TNBC represents a heterogeneous group of breast cancers that do not express ER-α, PgR and Her-2 receptors. Generally, these tumors are aggressive and more common in younger women. The aim of our study was to create a representative set of patients with TNBC, which could be analyzed and the data gathered to build basic epidemiological, molecular and clinical characteristics of Czech patients with TNBC. In particular, we focused on older patients (≥70 and ≥75 y.o.).

Methods: We retrospectively studied a consecutive cohort of 408 patients diagnosed and/or treated for TNBC at the Masaryk Memorial Cancer Institute between 2004 and 2010. Some clinical-pathologic/molecular correlations were performed to identify prognostically different groups of patients.

Results: The median age of patients was 56 years (25–88). A total of 9.3% of TNBC cases were diagnosed in patients under the age of 34, another 15.2% and 15.0% of cases were in the age group of 35 to 44 years and ≥70 years, respectively. In the group of patients aged ≥70 years (61), 59 % (36) were ≥75 y.o. Incidence of CK5/6+ and BRCA1 mutated tumors decreased with increasing age of patients, while the number of AR+ tumors increased (Chi-square test for trends: p=0.0245, p=0.0049 and p=0.0047, respectively). We confirmed the aggressive nature of this disease: in the follow-up period (median 77.2 months) we observed a relapse in 27.2 % (111) of patients: 71 % of deaths due to disease progression occurred within 2 years after diagnosis of the disease. Patients ≥70 and especially ≥75 years of age had, together with patients ≤30 y.o., the highest risk of death due to tumor progression. DFS and OS of patients ≥75 y.o. was significantly worse in comparison with other patients (OS: p=0.035, HR 0.515; DFS: p=0.0077, HR 0.475). Simultaneously, adjuvant chemotherapy and anthracyclines were much less frequently administered in this age group (p<0.0001), despite the fact that the distribution of clinical stages did not differ among the age groups. In the whole cohort, the most important negative prognostic factors in relation to disease specific OS were: higher clinical stage and pT (both p<0.0001), pN-positive status (p<0.0001), absence or early withholding of chemotherapy (p<0.0001) and minimal disease response to neoadjuvant treatment (TRG4-TRG5) (p=0.005). High levels of BCL2 expression predicted poor OS in basal-like TNBC patients treated with adjuvant anthracycline-based regimens (p=0.033, HR 3.04). Contrariwise, longer OS was associated with the presence of tumor infiltrating lymphocytes (p=0.0004, HR 2.40).

Conclusion: TNBC is an aggressive form of breast cancer, which may occur in patients of all ages, but more frequently in younger patients. Early detection and intensive treatment of these tumors gives a high chance of cure. Patients ≥70 and especially ≥75 years of age, together with patients ≤30 y.o., are at the highest risk of death due to tumor progression. While in younger patients it is likely due to the aggressiveness of disease, in older patients, in particular, because of an absence of adjuvant systemic therapy. The introduction of targeted therapies could potentially improve prognosis in both groups of patients (eg. PARP inhibitors, antiandrogens).

Supported by IGA MZ CR: NT14599-3.
Title: Breast cancer presentation in the over 70s

Murray J, Smith L and Lannigan A. Wishaw General Hospital, Wishaw, Lanarkshire, United Kingdom.

Body: Aims:
Life expectancy of the population is rising, and the risk of breast cancer increases with age. Older patients with breast cancer generally present later with more advanced disease for a variety of factors. We aimed to explore the presentation of the over 70s with breast cancer, along with their attitudes towards screening.

Methods:
A questionnaire was sent to patients aged over 70 at the time of their breast cancer surgery within the three hospitals across NHS Lanarkshire. This detailed reasons and timing of presentation, in addition to thoughts about screening.

Results:
Three hundred and fifty-two questionnaires were sent to women with a mean age of 76 years, 230 were returned (65%). Sixty-four percent routinely examined their breasts with more identifying a lump themselves (70%). Knowledge of signs and symptoms beyond a lump was explored, explicitly asking about nipple inversion, nipple discharge and skin changes with all three having similar degrees of awareness (40%, 35%, 25% respectively). Distressingly only 36% of our patients were aware of any of these signs, with only 9% being aware of all three. The majority of women sought medical attention early after identifying a problem, with 39% seeing someone within days and only 6% waiting over 6 months. Personal concern was the greatest prompt for women to see a doctor (68%) followed by family or friend concern (10%). With regard to screening, 83% routinely attended screening when they were invited. Ninety percent also said they would attend if the service was routinely offered to the over 70s. Although the majority (60%) were not aware that they could opt into the screening service once over 70.

Conclusions:
Despite the belief that most older women are felt to be less breast aware than younger most of our patients routinely examined themselves and identified the pathology, before promptly seeking medical advice (70% within weeks). This is the case despite most women having no knowledge of other signs and symptoms of breast cancer. Our cohort have also shown that they are keen to continue screening over the age of 70 if it was routinely offered.
Title: Breast cancer in young patients, twelve years of experience in a single institution


Body: Breast cancer is very rare in young women and has a more aggressive biological behavior and a worse prognosis than in older premenopausal women. This study was designed to determine and evaluate the features of breast cancer in young patients less than 35 years old at the Instituto Nacional de Enfermedades Neoplásicas, Lima - Perú.

Medical records of 115 patients less than 35 years old with breast cancer, whom were diagnosed and received a kind of treatment, even surgery, chemotherapy, radiotherapy or all of them at the Instituto Nacional de Enfermedades Neoplásicas Lima-Perú, between January 2000 and December 2012, were reviewed.

Triple negative breast cancer (38.3%) is the most common subtype cancer and its presentation is in a lower age compared with the others subtypes (27.1 years vs 28.5 years p: 0.047). The high histological grade was more frequent in triple negative and HER2 subtypes (80% both vs 30% and 48% for luminal A and luminal B p: 0.001). The 2 yr and 5 yr overall survival was 75.0% and 59.3% for luminal A, 71.4% and 55.1% luminal B, 67.7% and 54.9% triple negative, 58.3% and 41.7% HER2 (p: 0.434). The 2 yr progression free survival was 81.6%, 72.6%, 61.5% and 59.4% for each group respectively (p: 0.522).

As conclusion, the breast cancer in 35 yr old or less women is uncommon; the triple negative subtype is more common, also in relation with high histological grade and in lower age. The overall survival and the progression free survival are worse in patients with lower age.
Title: Spatial analyses of breast cancer in women 15-49 years-old in Rio Grande do Sul, southern Brazil


Body: Breast cancer (BC) rates under 50 years have been increasing over time in several countries. Although the exact impact of BC is not know in low and middle income countries, it's estimated that at least 20% of deaths caused by BC affect women under the age of 50 years. In Porto Alegre, the capital of Rio Grande do Sul (RS), Brazil's southernmost state, the incidence of BC in women aged 40-49 years is 165 cases per 100,000, while the mortality rate reaches 25 in 100,000 women. In order to understand BC profile in this age group in the entire state of RS, we established incidence, morbidity and mortality between 2002-2011 through crude and smoothed rates for each municipality. Furthermore, we perform the spatial analysis of these indicators. Incidence data were only available for the state capital, where the rate incidence was 19 and 160 cases per 100,000 women in the age groups of 15-39 and 40-49, respectively. Statewide mortality was 2 and 21 per 100,000 women in the age groups of 15-39 and 40-49, respectively. Hospitalar morbidity associated with BC was 0.6% and 2% in women 15-39 and 40-49 years, respectively. The distribution of morbidity and mortality showed regions with continuously high rates throughout this period with result above 3% and 20 deaths per 100,000 women. In the same areas we did not identify centers specialized in cancer treatment and care. Although there is specific epidemiological surveillance for cancer in Brazil, the coverage is insufficient, especially when it comes to incidence. Morbidity and mortality data showed regions continually affected by high rates suggesting that specific measures as well as specialized care for women in these regions are necessary. This result is particularly important since current guidelines of the Ministry of Health in Brazil recommend mammographic screening starting at the age of 50 years. The expansion of coverage and access to appropriate treatment is essential for the recognition of risk factors, adoption of effective strategies and reduction of cases and deaths in this group of women. Knowledge of the age distribution of BC cases and BC related deaths is important for the definition of health care policies, which will likely be different in different regions of the world.
**Title:** The smoking related risk of breast cancer and proportion of avoidable breast cancer cases due to passive and active smoking in middle-aged women in Norway in 2012: The Norwegian women and cancer study 1991-2012

Gram IT T, Little MA A, Lund E and Braaten T. UiT The Arctic University of Norway, Tromsø, Norway and University of Tennessee Health Science Center, Memphis, TN.

**Body:** Background: The burden of smoking on society may be underestimated as previous estimates of cancer due to smoking have generally not included breast cancer. We utilized the Norwegian Women and Cancer Study, a nationally representative prospective cohort study to examine the risk of breast cancer due to passive and active smoking. We also estimated the proportion of breast cancer attributable to passive and active smoking.

Material and methods: Our study included 130053 women, aged 34 to 70 years, who completed a baseline questionnaire between 1991 and 2007. We followed the women through linkages to the Cancer Registry of Norway and the Norwegian Central Population Register, to identify all cancer cases, emigrations, and deaths, respectively, using the unique national 11-digit personal identification number. Person-years were calculated from the start of follow-up to the date of any incident cancer diagnosis, emigration, death, or the end of follow-up December 31, 2012, whichever came first. Breast cancer cases were classified according to the original codes in the International Classification of Diseases, Seventh Revision including estrogen and progesterone hormone tumor receptor status. We used Cox proportional hazards models, adjusting for relevant confounders, to estimate multivariate adjusted hazard ratios (HRs) with 95% confidence intervals (CIs). Never smokers, excluding passive, served as the reference group. We estimated attributable fractions in smokers and in the population with 95% CIs.

Results: Ever compared with passive and never smokers were younger at breast cancer diagnosis, at first childbirth and at menopause; they were less likely to have higher education, more likely to have used hormonal contraceptives and postmenopausal hormone therapy and to consume alcohol. The alcohol drinkers were consuming more alcohol. During follow-up 4293 women developed invasive breast cancer confirmed by histology. Compared with never smokers, the multivariate adjusted breast cancer HR was for ever smokers 1.21 (1.08-1.34). Compared with parous never smokers, the HR estimate for breast cancer for ever smokers who had smoked five or more years before giving birth was 1.29 (1.14-1.46) after adjustment. A trend test for number of pack-years and breast cancer risk was significant (ptrend= 0.007). We found similar HR estimates when we stratified by menopausal and parous status at entry. The attributable fraction for breast cancer was 17.3 (7.4-25.4) for active smokers. The population attributable fraction of breast cancer for active smoking was 11.9 (5.3-18.1).

Conclusion: In smokers, one in six, and in the population almost one in eight breast cancer cases could have been avoided in the absence of smoking.
Title: Use of serum tumor markers and high cost imaging in women with metastatic breast cancer


Body: Background: Despite data on the sensitivity and specificity of serum tumor marker (STM) tests, there is no evidence to suggest that early changes in therapy related to rising tumor markers have an effect on survival. In fact, the limited data suggests no benefit to early change in therapy. The National Comprehensive Cancer Network recommends monitoring cancer burden in women with metastatic breast cancer (MBC) undergoing therapy; however, they do not provide specific recommendations regarding optimal frequency of STMs or of tumor imaging. We performed a population based analysis to evaluate serum tumor marker usage in patients with hormone sensitive MBC.

Methods: The Surveillance, Epidemiology, and End Results-Medicare database was used to identify female patients with hormone receptor positive MBC diagnosed between 2002 and 2011. For each patient, the dates of STMs (CEA and/or CA 15-3/CA 27.29) were recorded; if either or both CEA and CA 15-3/CA 27.29 were ordered on the same day they were counted as one test. We categorized regular STM use as the percentage of patients who had >4 tests in any year, amounting to tests less than 3 months apart; and very frequent STM use as the percentage of patients who had >12 tests in a year, amounting to tests less than 4 weeks apart. Multivariable analysis was performed to further examine patient characteristics associated with frequent STM use. Odds ratios were calculated comparing positron emission tomography (PET) scan use versus computed tomography (CT) use in women with frequent STM testing.

Results: We identified 3,251 eligible patients. Of these, 2,034 (62.6%) had ≥1 STM test in a given year. On average, patients who underwent STM testing were tested 4 times per year (SD 2.9) for an average of 3 years (SD 2.0). Over half of patients with STM testing had regular testing; 1,065 (52.2%) had STM less than every 3 months, 498 (24.5%) less than every 6 weeks, and 146 (7.2%) less than every 4 weeks apart in any given year. Regular STM evaluation was associated with younger age (65-74 vs 75-84) (OR 1.51, 95% CI 1.25-1.83), later year of diagnosis (OR 1.3, 95% CI 1.04-1.69), and high socioeconomic status compared to low socioeconomic status (OR 1.37, 95% CI 1.08-1.73). Similar factors were associated with very frequent STM use (>12 tests/year). Use of PET scan for tumor imaging compared to CT scan use was higher in women with regular STM evaluation (OR=1.97, 95% CI 1.65-2.35) and in women with very frequent STM evaluation (OR=3.77, 95% CI 2.51-5.66).

Conclusion: Regular use of STMs is common in women with hormone receptor positive MBC. Women who had very frequent STMs were almost 4 times more likely to have expensive tumor imaging. Given the rising costs of cancer care, and the increasing survival time in women with metastatic breast cancer, efforts should be made to determine the optimal timing and modality for evaluating response to treatment.
Title: Infectious disease hospitalization in breast cancer patients: Risk and impact on prognosis


Body: Background: Infection-related hospitalizations are a serious complication in breast cancer patients, resulting in treatment delay, prolonged hospitalization and future morbidity and mortality. Little, however, is known about the actual risk, clinical characteristics and outcomes of infection-related hospitalizations in this patient population.

Methods: We conducted a prospective population-based study including 7071 women diagnosed with primary invasive non-metastatic breast cancer between 2001 and 2008 in the Stockholm-Gotland region (Sweden), with complete follow-up until 2010. Standardized incidence ratios (SIRs) for infection-related hospitalizations (overall and by site) were estimated using background rates from the general female population, matched on age and calendar period. Associations with clinical characteristics and breast cancer outcomes (breast cancer death, distant metastasis and locoregional recurrence) were analysed using flexible parametric survival models.

Results: During a medium follow-up of 5.3 years, 657 hospital admissions with infections were observed and 1, 2 and 5-year cumulative risks were 3.4, 4.7 and 8.1% respectively. Rates of infection-related hospitalizations were increased compared to the general female population (SIR = 2.12; 95% CI = 1.96-2.29) and site-specific SIRs were most pronounced for sepsis (SIR = 3.65; 95% CI = 3.13-4.26) and skin infections (SIR = 2.93; 95% CI = 2.35-3.64). The overall risk of infections was highest during the first year (SIR = 5.06; 95% CI, 4.46-5.74), and recurrent disease contributed to the long-term risk observed. Older age at diagnosis, chemotherapy, axillary radiotherapy, comorbidities and markers of tumor aggressiveness (large tumors, estrogen receptor negative tumors and lymph node positive tumors) were independent predictors of infectious disease risk. Analyses evaluating the prognostic impact of infections revealed an independent effect on breast cancer death and distant metastasis, associations that were mainly driven by sepsis (HR = 4.53; 95% CI, 3.25-6.33 and 1.69; 95% CI, 1.04-2.79 respectively). No association was found with future risk of locoregional recurrence.

Conclusions: Physicians and patients should be aware of the risk of serious infections which persists beyond the initial treatment period. Infection-related hospitalizations are an independent marker of poor prognosis. Further research is needed to elucidate the role of sepsis in breast cancer progression.
Title: Prognostic impact of HER2 overexpression/amplification in women with pT1aN0M0 breast cancer with known screening status: Results from a multicenter population-based cancer registry study

Musolino A, Michiara M, Boggiani D, Sikokis A, Rimanti A, Pellegrino B, Silini EM M, Campanini N, Barbieri E, Sgargi P, Falcini F and Pinto C. Medical Oncology Unit and Cancer Registry of Parma Province, University Hospital of Parma, Parma, Italy; Section of Anatomy and Pathology, University Hospital of Parma, Parma, Italy; SSD Oncologia Medica Addarii, Policlinico S.Orsola-Malpighi, Bologna, Italy and Romagna Tumor Registry, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, IRCCS, Meldola, Italy.

Body: Introduction: Outcomes for women with pT1aN0M0 breast cancers (BC) may vary by biologic subtype. A higher proportion of HER2-positive BCs diagnosed in the interval between scheduled screening rounds has been proposed to account for the more aggressive behaviour of interval cancers (IC) compared with screen-detected (SD) tumors. No data are available on the prognostic role of HER2-positive status in a general population of pT1aN0M0 breast tumors with known screening status.

Methods: All incident pT1aN0M0 BCs (n=874), systematically collected by the Cancer Registries of Emilia Romagna Region (northern Italy) and diagnosed in women aged 50-69 from 2003 to 2009 were evaluated. Screening status was ascertained by reference to the Emilia Romagna Breast Cancer Screening Program (ERBSP) database. Patients unexposed to screening, with HER2 unknown primary tumor and/or who received adjuvant chemotherapy or trastuzumab were excluded from analysis.

Results: Twenty percent of patients had HER2-positive tumors. Fifty-three percent of the entire study population were SD cancers, while 18% were ICs. Tumors with high histologic grade, high proliferative rate, negative estrogen receptor status, or HER2-positive status were more likely to be diagnosed in the interval between screening. At a median follow-up of 84 months, the 5-year invasive disease-free survival (IDFS) rates were 89% and 95% in patients with HER2-positive and HER2-negative tumors, respectively (P = 0.025). Notably, HER2-positive ICs showed poorer IDFS than HER2-positive SD tumors (84% vs. 95%, respectively; P = 0.04). No difference in IDFS rates were observed between HER2-positive SD cancers and HER2-negative SD cancers. Multivariable analysis of candidate prognostic factors for IDFS will be reported.

Conclusions: In a general population of pT1aN0M0 early BCs with known screening status, IC detection may identify patients with HER2-positive pT1aN0M0 tumors in whom the rate of recurrence justifies consideration for systemic, anti-HER2, adjuvant therapy.
Increased risk of contralateral breast cancer after diagnosis of microscopically invasive breast cancer: SEER 1998-2012

Thomas A, Weigel RJ J, Leone JP P, Spanheimer PM M and Schroeder MC C. Carver College of Medicine, University of Iowa, Iowa City, IA and College of Pharmacy, University of Iowa, Iowa City, IA.

Body: Node negative microscopically invasive breast cancer (BC) is frequently associated with ductal carcinoma in situ (DCIS) and considered to have a similar prognosis. We evaluated women with T1micN0M0 (T1mic), DCIS and Stage I BC and report clinical characteristics, risk for subsequent contralateral breast cancer (CBC) and overall survival (OS).

Methods:
The study cohort included women diagnosed 1998-2012 and reported to Surveillance, Epidemiology, and End Result (SEER) data with DCIS, T1mic, or Stage I BC (not including T1mic). Subsequent CBCs were identified in patients with known laterality without contralateral mastectomy. Kaplan Meier models were used to estimate survival and time to CBC. Log-rank tests assessed differences in survival across groups.

Results:
During the study period, 9,785 women were diagnosed with T1mic. Clinical features and risk of CBC are shown in the Table 1. Women with DCIS and T1mic were younger than those with Stage I BC. T1mic was more likely to be hormone receptor (HR) negative. Women with T1mic underwent mastectomy significantly more often than women with DCIS or Stage 1 BC. T1mic occurred more frequently in non-white women. Women with T1mic were significantly more likely to develop subsequent CBC than women with Stage 1 BC with a trend for increased CBC compared to women presenting initially with DCIS. Of those who develop CBCs 5.9% (DCIS), 11.2% (T1mic), and 14.6% (Stage 1) developed within 1 year (YR) of diagnosis of the index cancer. At 10 YRS these numbers were 73.7%(DCIS), 82.7%(T1mic) and 83.2% (Stage 1) (DCIS vs T1mic, p<0.001 T1mic vs Stage 1, P=0.048). At 10 YRS OS for women with CBC after initial BC was 89.5%(DCIS), 86.6%(T1mic) and 84.3%(Stage1) (DCIS vs T1mic, p=0.077, T1mic vs Stage1 p=0.293), Table 2.

Conclusion:
Women with T1mic were at increased risk for subsequent CBC relative to women with Stage I BC. When subsequent CBC occurred it developed earlier in women with T1mic than those with DCIS. Time course for this second event and survival with CBC at 10 years matched more closely with women diagnosed with Stage 1 BC. These findings offer suggestions about the biology of T1mic and may have implications for counseling these women on risk reducing strategies.

Table 1 DCIS, T1mic, Stage I BC: Clinical Features and Contralateral Breast Cancer

<table>
<thead>
<tr>
<th></th>
<th>DCIS</th>
<th>T1mic</th>
<th>Stage 1 (excluding T1mic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>49,682</td>
<td>9,785</td>
<td>248,307</td>
</tr>
<tr>
<td>Median Age</td>
<td>58</td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>HR positive</td>
<td>85.0%</td>
<td>72.8%</td>
<td>86.5%</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-moderately differentiated</td>
<td>56.3%</td>
<td>61.1%</td>
<td>76.6%</td>
</tr>
<tr>
<td>Poorly-undifferentiated</td>
<td>43.7%</td>
<td>38.9%</td>
<td>23.4%</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>22.7%</td>
<td>36.7%</td>
<td>24.1%</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>79.7%</td>
<td>77.3%</td>
<td>84.2%</td>
</tr>
<tr>
<td>Black</td>
<td>10.8%</td>
<td>11.0%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Other</td>
<td>9.4%</td>
<td>11.6%</td>
<td>7.9%</td>
</tr>
</tbody>
</table>
Table 2 OS by Initial Stage and with CBC

<table>
<thead>
<tr>
<th></th>
<th>5 YR</th>
<th>10 YR</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OS</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>DCIS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>97.3%</td>
<td>88.4%</td>
<td></td>
</tr>
<tr>
<td>develop CBC</td>
<td>97.8%</td>
<td>89.5%</td>
<td>0.037</td>
</tr>
<tr>
<td>T1mic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>96.3%</td>
<td>88.9%</td>
<td></td>
</tr>
<tr>
<td>develop CBC</td>
<td>95.0%</td>
<td>86.6%</td>
<td>0.036</td>
</tr>
<tr>
<td>Stage1 (excluding T1mic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>95.9%</td>
<td>85.0%</td>
<td></td>
</tr>
<tr>
<td>develop CBC</td>
<td>86.9%</td>
<td>84.3%</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Comparing survival of the stage cohort (all) with women diagnosed with that stage who develop CBC

* Portion of full sample, **Of those who had a subsequent BC (ipsilateral or contralateral), ^ DCIS vs T1mic, ^^ stage I vs T1mic
Title: Patterns of multidisciplinary care in the management of nonmetastatic invasive breast cancer in the United States Medicare patient

Churilla TM M, Egleston BL L, Murphy CT T, Sigurdson ER R, Hayes SB B, Goldstein LJ J and Bleicher RJ J. Fox Chase Cancer Center, Philadelphia, PA.

Body: Background: Multidisciplinary care (MDC) in managing localized breast cancer is a resource-intensive treatment strategy that is anecdotally growing in prevalence, but is poorly characterized and thus cannot yet be defined as "standard care." We sought to determine the patterns of MDC care in the United States Medicare patient and assess if survival advantages exist for this paradigm.

Methods: Using the Survival, Epidemiology, and End Results (SEER)-Medicare linked dataset, we evaluated patients with non-metastatic breast cancer from 1992 to 2009. MDC was defined as a preoperative visit after breast cancer diagnosis with a surgeon, medical oncologist and radiation oncologist. Two separate analyses were performed: The first evaluated all MDC patients, and the second characterized the subset of patients who saw all three specialties on the same day. We tested for associations between MDC and clinical/demographic variables using logistic regression and evaluated outcomes using propensity score matching.

Results: A total of 87,984 invasive nonmetastatic breast cancer patients were included. MDC was utilized in 2.8% of patients, while 13% of these saw all three oncologic specialists on the same date. MDC use did not vary significantly according to AJCC stage. Patients receiving MDC overall were significantly more likely to be younger (continuous variable; OR [95% CI] = 0.99 [0.98-0.99]), black race (1.75 [1.50-2.05]), receive lumpectomy (1.15 [1.03-1.28], have fewer nodes examined (0.98 [0.98-0.99], and receive radiation therapy (1.37 [1.25-1.51]). MDC patients receiving care all on the same date were significantly more likely than non-MDC patients to have lobular histology (OR [95% CI] = 1.48 [1.06-2.06]), black race (3.09 [2.19-4.35], receive mastectomy (1.75 [1.34-2.30]) and receive radiation therapy (1.98 [1.52-2.60]). The use of MDC overall and on the same date increased over time (p < 0.001) and varied widely according to geographic region. There was a 20.8 odds increase in the use of same-date MDC in the Midwest compared to the South (p < 0.001). Patients in rural settings were less likely to receive MDC overall: OR [95% CI] = 0.57 [0.48-0.68] and on the same date (0.27 [0.16-0.48]). Survival data suggest improved outcomes for women undergoing MDC (Table 1). There were 117 breast cancer deaths in the MDC overall group but only 15 such events in the smaller MDC same-day subgroup (limiting its power).

Conclusions: The vast majority of Medicare patients having breast cancer did not undergo MDC during the period of study. MDC rates have increased over time, with widely varied MDC utilization across regions. Employing same-day MDC should be considered for patient convenience and may improve outcomes. While not yet widespread, efforts should be made to integrate MDC as standard care across the United States.

Table 1. Propensity score matched outcomes according to MDC.

<table>
<thead>
<tr>
<th></th>
<th>MDC Overall*</th>
<th>MDC on Same Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 2,491)</td>
<td>(n = 330)</td>
</tr>
<tr>
<td>HR [95% CI] p</td>
<td>HR [95% CI] p</td>
<td></td>
</tr>
<tr>
<td>Adjusted Overall Survival</td>
<td>0.94 0.80-1.09</td>
<td>0.400 0.36 0.18-0.72</td>
</tr>
<tr>
<td>Adjusted Breast Cancer Specific Mortality</td>
<td>0.75 0.58-0.96</td>
<td>0.024 0.42 0.15-1.18</td>
</tr>
</tbody>
</table>

* Includes MDC patients on same and different dates.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-07-26

Title: Marital status and overall mortality in breast cancer patients: Differences by socioeconomic status and race/ethnicity

Martinez ME Elena, Anderson K, Schwab R, Hurley S, Canchola AJ J, Keegan THM HM, Cheng I, Clarke C, Glaser S and Gomez SL L. Moores Cancer Center, University of California, San Diego, La Jolla, CA; University of California, San Diego, La Jolla, CA; Cancer Prevention Institute of California, Fremont, CA; School of Medicine, Stanford, CA and Stanford Cancer Institute, Palo Alto, CA.

Body: Background: Results of several studies show that married cancer patients have lower mortality than unmarried patients. However, data on differences by race/ethnicity are lacking. We assessed the risk of overall mortality associated with marital status among patients with breast cancer according to neighborhood socioeconomic status (SES) and race/ethnicity.

Methods: We used data from the population-based California Cancer Registry to include breast cancer cases diagnosed between 2000 and 2009 with follow-up through December 31, 2012 and identified 175,154 women with invasive breast cancer. We estimated hazard rate ratios (HR) and 95% confidence intervals (CI) for overall mortality by neighborhood SES and race/ethnicity.

Results: Among all cases, 42.6% were unmarried at the time of diagnosis. In adjusted models, the HR for all-cause mortality (95% CI) for unmarried compared to married women was 1.20 (1.17-1.22). There was no significant difference by SES. However, significant variation was observed by race/ethnicity. The HRs (95% CI) were 1.23 (1.20-1.26), 1.20 (1.11-1.30), 1.12 (1.04-1.21), and 1.05 (0.99-1.11) for NH Whites, NH Asians/Pacific Islanders (API), NH Blacks, and Hispanics, respectively. Among API subgroups, Chinese (HR=1.35; 95% CI, 1.12-1.62) and Filipinas (HR=1.25; 95% CI, 1.09-1.44) were the only groups with significantly higher risk of total mortality associated with unmarried status.

Conclusions: Our results show that not being married at the time of breast cancer diagnosis is associated with worse overall survival compared to being married. Significant variation in risk of mortality was shown by race/ethnicity but not by neighborhood SES. Targeted, culturally-appropriate interventions to support unmarried breast cancer patients through their cancer treatment and survivorship need to be developed to improve their survival.
Title: Systematic review of brain metastases in breast cancer in the United States, European Union, and Japan

Fenske DC, Price GL L, Nyhuis AW W and Hess LM M. Eli Lilly and Company, Indianapolis, IN; Eli Lilly and Company, Indianapolis, IN; Eli Lilly and Company, Indianapolis, IN and Eli Lilly and Company, Indianapolis, IN.

Body: Background: Prevalence of brain metastases (BRM) in breast cancer is increasing due to better detection methods and improved patient survival, presenting an unmet need. The exact prevalence is unknown due to a lack of national cancer registries that track BRM cases. Additionally, preceding research presents inconsistent results on survival outcomes, treatment regimens are not well-defined, and there is very limited data on the cost of treating breast cancer BRM. This study was designed to better understand the epidemiology, treatment patterns, cost, and overall survival (OS) of breast cancer patients with BRM in the US, EU, and Japan. Methods: A systematic review following PRISMA guidelines was conducted by searching PubMed, Ovid, and Embase from January 2003 to December 2013. Keywords, MeSH, and Emtree terms were used to define the search strategy. Studies of patients with breast cancer and BRM met eligibility criteria if peer-reviewed, observational, and published in English. Demographic, clinical, and outcomes data were extracted from the publications and entered into Excel. Descriptive statistics were generated with SAS version 9.2. Demographics were summarized, and treatment patterns and median OS were assessed by country. Results: The literature search identified 8,257 articles, of which 245 (n=105,871 breast cancer patients) met eligibility criteria. In these studies, 18,690 breast cancer patients from the US (58.9%), EU (29.5%), and Japan (11.6%) were reported with BRM. Median age of breast cancer patients with BRM was 51.5 years. Patient characteristics are summarized in Table 1. Median OS of breast cancer patients from the time of BRM diagnosis are presented in Table 2. The rate, by country, of radiation therapy among breast cancer patients with BRM ranged from 38.2% to 90.2%, systemic therapy ranged from 5.3% to 46.8%, and surgery was used in 0.8% to 16.2% of studies.

Table 1: Breast Cancer Patient Characteristics

<table>
<thead>
<tr>
<th>Demographics of BRM Population</th>
<th>(n=18,690)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age</strong>^1 (years)</td>
<td>51.5</td>
</tr>
<tr>
<td><strong>Gender</strong>^2 (%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18,399 (100.0)</td>
</tr>
<tr>
<td>Male</td>
<td>1 (0.0)^3</td>
</tr>
<tr>
<td><strong>Race</strong>^2 (%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>5 (0.4)</td>
</tr>
<tr>
<td>Black</td>
<td>98 (7.1)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8 (0.6)</td>
</tr>
<tr>
<td>White</td>
<td>1,191 (86.4)</td>
</tr>
<tr>
<td>Other</td>
<td>76 (5.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study Enrollment by Country and Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Studies Reported</strong></td>
</tr>
<tr>
<td><strong>Total Breast Cancer Population</strong></td>
</tr>
<tr>
<td><strong>Total BRM Population (%)</strong></td>
</tr>
<tr>
<td>EU</td>
</tr>
<tr>
<td>France</td>
</tr>
<tr>
<td>Germany</td>
</tr>
<tr>
<td>Italy</td>
</tr>
</tbody>
</table>
Table 2: Reported Median OS\textsuperscript{1} for Breast Cancer BRM by Treatment Type

<table>
<thead>
<tr>
<th>Country</th>
<th>Systemic Therapy</th>
<th>Radiation Therapy</th>
<th>Surgery</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>6.90</td>
<td>6.97</td>
<td>5.95</td>
<td>8.17</td>
</tr>
<tr>
<td>Germany</td>
<td>NR\textsuperscript{2}</td>
<td>15.00</td>
<td>46.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Italy</td>
<td>20.45</td>
<td>23.60</td>
<td>15.57</td>
<td>13.50</td>
</tr>
<tr>
<td>Spain</td>
<td>7.75</td>
<td>5.50</td>
<td>5.50</td>
<td>7.75</td>
</tr>
<tr>
<td>UK</td>
<td>5.40</td>
<td>4.70</td>
<td>13.50</td>
<td>9.50</td>
</tr>
<tr>
<td>Japan</td>
<td>11.40</td>
<td>8.80</td>
<td>10.10</td>
<td>8.80</td>
</tr>
<tr>
<td>US</td>
<td>10.60</td>
<td>11.50</td>
<td>11.20</td>
<td>11.45</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Reported in months as the median of medians. \textsuperscript{2}NR=not reported

**Conclusions:** Reported median OS and treatment patterns were highly variable across countries. Exposure to risk factors associated with BRM may help explain some of the geographic variability in survival. The lack of published cost data underscores the need to quantify the economic burden of BRM on patients and society.
Abstract Withdrawn
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-08-02

Title: CYP2D6 gene variants and effectiveness of adjuvant tamoxifen in breast cancer: A population-based case-control study

Weinmann S, Richert–Boe K, Goddard K, Chen C, Punj S, Schwarzkopf D, Kalter M and Richards CS.  Center for Health Research Northwest, Kaiser Permanente, Portland, OR and  Oregon Health Sciences University, Portland, OR.

Body: Tamoxifen, a cornerstone of adjuvant therapy for hormone-receptor-positive breast cancer, is metabolized to the active metabolite endoxifen through enzymatic activity of cytochrome P450 2D6. CYP2D6 has numerous alleles that affect metabolizing phenotype. Among women who take tamoxifen, those homozygous for inactive alleles (poor metabolizers) have lower levels of serum endoxifen than those with two functional alleles (extensive metabolizers). Several studies have reported increased risk of breast cancer recurrence or death in women homozygous for CYP2D6 inactive alleles, but others have found no association between CYP2D6 function and outcome. We explored this question in the large member population of the Kaiser Permanente Northwest (KPNW) integrated health plan. We conducted a population-based case-control study to evaluate the hypothesis that, after adjuvant tamoxifen treatment for breast cancer, women with CYP2D6 genotypes associated with poor metabolism of tamoxifen have an elevated risk of breast cancer recurrence compared to women with CYP2D6 genotypes associated with extensive metabolism of tamoxifen. We further hypothesized that women with CYP2D6 genotypes associated with intermediate metabolism of tamoxifen are at intermediate risk of recurrence. Study subjects were women who were diagnosed from 1980 to 2011 with hormone-receptor positive breast cancer, who received at least 180 days of adjuvant tamoxifen treatment, and for whom stored formalin-fixed paraffin-embedded (FFPE) normal tissue was available for laboratory analysis. Cases (358) were women with breast cancer recurrence recorded in the KPNW Tumor Registry and validated by medical record review. Randomly selected controls (833), without recurrent breast cancer, were matched to cases on tumor stage, diagnosis year, diagnosis age, race/ethnicity, and patterns of health plan membership. We collected data from medical records and from pharmacy, laboratory, tumor registry, and membership health plan databases. The Oregon Health & Science University Molecular Genetics Laboratory extracted genomic DNA from stored FFPE tissue blocks and performed allelic discrimination assays and pyrosequencing to accurately determine CYP2D6 variant status for the alleles, *3, *4, *5, *10, *17, and *41. All assays have been completed and study subjects have been categorized according to CYP2D6 metabolizer phenotype (poor, intermediate, extensive) and activity score (0-2). Based on the ethnicities in our study population, the CYP2D6 allele frequencies are in Hardy-Weinberg equilibrium, and the frequencies of the predicted metabolizer phenotypes also fall within the expected range. Using multivariable logistic regression analysis, we will assess CYP2D6 functional status and activity score in relation to breast cancer recurrence, taking into account factors that may alter the association, including tamoxifen dose and duration of use, as well as concomitant medications that alter the activity of the CYP2D6 enzyme. Results will be available by 12/1/2015.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-08-03

Title: Association analysis of single-nucleotide polymorphisms in FANCD2–DNA damage repair pathway genes with breast cancer risk

Chen F, Tang L and Huang J. Xiangya Hospital of Central South University, Changsha, Hunan, China.

Body: Purpose: The aim of the study was to estimate breast cancer risk conferred by individual single-nucleotide polymorphisms (SNPs) of breast cancer susceptibility genes in the monoubiquitinated FANCD2–DNA damage repair pathway.

Methods: We selected 48 tagging SNPs (tSNPs) from eight breast cancer susceptibility genes (TP53, PTEN, NBS1, BRIP1, PALB2, ATM, CHEK2 and RAD50) involved in the monoubiquitinated FANCD2–DNA damage repair pathway. The 48 tSNPs were genotyped by SNPscan in 734 women with breast cancer (534 sporadic cases and 200 early-onset and familial cases) and 672 sex- and age-matched healthy controls from Hunan and Sichuan Province of China. The odds ratio were calculated by logistic regression analysis under co-dominant model, dominant model and recessive model respectively.

Results: Forty-five tSNPs were successfully genotyped by SNPscan, and the call rates for each tSNP were above 98.9%. We found that 13 tSNPs of five genes (PALB2, TP53, NBS1, PTEN, and BRIP1) were significantly associated with breast cancer risk. A total of five tSNPs (rs2299941 of PTEN, rs2735385, rs6999227, rs1805812, and rs1061302 of NBS1) were tightly associated with breast cancer risk in sporadic cases, and five other tSNPs (rs1042522 of TP53, rs2735343 of PTEN, rs7220719, rs16945628, and rs11871753 of BRIP1) were tightly associated with breast cancer risk in early-onset and familial cases. We have not found significant tSNPs in the other three genes (ATM, RAD50, and CHEK2). Three tSNPs of TP53 (rs12951053, rs1042522 and rs8064946), three tSNPs of BRIP1 (rs16945628, rs7220719 and rs11871753) and rs2735343 of PTEN can significantly increase the risk of breast cancer, and most of these were under the analysis of early-onset and familial cases. Four tSNPs of NBS1 (rs2735385, rs6999227, rs1805812 and rs1061302), rs2299941 of PTEN and rs513313 of PALB2 can significantly decrease the risk of breast cancer, and most of these were under the analysis of sporadic cases.

Conclusions: Some of the tSNPs of five breast cancer susceptibility genes (PALB2, TP53, NBS1, PTEN, and BRIP1) involved in the monoubiquitinated FANCD2–DNA damage repair pathway were significantly associated with breast cancer risk in women from Hunan and Sichuan Province of China. Some locuses can increase breast cancer risk, and others can decrease breast cancer risk. But the majority of the tSNPs are located in the intron domain and their functions are unknown, so larger studies are needed to research the functions of these genes further.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-08-04

Title: Sources of polycyclic aromatic hydrocarbons associated with gene-specific promoter methylation in women with breast cancer

White AJ J, Chen J, McCullough LE E, Xu X, Cho YH, Conway K, Beyea J, Stellman SD D, Steck SE E, Mordukhovich I, Eng SM M, Terry MB, Engel LS S, Hatch M, Neugut AI I, Hibshoosh H, Santella RM M and Gammon MD D. University of North Carolina at Chapel Hill; Ichan School of Medicine at Mt. Sinai; Roche Product Development in Asia-Pacific; University of Montana; Consulting in the Public Interest; Columbia University; University of South Carolina and National Cancer Institute.

Body: Tobacco smoke, diet, and indoor and outdoor air pollution, all major sources of polycyclic aromatic hydrocarbons (PAHs), have been associated with breast cancer incidence. Aberrant methylation may be an early event in carcinogenesis, but whether PAHs influence the epigenome is unclear. Few studies have evaluated whether PAHs are associated with methylation, particularly in breast tumors where methylation changes are particularly relevant. In a population-based case-control study, we measured promoter methylation of 13 breast cancer-related genes in breast tumor tissue (n=765-851 cases) and global methylation in peripheral blood (1,055 cases/1,101 controls). PAH sources (current active smoking, residential environmental tobacco smoke (ETS), vehicular traffic, synthetic log burning, and grilled/smoked meat intake) were evaluated separately. Logistic regression was used to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs). When comparing methylated versus unmethylated genes, synthetic log use was associated with increased ORs for CDH1 (OR=2.28, 95%CI=1.07-4.83), HIN1 (OR=2.11, 95%CI=1.32-3.38) and RARβ methylation (OR=1.82, 95%CI=1.18-2.83) and decreased ORs for BRCA1 methylation (OR=0.44, 95%CI=0.30-0.65). Residential ETS was associated with decreased ORs for ESR1 (OR=0.74, 95%CI=0.56-0.99) and CCND2 methylation (OR=0.65, 95%CI=0.44-0.96). Current smoking and vehicular traffic were associated with decreased ORs for DAPK (OR=0.53, 95%CI=0.28-0.99) and increased ORs for TWIST1 methylation (OR=2.79, 95%CI=1.24-6.30), respectively. In controls, synthetic log use was inversely associated with LINE-1 methylation (OR=0.60, 95%CI=0.42-0.87). PAH sources were associated with hypo- and hypermethylation at multiple promoter regions in breast tumors and LINE-1 hypomethylation in blood of controls. Methylation may be a potential biologic mechanism for the association between PAHs and breast cancer incidence.
Expression levels of sialyl transferases and fucosyl transferases in breast cancer and their prognostic significance


Aberrant expression of fucose or sialic acid containing glycans is prevalent in various cancers, including breast cancer. The addition of fucose and sialic acid to glycans is mediated by fucosyl transferases (FUTs) and sialyl transferases (STs), respectively. To explore the roles of these FUTs and STs in breast cancer, we collected 123 paired breast cancer specimens (tumor tissue and non-tumor tissue) and determined the RNA expression levels of 13 FUTs (FUT1-13) and 13 STs (ST3Gal1-6, ST6Gal1, ST6GalNAc1-4, ST6GalNAc6, ST8Sia1 and ST8Sia4) by q-PCR. The expression of 10 FUTs and 10 STs is significantly higher in tumor tissue than in non-tumor tissue by 1.2-4.09 folds. Notably, expression of FUT1 and ST3Gal1 appeared to be highest in triple negative subtype. Further multivariate Cox regression analysis showed a significant correlation between higher hazard ratios (HRs) for five-year relapse-free survival (RFS) and higher ST3Gal1 expression (N = 16, HR = 2.54, p = 0.02) or lower ST3Gal3 expression (N = 88, HR = 3.44, p < 0.001). As to the joint effects of high expression of ST3Gal1 and low expression of ST3Gal3 (N = 10) compared with low expression of ST3Gal1 and high expression of ST3Gal3 (N = 82), the HR was 5.5 (95% CI = 2.16-14.02, p = 0.0004) for RFS. The two gene additive model displayed a statistically significant HR of 2.33 for RFS (95% CI = 1.48-3.69, p = 0.0003). These results suggest that FUTs and STs play important roles in tumor progression.
Body: Background: Due to better molecular classification and new treatment options, epidemiology and prognosis of mBC is rapidly changing. Clinical data extracted from randomized studies are only relevant to specific subpopulations and retrospective studies are prone to selection bias. SToRM is a prospective clinical trial that aims to create a cohort of 1500 mBC patients, with the ultimate goal of identifying germ line polymorphisms associated with prognosis of breast cancer (BC) and response to treatment in the metastatic phase.

Material and methods: Any newly (within 1 year) diagnosed mBC patients were eligible. Whole blood samples were drawn and germline DNA extracted for genetic analysis. Extensive epidemiologic data, disease history from primary diagnosis to metastatic spread, pathological characteristics and ER, PR and HER2 status were collected. Patients are prospectively followed until death. Genotyping using the HumanCoreExome chipset from Illumina is currently underway and will be completed in early summer 2015.

Results: 1502 patients were included from March 2012 to May 2014 from 71 French institutions. Median age at metastatic relapse was 60 years (range 26-93). Median time from primary diagnosis to metastatic relapse was 30 months (range 0-473) with 24% of patients already metastatic at initial diagnosis. 78% of patients were ER+, 18% were HER2+ and only 16% were triple negative. Molecular subtype classification derived from pathological data following St Gallen consensus recommendations is presented below:

<table>
<thead>
<tr>
<th>Subtype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A like</td>
<td>261 (22.2%)</td>
</tr>
<tr>
<td>Luminal B like HER2 negative</td>
<td>476 (40.5%)</td>
</tr>
<tr>
<td>Luminal B like HER2 positive</td>
<td>134 (11.4%)</td>
</tr>
<tr>
<td>HER2 positive non Luminal (ER-)</td>
<td>111 (9.5%)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>193 (16.4%)</td>
</tr>
<tr>
<td>Missing data</td>
<td>327</td>
</tr>
</tbody>
</table>

64% of the patients had received previous adjuvant treatment, among which 81% received adjuvant chemotherapy and 9% trastuzumab.

At metastatic relapse, loco-regional progression, liver, lung and bone metastasis were documented in 301 (20%), 494 (33%), 410 (27%) and 1017 (68%) patients respectively. 313 patients (21%) had bone only metastatic disease. First line treatment included:
chemotherapy (71%), endocrine therapy (50%) and anti-HER2 treatments (17%). Survival data will be presented at the meeting. **Conclusion:** Despite a theoretically better prognosis and widespread use of adjuvant hormonal treatment, ER+/HER2- breast cancer still account for more than 60% of mBC. The proportion of patients with HER2+ disease (18%) and triple negative disease (16%) is consistent with percentages observed in early BC populations. In comparison with a cohort of "cured", localized cancer, such as the SIGNAL/PHARE study, GWAS analysis will allow for the identification of genetic polymorphisms correlated with treatment resistance. Fundamentally, such variants will provide insight into the molecular mechanisms responsible for host-genetic influence on BC progression. From a clinical perspective, genetic variants that predispose to metastatic disease can serve as stratification variables in future clinical trials, particularly as the development of new treatment options for resistant BC is needed.
Body: Background: Evaluation of women with BC for germline mutations associated with hereditary breast and ovarian cancer (HBOC) has become increasingly common due to its impact on management. Guidelines for genetic evaluation indicate testing for cases with early onset, triple negative disease or family cancer history. However, the majority of breast cancer occurs in patients without these high risk characteristics. The prevalence of mutations associated with HBOC has not been well characterized in this population.

Methods: We performed a cross sectional study using DNA from blood samples from consecutive new invasive BC patients seen at the Dana-Farber Cancer Institute (01/01/2010 to 07/31/2012) who consented to research. Subjects were otherwise unselected. Mutations in 25 cancer genes were identified using a next generation sequencing based panel. Germline sequence variations and large rearrangements were classified for pathogenicity.

Results: 456 samples from eligible subjects were included. The mean age of BC diagnosis was 50 years. Mutations were found in 51 women, 49 of which were associated with breast cancer (10.8%, 95% CI 8.1-14.0). BRCA1/2 mutations were found in 6.6% [95% CI 4.5-9.2%] while mutations in other BC-associated genes were found in 4.4% [95% CI 2.7-6.7%], particularly CHEK2 (2.2%, 95% CI 1.1, 4.0). Of the 49 women with BC-related mutations, 21 (43%) had BC diagnosed after age 45. In univariate analyses, age at diagnosis, Ashkenazi Jewish ancestry, triple negative histology and family BC/ovarian cancer (OC) history were associated with BRCA1/2 mutations, but no factors were significantly associated with mutations in other genes. Among 261 women with no FDR/SDR with BC/OC, 26 (10.0%) had a mutation. Nineteen mutations (10 BRCA1/2) were found in the 256 women (7.4%) who had not had previous genetic testing.

Conclusions: In a single academic institution, 11% of new breast cancer patients had a germline mutation in a breast cancer predisposition gene: 6.6% were in BRCA1/2. The elevated prevalence compared to population-based series may reflect the practice composition of academic centers, which often attract women younger at BC diagnosis. In an academic practice with an active cancer genetics program, 10 women with BRCA1/2 and 9 with other mutations had not had genetic testing. Expanded testing identifies additional predisposing mutations, the utility of which are being defined for the care of breast cancer patients and their families.
Title: Heterozygous germline mutations in RAD50 among Korean patients with high-risk breast cancer negative for BRCA1/2 mutation

Kim H, Cho D-Y, Choi DH, Jung GH, Shin I, Park W, Huh SJ, Nam SJ, Lee JE, Gil WH and Kim SW. Hallym University Dongtan Sacred Heart Hospital, Hwaseong, Republic of Korea; LabGenomics Clinical Research Institute, LabGenomics, Seongnam, Republic of Korea; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea and Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

Body: Background: The MRE11-RAD50-nibrin (MRN) complex participates in pathways of double-strand break induced DNA repair and cell cycle checkpoint control. RAD50 interacts with the MRE11 and NBS proteins, is involved in the maintenance of genomic integrity. The association of RAD50 mutation and breast cancer susceptibility has been reported in European patients. However, the impact of RAD50 mutation on a breast cancer predisposition among Koreans remains uncertain. In the current analysis, we evaluated the incidence of RAD50 mutations among Korean patients with non-BRCA1/2 high-risk breast cancer.

Materials and Methods: A total of 247 Korean patients with high-risk breast cancer who tested negative for BRCA1/2 mutation were enrolled. The criteria for high-risk breast cancer were as follows: having a family history of breast or ovarian cancer in any relative; diagnosed at age 40 years or younger; bilateral breast cancer; and male breast cancer. All participants were screened for BRCA1/2 mutations using fluorescent-conformation sensitive capillary electrophoresis (F-CSCE) and traditional sequencing. The entire RAD50 gene of each patient was sequenced using F-CSCE. In silico analyses of the RAD50 variants was performed using PolyPhen-2 and SIFT.

Results: There were two novel deleterious mutations in RAD50 (p.Q426X, p.E1271del). These mutations were found in two patients, including one with p.Q426X and the other with p.E1271del. Besides, five sequence variants in RAD50 were identified: four exonic variants (p.I118T, p.R486C, p.L1264F, and p.R1279H) and one intronic variant (c.1246-11T>C). Among the four missense variants, p.R486C and p.L1264F were variants predicted to be deleterious by in silico analyses.

Conclusions: We found protein-truncating mutations in RAD50 gene in a small proportion of Korean patients with high-risk breast cancer. The contribution of the mutation to the development of breast cancer should be clarified in further researches.
Increased prevalence of luminal B subtype in Colombian women with breast cancer

Serrano-Gomez SJ J, Sanabria MC C, Hernández-Suarez GA A, Garcia O, Silva C, Romero A, Mejía JC C, Fejerman L, Antonia T, Miele L and Zabaleta J.  Instituto Nacional de Cancerologia, Bogota, Cundinamarca, Colombia;  Pontificia Universidad Javeriana, Bogota, Cundinamarca, Colombia;  Stanley S. Scott Cancer Center, New Orleans, LA;  UCSF, San Francisco, CA;  Moffitt Cancer Center, Tampa, FL; Department of Genetics, New Orleans, LA and  Department of Pediatrics, New Orleans, LA.

Body: Background: Breast cancer is the most frequent malignancy in women worldwide. Distinct intrinsic subtypes of breast cancer have different prognoses, and their relative prevalence varies significantly among ethnic groups. Hispanic/Latino (H/L) populations are a genetically admixed and heterogeneous group, with variable levels of European, Native American and African ancestries. Breast cancer in H/L patients is understudied from a molecular standpoint, and most studies reported so far include limited numbers of H/L patients and assign ethnicity based on self-reported data rather than ancestry. This is the first study to explore the prevalence of breast cancer intrinsic subtypes in Colombia and their association with clinicopathological data and genetic ancestry.

Methods: Immunohistochemistry surrogates from the 2013 St. Gallen International Expert Consensus were applied to classify breast cancer into intrinsic subtypes in 301 patients diagnosed between 2008 and 2012 at the Colombian National Cancer Institute. We analyzed the distribution of subtypes by age, histologic type, node status, margins at surgery, AJCC stage, tumor size, Bloom-Richardson grade, histologic features, administration and response to neoadjuvant therapy, adjuvant therapy and recurrence. Genetic ancestry was estimated from a panel of 80 ancestry-informative markers (AIM).

Results: Luminal B breast cancer subtype was the most prevalent in our population (47.5%), followed by luminal A (23.9%), non-basal triple negative (9.3%), basal-like (8.6%), HER2-enriched (8%), and unknown (2.6%). The average of age at diagnosis was 55 and the average tumor size was 4.08 cm. We found statistical significant differences in age at diagnosis, Bloom-Richardson grade, histologic features, adjuvant chemotherapy and recurrence according to intrinsic subtype. Consistent with North American and European observations, basal-like and non-basal triple negative were poorly differentiated tumors and more likely to be diagnosed at younger ages compared to luminal tumors. Patients diagnosed with HER2-enriched, basal and non-basal triple negative breast cancer had the highest African ancestry.

Conclusions: Luminal B tumors, a high risk subset of ER-positive breast cancer, occur with remarkably higher prevalence in Colombian women with breast cancer compared to North American and European populations. Triple-negative subtypes and HER2-enriched tumors appeared to be more frequent among patients with African ancestry, as observed in North American cohorts. Future studies analyzing the molecular profiles of breast cancer in Colombian women will help us understand the molecular basis of this subtype distribution and compare the molecular characteristics of the different intrinsic subtypes in Colombian Hispanic/Latina patients.
Title: Clinicopathological characteristics and clinical outcome in Egyptian female breast cancer patients with and without BRCA1/2 mutations

AbdelHamid SG G, El-Mesallamy HO O, AbdelAziz HM M and Zekri A-RN N. Faculty of Pharmacy, Ain Shams University, Cairo, Egypt; Faculty of Medicine, Ain Shams University, Cairo, Egypt and National Cancer Institute, Cairo University, Giza, Egypt.

Body: Background: Germline mutations in BRCA1 and BRCA2 genes confer high risk of developing breast cancer. We sought to examine for the first time in the Egyptian population, which has witnessed the world's oldest recorded breast cancer case, whether the clinicopathological characteristics and the clinical outcome differ in patients with and without BRCA mutations.

Patients and Methods: A series of 103 Egyptian female patients diagnosed with breast cancer before 2008 were recruited from Breast Cancer Unit, Clinical Oncology Department, Ain Shams University, Egypt. The enrolled patients, unselected for age of onset or family history, were tested for BRCA1/2 mutations using HRM analysis and direct sequencing. The clinical and pathological features of the patients were retrospectively assessed and comparisons were made between BRCA mutation carriers and non-carriers, respectively, using Chi-square. Disease free survival (DFS) was estimated by Kaplan–Meier method and compared in the two groups with log-rank. Survival Cox proportional hazards models were fit to determine the independent association of mutation status with outcome.

Results: The overall rate of BRCA1/2 mutations was 44% (46/103). Novel deleterious mutations were detected and submitted to NCBI Clinvar database. Deleterious mutations were identified in 29 cases and unclassified variants in 32 cases, 15 of which had a co-occurring deleterious mutation. Patients with BRCA mutations tended to have early onset breast cancer compared to non-carriers (P=0.002), more often premenopausal (P=0.006), with a familial history of breast cancer as well as other cancers (P=0.005). BRCA-related breast cancers were more likely to have T3-T4 stage than wild type (41% versus 28%, P=0.02), positive lymph node involvement (78 versus 53%, P=0.007) and develop bilateral breast cancers (24% versus 9%, P =0.007). Grade and histology were not associated with mutation status. The incidence of ER negative and PR negative tumors was higher in BRCA carriers, but not statistically significant (P=0.17 and 0.15, respectively). No difference in HER-2/neu status was observed (P=0.25). Multivariate logistic regression model showed that early age at onset, positive lymph node involvement, family history of any cancer (P=0.047, 0.05 and 0.05, respectively) are independent predictive factors for occurrence of BRCA1/2 mutations. Carriers of BRCA2 deleterious mutations were more likely to report positive family history of cancer other than breast compared to non-carriers (P=0.001). The median follow-up time for the cohort was 5.53 years (ranged from 4.7 to 20.4 years). Patients with BRCA mutations had poorer 5-year DFS compared to non-carriers (47.7% versus 67.4%, P=0.041); but Cox regression analysis failed to demonstrate a significant independent influence of BRCA mutation status on DFS.

Conclusion: This is the first study in the Middle East to show that BRCA-related breast cancers in the Egyptian population have distinctive clinical and tumor features as well as outcome. Early onset breast cancer, family history of cancer and positive lymph node involvement are predictors for BRCA mutation in this Egyptian cohort. This data has important health implications for guiding cancer control policies.
African American women have lower pathologic complete response rates to neoadjuvant chemotherapy compared to white women for triple negative and HER 2 positive breast cancer

Killelea BK, Chagpar AB, Horowitz NR, Pusztai L, Wang S, Mougalian S and Lannin DR. Yale University School of Medicine, New Haven, CT and Yale University School of Public Health, New Haven, CT.

**Body:**

**Introduction**

Although racial disparities in breast cancer treatment have been well documented, data regarding differences in response to neoadjuvant chemotherapy are few. In 2010 the National Cancer Database (NCDB) included a new variable, documenting pathologic complete response (pCR) after neoadjuvant chemotherapy. The purpose of this study was to explore racial differences in the rates of pCR by molecular subtype.

**Methods**

The NCDB was queried to identify women diagnosed with invasive, stage 1-3 breast cancer in 2010-2011 who received neoadjuvant chemotherapy. Univariate and multivariate logistic regression was performed to determine factors associated with likelihood of pCR.

**Results**

Out of 278,815 patients with known race and ethnicity, 27,300 (10%) received neoadjuvant chemotherapy. Of 17,970 where the outcome was known, 5,944 (33%) had a pCR. As seen in the table, there were no differences in response rate for ER/PR+ tumors, but compared to whites, non-hispanic black women had a lower rate of pCR for ER/PR- Her2+ and triple negative tumors. This difference persisted when adjusted for patient age, clinical T stage, clinical N stage, histology, grade, comorbidity index, facility type, geographic region, insurance status, and census-derived median income and education for the patient’s zip code (OR 0.84, 95% CI: 0.77-0.93).

**pCR rate by race and molecular subtype**

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Race</th>
<th>pCR number (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER/PR+, Her 2-</td>
<td>Non-Hispanic White</td>
<td>943/5129 (18.4%)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Black</td>
<td>204/1042 (19.6%)</td>
<td>0.367</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Asian/Pacific Islander</td>
<td>59/291 (20.3%)</td>
<td>0.420</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>121/609 (19.9%)</td>
<td>0.373</td>
</tr>
<tr>
<td>ER/PR+, Her 2+</td>
<td>Non-Hispanic White</td>
<td>852/2107 (40.4%)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Black</td>
<td>143/380 (37.6%)</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Asian/Pacific Islander</td>
<td>42/124 (33.9%)</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>92/224 (41.1%)</td>
<td>0.854</td>
</tr>
<tr>
<td>ER/PR-, Her 2+</td>
<td>Non-Hispanic White</td>
<td>698/1295 (53.9%)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Black</td>
<td>116/272 (42.6%)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Asian/Pacific Islander</td>
<td>66/112 (58.9%)</td>
<td>0.306</td>
</tr>
<tr>
<td></td>
<td>Hispanic</td>
<td>88/174 (50.6%)</td>
<td>0.409</td>
</tr>
<tr>
<td>ER/PR-, Her 2-</td>
<td>Non-Hispanic White</td>
<td>1318/3079 (42.8%)</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Black</td>
<td>416/1138 (36.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Asian/Pacific Islander</td>
<td>64/165 (38.8%)</td>
<td>0.310</td>
</tr>
</tbody>
</table>
Conclusions
Non-hispanic black women have a lower likelihood of pCR after neoadjuvant chemotherapy compared to white women for triple negative and Her 2 positive breast cancer. It is unknown whether this is due to biologic differences in chemosensitivity or whether it represents treatment or socioeconomic differences that cannot be adjusted for in the current analysis.
Higher incidence of second cancers in African American (AA) patients compared to Caucasian patients with a primary breast cancer

Diab N, Clark G, Hamlington B, Brzeskiewicz L, Langer L and Diab S. Vanderbilt University, Nashville, TN; Arry BioPharma, Boulder, CO; Rocky Mountain Cancer Centers, Aurora and Denver, CO and Compass Oncology, Portland, OR.

**Body:** Background: AA women with breast cancer have lower survival rates compared to Caucasian women. Since this lower survival rate may be related to genetic mutations, and environmental/socioeconomically factors, we hypothesize that the same factors may lead to a higher risk of secondary cancers after an initial diagnosis of breast cancer.

**Method:** Analysis of the Surveillance, Epidemiology, and End Results (SEER) Program data using Multiple Primary - Standardized Incidence Ratio parameters. The incidence of second cancer diagnoses in AA and Caucasian women previously diagnosed with breast was compared to the incidence of cancer in the general population matched by age, race, and year of diagnosis. Results are reported as the observed risk divided by the expected risk (O/E).

**Results:** For the 43,688 AA pts, the overall O/E and excess risks were 1.48 and 51.2 compared to 1.11 and 14.7 for the 428,103 Caucasian patients. The mean ages of diagnoses of initial breast cancer diagnosis and second cancer were 57.2 and 65 years for AA patients compared to 61.8 and 70.2 years for Caucasian patients. The following is a summary of statically significant (p <0.05) selected O/E by the site of second cancer:

<table>
<thead>
<tr>
<th>Site</th>
<th>AA</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Cavity and Pharynx</td>
<td>1.65</td>
<td>1.41</td>
</tr>
<tr>
<td>Digestive System</td>
<td>1.18</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory System</td>
<td>1.27</td>
<td>1.13</td>
</tr>
<tr>
<td>Skin excluding Basal and Squamous</td>
<td>1.93</td>
<td>1.33</td>
</tr>
<tr>
<td>Breast</td>
<td>1.67</td>
<td>1.18</td>
</tr>
<tr>
<td>Female Genital System</td>
<td>1.11</td>
<td>0.95</td>
</tr>
<tr>
<td>Urinary System</td>
<td>1.56</td>
<td>1.18</td>
</tr>
<tr>
<td>Endocrine System</td>
<td>1.52</td>
<td>1.38</td>
</tr>
<tr>
<td>All Lymphatic and Hematopoietic Diseases</td>
<td>1.26</td>
<td>1.05</td>
</tr>
</tbody>
</table>

This higher risk of second cancers occurred despite the lower relative survival rate for AA compared to Caucasian patients with a 5-year relative survival rate of 68.6% for AA and 78.3% for Caucasian patients.

**Conclusions:** To our knowledge, this is the first report of the incidence of second cancers in AA patients with breast cancer compared to caucasian. More research to understand the biological, genetic, therapeutic, and environmental factors leading to this higher risk of second cancers is warranted.
Socioeconomic characteristics of African American women with breast cancer

Winter A, Raska P, Ornstein M, Moore H, Montero A, Budd GT Thomas, Tullio K, Bailey J and Abraham J. Cleveland Clinic, Cleveland, OH.

Background: Breast cancer is the most common cancer among African American (AA) women. Despite having a lower incidence of breast cancer compared to white women (124.4 compared to 127.9 per 100,000), AAs have a higher death rate (30.2 compared to 21.3 per 100,000). One explanation for this discrepancy is that breast cancer in AAs is often detected at a later stage compared to white women. We conducted this retrospective study to examine socioeconomic characteristics among AA women with breast cancer to see if there were factors associated with stage of diagnosis which may contribute to the known disparities.

Methods: We identified all AA women diagnosed with any stage breast cancer from 2006-2014 within the Cleveland Clinic Cancer Data Warehouse and classified them into either early or late stage disease at time of diagnosis. Stages 0-II were classified as early and stages III-IV as late. We examined several variables at diagnosis including age, marital status, tobacco use, alcohol use, Medicaid insurance status, and breast cancer subtype which included HER-2 positive (HER+), hormone receptor positive/HER2 negative (HR+/HER-), and triple negative(TN). AA median income was obtained from US census data according to the zip code at diagnosis. We conducted univariate logistic regression for individual estimates and confidence intervals and multiple logistic regression and model selection to determine significant predictors of stage of diagnosis.

Results: Of the 771 AA women identified, 108 (14%) were diagnosed at a late stage of disease with a median age of 59 years. Receptor status distribution was 12.4%, 31%, and 16.6% for HER+, HR+/HER-, and TN respectively for early stage, and 15.7%, 27%, and 25% for late stage. Among early stage 50% were current or previous smokers and 2.6% had Medicaid insurance compared to late stage patients where 63% were current or previous smokers and 9.2% had Medicaid insurance. Multiplicative effect estimates and 95% confidence intervals from univariate logistic regressions identified the following significant factors: tobacco use 1.48 [1.11-1.96] and Medicaid 3.73 [1.56-8.51] (p-values<0.01), and TNBC 1.67 [1.02-2.68] (p-value<0.05). In a stepwise model selection, only tobacco use and Medicaid were retained in the model, as well as age at diagnosis. Conclusions: There are socioeconomic differences among AA women with breast cancer. Only tobacco use, Medicaid insurance, and age at diagnosis were predictive of late stage in this study.
Title: New Orleans has the highest incidence rates of triple negative breast cancer

Loch MM M, Li X, Hsieh M-C, Chen VW W and Wu X-C. Louisiana State University Health Sciences Center, New Orleans, LA and Louisiana State University School of Public Health, New Orleans, LA.

Body: Background: We previously demonstrated increased incidence rates of triple negative breast cancer (TNBC) in Black women (BW) in New Orleans (NO) and Louisiana (LA) compared with SEER 17. We explored the hypothesis that BW in NO had a higher incidence of TNBC than in BW other metro areas in the SEER Program.

Methods: We analyzed tumor characteristics of invasive female breast cancers diagnosed 2010-12 from SEER, focusing on racial disparities. We compared LA data with SEER 17 and metropolitan areas (Atlanta, Detroit, Los Angeles and San Francisco). Predictors of TNBC were identified in multivariate logistic regression.

Results: Overall incidence rate of TNBC in BW was significantly higher in NO (32 per 100,000) than in the rest of LA (24 per 100,000) and the SEER metro areas combined (23 per100,000). Detroit had the 2nd highest rates (27.3 per 100,000) followed by Los Angeles, San Francisco and Atlanta (23.2, 23, 22.4 respectively). Compared with SEER 17, the rate ratio of TNBC was 38\% higher for BW in NO (CI: 1.17-1.61) and 17\% higher for BW in Detroit (CI: 1.06-1.29). Compared with other LA metro areas, the rate ratio of TNBC was 44\% higher for BW in NO (CI: 1.10-1.91). Young age (<65) and black race predicted TNBC after adjusting for insurance status, tumor size, lymph node status, grade and derived AJCC stage.

Conclusions: The incidence rates and rate ratios of TNBC in BW in NO and LA are not only significantly higher than in SEER 17 but also higher than any other SEER metro area. We will explore this dataset further by analyzing BMI, comorbidities and SES. This disparity in BW in NO has clinical implications and translational research potential as it enables us to broaden the understanding and treatment of this aggressive disease.
Title: Survival disparities: Quality of care apparently not the answer

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Body: Introduction: In New Mexico (NM), Hispanic women have a 1.6-fold increased risk of breast cancer-specific death compared to non-Hispanic white women. In previous studies, race/ethnic minority women have been less likely to receive recommended adjuvant treatments, including radiation in women undergoing breast conservation, and hormonal therapy.

Objective: To determine whether non-receipt of recommended therapies contributed to disparate survival.

Methods: We conducted a case-cohort study of breast-cancer-specific survival within a population-based cohort of first invasive breast cancer diagnosed in white females from 1997-2009 in six NM counties, identified through Surveillance Epidemiology End Results (SEER). We selected fifteen percent of all women diagnosed with breast cancer and all breast cancer deaths. After IRB approval, data were collected from comprehensive medical chart reviews, supplemented by SEER information. Receipt of standard of care, vs. not, was defined based on age, diagnosis year and tumor characteristics, according to changes in treatment guidelines. Women who had a reported contraindication or refused therapy were omitted from assessment of quality of care for that therapy. Cox proportional hazards models for case-cohort were conducted using weighted estimates, with calculation of robust variance and hazard ratios (HR) and 95% confidence intervals (CI), using an alpha level of .05. Analyses were restricted to women of age 70 or less who survived at least 12 months. The proportional hazards assumption was verified by Schoenfeld residuals. All analyses were adjusted for age.

Results: Comprehensive medical records reviews were completed for 91% of eligible women (674 cohort members, 519 breast cancer deaths; median follow up 7.8 years). All others were omitted from analysis. Of women eligible for guideline-based treatment, receipt of guideline-appropriate therapy did not differ by Hispanic ethnicity for any treatment, and Hispanic women were slightly more likely overall to receive appropriate therapy (difference not significant). Among guideline-eligible women, at least 91% received radiotherapy, 78% received chemotherapy, 82% received endocrine therapy, and 89% received anti-HER2 targeted agents. After adjustment for other treatment, lack of receipt of guideline-appropriate therapy was related to an increased risk of breast cancer death for endocrine (HR 1.76; 95% CI 1.09-2.84) and radiation therapy (HR 2.05; 95% CI 1.14-3.69). The few HER2-positive women not treated precluded further assessment. After accounting for endocrine and radiation therapy the survival disparity HR of 1.6 in Hispanic women was reduced to 1.57 suggesting only 2% of the disparity was due to differences in receipt of these treatments.

Conclusion: Limitations include likely undercounts of appropriate therapy, thus proportions cited are minimal estimates. Appropriate therapy includes only documented receipt as therapy completion could not always be assessed. Hispanic women have a disproportionately higher breast cancer mortality despite apparently receiving adjuvant therapies to a similar degree as non-Hispanic white women. Equalizing standard of care and attempting to reduce treatment disparities may not be sufficient to address the disproportionate mortality in Hispanic women.
Body: Introduction
Although Hispanic women have lower rates of breast cancer compared to non-Hispanic whites (NHW), breast cancer remains the leading cause of cancer death among Hispanic women. Some studies suggest that Hispanic women have lower rates of breast cancer screening compared to NHW women, primarily due to lack of health insurance and socioeconomic disadvantages. This study aims to understand the demographic characteristics of Hispanic women in the U.S. who access and utilize the Avon Breast Health Outreach Program (BHOP), and how Hispanic women's mammography history differs by nativity. Avon BHOP supports community-based organizations to conduct education and outreach to over 50,000 low-income and uninsured women each year, linking them to routine breast cancer screening and care.

Methods
This study analyzed 2014 Client Intake Forms (CIF) for all women aged 40 and older, who were served by BHOP for the first time (~16,000 women). Descriptive analysis was conducted to summarize their demographic and breast health information, comparing Hispanics with NHW. Multivariate logistic regression was performed to examine the relationship between ethnicity and nativity and having a mammogram in the last two years. Lastly, a sub-analysis among foreign-born (FB) Hispanics only was conducted to assess how the length of time living in the U.S. (number of years) impacts the likelihood of having a mammogram in the last two years.

Results
Of the first time clients served through BHOP in 2014, 10.4% were US-born Hispanics, 45.2% were FB-Hispanics, and 44.4% were NHW. The majority of FB-Hispanic women were from Mexico (66.2%). FB-Hispanics are less likely to have insurance as compared to US-born Hispanic women (19.7% vs. 39.3%), and less likely to have a high school degree (34.3% vs. 44.8%). After controlling for demographic and socioeconomic variables, FB-Hispanics had significantly greater odds of having a mammogram in the past two years compared to US-born Hispanics (OR=1.6, 95% CI, 1.3-1.9), and NHW women (OR=1.4, 95% CI, 1.2-1.6). FB-Hispanics living in the U.S. for 5+ years had a 1.7 greater odds of having a mammogram in the past 2 years, as compared to recent immigrants living in the U.S. for less than one year (OR=1.7, 95% CI, 1.1 – 2.7).

Discussion
In 2014, the percentage of BHOP Hispanic women aged 40+, reporting a mammogram in the last two years (41.1%) was lower than the overall U.S. Hispanic rate (61.4%), suggesting confirmation that BHOP serves vulnerable and underserved populations, as it aims to do. This study also demonstrates how screening behaviors differ among Hispanic women served through BHOP, with FB-Hispanics having higher mammography utilization rates as compared to their US-born counterparts, with variation seen by the number of years living in the U.S. In conclusion, it is critical to view Hispanics as a heterogeneous group in order to best address their clinical needs. Specifically, the results point to how U.S.-born Hispanic BHOP clients may be at particularly high risk for low breast cancer screening.
Title: Effects of breast cancer treatment on markers of metabolic syndrome in a predominantly hispanic patient population

Gaur S, Ochoa C, Sanchez L and Nahleh Z. Texas Tech University Paul L Foster School of Medicine, EL Paso, TX.

Body: Background: Adjuvant chemotherapy improves survival in early breast cancer, however has been reported to contribute to weight gain and insulin resistance. Hispanics are reported to have higher levels of insulin resistance and features of metabolic syndrome as compared to caucasians and as such may be at higher risk of metabolic decompensation during treatment of their cancer. We sought to evaluate the effects of adjuvant/neo-adjuvant breast cancer treatment on markers of metabolic syndrome in a predominantly hispanic population. Study was funded by the institutions department of medicine seed grant funds.

Methods: We enrolled 35 consecutive patients who were about to commence adjuvant or neo-adjuvant chemotherapy for breast cancer. Patients with diabetes mellitus or hyperlipidemia were excluded. Fasting glucose, HBA1C, insulin levels, HDL cholesterol, triglyceride levels, waist circumference and blood pressure were measured before starting chemotherapy and then every 3 months for 1 year. Results were analyzed using repeated measures ANOVA for normally distributed data. For data that was not normally distributed, Friedmans non parametric test was utilized.

A survey of dietary habits, exercise frequency and life style factors was administered before initiating treatment and at completion of the study.

Results:

Baseline characteristics: Of the 35 patients enrolled 31 were hispanics (89%). Median age was 47 years (33-68). 31 (82.8%) were over weight or obese. 13 (37%) had insulin resistance as assessed by HOMA (homeostatic model assessment)-IR, and 12 (34%) met the international diabetic federation (IDF) criteria for metabolic syndrome. 17% had stage 1, 52% had stage 2 and 31% had stage 3 disease. Most common chemotherapy regimen used was dose dense doxorubicin, cyclophosphamide and weekly paclitaxel.

No significant change was noted in the fasting glucose, HBA1C levels, insulin levels, HOMA-IR, weight or waist circumference at any point during the 1 year follow up. Triglyceride levels increased from a mean of 162.2mg/dl prior to therapy to 202.8mg/dl by 3 months, p=0.014. HDL-cholesterol fell from a mean of 50.6 mg/dl to 44mg/dl by 3 months, p=0.04.

Both triglyceride levels and HDL levels returned to baseline by 9 months and there was no change noted by 12 months. Overall 12 patients (34%) met the IDF criteria for metabolic syndrome before initiating adjuvant therapy as compared to 14 (40%) at 1 year.

Subgroup analysis of patients with preexisting metabolic syndrome, obesity or insulin resistance (HOMA-IR >3.8) showed similar results.

Analysis of the survey data showed 22 of the 35 patients (62%) had improved their dietary and exercise habits over the course of the study.

Conclusions: Contrary to other studies, we did not find a significant difference in most of the parameters of metabolic syndrome in a predominantly hispanic patient population. A transient increase in triglyceride levels and a decline in HDL cholesterol level was noted at 3 months, however resolved by the 9th month of treatment. Our data suggests that life style modification may mitigate most of the metabolic adverse effects of therapy and women, at the time of diagnosis, may be particularly motivated to make such changes.
Title: Does adverse tumour biology contribute to inferior outcomes for Indigenous Australians diagnosed with breast cancer?

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Body: Background: Analyses across multiple Australian states have consistently demonstrated significantly inferior breast cancer survival for Indigenous patients (IPs). Studies compensating for increased remoteness, socioeconomic disadvantage and later presentation demonstrate a residual unexplained detriment. This survival disadvantage is confined to the first five years, akin to the inferior outcomes demonstrated by higher risk biological breast cancer subtypes. We postulated that a preponderance of such higher risk subtypes could explain the disparate mortalities.

Methods: The distribution of breast cancer subtypes in Western Australian IPs diagnosed between 2001 and 2010 was assessed to explore the contribution of adverse prognostic subtypes to poorer outcome. This was a retrospective cohort study of Indigenous women (n=114) and 3:1 age and remoteness matched non-Indigenous women (n=310) diagnosed with invasive, non-metastatic, unilateral breast cancer, who underwent definitive local treatment. Subtypes were assigned as luminal A, B, HER2 enriched and triple negative by ER, PR, HER2 and tumour grade comparisons. Differences in basic tumour demographics and biological sub-types were analysed and racial survival discrepancies explored within biological subtype cohorts.

Results: Hazards for overall and breast cancer-specific mortality in IPs were 4.07 (95% CI 2.55-6.49) and 4.19 (95% CI 2.42-7.25). IPs were significantly more likely to have grade 3 tumours (41 v 25%, p<0.001), LN positive disease (39 v 27%, p<0.001) and larger tumours (median 20 v 10 mm, p<0.001). No significant differences in proportions of classical histological sub-types (ductal v lobular) or in tumours showing ER, PR or HER2 positivity were observed. There were no significant differences in biological sub-type proportions although IPs were diagnosed with numerically more non-Luminal A subtypes (56 v 44%, p=0.08), accounted for by increased Luminal B (21 v 15%) and HER2 enriched (10 v 5%) sub-types. The significant relative five-year survival deficit for IPs noted overall (94 v73%, p<0.0001) was observed for each sub-type with the exception of HER2 positive patients. This extended from the relatively low risk luminal A sub-type where oral anti-estrogens are the mainstay of treatment (98 v 82%, p=0.0002) to the high risk triple negative sub-type where intravenous chemotherapy is the standard adjuvant therapy (94 v 50%, p=0.0014).

Conclusions: The contribution of adverse tumour biological subtype to poorer outcomes for Indigenous women is modest. Indigenous women with almost all biological subtypes fare significantly worse than their non-Indigenous contemporaries. Ongoing work includes more detailed biological comparisons of RNA expression and DNA mutation between groups as well as an exploration of potentially disparate treatment patterns.
2015 San Antonio Breast Cancer Symposium

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Title: Role of endocrine therapy in premenopausal patients with hormone receptor-positive metastatic breast cancer, compared with postmenopausal patients: Diachronic analyses from nationwide cohort in Korea (KCSG BR 14-07)

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Body: Background

Endocrine therapy (E) has a major role in treatment of hormone receptor (HR)-positive metastatic breast cancer (MBC). However, in contrast to western countries, premenopausal patients (PRE) more prevalent (50% of all breast cancer patients) and have less options of E than postmenopausal patients (POST) in Korea where the use of LHRH agonist in combination aromatase inhibitors (AIs) in PRE is restricted. Recently we have been successfully established nationwide cohort for the patients MBC (575 patients from 26 institutes). This study was designed to evaluate the role of E especially in PRE.

Methods

The patients with MBC were prospectively or retrospectively enrolled between September 2014 and May 2015. Only menopausal status-confirmed patients (296) were analyzed. Postmenopause was defined, based on NCCN guideline. Total duration of treatment was defined as the time from start day of any first treatment to end of any last treatment. Total duration of E was defined as the sum of time duration of each E. Overall survival was calculated from the start day of any treatment for MBC to any causes of death. This work is supported by National Strategic Coordinating Center for Clinical Research (H110C2020).

Results

A total of 296 patients with HR-positive MBC were analyzed [PRE, 169 (57.1%) and POST, 127 (42.9%)]. Except age (mean 44 and 60 years), baseline characteristics including in pathology, HER2 status, initial pathologic stage, de novo metastasis versus recurrence, surgery and adjuvant treatment (chemotherapy, endocrine therapy and radiotherapy) were well balanced. 92 (54.4%) of PRE and 77 (60.6%) of POST received at least one or more E through all treatment course. 41 (24.2%) of PRE and 44 (34.6%) received E as 1st-line treatment (p=0.034). Among PRE who received 1st-line of E, 30 (71.4%) and 9 (21.4%) of PRE received 2nd- and 3rd-line E. 20 (45.4%) and 10 (22.7%) of POST received 2nd- and 3rd- or more line of E. Most of PRE (54%) received tamoxifen+/-goserelin and 32% of PRE received AIs along with ovarian suppression. 71% of POST received AIs. As initial treatment, E was more frequently used in POST than in PRE (34.6% and 24.3%, p=0.053). Overall survival (OS) of all patients was 18.2 months (95% CI, 14.8-21.5). There was no difference in OS between PRE (17.8 months, 10.9-24.8) and POST (18.5 months, 95% CI, 13.2-23.9) (P=0.337). No difference of OS was observed (E, 18.1 moths, 95% CI, 13.0-23.3; chemotherapy 21.2 moths, 95% CI, 16.8-25.5), regardless of initial treatment. Total duration of treatment of PRE and POST were 15.2 and 13.6 months, respectively with no significant difference (p=0.389). PRE (8.3 moths, 95% CI,5.7-10.8) showed the trend toward longer duration of E in comparison with POST (5.5 moths, 95% CI,4.4-6.7), however the difference did not reach statistical significance (p=0.051).

Conclusion

E was more commonly used as 1st-line therapy in POST than in PRE. Although PRE had limited options of E, E was used in long duration of treatment especially in PRE. These findings suggested that E had a role in treatment for PRE with HR-positive MBC and could be used in treatment for PRE with good efficacy.
Title: Mammographic density: Its inherent epidemiology in 12000 women from 22 diverse countries

McCormack VA A. International Pooling Project on Mammographic Density Consortium.

Body: The International Pooling Project on Mammographic Density Consortium

Background: Over the past 25 years, the epidemiologic knowledge for mammographic density (MD), as a strong marker of breast cancer risk, has expanded greatly, aided by the availability of mammograms in countries with wide-scale mammography-based breast cancer screening programs. The known epidemiology of this breast-tissue specific marker is thus that of women in high incidence countries, and not of that in countries with lifestyles that are characterized by very different life-long hormonal influences, especially during the reproductive years. The International Pooling Project on Mammography Density (IPPMD) aimed to examine the epidemiology of MD internationally, to benefit from breast cancer risk and breast cancer risk factor heterogeneity.

Methods: From diverse breast cancer risk populations, IPPMD sought to include samples of 200 premenopausal and 200 postmenopausal general population women for which risk factor data and mammograms, not taken for symptomatic reasons, were available. To date we have included 11447 women from 22 countries, consisting of 40 ethnicity and location-specific population groups. 5 to 6 countries were included in each of Europe, Africa and the Middle East, Asia and the Americas. Populations not previously studied in terms of MD include South Africa, Kenya, Turkey, Egypt and Brazil.

Digitized/digital films for every woman were anonymised, transferred to the study and read using Cumulus 6 by 3 readers, to obtain standardized quantitative and comparable MD estimates across the entire sample. Readers were blind to all woman and study/country identifiers. Normal errors regression models were used to analyse square root percent density and absolute dense area, after standardizing reader-specific values to a single reader’s distribution.

Results: All risk factor data and MD readings have recently been pooled. Initial findings are as follows. Mean percent MD and absolute dense area varied over 2-fold between the 40 population groups, after adjusting for age and BMI. There is striking consistency in several epidemiologic features of MD across all population groups, including the rate of decline of density at both pre and post-menopausal ages and the decline associated with the menopausal transition. We are exploring to what extent these and other factors such as menarche, parity, age at first birth and breastfeeding, account for between population-group differences in MD.

Conclusions: This international perspective provides a valuable insight to the epidemiology of MD worldwide.
Title: A randomized, double-blind trial to compare the efficacy and safety of proposed biosimilar pegfilgrastim (LA-EP2006) with reference pegfilgrastim in patients with breast cancer (PROTECT1)

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Body: Background: An abbreviated pathway for biological products shown to be biosimilar to the reference product exists in Europe and the US. The randomized PROTECT1 trial compared the efficacy and safety of the proposed biosimilar pegfilgrastim with reference pegfilgrastim.

Methods: In this multinational, prospective, double-blind trial, chemotherapy-naïve women aged ≥18 years with histologically proven breast cancer received up to 6 cycles of (neo)-adjuvant TAC chemotherapy (docetaxel 75 mg/m², doxorubicin 50 mg/m², cyclophosphamide 500 mg/m²). Patients were randomized to a single 6 mg SC injection of the proposed biosimilar pegfilgrastim (LA-EP2006) or the reference (Neulasta®) on day 2 of each cycle. Primary endpoint was duration of severe neutropenia (DSN) during Cycle 1, defined as number of consecutive days with an absolute neutrophil count (ANC) <0.5 x 10⁹/L. The study was powered at 90% and had a hierarchical testing procedure utilizing a ±1 day margin to test for equivalence (2-sided 95% confidence interval [CI]) and a subsequent −0.6 day non-inferiority margin (1-sided 97.5% CI) for DSN during Cycle 1. DSN was analyzed with an ANCOVA model adjusted for treatment, chemotherapy, region and baseline ANC. Secondary efficacy assessments were: time to ANC recovery, ANC nadir, incidence of febrile neutropenia, number of days of fever, frequency of infections and mortality due to infection. Safety was assessed at 4 weeks and 6 months after the last pegfilgrastim administration. Immunogenicity was assessed by testing for neutralizing anti-pegfilgrastim antibodies.

Results: A total of 316 patients were randomized and included in the full analysis set (LA-EP2006: n=159; reference: n=157). Baseline demographics were similar in both groups (mean±SD age: LA-EP2006 49.9±9.53, reference 50.5±10.87 years; breast cancer stage II-III: LA-EP2006 n=155 [97.5%], reference n=151 [96.2%]). Mean±SD DSN in Cycle 1 was 0.75±0.88 days with LA-EP2006 and 0.83±0.90 days with reference, with a treatment difference of 0.07 days (95% CI: −0.12, 0.26); LA-EP2006 was both equivalent and non-inferior to the reference. There were no clinically meaningful differences between LA-EP2006 and reference in incidence of febrile neutropenia (3.8% vs 7.0% in Cycle 1, 5.7% vs 7.6% across all cycles), days with fever, depth of ANC nadir in Cycle 1, time to ANC recovery in Cycle 1, or frequency of infections in Cycle 1 and across all cycles. Treatment-emergent adverse events (TEAEs) were similar across groups and consistent with the known safety profile of pegfilgrastim. Most frequently reported TEAEs related to treatment were musculoskeletal and connective tissue disorders (LA-EP2006 4.4%, reference 5.7%). Serious TEAEs were reported in 10.1% of LA-EP2006 and 13.4% of reference patients. No neutralizing anti-pegfilgrastim antibodies were detected.

Conclusions: Proposed biosimilar pegfilgrastim (LA-EP2006) met the primary endpoint demonstrating both equivalence and non-inferiority to the reference. LA-EP2006 and the reference are similar with no clinically meaningful differences regarding efficacy and safety in breast cancer patients receiving (neo)-adjuvant myelosuppressive chemotherapy.
Title: Usage of epoetin alfa biosimilars in the management of chemotherapy-induced anemia in patients with breast cancer: A subanalysis of the ORHEO study

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Body: Introduction
Chemotherapy-induced anemia (CIA) is a frequent complication of breast cancer patients that is associated with fatigue and impaired quality of life. Biosimilars of erythropoietin-stimulating agents have now been approved to treat CIA in Europe. The ORHEO (biOsimilaRs in the management of anaemia secondary to chemotherapy in HaEmatology and Oncology) study was an observational study conducted in France to evaluate the efficacy and safety of a biosimilar epoetin alfa in an oncology setting. The large number of patients who enrolled in the study permitted indication-specific subanalyses to be undertaken. Herein we report the results of the subanalysis that focuses on the subpopulation of breast cancer patients.

Methods
The ORHEO study was a prospective, observational, longitudinal, multicenter postmarketing study. Patients ≥18 years with CIA (hemoglobin [Hb] <11 g/dL)-associated solid tumors, lymphomas, or myelomas who were eligible for treatment with an epoetin alfa biosimilar were enrolled. The primary endpoint was Hb response (defined as Hb reaching ≥10 g/dL, an increase of Hb ≥1 g/dL since inclusion, or reaching target Hb) measured at 3 and 6 months (M3 and M6, respectively). Secondary endpoints included safety and tolerability. Only breast cancer patients with CIA were included in this subanalysis.

Results
The ORHEO study enrolled 2333 patients; of these, 266 patients presented with CIA associated with breast cancer and were included in this subanalysis. The mean age was 61 years, the majority had ECOG PS 1 (51.5%) and metastatic disease (56.0%), and 99.6% received an epoetin alfa biosimilar. At baseline, the mean Hb level was 9.9 g/dL. At the M3 and M6 time points, an Hb response was observed in 86.8% and 91.7% of patients, respectively; average Hb levels increased by 1.3 g/dL (M3) and 1.8 g/dL (M6). By M3, 44% of patients reached their target Hb level; this increased to 53.7% at M6. At M3 and M6, of those patients who definitively or temporarily discontinued treatment, over half did so because the target Hb had been reached (M3: 55.6% [n = 74/133]; M6: 53.2% [n = 81/152]). Clinically significant adverse events (AEs) were observed in 9.9% of patients; the most common AE was infection (7.9%), while thrombotic events were only reported in 0.8% of patients. High baseline systolic blood pressure, high hematocrit levels at M3, and clinical improvement by M3 were identified as potential indicators of a positive response to an epoetin alfa biosimilar.

Conclusion
This subanalysis of the large ORHEO observational study has demonstrated the utility of biosimilar epoetin alfa in a real-world setting. Biosimilar epoetin alfa was seen to be efficacious and well tolerated in patients with breast cancer who presented with CIA, and no new safety signals were identified in this subpopulation. These data support the results observed in the parent ORHEO study, and confirm that biosimilar epoetin alfa (Hospira) is a therapeutic alternative for treating CIA in patients with breast cancer. Several potential prognostic indicators were identified that may warrant further study.
Biosimilar filgrastim in the treatment of patients with breast cancer undergoing neutropenia-inducing chemotherapy: A subanalysis of the NEXT study

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Body: Introduction
Febrile neutropenia (FN) is a frequent and serious complication in breast cancer patients undergoing chemotherapy. In addition to being a major risk factor for infection-related morbidity/mortality and disease progression, FN can lead to dose delays and reduction. Nivestim™ (Hospira Inc), a biosimilar filgrastim, is a granulocyte colony-stimulating factor licensed for the treatment of neutropenia and FN induced by myelosuppressive chemotherapy.

The NEXT (Nivestim safety profile in patiEnts treated with cytotoXic CT in real-life clinical practIce) study was a prospective real-world trial assessing the safety of curative or prophylactic biosimilar filgrastim in the treatment of patients undergoing chemotherapy. The large size of this study enabled a subanalysis to be performed in breast cancer patients; the results are presented herein.

Methods
NEXT was a prospective, noninterventional, longitudinal, multicenter French study (NCT01574235). Patients with a solid tumor or a malignant hemopathy (excepting chronic myeloproliferative and myelodysplastic syndromes) undergoing or starting neutropenia-inducing chemotherapy were enrolled. Objectives were to evaluate safety and incidence of FN and infection. Patients received biosimilar filgrastim (Hospira) and were monitored for 1–6 chemotherapy cycles, with 3 visits at inclusion, during, and following treatment.

Results
A total of 2114 patients were enrolled in this study; of these, 463 patients had breast cancer and were included in this analysis. Mean age was 58.4 years, 97% were female, 95.8% had an ECOG PS of 0–1, and 19.4% had metastatic disease. The majority of patients (n = 454; 98.1%) received biosimilar filgrastim as prophylaxis (primary, 94.1%; secondary, 5.9%); 9 (1.9%) patients received curative biosimilar filgrastim. Median time to initiation of biosimilar filgrastim was 7.5 days for curative patients and 4 days for prophylactic patients. Mean treatment duration was: 6.0±1.7 days (curative) and 5.6±2.6 days (prophylactic). Biosimilar filgrastim-related adverse events (AEs) were reported in 119 patients (curative, n = 1; primary prophylaxis, n = 111; secondary prophylaxis, n = 7). The rate of AEs was higher in breast cancer patients (26.1%) compared with the entire NEXT study population with solid tumors (21.1%); similar rates of FN were reported but breast cancer patients had higher rates of AEs and bone and muscle pain compared with the total study population. Considering the prophylactic group only, 4.7% experienced FN and 1.6% reported an infection. The incidence of FN and/or infection resulted in hospitalization in 4.7% of patients (mean duration: 4.6±2.5 days), chemotherapy dose reduction in 2.7% of patients, and a delay of chemotherapy in 5.2% of patients.

Conclusion
These data demonstrate the safety and efficacy of biosimilar filgrastim in a real-world setting. The subanalysis illustrated that biosimilar filgrastim was effective and well tolerated in breast cancer patients in the curative and prophylactic setting. These results mirror those of the parent NEXT study, and show that biosimilar filgrastim is a valid alternative therapeutic option for chemotherapy-induced neutropenia in breast cancer patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-10-04

Title: A prospective study of patterns of chemotherapy, colony-stimulating factor use, and burden of colony-stimulating factor injections in patients with early-stage breast cancer


Body: Background: Febrile neutropenia (FN) is a common side effect of myelosuppressive chemotherapy. Primary prophylaxis with colony-stimulating factors (CSFs) can reduce FN incidence and is recommended when a patient has a high risk of FN (>20%). In the prophylactic setting, CSFs should be administered at least 24 hours after chemotherapy completion. Patient burden associated with CSF administration is not well understood. Here we describe current patterns of chemotherapy use and burden of CSF injections for patients with early-stage breast cancer (ESBC) in US clinical practice.

Methods: This was a prospective cohort study of adult ESBC patients receiving their first chemotherapy course who had a high risk of FN based on high- or intermediate-risk chemotherapy regimen and individual FN risk factors. The burden associated with CSF injections was assessed via questionnaires among patients who received CSF, and a subset analysis of patient burden in the first cycle of chemotherapy is reported.

Results: 598 patients completed the "burden of CSF injections" questionnaire following the first cycle of chemotherapy. Most patients were < 65 years old (76.8%), had a BMI < 30 kg/m² (54.9%), and had few comorbidities (see table for additional characteristics and comorbidities). The three most common chemotherapy regimens received were ddAC-T (34.4%), TC (23.4%), and TCH (15.6%). 98.3% of patients received prophylaxis with CSF in the first chemotherapy cycle: 94.6% of these received pegfilgrastim, and 5.4% received filgrastim. Among all patients who received CSF, mean (SD) one-way travel time for a single CSF injection was 31 (25) minutes; mean (SD) time in office to receive a CSF injection was 41 (68) minutes. Across the first chemotherapy cycle, mean (SD) time missed from work for CSF administration was 3.1 (9.3) hours, and mean (SD) time missed from non-work activities was 5.5 (14.4) hours. 66.3% of patients had someone else assist them with travel to the clinic to receive CSF, of which 98.8% were helped by an unpaid caregiver. When patients were questioned about the subjective burden of CSF injections, 25.4% reported some degree of bother, and 15.9% reported at least moderate inconvenience.

Conclusions: Among the high- and intermediate-risk regimens investigated here, dose-dense and taxane-based chemotherapy regimens were common. As many high-risk patients with ESBC receive primary prophylaxis with CSF, travel and time needed to receive CSF can contribute to patient and caregiver burden.

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>N = 598</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD) years</td>
<td>55.1 (11.3)</td>
</tr>
<tr>
<td>BMI, mean (SD) kg/m²</td>
<td>30.7 (7.5)</td>
</tr>
<tr>
<td>HER2+</td>
<td>23.2%</td>
</tr>
<tr>
<td>Luminal Aα</td>
<td>52.0%</td>
</tr>
<tr>
<td>Triple negative</td>
<td>21.6%</td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24.6%</td>
</tr>
<tr>
<td>2</td>
<td>53.7%</td>
</tr>
<tr>
<td>3</td>
<td>21.2%</td>
</tr>
<tr>
<td>Missing</td>
<td>0.5%</td>
</tr>
<tr>
<td>Comorbidities &gt; 10%</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>37.5%</td>
</tr>
<tr>
<td>Condition</td>
<td>Percentage</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>24.7%</td>
</tr>
<tr>
<td>Depression</td>
<td>13.9%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>13.4%</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>12.7%</td>
</tr>
<tr>
<td>Gastroesophageal reflux disease</td>
<td>12.2%</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>12.0%</td>
</tr>
<tr>
<td>Anxiety</td>
<td>11.9%</td>
</tr>
</tbody>
</table>

*Hormone receptor positive but HER2 negative.*
Title: Randomized phase 2, open-label, dose-ranging study of a novel, long-acting G-CSF (SPI-2012) or pegfilgrastim for the management of neutropenia in patients with breast cancer (BC) treated with (Neo) adjuvant chemotherapy with docetaxel + cyclophosphamide (TC)

Body: Background: SPI-2012 is a distinct biologic that uses the innovative proprietary long-acting protein/peptide discovery technology (LAPSCOVERY™) to enhance the activity of G-CSF. SPI-2012 consists of a novel, modified recombinant human G-CSF conjugated to the Fc fragment of IgG4 via a polyethylene glycol linker to produce a new, more potent, longer-acting G-CSF with a potentially unique distribution to areas rich in Fc receptors. To assess the effect of SPI-2012 in supporting patients with breast cancer receiving myelosuppressive chemotherapy with TC, we conducted a randomized Phase 2 study of 3 SPI-2012 doses versus pegfilgrastim.

Methods: This was an open-label, global, multicenter, dose-ranging study designed to compare the safety and efficacy of SPI-2012 relative to a fixed, standard dose of pegfilgrastim as a concurrent active control. The study included 4 treatment arms: 3 dose levels of SPI-2012 (45 µg/kg, 135 µg/kg, and 270 µg/kg) vs pegfilgrastim (6 mg.). The primary objective of the study was the Duration of Severe Neutropenia (DSN) in patients treated with SPI-2012 compared to pegfilgrastim.

Results: A total of 147 evaluable patients were enrolled. Patient and tumor characteristics were comparable across all 4 treatment arms. Mean age was 58.2 years (range 32 to 77 years); most patients were <65 years (68%), female (98%) and white (95%). The study met its primary endpoint with DSN in patients treated in the 135 µg/kg and 270 µg/kg SPI-2012 treatment arms showing non-inferiority to pegfilgrastim (p=0.002 and p<0.001, respectively). In addition, superiority was demonstrated in patients treated with 270 µg/kg SPI-2012 compared to pegfilgrastim (p=0.023). Non-inferiority in DSN was also observed in Cycles 2 to 4 in both the 135 µg/kg and 270 µg/kg SPI-2012 treatment arms compared to pegfilgrastim.

Duration of Severe Neutropenia in Cycle 1 of TC chemotherapy by Treatment Arm

<table>
<thead>
<tr>
<th></th>
<th>45 µg/kg SPI-2012 (N=39)</th>
<th>135 µg/kg SPI-2012 (N=36)</th>
<th>270 µg/kg SPI-2012 (N=36)</th>
<th>Pegfilgrastim (N=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSN Mean (SD)(days)</td>
<td>1.03 (1.5)</td>
<td>0.44 (1.3)</td>
<td>0.03 (0.2)</td>
<td>0.31 (0.8)</td>
</tr>
<tr>
<td>Difference with pegfilgrastim</td>
<td>0.72</td>
<td>0.14</td>
<td>-0.28</td>
<td>NA</td>
</tr>
<tr>
<td>Non-inferiority p-value</td>
<td>0.296</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>NA</td>
</tr>
<tr>
<td>Superiority p-value</td>
<td>0.006</td>
<td>0.528</td>
<td>0.023</td>
<td>NA</td>
</tr>
</tbody>
</table>
The common treatment-emergent adverse events observed in ≥20% of patients were similar across all 4 study arms with similar or lower incidence in the SPI-2012 treatment arms, and included fatigue, nausea, alopecia, diarrhea, and bone pain.

**Conclusions:** All doses of SPI-2012 administered in this Phase 2 study were well tolerated, and no new or significant dose-related toxicities were observed. Most reported adverse events were mild and similar to those previously reported in clinical trials with filgrastim and pegfilgrastim in patients receiving myelosuppressive chemotherapy. In Cycle 1, the 135 µg/kg dose of SPI-2012 was non-inferior compared to pegfilgrastim, and the 270 µg/kg dose was superior in terms of DSN. Additional efficacy and safety data for SPI-2012 will be collected in planned Phase 3 clinical trials.
Title: Nausea control and quality-of-life benefit with NEPA, the first combination antiemetic agent, in patients with breast cancer receiving anthracycline/cyclophosphamide (AC) chemotherapy

Rugo HS S, Aapro M and Rizzi G. University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Clinique de Genolier, Genolier, Switzerland and Helsinn Healthcare, SA, Lugano, Switzerland.

Body: Background: Patients (pts) with breast cancer (BC) are at high risk for developing chemotherapy-induced nausea and vomiting (CINV) due to the emetogenicity of chemotherapy (often AC-based) and predisposing risk factors including young age and female gender. For pts receiving AC, antiemetic guidelines recommend prophylactic administration of an NK₁ receptor antagonist (RA), a 5-HT₃ RA, and dexamethasone (DEX). NEPA is the first fixed combination agent approved in oncology; comprised of a highly selective NK₁ receptor antagonist (RA), netupitant (300 mg), and the pharmacologically/clinically distinct 5-HT₃ RA, palonosetron (PALO 0.50 mg). NEPA has shown superior complete response (no emesis/no rescue use) rates compared with oral PALO in a Phase 3 trial in pts receiving AC (Aapro, Ann Oncol 2014) and in that study's BC subset. Despite progress in prevention of vomiting, nausea control remains suboptimal, particularly in the delayed phase (days 2-5), and debate exists whether NK₁ RAs improve nausea control. The objective of this post-hoc analysis was to evaluate whether NEPA showed nausea and associated quality-of-life (QOL) benefits in the subset of patients with BC in this trial.

Methods: The subset of chemotherapy-naïve BC pts from this multinational, randomized, double-blind Phase 3 study were included in this analysis. Patients received either a single dose of NEPA or oral PALO prior to AC along with oral DEX 12 mg (NEPA) or 20 mg (PALO). No significant nausea (NSN: max <25 mm on 100 mm visual analog scale) rates in the acute (0-24h), delayed (25-120h) and overall (0-120h) phases following chemotherapy during cycles 1-4 were calculated. QOL was assessed by the Functional Living Index—Emesis (FLIE) during cycle 1 overall phase; the percentage of pts with "no impact on daily life" (NIDL) was calculated. Comparisons between groups were performed using a Cochran-Maentel-Haenszel test.

Results: 1412 patients with BC were included for a total of 5839 AC cycles. NSN rates were similar for both groups in the acute phase, superior for NEPA during cycles 1, 2 and 4 in the delayed phase, and superior for NEPA during cycles 1-4 in the overall phase.

<table>
<thead>
<tr>
<th>Overall NSN (0-120h)</th>
<th>NEPA + DEX</th>
<th>Oral PALO + DEX</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>74.2% (N = 708)</td>
<td>68.5% (N = 704)</td>
<td>0.016</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>77.0% (N = 621)</td>
<td>71.1% (N = 636)</td>
<td>0.015</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>78.2% (N = 586)</td>
<td>72.7% (N = 594)</td>
<td>0.027</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>79.9% (N = 542)</td>
<td>74.9% (N = 550)</td>
<td>0.040</td>
</tr>
</tbody>
</table>

This corresponded with a significantly greater proportion of NEPA patients reporting NIDL compared with PALO due to nausea (71% vs 65%; p=0.007) in cycle 1.

Conclusions: While other NK₁ RAs have not consistently shown benefit in improving nausea control over 5-HT₃ RA + DEX, in this study NEPA significantly improved prevention of nausea over oral PALO in BC patients receiving AC. In addition, NEPA was superior to PALO in reducing the negative impact of nausea on patients’ daily functioning. As the first fixed antiemetic drug combination, NEPA is highly effective and offers the convenience of a single dose administered with DEX on Day 1 only.
**Title:** Phase 3 comparison of APF530 versus ondansetron, each in a guideline-recommended 3-drug regimen for prevention of chemotherapy-induced nausea and vomiting due to anthracycline + cyclophosphamide (AC)–based highly emetogenic chemotherapy (HEC) regimens: A post hoc subgroup analysis of the MAGIC trial

Schnadig I, Agajanian R, Dakhil S, Taylor C, Wilks S, Cooper W, Mosier M, Payne Y, Klepper M and Vacirca J. Compass Oncology, US Oncology Network, Tualatin, OR; The Oncology Institute of Hope and Innovation, Whittier, CA; Cancer Center of Kansas, Wichita, KS; Tulsa Cancer Institute, Tulsa, OK; Cancer Care Centers of South Texas, San Antonio, TX; TFS International, Flemington, NJ; EMB Statistical Solutions, LLC, Overland Park, KS; Heron Therapeutics, Redwood City, CA; Drug Safety Navigator, LLC, Durham, NC and North Shore Hematology Oncology, East Setauket, NY.

**Body:**

**Background:** Managing delayed chemotherapy-induced nausea and vomiting (CINV) associated with HEC is an unmet need. AC-based HEC is often administered to breast cancer patients (pts), a mostly female, high-CINV-risk population. APF530, an extended-release formulation of granisetron, demonstrated superior complete response (CR; no emesis [vomiting, retching] + no rescue medication use) in delayed-phase (>24-120 h) CINV with HEC (ASCO criteria) vs ondansetron (Ond) (65% vs 57%, \( P=0.014 \)), each combined with a neurokinin-1 antagonist and dexamethasone (Dex) (NCT02106494). This post hoc analysis evaluated efficacy and safety of APF530 in pts receiving AC-based therapy.

**Methods:** In this randomized, double-blind, multicenter trial, pts scheduled to receive single-day HEC were stratified by cisplatin \( \geq 50 \text{ mg/m}^2 \) yes/no and randomized 1:1 to APF530 500 mg SC (granisetron 10 mg) or Ond 0.15 mg/kg IV. Pts received concomitant Dex 12 mg IV and fosaprepitant 150 mg IV on day 1 and oral Dex on days 2-4. The primary end point was CR in the delayed phase. Secondary and other end points included CR in acute (0-24 h) and overall (0-120 h) phases, and complete control (CC; CR and no more than mild nausea) and total response (TR; CR and no nausea) in acute, delayed, and overall phases. Rates were compared using 95% confidence intervals (CIs) for treatment differences; post hoc analysis was not powered to detect treatment differences in the AC subgroup. Safety assessments included adverse events (AEs), injection-site reactions (ISRs), laboratory parameters, and vital signs.

**Results:** A total of 589/902 pts (65%) in the modified intent-to-treat population received AC-based HEC (APF530 291, Ond 298). Baseline demographics were balanced between treatment arms. The majority of pts in the AC subgroup were female (APF530 99%, Ond 98%). Delayed-phase CR was higher with APF530 vs Ond, approaching statistical significance (APF530 64%, Ond 56%; \( P=0.062 \)) in the AC subgroup, similar to the benefit seen in the larger study. No appreciable benefit of APF530 vs Ond was observed in the acute phase, and trends favorable to APF530 were observed in the overall phase (Table). APF530 was well tolerated. Most AEs were ISRs, generally mild or moderate, and resolved by end of study.

<table>
<thead>
<tr>
<th>Phase, n (%)</th>
<th>APF530 N=291</th>
<th>Ondansetron N=298</th>
<th>Treatment Difference (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complete response</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>185 (64)</td>
<td>167 (56)</td>
<td>8 (-0.4, 15.4)</td>
</tr>
<tr>
<td>Overall</td>
<td>163 (56)</td>
<td>153 (51)</td>
<td>5 (-3.4, 12.7)</td>
</tr>
<tr>
<td>Acute</td>
<td>205 (70)</td>
<td>204 (69)</td>
<td>1 (-5.4, 9.4)</td>
</tr>
<tr>
<td><strong>Complete control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>171 (59)</td>
<td>156 (52)</td>
<td>7 (-1.6, 14.4)</td>
</tr>
<tr>
<td>Overall</td>
<td>149 (51)</td>
<td>143 (48)</td>
<td>3 (-4.9, 11.3)</td>
</tr>
<tr>
<td>Acute</td>
<td>193 (66)</td>
<td>191 (64)</td>
<td>2 (-5.5, 9.9)</td>
</tr>
<tr>
<td><strong>Total response</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed</td>
<td>119 (41)</td>
<td>107 (36)</td>
<td>5 (-2.9, 12.8)</td>
</tr>
<tr>
<td>Overall</td>
<td>100 (34)</td>
<td>94 (32)</td>
<td>2 (-4.8, 10.4)</td>
</tr>
</tbody>
</table>
**Conclusions:** APF530 demonstrated an apparent clinical benefit in delayed-phase CR in pts receiving AC-based HEC, concordant with the statistically significant benefit seen in the overall study population. Prevention of CINV in this patient population continues to be a treatment challenge and further investigation is needed.
Title: Effect of a structured group intervention on obesity in breast cancer survivors

Kitagawa H, Hosaka T, Takeda N, Matsumoto N, Tomita M, Takahashi M and Yamauchi H. St. Luke's International Hospital, Chuo-ku, Tokyo, Japan; St. Luke's International Hospital, Chuo-ku, Tokyo, Japan; Breast Center/Oncology Center, St. Luke's International Hospital, Chuo-ku, Tokyo, Japan; St. Luke's International Hospital, Chuo-ku, Tokyo, Japan and Division of Cancer Survivorship Research, National Cancer Center, Chuo-ku, Tokyo, Japan.

Body: Background: Obesity is associated with an increased risk of breast cancer and poor outcomes. Endocrine therapy, including aromatase inhibitors (AI) is a key part of adjuvant treatment. However, it may produce some side effects, including musculoskeletal pain and weight gain. This study aimed to examine the effect of a structured group intervention for breast cancer patients with obesity and abnormal lipid metabolism during adjuvant endocrine therapy.

Methods: The subjects were 32 breast cancer survivors with obesity who were undergoing endocrine therapy (AI or tamoxifen) and had undergone surgery at least 12 months before enrollment. Median age was 51 years (range 37-67). We performed a single-arm pre-post study of combined interventions. The intervention consisted of 15 minutes of nutrition education delivered by a registered dietitian, a 30-minute group health coaching program by a coaching staff, and a 45-minute group aerobic exercise. Before and after the intervention, body weight (BW), body mass index (BMI), mid-upper arm circumference, triceps skinfold, liver enzyme levels (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), total cholesterol (CHO) level, and triglyceride (TG) level were determined and compared. We also compared the results of the Japanese version of K6, Cancer Fatigue Scale (CFS), and Self-efficacy Scale for Cancer Patients (SES) before and after intervention. These weekly structured interventions were held in 3 consecutive weeks. Participants were asked to practice to exercise at home with a DVD, performing the same activities. Pre- and post-intervention data were compared by using paired t tests with IBM SPSS version 21.

Results: Significant decreases were found in BW (p < 0.01), BMI (p < 0.01), TG level (p < 0.05), CHO level (p < 0.01), K6 result (p < 0.05), and CFS result (p < 0.01). In contrast, no statistically significant change at the 5% level was observed in the mid-upper arm circumference, triceps skinfold, AST level, ALT level, and SES result.

Comparison of measured values between pre- and post-group intervention

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted mean ± standard deviation</th>
<th>Unadjusted mean ± standard deviation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before intervention</td>
<td>After intervention</td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td>60.5 ± 10.9</td>
<td>59.5 ± 11.0</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.5 ± 4.4</td>
<td>24.0 ± 4.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Mid-upper arm circumference (cm)</td>
<td>28.8 ± 3.7</td>
<td>28.5 ± 3.7</td>
<td>0.104</td>
</tr>
<tr>
<td>Triceps skinfold (cm)</td>
<td>33.6 ± 10.4</td>
<td>31.7 ± 10.5</td>
<td>0.143</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>121.1 ± 62.3</td>
<td>101.2 ± 43.3</td>
<td>0.044</td>
</tr>
<tr>
<td>CHO (mg/dL)</td>
<td>206.6 ± 34.7</td>
<td>192.9 ± 33.7</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Comparison of self-administered questionnaires between pre- and post-group intervention

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted mean ± standard deviation</th>
<th>Unadjusted mean ± standard deviation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before intervention</td>
<td>After intervention</td>
<td></td>
</tr>
<tr>
<td>K6</td>
<td>4.9 ± 5.1</td>
<td>3.3 ± 3.8</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Conclusion: This study showed that a short-term, three-session, structured intervention is effective for behavioral changes to promote health among breast cancer survivors, resulting in favorable changes in not only obesity but also in TG/CHO levels. These results suggest its potential as an effective intervention for cancer-related fatigue and mental health of survivors. Further studies with more participants and with a control group are needed to demonstrate the long-term effects of the structured intervention, particularly its possible impact on breast cancer prognosis.
Title: Are patients with breast cancer undergoing adjuvant treatment able to follow an exercise program with a moderate to high intensity?

May AM M, Boer JH H, Velthuis M, Steins Bisschop CN N, Los M, Erdkamp F, ten Bokkel Huinink D, Bloemendal HJ J, Rodenhuis C, de Roos MAJ AJ, Verhaar M, van der Wall E and Peeters PHM HM. University Medical Center Utrecht, Utrecht, Netherlands; Comprehensive Cancer Organisation (IKNL), Utrecht, Netherlands; St. Antonius Hospital, Nieuwegein, Netherlands; Orbis Medisch Centrum, Sittard, Netherlands; Diakonessenhuis, Utrecht, Netherlands; Meander Medical Center, Amersfoort, Netherlands; Hospital Rivierenland, Tiel, Netherlands and Holpoort Hospital, Woerden, Netherlands.

Body: PURPOSE: We recently showed in a randomized trial, the Physical Activity during Cancer Treatment (PACT) study, that an 18-week exercise program reduced complaints of fatigue and improved physical fitness in newly diagnosed breast cancer patients undergoing adjuvant treatment. The beneficial effects were probably underestimated due to high levels of physical activity in the control group that received usual care only. Another possibility for dilution of the effect might be limited participation of the intervention group in the supervised exercise program or low compliance, i.e., an adjustment of the prescribed exercise protocol. We set out to study participation and compliance and to find determinants of reduced compliance.

METHODS: 102 patients in the PACT study were randomized into the intervention group that received a supervised exercise program 2 times a week for 18 weeks (36 sessions in total). Each session had a duration of 60 minutes and included a pre-specified period of aerobic interval exercises of specific intensities as well as muscle strength exercises. Sessions were supervised by physiotherapists, intensity was based on individual fitness characteristics and results were kept in a log. We computed attendance (percentage of total sessions attended) and compliance (adherence to the prescribed duration and intensity of the aerobic part and to the muscle strength part of each attended session). We computed for each woman the percentage of sessions the women complied with the protocol, and report median percentages for compliance with the aerobic exercises, duration and intensity, and with the muscle strength exercises separately. Determinants of low compliance that were included in linear regression models were: age, behavioral, physical and psychosocial factors.

RESULTS: For 92 patients exercise logs were available. Patients were, on average, 50.2±7.8 years of age, all patients received chemotherapy and 70% received radiotherapy. Participation was high: patients participated in 83% (interquartile range 69-91%) of the sessions offered. Overall, also compliance was high: in 88% (63-97%) and 84% (65-94%) of all attended sessions patients were able to complete the aerobic (duration) and muscle strength program, respectively, as prescribed in the protocol. Compliance to the high-intensity part of the aerobic program was lower: in 50% (22-82%) of the sessions the intensity of the aerobic exercises was adjusted. Especially patients who received radiotherapy in addition to chemotherapy and patients who were more physically fatigued at baseline had a lower compliance to the high-intensity part of the aerobic exercises (β=-5.3 (confidence interval -9.4;-1.2) and β=-0.6 (-1.0;-0.1), respectively).

CONCLUSIONS: Participation in and compliance to an 18-week aerobic and muscle strength exercise program was high. Thus, patients are well capable to exercise during adjuvant treatment for breast cancer. This study shows that preferably high intensity aerobic exercises were adjusted in a significant number of participants rather than the duration or the strength exercises. This has to be taken into account when developing training programs, especially in those patients who receive both, radiotherapy and chemotherapy.
Title: An integrative intervention to change breast cancer patients' lifestyle: A medical challenge. A randomize controlled trial


Body: Background. Physical exercise increases breast cancer (BC) patients' survival. However, only about two thirds of them follow the American Guidelines of Exercise to cancer survivors. The aim of this study was to examine the effect of an exercise intervention in breast cancer patients' lifestyle.

Methods. A randomized clinical trial evaluated an intervention (EXE) vs. a control (CON) group in early stage BC patients who recently finished the chemo and radio (neo) adjuvant. Intervention consists on exercise group classes combining aerobic and resistance activities designed specially for the necessities of these patients. Intensity was increasing gradually and intervention was controlled by a qualified in oncologic exercise specialist. CON group maintained their lifestyle without changes. Leisure-time exercise levels (LTEL), quality of life (QoL), grip strength index (GSI), physical capacity (VO2max) and fatigue, were assessed at baseline and after 3 months in both groups. Women who had been participated in the intervention group were followed up after six months to know if lifestyle levels were maintained.

ANOVA and Pearson Test were used to analyze the continuous variables of baseline and final data and ANOVA test was used to analyze the follow-up data. A 95% of CI was calculated and p < 0.05 was determined as statistically significant.

Results 89 women, aged 49.06±8.75, completed the study, 44 in EXE group and 45 in CON group. Adherence rate was on average of 89%. Main results are presented in the Table.

<table>
<thead>
<tr>
<th>MEASURE</th>
<th>EXE Group</th>
<th>EXE Group</th>
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<tbody>
<tr>
<td></td>
<td>BL</td>
<td>F</td>
<td>FU</td>
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<tr>
<td>QoL*+</td>
<td>107.53±17.99</td>
<td>112.88±17.74</td>
<td>110.96±14.40</td>
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<tr>
<td>LTEL*+</td>
<td>18.73±20.53</td>
<td>45.11±14.61</td>
<td>34.56±19.51</td>
<td>16.02±8.52</td>
<td>15.04±4.75</td>
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<tr>
<td>GSI*+</td>
<td>2.07±1.08</td>
<td>2.60±0.83</td>
<td>2.48±0.58</td>
<td>2.03±0.72</td>
<td>2.12±0.69</td>
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<tr>
<td>VO2max*+</td>
<td>26.99±4.35</td>
<td>32.58±4.96</td>
<td>32.11±7.10</td>
<td>27.73±4.82</td>
<td>27.08±3.73</td>
</tr>
<tr>
<td>Fatigue *+</td>
<td>130.09±19.68</td>
<td>135.94±18.20</td>
<td>138.24±17.49</td>
<td>124.83±24.66</td>
<td>124.00±24.20</td>
</tr>
</tbody>
</table>

BL=baseline; F= Final; FU= Follow-Up. *Significant differences between EXE and CON. + Significant differences between BL and FU.

There was a significant improvement in LTEL (p=0.0001) and in QoL (p=0.0001) comparing EXE vs. CON group. In addition, results showed a correlation between these two variables in EXE group (r=0.22; p=0.013). These significant improvements, as well as the mentioned correlation, were maintained in the 6-months follow-up assessment in EXE group.

Significant differences between groups were observed in GSI (p=0.004), and in VO2max levels (p=0.001). EXE group showed a significant improvement in fatigue levels compared with CON group (p=0.0001). All these significant improvements were maintained in the 6-months follow-up assessment in EXE group, as well as previous variables.

Conclusion. These results suggest that an exercise intervention increases LTEL correlated to a better QoL, improving patients' lifestyle that could be long lasting. These changes may ameliorate psychological and physical BC treatments side effects, such as fatigue, in patients with early breast cancer that has recently finished adjuvant treatments.
**Title:** Prognostic value of non-alcoholic fatty liver disease in stage II/III breast cancer patients who received neoadjuvant chemotherapy

Yang Y, Lee K-H, Kim T-Y, Han S-W, Oh D-Y, Kim T-Y, Han W, Moon H-G, Park IA, Noh D-Y and Im S-A. Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea; Cancer Research Institute, Seoul National University, Seoul, Korea; Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea and Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Korea.

**Body: Background**
Metabolic syndrome is associated with various malignancies, including breast cancer. Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome. NAFLD is frequently observed during chemotherapy, but its clinical implication is unclear. The purpose of this study is to evaluate the prognostic value of NAFLD in patients with stage II/III breast cancer who received neoadjuvant chemotherapy (NAC Tx).

**Methods**
The patients with clinical stage II/III breast cancer who received NAC Tx with docetaxel and doxorubicin were enrolled. Treatment sequences were: 3 cycles of NAC Tx → surgery → 3 cycles of adjuvant chemotherapy (AC Tx), or 6 cycles of NAC Tx → surgery, allowing AC Tx at the physician's discretion. NAFLD was determined by ultrasonography (radiologists' decision), non-constrast computed tomography [CT] (the attenuation of liver is less than that of spleen or <49 hounsfield unit [HU]) or contrast CT (the difference between liver and spleen attenuation < -18.5 HU). The presence of NAFLD was evaluated 3 times, at diagnosis, after NAC Tx and after the completion of AC Tx. Improvement of NAFLD during NAC Tx was defined as the disappearance of NAFLD after NAC Tx.

**Results**
Between 2002 and 2008, 269 patients were enrolled. The median age was 45 (range 18-69), and 51.7% and 28.6% were with hormone receptor and HER2 positive tumors, respectively. The median number of NAC Tx was 3 cycles (range 1-6). NAFLD was observed in 33 (12.4%) patients at diagnosis, 52 (19.3%) after NAC Tx and 71 (26.4%) at the completion of AC Tx. The patients with NAFLD at diagnosis showed shorter overall survival than those without NAFLD (HR=2.688, 95% CI 1.259-5.747, \( P = 0.011 \)). The improvement of NAFLD during NAC Tx was observed in 28 (10.4%, group A) and persistence of NAFLD observed in 24 (8.9%, group B). Group A showed better prognosis in both PFS (HR 0.125, 95% CI 0.016-0.962) and OS (no event), whereas group B showed worse PFS (HR 2.329, 95% CI 1.280-4.237) and OS (HR 3.721, 95% CI 1.727-8.015) compared to the patients without NAFLD at diagnosis.

**Conclusions**
NAFLD at diagnosis was a poor prognostic marker of OS in patients who received NAC Tx. Improvement of NAFLD during NAC TX was associated with good prognosis in terms of PFS and OS.
Title: Biopsychosocial concerns of adolescent young adults (AYA) with breast cancer

Smith R, Mortimer J, Loscalzo M and Clark K. City of Hope National Medical Center, Duarte, CA.

Body: Background: The natural history of breast cancer in the AYAs with breast cancer is reported to be more aggressing than that observed in older women. We wanted to determine the level of biopsychological distress in this population compared with women in other age groups.

Methods: All new patients seen at City of Hope complete a tablet-based self report biopsychosocial screening questionnaire (SupportScreen®). Patients are asked to score a series of problems on a 5-point Likert scale ("not a problem" to "very severe problem") and are asked if they are interested in obtaining help for that problem. Results: To date 1,159 women have undergone screening; 79 pts (6.9%) were age 18-39 yrs (AYA), 807 (69.6%) age 40-64 yrs, and 273 (23.6%) > 65 yrs. The concerns that were unique to the AYA population included: Ability to have children (p=0.001) and physical appearance (p=0.047 for 40-64 yrs and p=0.018 for age > 65 yrs). Older women were more concerned that AYAs or middle aged women about transportation (p=0.008), walking, climbing stairs (p=0.000). Compared to older women, those age 40-64 yrs were more likely to identify feeling anxious or fearful (p=0.032) and managing work, school or home life (p=0.018). In comparison to AYAs, those age 40-64 yrs had more distress related to recent weight change (p=0.034) and difficulty sleeping (p=0.006).

Conclusions: Biopsychosocial concerns change over the continuum of age. Compared to other age groups, the AYA population was more concerned about their ability to have children and their physical appearance. In other domains of distress, they were comparable to women age 40-65 yrs.
Ethnic differences in quality of life, anxiety and depression and fatigue in breast cancer survivors

Ho PJ, Verkooijen HM M, Gernaat S and Hartman M. Saw Swee Hock School of Public Health, National University of Singapore, Singapore; Imaging Division, University Medical Center Utrecht, Netherlands and Julius Center, University Medical Center Utrecht, Netherlands.

Introduction: With the sharp increase in incidence and improving survival of breast cancer in Asia, survivorship issues, like fatigue, anxiety and depression and health related quality of life are becoming increasingly important. In the multi-ethnic setting of South East Asia, these items have been understudied.

Methods: This cross-sectional study included a random sample of 377 breast cancer patients visiting the breast care clinic of the National University Hospital for routine follow-up between April 2014 - April 2015. Patients were at least 12 months post diagnosis. Patient characteristics were collected from medical records. Malay (n=72) and Chinese (n=305) breast cancer patients were compared in terms of physical function, fatigue, and financial difficulties (as reported in the EORTC-QLQ-C30), and anxiety and depression (Hospital Anxiety and Depression Scale, HADS). Clinically relevant fatigue (CRF) was defined as a score ≥40, and clinically relevant anxiety and depression were defined as scores of ≥8. Mann-Whitney U test and Fisher's exact test were used to compare patient reported outcomes between Chinese and Malays.

Results: The mean age at time of survey was 56.5 (range: 27-79), with 305 (74%) Chinese and 72 (18%) Malays. Median (Interquartile range) ages were 58 (52-64) for Chinese and 52 (46-57) for Malays; the distribution of age was significantly different (p<0.001). Forty five (15%) Chinese and 8 (11%) Malays were originally diagnosed with in situ cancer, 98 (33%) and 18 (25%) with stage 1, 130 (43%) and 33 (46%) with stage 2, and 29 (10%) and 13 (18%) with stage 3 or 4; the distribution of stage was not different for Chinese and Malays (p=0.143). Overall quality of life was not significantly different for ethnicity, median were 67 (58-83) for Chinese and 75 (58-83) for Malays (p=0.773).

Ninety seven (26%) patients reported scores 8 and above for anxiety; with 80 (26%) Chinese and 17(24%) Malays (p=0.765). Sixty two (24%) patients reported scores of 8 and above for depression, with 55 (18%) Chinese and 7 (10%) Malays (p=0.111). Sixty two (24%) patients experienced clinically relevant fatigue, 44(14%) Chinese and 18 (25%) Malays (p=0.034). Malays experienced lower level of physical function than Chinese, 87 (73-93) vs 93 (87-100) respectively (p=0.004), and more financial difficulty, 33 (0-67) vs 0 (0-33) respectively (p<0.001).

Conclusion:
One in 4 patients experience anxiety, depression, and/or clinically relevant fatigue. Malay breast cancer survivors, experience more clinically relevant fatigue and reduced physical functions compared to Chinese breast cancer survivors. In addition, they experienced more financial difficulties.
Title: Meeting the information and psychosocial needs of young Jewish women at increased risk for or diagnosed with breast cancer

Silber E, Fleischmann AK, Shoretz R, Johnson AC, Murphy SE, Mays D, O'Neill SC and Tercyak KP. Sharsheret, Teaneck, NJ and Division of Population Sciences, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC.

Body: Background: Approximately 12% of women living in the United States will be diagnosed with breast cancer during their lifetimes. Although breast cancer survival rates are improving, there remain significant impacts upon women's length and quality of life. This includes women already diagnosed with the disease, as well as those who are at increased risk owing to hereditary breast cancer-causing mutations (e.g., BRCA1/2 genes). While all women face formidable challenges posed by the threat of living with or being at increased risk for breast cancer, those of Ashkenazi and Central or Eastern European Jewish descent face additional challenges owing to higher BRCA1/2 mutation prevalence in this population. Amidst calls for population-based screening for hereditary breast cancer risk, much can be learned from the experiences of young Jewish women at risk for and surviving with breast cancer about their information and psychosocial needs.

Methods: The present study is a secondary analysis of survey data from 2010 to 2014 originally collected by Sharsheret, a non-profit organization dedicated to serving women of all Jewish backgrounds facing or at risk for breast cancer. The evaluation included measures and metrics of community referral practices, social service/program engagement and satisfaction, and resource needs among the target population (N=555). Only survey items administered across all data collection years were analyzed.

Results: Respondents had a mean age of 50 years and ~90% identified as Jewish. Over 1/3 were referred to the organization by family or friends, most often after a breast cancer diagnosis. Within the surveyed population, 25% reported being at risk or confirmed carriers of a BRCA1/2 mutation. Of the education and support programs offered, the greatest level of engagement occurred in the 1-on-1 peer emotional support and health care symposia education programs. Women reported very high levels of satisfaction with the programs and services available, sought additional information and social support services, and noted a strong desire to give back to the community by participating as a peer supporter. Importantly for this high-risk population, women who participated in the organization's breast cancer genetics program were >98% satisfied (on average) with the various evaluated components of the program. Women identified genetic risk information as one of their top needs for future engagement.

Conclusion: These data affirm success of the organization's programs, especially in educating Jewish women about breast cancer genetics. Genetic information is increasingly salient among members of the Jewish community, extending to their cultural needs and need for peer support. Women likely responded well to Sharscheret's peer support program as it connects women to those with similar backgrounds, including culture, lifestyle, and medical similarities. Implications of these findings assert that culturally-relevant information and psychosocial services for young Jewish women living with or at risk for breast cancer can be enhanced for larger dissemination to meet the expected growing demand in this high risk community.
Title: Breast cancer survivorship support services: Evaluation findings of the thriving again survivorship program

Silber E, Stahl S and Fleischmann AK K. Sharsheret, Teaneck, NJ.

Body: Background. While a breast cancer diagnosis can be daunting for women of any age, studies have shown that young breast cancer survivors exhibit more emotional and psychological distress because of their relatively young age and life stage at diagnosis. In September 2011, the Centers for Disease Control and Prevention funded seven organizations, including Sharsheret, a national not-for-profit organization supporting young Jewish women and their families facing breast cancer, to develop support services and educational awareness activities for young breast cancer survivors. With this funding, Sharsheret developed the Thriving Again® (TA) survivorship program, which provides support services and resources, including a tailored survivorship kit with a survivorship care plan template, exercise DVD and healthy living cookbook. Participants were asked to complete an evaluation of services received to further enhance the program.

Methods. Breast cancer survivors who received a TA survivorship kit were asked to complete an online or paper survey evaluating Sharsheret's survivorship support services. Among 972 women who received the TA survivorship kit and were invited to complete an evaluation, 164 women returned the evaluation survey and 85 completed it in its entirety. Descriptive statistics on demographics and factors related to utilizing the survivorship care plan template and Sharsheret's services were calculated and analyzed. The small sample size limited additional analyses.

Results. Of the 85 women who completed the survey, 46% were <45 years of age and 43% were of Jewish descent. 62% of respondents reported that they received the kit and care plan template at the time they needed it most in their survivorship journey. These women more often reported completing their survivorship care plan template either themselves or with a member of their medical team. Care plan completion was also high among women considering themselves as "survivors" (89%). The vast majority of women participating in the Genetics for Life® program (76%) had spoken with a Sharsheret clinical team member at the time they ordered their TA kit. Other factors, such as stage at diagnosis and age, were unrelated to use of the care plan and Sharsheret's programs.

Conclusion. Although breast cancer survivors may identify themselves as "survivors" at any point post-diagnosis, women responding to the survey who were satisfied with the timing of receiving the TA kit during their survivorship journey and who self-identified as a "survivor" were more likely to complete the care plan template. Women who reviewed the kit and learned more about TA with a member of Sharsheret's support team were more likely to engage in other Sharsheret programs, most notably, Genetics for Life®. Although the sample size was small, the findings from this evaluation may be helpful to other survivorship programs. Most notably, other programs may need to be aware of how patients perceive themselves as survivors and offer care plans and resources when patients feel they most need them, even if patients have not yet completed treatment.

The findings in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Title: Improving provider adherence to breast cancer care quality metrics: Use of a novel care planning tool


Body: Background: With rapid advances in research clinicians often struggle to remain current on evolving care guidelines as well as current National Quality Standards (NQS) relevant to breast cancer management. Adherence to NQS now drives much reimbursement for cancer center services but clinical workflow processes and IT solutions are often not in place to effectively document adherence. The On Q Care Planning System™ (CPS), an evidence-based patient assessment and care planning software, has been designed to close gaps in quality cancer care and facilitate data collection to help centers both better understand and document their adherence rates to quality care standards.

Methods: This multi-site study will enroll approximately 150 non-metastatic breast cancer patients, presenting for no greater than their second medical oncology visit, across five cancer centers. Patients must be planned for but not yet receiving chemotherapy treatment. A between subject design using 150 matched historical controls will be used to assess the impact of the 2-part intervention, at both the patient and provider level, on select quality metrics. At two consecutive clinical visits, patients will engage with the On Q CPS to assess family and medical history and current symptoms and receive two separate care plans. Care plans include (when applicable) recommendations for symptom management and appropriate referrals (i.e. genetic counseling for those at increased hereditary risk, and/or reproductive endocrinology for those interested in preserving fertility). To augment the effectiveness of the On Q CPS, providers will also participate in certified continuing medical education activities designed to educate about evidence-based assessment, decision-making, and management strategies for breast cancer patients. The primary aim is to evaluate provider adherence to select quality metrics among recently diagnosed breast cancer patients following the intervention, and compare to adherence rates for historical controls from the pre-intervention period. Metrics of primary interest include distress screening and management, complete family history assessment, genetic counseling referral, discussion of infertility risk, and discussion of fertility preservation options and/or referral to a specialist. These metrics have been chosen as the primary endpoints given that they have been historically documented as being resistant to change. Outcomes will be assessed by chart abstraction using a score card method of select quality metrics for both enrolled patients and matched historical controls.

Analysis/Results: Patient enrollment begins in June 2015 and thus data will be presented at time of symposium. Patient characteristics and primary outcomes will be analyzed using a multi-step approach to first describe and then compare, at the individual patient level, provider adherence to the select quality metrics evaluated in this study. Descriptive statistics will also be estimated within a mixed model approach for the rate with which each single metric was achieved across patients.

Conclusions: The On Q CPS, a care planning software tool, has the potential to both improve provider adherence to NQS and allow institutions an easy and accessible way to document that adherence.
Mailliez A, Bregegere S and Bonneterre J. Centre Oscar Lambret, Lille, France.

**Body:** 2344 patients (pts) younger than 40 years of age have been diagnosed for breast cancer in France in 2012. Almost all were premenopausal and some of them had not completed their family. We conducted a retrospective study to evaluate the level of information on the impact of breast cancer treatment on fertility. Patients: We selected breast cancer patients without relapse less than 40 treated in our institution between 2003 and 2012 who received (neo)adjuvant chemotherapy (3FEC100-3 Docetaxel). From 2008, only pts with hormone receptor (HR) negative tumours were considered. HR positive pts were receiving hormonotherapy and thus could not be pregnant. A questionnaire was developed and included 18 items on gynaecologic concerns before and after cancer diagnosis and information on the impact of breast cancer treatment on fertility.

Results: 121 met the inclusion criteria. 64 pts returned the questionnaire (52,8%). Median age at diagnosis was 34,3y (25-39). Before the diagnosis of cancer, 90% were married. 76,6% had at least one child, five of them with assisted reproductive technology. 18,8% wanted a pregnancy at the time of cancer diagnosis. 79,7% had regular menstrual cycles. 78,1% had contraception (oral contraceptive 68%, copper intra uterine device (IUD) 8%, Levonorgestrel-impregnated IUD 19%, condoms 6%).

Only 48,4% considered to be informed on the impact of breast cancer treatment on their fertility and 22 % were addressed to a physician of reproductive medicine. Four patients had fertility preservation.

After cancer treatment, only 14,1% had regular menstruations. 75% had a contraception (oral contraceptive 2%, copper intra uterine device (IUD) 63%, Levonorgestrel-impregnated IUD 4%, tubal ligation 2%, condoms 29%). 35,9% decided not to become pregnant. 16 patients (25%) wished to become pregnant. Seven of them had not received any information neither on the impact of treatment on fertility nor on fertility preservation techniques.

18,8% had a pregnancy (unwanted for 3 patients who requested an abortion) without fertility preservation or assisted reproductive technology. Only 9,4% had at least a live birth.

Several patients complained about chemotherapy-induced premature ovarian failure and the ensuing disturbances such as decreased libido, amenorrhea and menopause.

Conclusion: About 50% of the pts considered not to have received adequate information on fertility and other gynaecological consequences of anti cancer treatment.

This study shows how important gynaecologic considerations are for young breast cancer patients. Information about impact of treatment, fertility preservation techniques and symptoms of premature ovarian failure should be part of the treatment of these patients. Specific time should be devoted during the different consultations before, during and after treatments.
Title: Contraception use in young women with breast cancer

Brigham and Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Mayo Clinic, Rochester, MN and Kaiser Foundation Research Institute, Oakland, CA.

Body: Background: Young women with breast cancer need highly effective contraception given the potential implications of unplanned pregnancy for optimal treatment, and the teratogenic risks. We sought to determine the contraceptive methods used by young women after diagnosis (dx) of breast cancer and factors associated with use of less effective methods or no contraceptive method, which confers a 6-90% annual risk of pregnancy in sexually active women in contrast to highly effective methods (risk <1%).

Methods: As part of a randomized trial conducted in 54 sites to test an education and support intervention for young women with breast cancer and their oncologists, we surveyed women about their pre-dx, current, and planned contraceptive use, and about communication with their providers regarding contraception. Women enrolled within 3 months of dx; contraception items were included on 3- and 12-month post-enrollment surveys. Intrauterine device (IUD) use, tubal sterilization, hysterectomy or bilateral salpingo-oophorectomy (hyst/BSO) after dx, or male partner vasectomy were classified as highly effective methods; all other methods and non-use were categorized as less effective. We excluded women not at risk of pregnancy: hyst/BSO prior to dx, or no indication for contraception. We used logistic regression to explore factors associated with use of less effective methods.

Results: Of 424 women who completed the 3-month post-enrollment survey, median age at dx was 39 (range 22-45). 312 women at risk of pregnancy were included in this analysis, including 291 reporting sexual activity with a male partner within the last 6 months, and 21 reporting no recent sexual activity but reporting use of birth control. 123 women (39%) used highly effective contraceptive methods prior to dx; after dx, 161 (52%) reported current use of or a plan to use a highly effective method. 19 women (6%) reported use of a hormonal birth control method since dx; 7 (2%) reported withdrawal as their only contraceptive method; 25 (8%) reported no contraception. 30% of women did not recall a discussion of avoiding pregnancy or need for contraception during treatment with their providers. In multivariable analyses (N=310), desire for additional biologic children (OR 7.54, 95% CI 3.88-14.66) and provider discussion of contraception and pregnancy (OR 2.13 95% CI 1.20-3.78) were associated with use of less effective contraception. Age, race/ethnicity, disease stage, and partner status were not significantly associated with use of less effective methods.

Conclusion: About half of women who are at risk of pregnancy reported use or planned use of less effective contraceptive methods or no method of contraception following dx of breast cancer. Women with breast cancer and their providers may benefit from targeted education on contraceptive options and method effectiveness.
Title: Skin, and nail, infections associated with the addition of pertuzumab to trastuzumab-based chemotherapy


Body: Objectives: We have maintained a local registry of skin and nail infections in patients receiving pertuzumab and trastuzumab as treatment for HER2 positive breast cancer. Over the past 16 months, we have continued to observe an increase in infectious complications in patients receiving the combination of pertuzumab and trastuzumab with or without chemotherapy. We expand a series of prospectively identified patients who developed infections while on these regimens. Methods: We became concerned about an increased incidence of infections shortly after the FDA approval of pertuzumab, and created an IRB approved registry of these patients. Results: Twenty-eight women were identified to have 32 separate infections (often at more than one site); 9 after cycle 1; 6 after cycle 2, 9 after cycle 3 and 8 after 4 or more cycles. The median age was 51 (Range 25-67); 14 received pertuzumab, trastuzumab, carboplatin, and docetaxel (PTCH); 5 pertuzumab, trastuzumab, and docetaxel, 7 pertuzumab, trastuzumab, and nab-paclitaxel, and 2 pertuzumab and trastuzumab. Folliculitis of the scalp, abdomen, and/or buttocks was observed in 19 patients, abscesses in 8 patients (4 of whom required incision and drainage) and cellulitis in 2. Severe paronychial infections involving one to 16 digits were observed in 4; 2 pt required surgical removal of 2 nails. Quantitative immunoglobulins were found to be low in 8 of 17 women tested; 2 patient had low total protein but did not have an assessment of quantitative immunoglobulins. All patients were initially treated with oral antibiotics, and 6 required hospitalization. Cultures were obtained in 10 patients; Staphylococcus aureus was identified in 4, methicillin resistant Staphylococcus aureus (MRSA) in 5, Enterococcus faecalis in 1. A 57 year old pt receiving neoadjuvant PTCH died on cycle 2 day 7. Autopsy was consistent with sepsis and gram positive cocci were identified. A 62 year old became septic and developed renal failure. Skin biopsies were performed in 3 patients and are consistent with changes associated with EGFR inhibition. Conclusions: We believe these infections are a result of combining pertuzumab with trastuzumab as 2 pts received no concurrent chemotherapy. An awareness of this complication is critical as some infections may be life-threatening. We have initiated patient education to ensure awareness of this potential complication.
Importance of the patient voice in drug development: Early-stage breast cancer and measurement gaps concerning the treatment experience


Background: Most early-stage breast cancer (EBC) patients (pts) do not experience signs or symptoms of disease; approximately 90% of women diagnosed in breast screening are asymptomatic in the US (Ryerson et al. 2015). Rather, side effects of cancer therapy have the greatest impact and can be burdensome to pts on and after treatment. Bother and impact have not been thoroughly assessed from the patient perspective in trials. Qualitative research with 56 pts undergoing or completing (after 3 and within 24 mos of) systemic treatment were conducted to assess the need for EBC-focused patient-reported outcome (PRO) measures.

Methods: Semi-structured interviews were conducted to better understand the treatment experience; the interview guide was developed in consultation with breast cancer advocates who were former pts. The interview sample was determined to capture findings across EBC therapies (HER2-targeted [HER2], hormone/endocrine [H/E], and/or chemotherapy [CT]). Treatment experience, including treatment-related symptoms and treatment impact (e.g. on activities of daily living, emotional aspects) were discussed in each 90-minute session. Pts rated level of bother of symptoms and impacts on an 11-point scale. Disease stage, treatment received, surgery, and other health information was collected from medical charts. Qualitative analysis was conducted with ATLAS.ti software. Symptom data was reviewed to appropriately analyze therapy subgroups.

Results: Stage Ia (17.9%), Ib (14.3%), Ila (32.1%), Ilb (25.0%), or IIa (7.1%) pts that received adjuvant (75%) or neoadjuvant (25%) therapy participated; 106 unique treatment-related symptoms were reported. Symptoms most frequently reported included hair loss (86.7%), change in taste (73.3%), and tiredness/fatigue (71.1%) on CT (n=45); tiredness/fatigue (34.8%), runny nose (26.1%), and watery eyes (21.7%) on HER2 (n=23); and hot flashes (50.0%), joint pain (37.5%), and weight gain (20.1%) on H/E (n=24). The most common symptoms reported after therapy completion included memory loss (63.6%), symptoms of neuropathy (numbness, tingling, and pain in fingers, 63.6%), and tiredness/fatigue (45.5%) (n=11). CT symptoms rated by ≥ 25% of pts that were most bothersome included tiredness/fatigue (x̄ =8.2, n=18**), hair loss (x̄ =8.2, n=32**), and memory loss (x̄ =7.7, n=15**). HER2 and H/E ratings of bother were less frequent. EBC treatment was associated with significant impact on pts’ lives; categories described are below:

<table>
<thead>
<tr>
<th>Impact category</th>
<th>Average bother rating* (n**)</th>
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</thead>
<tbody>
<tr>
<td>Concerns with treatment</td>
<td>9.5 (2)</td>
</tr>
<tr>
<td>Physical/functional</td>
<td>7.7 (36)</td>
</tr>
<tr>
<td>Work or school</td>
<td>7.5 (37)</td>
</tr>
<tr>
<td>Sleep</td>
<td>7.5 (21)</td>
</tr>
<tr>
<td>Daily tasks and activities</td>
<td>7.4 (95)</td>
</tr>
<tr>
<td>Emotional</td>
<td>7.4 (62)</td>
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<tr>
<td>Sexual behavior</td>
<td>7.1 (22)</td>
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<tr>
<td>Cognitive function</td>
<td>6.8 (6)</td>
</tr>
<tr>
<td>Social</td>
<td>6.7 (54)</td>
</tr>
<tr>
<td>Appearance</td>
<td>6.5 (32)</td>
</tr>
</tbody>
</table>

* Rating on 11-pt scale; 0=none to 10=extremely bothersome ** n=number of patients rating the level of bother
Conclusion: Treatment-related symptoms and associated degree of bother differed by treatment group. Pts' descriptions of treatment impact provided additional insight into the burden of EBC. EBC-specific PROs included in trials that gain pts' perspective on experience with treatment would further inform pts and may also inform therapy choice.
Title: Voice of cancer patients: Analysis of concerns of patients receiving adjuvant chemotherapy for breast cancer

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Body: Introduction: There is a growing interest in understanding the concerns of patients undergoing cancer therapy. Many patients share their experiences on online support forums, which contain millions of freely shared messages that can be used to analyze patient concerns. Unfortunately, these data are unstructured, which makes them difficult to analyze. In this project we organize the data on these forums using methods from Big Data Science (BDS), and then analyze these data by creating a Decision Support System (DSS): an interactive interface that can be used by both patients and providers to understand patient concerns about their cancer therapies.

Method: We collected approximately 10 million unique messages from 20 unrestricted breast cancer forums that provide information about diagnoses, treatments, side effects, supportive therapies, and specific experiences. After using domain knowledge of breast cancer to build custom ontologies for regimens, side effects, and supportive therapy, we use the following techniques from BDS in order to create our DSS:

• Topic Modeling to find keywords that best represent a given theme
• Information Retrieval to filter for messages that are related to this theme
• Natural Language Processing to extract the relevant data from these messages
• Token Windows and Co-occurrence-based Algorithms to associate regimens with their side effects and supportive therapies.

To use the DSS, a user provides disease-related parameters and the treatment. The DSS then gives the percentage of messages discussing side effects for a similar cohort of patients and the percentage of messages that discuss supportive therapies for each of these side effects.

Results: We retrieved 84938 messages from patients receiving adjuvant chemotherapy with the regimens listed below, and then analyzed the percentage of people mentioning each side effect. The results are summarized in the following table.

<table>
<thead>
<tr>
<th>Side-Effect</th>
<th>AC/T, %</th>
<th>C/T, %</th>
<th>TCH, %</th>
<th>AC, %</th>
<th>AC/Taxotere, %</th>
<th>FEC/Taxotere, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>56.6</td>
<td>51.4</td>
<td>50</td>
<td>51.5</td>
<td>57</td>
<td>56.4</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>6.4</td>
<td>4.6</td>
<td>6.1</td>
<td>7.5</td>
<td>4.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Nausea</td>
<td>12.6</td>
<td>11.2</td>
<td>13.4</td>
<td>14.5</td>
<td>10</td>
<td>14.6</td>
</tr>
<tr>
<td>Alopecia</td>
<td>2.5</td>
<td>3.6</td>
<td>3.2</td>
<td>2.6</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Nail changes</td>
<td>6.8</td>
<td>7.1</td>
<td>9.3</td>
<td>7</td>
<td>5.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Heart related</td>
<td>17.8</td>
<td>20.4</td>
<td>17</td>
<td>18</td>
<td>17.4</td>
<td>16.4</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8.6</td>
<td>9.8</td>
<td>8.6</td>
<td>10.5</td>
<td>7.9</td>
<td>9.2</td>
</tr>
<tr>
<td>Swelling</td>
<td>5</td>
<td>5.7</td>
<td>7.2</td>
<td>5</td>
<td>6.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Rash itching</td>
<td>7.3</td>
<td>9.6</td>
<td>8.9</td>
<td>9</td>
<td>8.1</td>
<td>7.6</td>
</tr>
<tr>
<td>Total count</td>
<td>21096</td>
<td>28788</td>
<td>13697</td>
<td>11058</td>
<td>7219</td>
<td>3080</td>
</tr>
</tbody>
</table>

These statistics reflect patient concern about a particular side effect, instead of the true incidence of that side effect. For example, although about 90% of patients receiving the above regimens experience alopecia, only between 2% and 4% of messages on online forums mention alopecia.

Our system can also associate drugs to their side effects and suggest supportive therapies. For instance, 5428 (20.6%) of 26370
messages on Neulasta, mention it as the cause of bone pain. Out of these, 1262 (23.2%) mention Loratidine in context with Neulasta as a suggestion to alleviate bone pain (p < 0.00001).

**Conclusion:** Using methods from BDS, our DSS reliably associates side effects to a particular drug or regimen and suggests a supportive therapy.

Our results reflect the concerns of patients undergoing cancer therapy, which might help the medical community identify areas of resource allocation and unmet needs.
Title: Evaluating the incidence of supportive care referrals generated using patient reported data from the Athena health questionnaire system


Body: Background
Patients at risk for or diagnosed with breast cancer have many symptoms and need for supportive care services. As part of the Athena Breast Health Network (a University of California-wide collaboration), the UCSF Breast Care Center (BCC) has incorporated an electronic health questionnaire system (HQS) prior to new patient and follow-up clinic visits, allowing patients to provide information on their personal health and family history, physical and psychological symptoms, and lifestyle. Based on these patient-reported outcomes (PRO), automated referrals for services including genetic counseling, psycho-oncology, social work, fertility preservation, and smoking cessation are generated. Algorithms defining thresholds to trigger these referrals were developed by clinicians and supportive care providers to proactively meet patients’ needs.

Objectives
To evaluate the incidence and outcomes of supportive care referrals based on existing algorithms, and identify reasons for non-utilization of the services offered. The ultimate goal for this evaluation is to modify the existing algorithms to better meet patients’ needs.

Methods
Patients initiating care at the UCSF BCC are invited by email to complete an HQS that provides information relevant to their clinical care. Patients sign an electronic consent, agreeing to have their PRO stored and accessed for research purposes. Family history, health behaviors, desired services, and responses to National Cancer Institute Patient Reported Outcomes Measurement Information System (PROMIS) items are processed through algorithms, generating referrals based on defined thresholds. A clinician summary report is generated and scanned into the electronic medical record (EMR), identifying services for which the patient has met thresholds. Referrals are sent to the clinician as pended orders through the EMR. Once signed by the care provider (physician or nurse practitioner), the order is routed through the EMR to the appropriate service and the patient is offered a visit or phone consultation when appropriate.

Results
Between 1/1/14 and 12/31/14, 1297 patients initiating care at the UCSF BCC completed an HQS prior to their clinic visit. 1108 patients (85.4%) agreed to have their data used for research. 623 patients (56.2%) were referred to at least one supportive care service. The table below summarizes the percentage of patients who met the defined referral thresholds:

<table>
<thead>
<tr>
<th>Referral Type</th>
<th>Number of Referrals</th>
<th>Percent of Patients Referred (n=1108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Counseling Services</td>
<td>443</td>
<td>40.0%</td>
</tr>
<tr>
<td>Psychological Services</td>
<td>257</td>
<td>23.2%</td>
</tr>
<tr>
<td>Social Work</td>
<td>137</td>
<td>12.4%</td>
</tr>
<tr>
<td>Smoking Cessation</td>
<td>34</td>
<td>3.1%</td>
</tr>
<tr>
<td>Fertility Preservation</td>
<td>29</td>
<td>2.6%</td>
</tr>
</tbody>
</table>

Ongoing analyses are underway to determine the percentage of patients who received services, explore barriers to accessing these services, and evaluate patients’ preferences regarding provision of services in alternate formats, including webinars, online content, and group sessions.
Conclusions
Effective use of PRO identifies a high percentage of patients in need of supportive care services. Through analysis of utilization of services based on our existing thresholds, we hope to optimize our algorithms to better serve our patients' needs throughout the continuum of cancer care.
Title: Bladder symptoms in women with newly diagnosed breast cancer

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Body: Background: Changes in bladder function are recognized effects of aging and menopause but have not generally been reported in women treated for breast cancer. We initiated a prospective trial to assess the impact of (neo)adjuvant therapy on women with early stage breast cancer.

Methods: Women with newly diagnosed invasive breast cancer who were to initiate (neo)adjuvant chemotherapy or endocrine therapy were approached for study participation. At baseline a urinalysis, urine culture, and self-assessment quality of life questionnaires were completed. The Urogenital Distress Inventory (UDI-6) assesses bladder symptoms and the Incontinence Impact Questionnaire (IIQ-7) assesses the impact of bladder symptoms on quality of life. Three months after initiation of (neo)adjuvant therapy, the quality of life questionnaires were repeated. We report the results of the pretreatment questionnaires.

Results: Between February and June, 2015, forty-nine women with newly diagnosed breast cancer were enrolled on study. The median age was 54 (Range 25-78); 21 were premenopausal and 28 postmenopausal. Twenty nine (59%) were treated in the adjuvant setting; 12 with chemotherapy and 17 with endocrine therapy. Twenty patients, (41%) were treated in the neoadjuvant setting with chemotherapy. Prior to initiation of therapy, "Frequent urination" was reported in 38 (65%), " Leakage related to urgency" in 5 (10%), " Leakage with physical activity" in 32 (55%) and "Small amounts of leakage" in 32 (55%). Bladder symptoms impacted the ability to perform household chores in 8 (16%), Physical recreation in 10 (20%), social activities in 9 (18%), and Emotional health in 5 (10%).

Conclusions: Symptoms of bladder dysfunction are common in women with newly diagnosed breast cancer even before therapy is initiated.
Title: Sarcopenia and toxicities in patients affected by breast cancer in adjuvant treatment

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Body: Purpose
Recent evidences suggest that severe depletion of skeletal muscle, known as sarcopenia, is associated with poor prognosis and toxicities of anti-cancer therapy. Sarcopenia, often unrecognized, affects patients with low, normal or high body mass index (BMI). The aim of our study was to evaluate the association between lumbar skeleton muscle status (LSMI) and toxicities in breast cancer patients receiving standard adjuvant chemotherapy.

Patients and Methods
Twenty-two breast cancer patients (mean age = 55.27; standard deviation, SD = 9.83) receiving epirubicin-based chemotherapy were enrolled in a prospective study. Skeletal muscle cross-sectional area at the third lumbar vertebra was measured by computerized tomography (CT) and sarcopenia was defined using the cut off point for LSMI of <38.5 cm2/m2. BMI and BSA were measured at every cycle of chemotherapy. CT scan was performed before cycle 1 and after cycle 4 of chemotherapy. Toxicity was assessed after every cycle of treatment and it was graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0. Serum samples were withdrawn at every cycle to determine drug concentrations.

Results
Before cycle 1 of chemotherapy, 86.36% of patients were classified as sarcopenic. The sarcopenia mean value was 32.22 cm2/m2 (SD, 5.78; range, 20.87-43.87), and no differences by age and BMI were found. The BMI mean value was 23.97 (SD, 4.32; range, 18.00-33.70).

After cycle 4 of chemotherapy the BMI mean value was 24.19 (SD, 4.74; range, 16.80-33.70), and the sarcopenia mean value was 32.18 (SD, 5.68; range, 22.41-45.43). 18.18% of patients reported severe toxicities (grade 3 or 4). Sarcopenia mean values were found significantly different (p-value 0.048) in patients with severe toxicity compared to patients with absent/mild toxicities (mean value, 27.17; SD, 3.27 versus 33.30; SD, 5.55; respectively). Changes in LSMI were associated with significant changes in toxicities (p-value 0.004). In 18% of patients there was an improvement in toxicity grade, and all of them reported an improvement in LSMI. Among the 40% of patients that reported a worsening in toxicity there was also a worsening in LSMI.

Conclusion
Preliminary analysis of available data showed an association between changes in sarcopenia and toxicities, suggesting that sarcopenia could be considered an early condition in breast cancer. We are presently analyzing data on serum drugs concentrations to detect eventual relation between actual drug level, sarcopenia and toxicity. If this findings will be confirmed in larger population, the measure of body composition could be used to personalized dosing of chemotherapy.
Title: Intrathoracic paclitaxel chemotherapy for malignant pleural effusion in breast cancer

Yeu KJ, Park J, Choi JE, Kang SH and Lee SJ. Yeungnam University College of Medicine, Daegu, Republic of Korea.

Body: Background: Malignant pleural effusion in breast cancer has been associated with poor prognosis which median survival rate is 5–16 months. The response rate of local treatment has been very low and in some case, complications have resulted in death.

Patients and methods: We investigated the efficacy and safety of paclitaxel, as an intrapleural chemotherapeutic agent. From January 2006 to June 2015, total 35 times of intrapleural chemotherapy were performed in 26 breast cancer patients who had developed malignant pleural effusion. They were infused 120mg/m2 of paclitaxel through a chest tube, which was clamped for 48 hours. The chest tube was maintained until drainage was reduced to less than 50-100 mL/day.

Results: Mean follow up period after intrapleural chemotherapy was 11 months. The average time of indwelling with a chest tube after intrapleural chemotherapy was 9.7 days.

Mean progression free survival was 7.8 Months. During the follow-up period, 5 patients had no progression of pleural effusion and 2 of them were free from progression for more than 36 months. In 26 attempts, there were effective and could remove chest tube otherwise in 9 attempts, there were no improvement after intrapleural chemotherapy. 1 patients received a second round of ipsilateral intrapleural chemotherapy and had no response unlike earlier attempts. There were 3 severe adverse effect related death caused by respiratory failure with or without G4 neutropenia.

Conclusion: Intrapleural paclitaxel chemotherapy is helpful for some cases of uncontrolled pleural effusion in that reduce the duration of hospital stay and improve quality of life. But it will be determined carefully, considering the side effects and response rate of treatment.
Title: Frozen glove could be a new hope for prevention of chemotherapy induced peripheral neuropathy

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Body: Introduction
Chemotherapy induced peripheral neuropathy (CIPN) is a major problem for patients who receive chemotherapy, and it sometimes deteriorate patients' QOL. Many CIPN prevention trials have been conducted, but no one succeeded to date.

Objectives
To investigate if frozen glove (FG) prevents peripheral neuropathy induced by nanoparticle albumin-bound paclitaxel (nab-PTX).

Methods
We conducted CIPN prevention study using FG, as part of multi-institutional phase II study which analyze efficacy and safety of nab-PTX (260mg/m2 q3w) followed by FEC (500/100/500 mg/m2, q3w) in pre-operative setting (KBCSG-TR 1213 trial). Each patient wore an FGs for a total of 60 minutes (15mins before and after nab-PTX treatment) on both hands. CIPN were assessed during treatment period with nab-PTX by the Patient Neurotoxicity Questionnaire (PNQ) and the FACT/GOG (Gynecologic Oncology Group) Neurotoxicity (Ntx) subscale. Patients were asked to access PNQ and FACT/GOG Ntx on a daily basis and recorded in the CIPN diary.

Results
Sixty two patients were registered for KBCSG-TR 1213 trial. And forty two pts (68%) who turned in the diary were analyzed. Median age and median body mass index (BMI) was 48 years old and 21.6 kg/m2, respectively. We analyzed following 6 categories, 1) symptoms of hands and arms, 2) symptoms of foots, 3) symptoms of general, 4) symptoms of ears 5) muscle weakness of hands and arms and 6) muscle weakness of foots. Median time to each event was 1) 25.5 days, 2) 5days, 3) 3days, 4) not available, 5) 46.5days, 6)4 days. By using FG, time to event of hands and arms was much longer compared with that of foots.

Conclusions
CIPN could be prevented or lessened by FG. Randomized phase II CIPN prevention study has been just launched.
Title: Self-reported symptoms and interference issues in breast cancer patients


BACKGROUND: Breast cancer and its treatments produce multiple symptoms that significantly impact patient quality of life (QOL). Distress and impaired function are the most commonly referred symptoms [Cleeland CS, 2007]. Routine cancer care assessment of patient-reported outcomes (PROs), including symptoms, function, and QOL, has been shown to improve symptom management, identification of psychosocial problems, and patient-provider communication. The Symptom Inventory Tool (SIT) is an assessment tool that captures the patients' perceived symptom burden for real-time clinical intervention, taken at the point of no intervention (baseline) and every 21 days or greater. The SIT is comprised of 27 questions utilizing the M.D. Anderson Symptom Inventory tool (MDASI) [Cleeland CS, Cancer 2013], and validated assessment instrument with 8 questions added and a free text box by Cancer Treatment Centers of America (CTCA). CTCA is a national network of five hospitals that specialize in cancer treatment and integrative oncology.

PATIENTS & METHODS: Patients reported symptoms intensity using 19-item MD Anderson Symptom Inventory (MDASI) and 8 additional questions created by CTCA (constipation, swelling, mouth soreness, bleeding, sexual interest, family, hope & QOL). Symptoms were rated "at the worst" on an 11-point numeric scale ranging from 0 ("no present") to 10 ("as bad as you can imagine") in the previous 24 hours. SIT became an integral part of patient care at CTCA beginning in 2012.

RESULTS: From July 2012 to February 2015, a total of 3,740 outpatients with breast cancer were evaluated at CTCA. A total of 13,852 assessments were analyzed. The assessments consisted of 3,513 completed at baseline, 2,237 completed at the 2nd follow up (FU), and 8,014 completed at 3rd FU or greater. Median age was 50 (range, 17-88), 60% of patients were ER+. Race: White (68%), Black (29%), and other (3%). Disease extension: locoregional (86%) and metastatic (13.6%). The average time since cancer was diagnosed were 35 months, and 50.7% of the patients received prior systemic therapy: chemotherapy (55%), hormone-therapy (41%), and immunotherapy (4%). Mean, standard deviation and inter quartile ranges at baseline assessment are depicted in Table 1.

<table>
<thead>
<tr>
<th>Patient Reported Symptom</th>
<th>Baseline assessment statistics</th>
<th>Percentage of patients with severe symptoms at baseline and reporting a clinically significant change (2 points) at 2nd SIT assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean +/- STD</td>
<td>Interquartile range (IQR)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Significant decrease</td>
</tr>
<tr>
<td>Distress</td>
<td>3.0 +/- 3.0</td>
<td>[0.5]</td>
</tr>
<tr>
<td>Sadness</td>
<td>2.5 +/- 2.9</td>
<td>[0.4]</td>
</tr>
<tr>
<td>Disturbed Sleep</td>
<td>3.3 +/- 3.2</td>
<td>[0.6]</td>
</tr>
<tr>
<td>Mood</td>
<td>2.6 +/- 2.7</td>
<td>[0.4]</td>
</tr>
<tr>
<td>Pain</td>
<td>2.7 +/- 3.0</td>
<td>[0.5]</td>
</tr>
</tbody>
</table>

* IQR is a measure of variability, based on dividing a data set into quartiles. Quartiles divide a rank-ordered data set into four equal parts.

CONCLUSIONS: The SIT was successful in identifying symptoms burden and interference with life issues in breast cancer patients. Distress, sadness, disturbed sleep, mood and pain were the most common reported symptoms. Early identification of patient burden symptoms allowed immediate intervention and improvement in approximately a quarter of patients.
Cultural and religious differences during breast cancer treatment between Dutch and non-Western immigrant women

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Body: Background
Twelve percent of the Dutch population consists of non-Western immigrant women with an incidence of breast cancer that has risen from 2.9% in 2005 to 5% in 2015. For the second generation of these women the incidence of breast cancer is expected to meet the native Dutch population rates soon, partly due to adoption of Western style dietary habits and physical activity. Chemotherapeutic treatment (CT) for breast cancer is associated with increased body fatness and interventions to prevent this increase are currently explored. Whether perceptions on cancer and treatment differ between non-Western immigrant women than for Dutch women needs to be evaluated before these interventions can be set up. This study aimed to explore cultural and religious differences on women’s perceptions of the diagnosis of breast cancer and changes in physical activity and eating habits during chemotherapy treatment.

Methods
A longitudinal qualitative multiple case study was conducted. Newly diagnosed women with breast cancer were recruited and purposively selected (n=23, non-immigrant) from six hospitals in the Netherlands. Semi structured interviews were conducted three times (in total 69): before start of CT, halfway and after CT. In addition 38 women (20 non-immigrant and 18 immigrant) were recruited and interviewed after finishing CT. All interviews (n=107, from 61 women) were audiotaped and transcribed verbatim. A thematic content analysis approach was used.

Results
All 43 non-immigrant women, mean age 51.1 yrs. and 18 immigrant women mean age 43.2 yrs., experienced known side effects from CT. Loss of hair and sometimes the breast appeared to be especially for immigrant women a shocking experience and for some even a loss of femininity. Most of the women perceived to have received incomplete and often unclear information from hospitals about weight and CT treatment. Immigrant women participating the Ramadan during treatment encountered resistance from their physicians. Weight gain during period of CT was higher among immigrant women (mean 13.1 kg, 4-28 kg) than among non-immigrant women (mean 2.5 kg, 2-9 kg). Although both groups said to be less physically active and complained about fatigue, non-immigrant women trying to maintain daily structure and were more active with their diet than most immigrants. Immigrant women expressed cancer as a taboo in their culture impeding them to talk openly about their illness, only when side effects of CT such as hair loss were visible they found it inevitable. Cancer was associated with death at time of diagnosis. For most immigrants and a few non-immigrants this was considered a religious ordeal from Allah or God, these women perceived less influence on their behavior during treatment. Most non-religious women perceived breast cancer as bad luck, stress or heredity.

Conclusion
Especially immigrant women experienced little respect for their culture and insufficient information about treatment. Non-immigrants had a need to actively contribute to their treatment while this need was less obvious for immigrants. Probably because they felt not encouraged by their religion and culture.
Title: Randomized trial of aromatherapy vs. conventional care for breast cancer patients during perioperative periods

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Body: PURPOSE: The aromatherapy has been performed as palliative care of the breast cancer patients but no studies have been reported regarding whether the therapy improved QOL of the patients during perioperative periods or not. Therefore, in this study, we compared the effects on QOL between aromatherapy and conventional therapy during perioperative periods of the patients.

METHODS: We examined Japanese breast cancer patients operated at Nahanishi Clinic, Okinawa, JAPAN. The patients were randomly assigned to a 2:1 ratio to aromatherapy or usual care following the informed consents were obtained. The aromatherapy group had aroma-oil (lavender, orange or ylang-ylang) placed at the bedside from 9 pm until 6 am. QOL of the patients was the primary endpoints and the changes of vital signs and the rate of hypnotic usage as secondary endpoints. QOL was assessed using the EORTC QLQ-C30, in which the Patients completed a baseline QOL assessment at the time of admission, of surgery day (AM7:00) and at the morning of post-operative day 1. Vital signs and hypnotic usage were also recorded at the same time.

RESULTS: Among 249 women screened, we randomized 153 women, with 102 to aromatherapy and 51 to conventional care. QOL tended to be improved among aromatherapy groups in physical functioning and role functioning at the morning of post-operative day 1, but the differences did not reach statistical significance (P = 0.08 and 0.09, respectively). There were no statistically significant differences between two groups in the other points of QOL assessment. Aromatherapy did by no means improve vital signs and the rate of hypnotic usage of the patients.

Global health status, functional scales and symptomatic scales of aromatherapy and usual care assessed by the EORTC QLQ-C30 questionnaire

<table>
<thead>
<tr>
<th></th>
<th>Aromatherapy</th>
<th>Usual care</th>
<th></th>
<th></th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good (≥66.7)</td>
<td>Average (33.3-66.6)</td>
<td>Poor (&lt;33.3)</td>
<td>Good (≥66.7)</td>
<td>Average (33.3-66.6)</td>
<td>Poor (&lt;33.3)</td>
</tr>
<tr>
<td>After operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global health status</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>QOL</td>
<td>47.1</td>
<td>43.1</td>
<td>9.8</td>
<td>33.3</td>
<td>52.9</td>
<td>13.7</td>
</tr>
<tr>
<td>Functional scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>76.5</td>
<td>18.6</td>
<td>4.9</td>
<td>58.8</td>
<td>31.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Role functioning</td>
<td>81.4</td>
<td>8.8</td>
<td>9.8</td>
<td>70.6</td>
<td>21.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Emotional functioning</td>
<td>81.4</td>
<td>16.7</td>
<td>1.9</td>
<td>76.5</td>
<td>19.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Cognitive functioning</td>
<td>85.3</td>
<td>13.7</td>
<td>1.0</td>
<td>90.2</td>
<td>7.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Social functioning</td>
<td>82.4</td>
<td>12.7</td>
<td>4.9</td>
<td>68.6</td>
<td>19.6</td>
<td>11.8</td>
</tr>
<tr>
<td>Symptom scales/items</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>10.8</td>
<td>56.9</td>
<td>32.4</td>
<td>15.7</td>
<td>58.8</td>
<td>25.5</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>2.9</td>
<td>22.5</td>
<td>74.5</td>
<td>2.0</td>
<td>13.7</td>
<td>84.3</td>
</tr>
<tr>
<td>Pain</td>
<td>11.8</td>
<td>53.9</td>
<td>34.3</td>
<td>19.6</td>
<td>49.0</td>
<td>31.4</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>3.9</td>
<td>27.5</td>
<td>68.6</td>
<td>5.9</td>
<td>33.3</td>
<td>60.8</td>
</tr>
<tr>
<td>Insomnia</td>
<td>22.5</td>
<td>48.0</td>
<td>29.4</td>
<td>35.3</td>
<td>39.2</td>
<td>25.5</td>
</tr>
</tbody>
</table>
### Conclusion

Aromatherapy did not improve the QOL including vital signs and the rate of hypnotic usage during perioperative periods but no adverse effects also detected. Therefore, aromatherapy may not be prohibited during perioperative periods of the patients when they asked to the physicians.

<table>
<thead>
<tr>
<th>Appetite loss</th>
<th>8.8</th>
<th>39.2</th>
<th>52.0</th>
<th>7.8</th>
<th>39.2</th>
<th>52.9</th>
<th>.98</th>
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<tbody>
<tr>
<td>Constipation</td>
<td>8.8</td>
<td>34.3</td>
<td>56.9</td>
<td>9.8</td>
<td>37.3</td>
<td>52.9</td>
<td>.90</td>
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<tr>
<td>Diarrhoea</td>
<td>1.0</td>
<td>8.8</td>
<td>90.2</td>
<td>3.9</td>
<td>3.9</td>
<td>92.2</td>
<td>.27</td>
</tr>
<tr>
<td>Financial functioning</td>
<td>16.7</td>
<td>42.2</td>
<td>41.2</td>
<td>25.5</td>
<td>35.3</td>
<td>39.2</td>
<td>.41</td>
</tr>
</tbody>
</table>
Title: Effect of mind and beauty education on body image among young breast cancer patients: A randomized controlled trial

Lee JK, Cho J, Park SK, Kim I-R, Yoon J-H, Choi E-K, Cho S-Y, Lee S-K, Lee JE, Kim S, Nam S-J, Park YH, Ahn JS and Im YH. Cancer Education Center, Samsung Comprehensive Cancer Center, Samsung Medical Center, Sungkyunkwan University School of Medicine; Health Science and Technology, SAHIST, Sungkyunkwan University; Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine and Hematology/Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine.

Body: Background
The proportion of young age-onset breast cancer in Korea is relatively higher than Western countries. Young breast cancer patients are more likely to suffer from altered appearance due to cancer treatment such as breast disfiguration, hair loss, skin change and experience poor body image. This randomized controlled trial (RCT) is designed to evaluate the effect of mind and beauty education program on body image among breast cancer patients under 40-years old.

Methods
A total of 109 eligible breast cancer patients aged 18-40 years old, who had surgery and/or chemotherapy within 18 months and who reported poor body image (<66 EORTC QLQ-BR23 body image score) were recruited and randomly assigned to intervention and control group from July 2014 and April 2015 at an university-based hospital in Seoul, Korea. Intervention group received a structured 8 hours education (2 hours for 4 weeks, 1 hour for mind control and 1 hour for altered appearance management) and control group had education after outcome evaluation. Body image as primary outcome was assessed using both EORTC QLQ-BR23 and body image scale (BIS). In addition, socio-demographic characteristic, self-esteem, quality of life, anxiety, and depression were assessed. Outcomes were evaluated before the intervention, right after the intervention (visit 2), and 3 (visit 3) and 6 months (visit 4) after the intervention. T-test and intention-to-treat analysis performed to compare the outcomes of the two groups.

Results
A total of 54 and 55 patients were assigned to intervention and control group respectively with block randomization. Among the intervention group, 43 participants (79.6%) attended for more than 6 hours of education. Total 46 participants (85.2%) in intervention group and 53 participants (96.4%) in control group completed the questionnaire at visit 2.

Mean age of the study population was 35.5 years old and there were 53 (48.6%), 32 (29.3%), 23 (21.1%) stage I, II, and III breast cancer patients respectively. At baseline, none of the socio-demographic, clinical, psycho-social characteristics were different between two groups. While there was no difference with the body image at baseline between intervention (57.69±20.57) and control group (53.09±26.98) (P=0.327), intervention group reported significantly improved body image than control group (EORTC QLQ-BR23 - Intervention; 71.69±20.27 and Control; 55.97±23.07, P<0.001). The results were similar with BIS measured body image (BIS - Intervention; 17.77±6.29 and Control; 21.29±6.94, P=0.012).

Conclusion
This study provided evidence supporting that mind and body education program would be beneficial to young women with breast cancer who would suffer from low body image. Active education program and psychosocial support related to altered appearance would help young breast cancer patients to make a smooth transit when they return to usual life.

Trial registration: This study is registered in Korean Clinical Research Information Service (CRIS) with registration number KCT0001191.

Funding: This study was supported by grants from Amorepacific.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-10-31

Title: Impact of increased physical activities after diagnosis on fatigue and overall pain during cancer treatment: A prospective cohort study

Lee JK, Kang D, Choi E-K, Kong S, Lee S-K, Lee JE, Han W, Park YH, Ahn JS, Im YH, Noh D-Y, Nam S-J and Cho J. Cancer Education Center, Samsung Comprehensive Cancer Center, Samsung Medical Center; Health, Behavior and Society, SAHIST, Sungkyunkwan University; Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine; Surgery, Seoul National University Hospital and Hematology/Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine.

Body: Background
Existing evidence strongly suggests that exercise is not only safe but also feasible during cancer treatment. Physical activity is recommended for improving multiple post-treatment adverse effects on bone health, muscle strength, and other quality-of-life measures. Yet, limited evidence exists regarding effect of increased physical activity after diagnosis on symptoms management of breast cancer patients.

Methods
A total of 422 patients were recruited from July 2010 to July 2011 at two cancer hospitals in Seoul, Korea. Physical activity in sports (PAS) was assessed using Minnesota Leisure Time Physical Activity Questionnaire before and 2 weeks, 3-, 6-, 12-, 24- and 36-months after diagnosis. Physical symptoms including fatigue, pain, arm symptom, and insomnia were measured using EORTC-C30 and BR23. Growth mixture models were used to identify trajectory classes of physical activity patterns. Multivariate analysis was used to find impact of PAS on symptom management using SAS.

Results
Three distinct PAS groups were identified according to 3-year change patterns: moderate to moderate (MM): 40.8%, none to moderate (NM): 31.1% and moderate to high (MH): 28.1%. The LM and MH group increased PAS from diagnosis but it began to decrease from 1 year after diagnosis. Compared to the MM, the NM and MH reported significantly lower level of fatigue (MM:40.7, NM:32.2, MH:33.7), pain(MM:28.0, NM:25.6, MH:20.6), systemic therapy side effects (MM:26.9, NM:22.6, MH:21.8), and breast symptoms (MM:25.4, NM:21.7, MH:20.2) during active treatment (6 months after diagnosis)

Change patterns of quality of life according to trajectory groups

<table>
<thead>
<tr>
<th>At diagnosis</th>
<th>2 weeks</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>24 months</th>
<th>36 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatigue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>31.3±1.9</td>
<td>30.2±1.9</td>
<td>35.3±2.0¹</td>
<td>40.7±2.1¹</td>
<td>37.8±2.1¹</td>
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</tr>
<tr>
<td>NM</td>
<td>30.2±1.9</td>
<td>28.2±1.9</td>
<td>31.9±2.1</td>
<td>32.2±2.2²</td>
<td>33.6±2.1</td>
<td>35.6±2.2¹</td>
</tr>
<tr>
<td>MH</td>
<td>28.8±2.3</td>
<td>27.4±2.2</td>
<td>33.0±2.4</td>
<td>33.7±2.5¹²</td>
<td>33.9±2.4¹</td>
<td>36.2±2.5¹</td>
</tr>
<tr>
<td><strong>Pain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>15.1±1.5</td>
<td>31.3±1.9¹</td>
<td>23.2±2.0¹</td>
<td>28.0±2.0¹</td>
<td>23.5±2.0¹</td>
<td>22.0±2.0¹</td>
</tr>
<tr>
<td>NM</td>
<td>15.4±1.5</td>
<td>32.4±2.0¹</td>
<td>23.3±2.0¹</td>
<td>25.6±2.1</td>
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<td>21.1±2.1</td>
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<tr>
<td>MH</td>
<td>17.2±1.8</td>
<td>28.4±2.3¹</td>
<td>21.2±2.3</td>
<td>20.6±2.4²</td>
<td>19.7±2.3</td>
<td>21.1±2.3</td>
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<tr>
<td><strong>Systemic therapy side effects</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>16.8±1.1</td>
<td>14.3±1.2¹</td>
<td>33.2±1.7¹</td>
<td>26.9±1.5¹</td>
<td>25.4±1.5¹</td>
<td>26.2±1.6¹</td>
</tr>
<tr>
<td>NM</td>
<td>15.0±1.1</td>
<td>14.5±1.2</td>
<td>35.2±1.7¹</td>
<td>22.6±1.6¹</td>
<td>22.0±1.6¹</td>
<td>24.5±1.7¹</td>
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<tr>
<td>MH</td>
<td>15.6±1.4</td>
<td>12.9±1.4¹</td>
<td>34.4±2.0¹</td>
<td>21.8±1.8¹²</td>
<td>21.8±1.7¹</td>
<td>22.1±1.9¹</td>
</tr>
<tr>
<td><strong>Breast symptoms</strong></td>
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<td></td>
<td></td>
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<tr>
<td>MM</td>
<td>13.8±1.2</td>
<td>26.4±1.6¹</td>
<td>20.8±1.5¹</td>
<td>25.4±1.6¹</td>
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<td>19.2±1.7¹</td>
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<tr>
<td>NM</td>
<td>13.0±1.2</td>
<td>24.7±1.6¹</td>
<td>19.6±1.5¹</td>
<td>21.7±1.6¹²</td>
<td>22.3±1.7¹</td>
<td>19.9±1.8¹</td>
</tr>
<tr>
<td>MH</td>
<td>16.0±1.4</td>
<td>24.1±1.8¹</td>
<td>19.4±1.8</td>
<td>20.2±1.8¹²</td>
<td>17.8±1.8²</td>
<td>17.4±2.0</td>
</tr>
</tbody>
</table>
Conclusion
The results of the study confirmed that increased physical activity after diagnosis, even with patients who did not exercise at all before diagnosis, helps to control fatigue, pain, systemic side effects, and breast symptoms during treatment. It is necessary to find ways to promote physical activity after diagnosis and help patients to stay active during treatment.
Title: Depression and anxiety after adjuvant ovarian function suppression in premenopausal breast cancer patients


Body: Purpose
The results of the Suppression of Ovarian Function Trial (SOFT) and the Tamoxifen and Exemestane Trial (TEXT) showed that ovarian function suppression (OFS) in premenopausal early breast cancer patients improves disease control. However, mood swings after OFS is one of the chief complaints to make patients stop undergoing endocrine therapy. Studies about complications of OFS in breast cancer patients are not established well. We designed this randomized controlled trial to evaluate psychological functioning of patients after undergoing adjuvant OFS by goserelin.

Patients and Methods
We randomly assigned 64 premenopausal women with hormone receptor positive early breast cancer to the tamoxifen or tamoxifen plus goserelin group for a period of 1 year. Participants were screened for depression and generalized anxiety disorder using Hamilton Rating Scale for Depression (HAMD), Hamilton Rating Scale for Anxiety (HAM-A), Anxiety Sensitivity Index (ASI) and Albany panic and phobia questionnaire (APPQ) at baseline, 6 months and 12 months. Brain-derived Neurotrophic Factor (BDNF) levels were measured, as well. The results were analyzed by using a linear mixed model and a generalized linear mixed model.

Results
Thirty two patients were distributed in each group, equally. Linear mixed-mixed model analyses revealed that, compared with HAM-A scores of each group at baseline, HAM-A scores at 12 months showed increments (p=0.0078). Among HAM-A questions, Questions for intellectual, sensory and autonomic status were scored significantly high at 12 months (p=0.0018, p=0.0132, p=0.0006). Platelet BDNF levels reported a statistically significant rise at 12 months (p=0.0006). There was no significant time-by-study group effect in all scales.

Conclusion
Compared with the patients without OFS, patients with Goserelin showed no difference in anxiety or depression scales. Thought the levels of anxiety of each group at 12 months were increased, they do not indicate medical interventions. Patients with increased levels of BDNF at 12 months are expected to have good recovery from anxious and depressive symptoms.
Title: Psychological morbidity in breast cancer survivors: Prevalence rates and determinants

Sztankay M, Oberguggenberger A, Meraner V, Egle D, Mangweth-Matzek B, Beer B, Huber N, Sperner-Unterweger B and Hubalek M. Innsbruck Medical University, Innsbruck, Austria; Innsbruck Medical University, Innsbruck, Austria and Institute of Legal Medicine and Core Facility Metabolomics, Innsbruck Medical University, Innsbruck, Austria.

Body: Background: The number of breast cancer survivors (BCS) is steadily increasing due to improved treatment options, early detection and younger age at diagnosis. Thus, it is increasingly important to determine and better understand the psychological outcome following a cancer diagnosis and treatment in long-term. This might contribute to meeting the long-term health care demands of cancer survivors. We aimed at investigating levels and determinants of anxiety and depression (AD) in BCS.

Patients and Methods: We included BCS with a non-metastatic disease in the stage of after-care. AD was determined as part of a cross-sectional, comprehensive patient reported outcome (PRO) assessment (incl. Functional Assessment of Cancer Therapy-G/+B/+ES, Eating Disorder Examination-Questionnaire, Sexual Activity Questionnaire and Body Image Scale) using the Hospital Anxiety and Depression Scale (HADS). Prevalence rates of AD and sample characteristics are presented descriptively using percentages, means and standard deviations. Predictors of anxiety and depression are identified by means of regression analysis.

Results: A final sample of 743 breast cancer survivors who were on average 2.9 years post diagnosis (range: 0.1-11.3 years) participated in the study. Mean patient age was 56.4a (SD 11.5a), 2/3 of patients were postmenopausal. 22.5% of patients reported clinically relevant levels of anxiety and 11.2% of depression. Older age (β=0.012, t=2.53, p<0.05), higher endocrine symptoms (β=-0.037, t=-8.89, p<0.01) and reduced functional well-being (β=-0.034, t=-7.73, p<0.01) were predictive for anxiety and depression in the regression model. The model explained 39.3% of the variance of anxiety and depression.

Conclusion: A distinct proportion of BCS report clinically relevant, long-term psychological morbidity. Especially older BCS, experiencing higher levels of endocrine symptoms and reduced functional well-being, seem to be at risk for psychological morbidity. A routine PRO-screening for psychological morbidity including the assessment of associated risk factors in this patient population might contribute to the identification of those women in need for psychological/ psychiatric treatment and in conjunction, improve cancer care.
Patient reporting pain intensity immediately after surgery can be associated with underlying depression in women with breast cancer

Kim YS, Lee JW, Kim J, Lee SB, Yu J, Ko BS, Kim HJ, Son BH and Ahn SH. Chosun University College of Medicine, Gwangju, Korea and University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

**Body: Objective**
The aims of this study were to determine the prevalence of severe, definite depression symptoms, as measured using the Center for Epidemiological Studies Depression Scale (CES-D), and the association between high CES-D scores (i.e., ≥25) and sociodemographic and perioperative factors during perioperative period.

**Methods**
Among 1690 consecutive breast cancer patients who were admitted for definitive breast surgery during the study period, 1499 patients were included in this study. Patients with a past medical history of psychiatric medication or support, a plan for elective surgery due to locoregional recurrence or any metastatic disease were excluded. The CES-D score was checked 1 day before definitive surgeries. The sociodemographic data and perioperative data were analyzed.

**Results**
The mean CES-D score was 18.5, with 24.1% (362/1499) and 56.7% (850/1499) having high CES-D scores of ≥25 and ≥16, respectively. Multivariate analysis revealed that the number of family members with any malignancy (≥2 vs 0), sedative medication (yes vs no) and postoperative numeric rating scale (NRS) scores (persistent, severe pain vs stably mild pain) were significant associated factors for severe, definite depression symptoms [CES-D score of ≥25: adjusted odds ratio (OR)=1.56, 95% confidence interval (CI)=1.10–2.21, P=0.013; adjusted OR=1.65, 95% CI=1.00–2.71, P=0.048; and adjusted OR=2.14, 95% CI=1.15–3.95, P=0.016, respectively].

**Conclusion**
Depression may increase the intensity of postoperative acute pain. Self-reporting of persistent postoperative pain intensity is potentially useful in detecting hidden depression symptoms in breast cancer patients during the perioperative period.
**Title:** Long-term patient satisfaction with cosmetic outcome and psychosocial wellbeing after breast conserving therapy is affected only by lumpectomy volume


**Body:** *Introduction:* Breast conserving therapy (BCT) is considered the treatment of choice for early stage breast cancer by National Cancer Institute guidelines. Little data exists on patient-reported satisfaction and quality of life outcomes after lumpectomy with radiation. This study aims to identify factors influencing satisfaction with cosmetic outcome and quality of life in patients receiving BCT using a validated instrument.

**Methods:** All patients treated with lumpectomy and radiation for breast cancer at our institution from 1997-2012 received a mailed questionnaire containing the BREAST-Q breast conservation module (graciously provided by Dr A. Pusic, Memorial Sloan Kettering Cancer Center), a validated quality of life survey instrument. A retrospective chart review was performed for survey responders for demographic, treatment, and staging information. Scores were calculated for satisfaction with appearance of the breast, adverse effects of radiation, sexual wellbeing, psychosocial wellbeing and physical wellbeing: upper body and arm. Pearson correlation coefficients were obtained. Wilcoxon rank-sum and one-way ANOVA were used to identify associations between patient variables and satisfaction scores. Multivariate regression was used to assess confounding variables.

**Results:** A total of 110 questionnaires (response rate of 29.5%) fit criteria for analysis. The mean age of respondents was 65.9±11.2 yrs, and mean time since diagnosis was 91.8±53.1 mos. We observed the strongest correlations between satisfaction with breast appearance and sexual wellbeing (r=0.66, p<0.01), breast appearance and psychosocial wellbeing (r=0.62, p<0.01), and fewer effects of radiation and physical wellbeing (r=0.65, p<0.01). Lumpectomy volume was associated with decreased satisfaction with breast appearance (r=-0.32, p <0.01) and psychosocial wellbeing (r=-0.19, p<0.05). There was no correlation between satisfaction with breast appearance and patient age, time since surgery, history of re-excision, stage or localization technique. Patients with older age at diagnosis reported significantly fewer effects of radiation and better psychosocial, physical, and sexual wellbeing (all p<0.05) (Table1). The incidence of recurrence was 2.7% and did not impact satisfaction scores.

**Conclusions:** In women undergoing BCT, patient satisfaction with appearance of the breast and psychosocial wellbeing at 7.6 years of follow-up correlated with the volume of tissue removed but no other patient or tumor characteristics. Increasing age at diagnosis was associated with greater satisfaction in multiple domains. These results emphasize the importance of precise surgical technique and patient selection in order to achieve long-term patient satisfaction with BCT.

### Distribution of Satisfaction/Quality of Life Outcomes by Age at Diagnosis

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Breast</th>
<th>Adverse Effects of Radiation</th>
<th>Psychosocial</th>
<th>Sexual</th>
<th>Physical</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45</td>
<td>14</td>
<td>58±22</td>
<td>82±19</td>
<td>73±19</td>
<td>45±20</td>
<td>74±16</td>
</tr>
<tr>
<td>45-55</td>
<td>32</td>
<td>64±26</td>
<td>83±19</td>
<td>80±20</td>
<td>54±25</td>
<td>79±17</td>
</tr>
<tr>
<td>56-60</td>
<td>26</td>
<td>67±17</td>
<td>94±9</td>
<td>83±23</td>
<td>70±18</td>
<td>85±13</td>
</tr>
<tr>
<td>61-65</td>
<td>12</td>
<td>67±29</td>
<td>89±23</td>
<td>83±20</td>
<td>53±29</td>
<td>83±17</td>
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<tr>
<td>&gt;65</td>
<td>26</td>
<td>70±23</td>
<td>91±12</td>
<td>88±16</td>
<td>63±25</td>
<td>88±25</td>
</tr>
<tr>
<td>All</td>
<td>110</td>
<td>65±23</td>
<td>88±16</td>
<td>82±20</td>
<td>58±24</td>
<td>82±19</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.159</td>
<td>0.019</td>
<td>0.014</td>
<td>0.030</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Rasch scores range from 0-100 where 100 indicates highest satisfaction*
Title: Chemotherapy-induced alopecia prevention and effects on quality of life among women with breast cancer: A study of a scalp-cooling system used in a Mexican public hospital

Nurses' ratings indicated that hair loss frequency was constantly lower, at each cycle of chemotherapy, in study patients with scalp-cooling system (n = 110) than in those without (n = 100). Differences between the two groups were statistically significant at cycles 1 and 4 (P < 0.047). Scalp cooling was generally very well tolerated; only four of 110 patients discontinued use of the cold cap due to discomfort. Alopecia was considered among the most distressing problems and a trend towards higher well-being was found in successfully scalp-cooled patients, as indicated by a general better health-related quality of life, whereas unsuccessfully scalp-cooled patients reported lowest well-being.

Conclusions: This study demonstrates that scalp cooling was an effective and safe method of protection against hair loss caused by chemotherapy and contributes to the well-being of the patients. Its routine use as part of neo or adjuvant chemotherapy should be seriously considered and should be clinically evaluated in a randomized trial and in studies using other chemotherapy regimens to determine optimal temperatures and durations of cooling for maximal efficacy.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P1-11-06

**Title:** The experience of caregivers of women with metastatic breast cancer: Insights from the Make Your Dialogue Count survey

Mayer M, Sampayo I, Bell Dickson R, Citron ML L and Brufsky AM M. AdvancedBC.org, NY, NY; SHARE, NY, NY; Harris Poll, NY, NY; ProHEALTH Care Associates, Lake Success, NY and University of Pittsburgh Medical Center, Pittsburgh, PA.

**Body:**

Introduction: Caregivers of patients with breast cancer have demonstrated persistent unmet needs, including reduced physical and psychosocial well-being. This may be particularly acute in caregivers of patients with metastatic breast cancer (MBC), whose ongoing treatments and increasing disability with disease progression offer particular challenges. While prevalence of MBC is currently unknown, caregivers of women with MBC represent a sizable group.

Objective: To explore the emotional, psychological, and social impact on caregivers of patients with MBC and to identify gaps in communications between patients and their caregivers and oncologists.

Methods: The "Make Your Dialogue Count" survey was conducted online, by paper, and by telephone (June-August 2014) among caregivers in the United States (age \(\geq 18\) y) who attended \(\geq 50\%\) of doctor visits of women with MBC (age \(\geq 21\) y). Survey responses were single- or multiple-response, numeric text, or rated on a 4-point Likert scale. Caregiver data were unweighted and representative only of those responding. Statistically significant differences between groups were determined by standard t-test of column proportions and means at the 95% confidence level.

Results: 234 caregivers responded; 73% were men, 44% were the patient's spouse/partner, and 27% were nonwhite. Median age was 44 y. The caregivers' loved ones had a median time from initial breast cancer diagnosis of 44 months prior, and 51% had recurred after early breast cancer. Most caregivers (76%) considered themselves to be extremely or very involved in treatment decisions, but a sizable fraction of caregivers were unaware of the HR (20%) or HER2 (29%) status of the patient's cancer, indicating a lack of basic information needed for informed decision-making. While most (93%) said they felt comfortable speaking with the treatment team about MBC treatment, 41% reported communication barriers. Over half of caregivers (53%) felt that nobody understands what they're going through, and most (86%) reported that their lives had been negatively affected in some way (such as sleep habits, relationships and social life, hobbies and personal time, and financial stability and employment). Most caregivers considered caregiving to be an emotional (77%) and physical (56%) burden and 36% felt unappreciated. At the time of initial MBC diagnosis, 69% of caregivers felt it was important/very important for their loved one's doctor to refer them to support services, but only 25% of caregivers reported receiving such a referral. Subgroup analysis of caregiver's gender revealed some differences in responses relating to communications with oncologists, treatment experience, and emotional impact.

Conclusions: While committed to their roles, these caregivers often found their role to be a physical and emotional burden, and many reported feeling isolated and unappreciated for their caregiving. Our findings indicate a strong need for support services specifically tailored to caregivers, including outreach to address emotional, financial, and practical needs stemming from caring for a loved one with MBC. They also indicate a need for improved disease and treatment information exchange between caregivers, patients, and healthcare providers.
The relationship between breast cancer progression and workplace productivity in the US


Background: A significant proportion of women with breast cancer leave employment due to their disease. Little is known about the effects of breast cancer progression on productivity among those who remain employed. We sought to determine the effect of disease progression on workplace productivity among women with breast cancer.

Methods: By linking health insurance claims data to workplace productivity data, a longitudinal dataset of women with breast cancer was constructed. The study cohort consisted of commercially insured women aged 18 to 64 in the US who were treated for any type of breast cancer between 2005 and 2012. Disease stage was measured through diagnosis codes and treatments observed, to classify women into the following breast cancer groups in each 90-day quarter: local; locally advanced; other non-metastatic; metastatic, 1st line therapy; metastatic, 2nd line therapy; metastatic, ≥ 3rd line therapy; metastatic, end-of-life care. Progression was defined as movement to a more advanced disease stage. Workplace productivity was measured as employment status and total hours away from work per quarter. Covariates included employer industry, comorbidities, age, region of residence, and a time trend. Reduced workplace productivity was valued using average U.S. wages by industry. Kaplan Meier analysis was used to test whether women whose cancer progressed were more likely to drop out of our employment-based sample. Linear and Heckman models were used to measure the effect of disease progression on workplace hours missed. The Heckman model was used to correct for selection bias, given that healthier women may be more likely to remain in our employment-based dataset.

Results: The study cohort included 6,409 women. Mean patient age was 52.0 years (SD: 7.7). The mean number of Charlson comorbidities was 0.52 per patient (SD: 2.9). The majority of our employment-based sample had non-metastatic breast cancer (90.7%). Breast cancer progression was associated with a lower probability of employment (hazard ratio = 0.65, P<0.01). Patients who left our employment-based dataset by the 12th quarter had a greater number of comorbidities (P<0.01) and missed a greater number of hours in the first two quarters (P<0.1), compared with those who remained. This indicated that patients leaving our employment-based sample were less healthy than those who stayed, supporting the use of the Heckman model. According to the Heckman results, progression was associated with increased workplace hours missed per quarter, both when comparing early versus late stage (P<0.001), and first-line versus later-line metastatic therapy (P<0.05). Linear results were similar. Using the Heckman results, the annual valuation of work missed per patient was $29,881 for patients without metastases and $34,141 for patients with, indicating that progression to metastatic cancer adds an additional $6,500 of lost work time, or about 14% of average US wages.

Conclusions: Breast cancer progression leads to increased workplace hours missed, with greater hours missed among those with more advanced disease. Avoiding or delaying disease progression could bring productivity gains to the workplace in addition to the benefits to the patient.

Support: This study was funded by Pfizer Inc.
Title: Abstract Withdrawn
Sequential versus concurrent administration of epirubicin and docetaxel as adjuvant chemotherapy in women with high-risk axillary lymph node negative early breast cancer. An interim analysis of a multicenter randomized study from the Hellenic oncology research group


Purpose: To compare the sequential versus the concurrent administration of epirubicin and docetaxel as adjuvant therapy in high risk axillary node negative women with early breast cancer.

Patients and treatment: Women 18-75 years old with invasive breast adenocarcinoma surgically resected with no infiltrated axillary lymph nodes and absence of metastatic disease were randomized to receive 4 cycles of epirubicin 90mg/m2 followed by 4 cycles of docetaxel 75mg/m2 (sequential regimen) or 6 cycles of epirubicin 75mg/m2 followed by docetaxel 75mg/m2 (concurrent regimen). All chemotherapy cycles were administered every 21 days with prophylactic G-CSF support for days 3-10 only for the concurrent regimen. Stratification was based on menopausal status, tumor size and hormone receptor expression. By protocol amendment in 2008 women with HER2 positive tumors were excluded. The primary endpoint of the study was to compare the disease-free survival (DFS) at 5 years and 329 patients were scheduled to enroll on each arm.

Results: Between 2001-2013, 658 women were randomized and received the sequential (n=329) or the concurrent (n=329) regimen. The median age was 53 and 52 years, premenopausal status 43.8% versus 44.1%, tumor size <2cm in 44.1% versus 44.4%, histological grade 3 tumor in 52% versus 53.5% and hormone receptor negative disease in 33.1% versus 37.4% of patients in the sequential and concurrent regimens, respectively. After a median follow up of 70.5 and 70 months, there were 29 (8.8%) versus 42 (12.8%) disease relapses (p=0.102) and 11 (3.3%) versus 19 (5.8%) deaths (p=0.135), in the sequential and concurrent arms, respectively. The median DFS has not yet been reached in either arm (p=0.053) and the 5-year DFS rates were 92.6% versus 88.2% for sequential and concurrent arms, respectively. Dose reduction was required in 1.2% versus 3% (p=0.001) of the treatment cycles in the sequential and concurrent arms, respectively. Toxicity included grade 2-4 neutropenia in 54% versus 41% (p=0.001), febrile neutropenia 2.7% versus 6.1% (p=0.06), anemia 12% versus 17% (p=0.07), nausea/vomiting 18.5% versus 12.4% (p=0.03) of patients in the sequential and concurrent arms, respectively. There were no toxic deaths.

Conclusion: In this interim analysis both the efficacy and the toxicity profile seem to favor the sequential over the concurrent regimen.
Title: Short term quality of life with epirubicin-fluorouracil-cyclophosphamid (FEC) and sequential epirubicin/cyclophosphamid-docetaxel (EC-DOC) chemotherapy in patients with primary breast cancer – Results from the prospective multi-center randomized Adebar trial

Schwentner L, Harbeck N, Singer S, Eichler M, Rack B, Forstbauer H, Wischnik A, Scholz C, Fink V, Huober J, Friedl T, Weissenbacher T, Härtl K, Kiechle M and Janni W. University Ulm, Germany; Breast Cancer Center, University of Munich, Germany; IMBEI, University of Mainz, Germany; University Munich, Germany; Oncology Rhein-Sieg, Germany; Hospital Augsburg, Germany; Fresenius University of Applied Science, Germany and Technical University Rechts der Isar, Munich, Germany.

Body: Background:
The grade of recommendation for adjuvant dose-dense chemotherapy in patients with high risk primary breast cancer is heterogeneous among international guidelines. Understanding the impact on quality of life (QOL) by adjuvant dose dense chemotherapy in comparison to standard adjuvant chemotherapy is thereby a crucial factor, especially if the benefit is potentially low. This study aims to assess the impact on QOL by adjuvant dose dense chemotherapy in the prospective randomized multi-center ADEBAR trial.

Methods:
QOL was assessed at baseline (t1), before cycle 4 FEC (Epirubicin 60mg/m2 i.v. d 1 + 8, 5-Fluoruracil 500mg/m2 i.v. d 1 + 8, Cyclophosphamide 75mg/m2 p.o. d 1–14, q4w x 6) and cycle 5 EC-DOC (Epirubicin 90mg/m2 plus Cyclophosphamide 600mg/m2 q3w x 4, sequentially followed by Docetaxel 100mg/m2 q3w x 4) (t2), 4 weeks after chemotherapy (t3), 6 weeks after radiation (t4) and 1 year after baseline (t5) using the European Organization for Research and Treatment for Cancer (EORTC) Quality of Life Core Questionnaire (QLQ-C30) and the Breast Cancer-Specific Module (QLQ-BR23). A multivariate mixed model was fitted to test for differences between the two treatment arms. Primary endpoint was global QOL, secondary endpoints physical functioning, nausea&vomiting, fatigue and systemic therapy side effects. A minimum clinically meaningful difference was considered to be 10 points.

Results:
1306 patients were recruited between 3/2002 and 5/2005 675 were assigned to the FEC and 688 to the EC-DOC arm. Compliance to QOL assessment was 74% at baseline and 58% four weeks after therapy, but dropped to 11% after one year follow up. After the beginning of treatment global QOL dropped in both arm by 3 to 4 points. In the EC-DOC arm QOL dropped further at t3 by 7 points and stayed stable in the FEC arm. 6 weeks after radiation QOL exceeded baseline in both arms by 6 to 8 points. The differences between treatment arms were strongest at t3 (54.1 vs. 49.7) but did not reach clinical relevance at any point in time. Physical functioning, nausea vomiting, fatigue and systemic therapy side effects followed with some minor exceptions similar patterns, but showed higher amplitudes.

Conclusion:
In conclusion we could not detect a statistically significant difference between the two treatment arms in QOL parameters, indicating that dose dense adjuvant chemotherapy did not impact QOL at a clinically relevant level compared to standard adjuvant chemotherapy.
**Title:** A phase 2 study of eribulin in breast cancer not achieving a pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC)

Yardley DA A, Peacock N, Shroff S, Molthrop, Jr DC C, Anz B, Daniel BR R, Young RR R, Weaver R, Harwin W, Webb CD D, Ward P, Shastry M, DeBusk LM M, Midha R, Hainsworth JD D and Burris III HA A. Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN; Florida Hospital Cancer Institute, Orlando, FL; Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Chattanooga, TN; The Center for Cancer and Blood Disorders, Fort Worth, TX; Sarah Cannon Research Institute/Florida Cancer Specialists, St Petersburg, FL; Sarah Cannon Research Institute/Florida Cancer Specialists, Fort Myers, FL; Baptist Health Louisville, Louisville, KY; Sarah Cannon Research Institute/Oncology Hematolog Care, Inc, Cincinnati, OH and Sarah Cannon Research Institute, Nashville, TN.

**Body:**

**Background:** Residual breast cancer after NAC is associated with a high risk of recurrence. Little evidence supports the use of further chemotherapy in this setting. Eribulin, an inhibitor of microtubule dynamics, demonstrated a survival advantage in patients with metastatic breast cancer who had progressed after previous anthracycline and taxane therapy. This phase 2 trial assessed the efficacy of eribulin (2-yr disease-free survival) administered postoperatively to breast cancer pts not achieving a pCR following standard NAC.

**Methods:** Women with invasive breast cancer (stage T1-4b, N0-2, M0 at diagnosis) and evidence of residual cancer (>5 mm) in the breast or axillary lymph nodes (LN) following ≥4 cycles of standard anthracycline and/or taxane-containing NAC were eligible. Additional eligibility criteria: age ≥18 yrs, peripheral neuropathy ≤ 1, adequate hematologic, hepatic, and renal function. 3 groups were studied: Cohort A-triple negative (TN), Cohort B-HR+/HER2-, Cohort C-HER2+. After recovery from definitive surgery, all pts received eribulin mesylate 1.4mg/m^2^ IV on days 1 and 8 every 21 days for 6 cycles. Cohort C pts also received trastuzumab 6mg/kg IV day 1 every 21 days for a total of 1 yr from start of NAC. Adjuvant hormonal therapy and loco-regional radiotherapy were administered per institutional guidelines. We hypothesized post-operative eribulin would result in a 40% increase over the reported 40% 2 yr DFS for TN, and a 15% increase over the reported 80% 2 yr DFS for HR+/HER2- pts who did not achieve pCR following standard NAC.

**Results:** 127 pts were enrolled (54, Cohort A; 42, Cohort B; 31, Cohort C). Pts on Cohort C continue with study treatment. Here, we present the results of 95 pts treated on Cohorts A and B. Median age-52 yrs (range, 27-74). 87 pts (92%) had invasive ductal adenocarcinoma, 6 (6%) invasive lobular, 1 (1%) mucinous, and 1 (1%) unknown; 34 pts (36%) had T3 or T4 tumors and 65 (68%) had N1-2 disease at diagnosis. NAC with anthracyclines was administered to 74 pts (78%), taxanes to 88 (93%), and 72 (76%) received both. 71 pts (75%) had mastectomies, 24 (25%) had breast conserving surgery. Median residual tumor was 17.5 mm (range 0.1 to 80); 60 pts (63%) were LN+. 78 pts (81%) completed the planned 6 cycles of eribulin. Adjuvant radiation was administered in 28 pts (30%). 3 pts discontinued treatment due to toxicity (1 each with G3 neutropenia, G3 nausea, and unknown grade neuropathy). The most common treatment-related G3/4 adverse events were neutropenia [29 pts (31%)] and leukopenia [10 pts (11%)]. 3 pts (3%) had G3/4 febrile neutropenia and 2 pts (2%) had G3/4 neuropathy. Growth factors were administered to 22 pts (24%). There were no treatment-related deaths. With a median follow up of 19.2 and 14.9 months for Cohorts A and B respectively, the 2 yr DFS probabilities calculated from date of surgery were 61.1 % (95% CI-41.2-76.0) for Cohort A; 82.2% (95% CI-60.2-92.7) for Cohort B.

**Conclusions:** The addition of eribulin is safe and feasible in pts who do not achieve pCR following anthracycline and/or taxane based NAC. At a median follow up of 19.2 months, a statistically significant improvement in the estimated 2 yr DFS was evident in the TN (Cohort A) pts.
Phase 2 study of dose-dense doxorubicin and cyclophosphamide followed by eribulin mesylate with or without prophylactic growth factor for adjuvant treatment of early-stage breast cancer

Cadoo K, Kaufman PA A, Hudis C, Chang C, Berrak E, Song J, Seidman AD D and Traina TA A. Memorial Sloan-Kettering Cancer Center, NY, NY; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH and Eisai inc, Woodcliff Lake, NJ.

Body: Background: Eribulin has demonstrated antitumor activity and significantly improved overall survival (OS) in patients (pts) with heavily pretreated locally advanced/metastatic breast cancer (BC). This trial assessed the feasibility of eribulin as adjuvant therapy following dose-dense doxorubicin and cyclophosphamide (AC) for pts with human epidermal growth factor receptor 2 (HER2)-negative early-stage BC.

Methods: Pts with HER2(-), stage I–III, invasive BC were enrolled. Pts received dose-dense AC (doxorubicin 60 mg/m² IV and cyclophosphamide 600 mg/m² IV) on D1 of each 14-day cycle for 4 cycles with pegfilgrastim, followed by 4 cycles of eribulin (1.4 mg/m² IV) on D1 and D8 every 21 days. Pts were divided into 2 cohorts: Cohort 1 did not receive any prophylactic growth factor (GF); Cohort 2 received a short course of prophylactic GF (filgrastim) on days 3, 4, 10, and 11 of each eribulin cycle. Primary endpoint of feasibility was determined as %pts who completed eribulin portion of the regimen without a dose delay (>2 days) or reduction due to eribulin-related adverse event (AE). Based on similar previous studies, the target for feasibility was 80%. Relative dose intensity of eribulin and toxicities were also summarized by cohort. Exploratory objectives include efficacy endpoints of 3-yr disease-free survival and OS.

Results: We report data from 81 pts (55 Cohort 1; 26 Cohort 2) enrolled in the study, of whom 88% completed study treatment. Pt characteristics include median age 49 yrs (range 26–69), ECOG status 0 (85%), BC stages 1/2/3 (21%/57%/22%). Of 90% (73/81) pts evaluable for feasibility, 27% and 40% of pts in Cohorts 1 and 2, respectively, had dose delay or reduction during eribulin treatment, indicating the primary endpoint was not met. Overall, results were similar between the 2 cohorts (Table). Median duration of treatment with eribulin was 10.14 weeks in both cohorts (vs 10 weeks planned). Most eribulin-related dose delays were due to grade 3 (n=18) or grade 4 (n=7) neutropenia. Non-fatal serious AEs were observed in 11% of pts in Cohort 1 and 15% in Cohort 2. Discontinuations due to AEs occurred in 6% of pts in Cohort 1 and 0 in Cohort 2. Neutropenia (all grades) was reported in 36% of pts in Cohort 1 and 42% in Cohort 2. Most common AEs (all grades) were fatigue (96%), nausea (75%), alopecia (73%), hot flush (63%), and constipation (57%).

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>Eribulin</th>
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<tr>
<td></td>
<td>Cohort 1*</td>
<td>Cohort 2*</td>
</tr>
<tr>
<td>Relative dose intensity, mean</td>
<td>99.5%</td>
<td>99.0%</td>
</tr>
<tr>
<td>Completed all planned doses</td>
<td>98.2%</td>
<td>96.2%</td>
</tr>
<tr>
<td>Dose modification†</td>
<td>12.7%</td>
<td>15.4%</td>
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</table>

GCSF, granulocyte-colony simulating factor. *With pegfilgrastim 6 mg given subcutaneously on D2 of each AC cycle; † including dose delays (>2 days)/reduction/interruptions, missing, and permanent discontinuation due to AE.

Conclusions: The primary study endpoint of >80% feasibility of planned dose delivery without any dose delays or reduction was not met. However, adjuvant treatment with dose-dense AC-eribulin was given safely, with two-thirds (67%) of pts achieving full dosing with no dose delay or reduction. Investigation into alternative dosing schedules or GF support is recommended.
Title: UCBG intergroup: 3-years efficacy results of the Unicancer-PACS08 trial including poor prognosis patients treated with docetaxel or ixabepilone in adjuvant setting

Campone M, Lacroix-Triki M, Roca L, Spielmann M, Wildiers H, Cottu P, Kerbrat P, Levy C, Mayer F, Bachelot T, Wiston T, Eymard J-C, Uwer L, Machiels J-P, Verhoeven D, Jaubert D, Facchini T, Orfeuvre H, Canon J-L, Asselain B, Lemonnier J and Roché H. ICO Saint Herblain, Saint Herblain, France; IUCT Claudius Régaud, Toulouse, France; ICM, Montpellier, France; Gustave Roussy, Villejuif, France; Catholique Universiteit, Leuven, Belgium; Institut Curie, Paris, France; Centre Eugène Marquis, Rennes, France; Centre François Baclesse, Caen, France; Centre Georges-François Leclerc, Dijon, France; Centre Léon Bérard, Lyon, France; Mayo Clinic Florida, Jacksonville, FL; Institut Jean Godinot, Reims, France; ICL Centre Alexis Vautrin, Nancy, France; Saint Luc University, Bruxelles, Belgium; AZ Klina Oncology, Brasschaat, Belgium; Clinique Tivoli, Bordeaux, France; Polyclinique de Courlan, Reims, France; Centre Hospitalier de Fleyriat, Bourg en Bresse, France; Grand Hopital de Charleroi, Charleroi, Belgium and R&D UNICANCER, Paris, France.

Body: Background: Ixabepilone, an epothilone B analog, has demonstrated single-agent activity in metastatic and neoadjuvant settings. The PACS08 trial aimed to compare adjuvant FEC100-Docetaxel regimen to FEC100-Ixabepilone in poor prognosis early breast cancer (BC) composed of patients presenting with triple-negative (TN) [i.e. estrogen receptor (ER)-/progesterone receptor (PR)-/HER2-] or ER+/PR-/HER2- tumor, which are subgroups significantly associated with worse prognosis.

Patients and methods: Between 2007 and 2010, 762 patients with unilateral TNBC (n=592, 78%) or node-positive ER+/PR-/HER2- BC (n=170, 22%) were enrolled. Recruitment was interrupted due to BMS decision to stop ixabepilone development in adjuvant setting. Main inclusion criteria were: age<70 years, normal cardiac, hepatic, haematological and renal functions. Arm A: pts received 3 cycles of FEC100 (F and C, each at 500 mg/m², E 100 mg/m², every 3 weeks) followed by 3 cycles of Docetaxel (100 mg/m² every 3 weeks); Arm B, Ixabepilone 40 mg/m² replaced Docetaxel. Radiotherapy was mandatory after conservative surgery and endocrine therapy was given to ER+ pts. ER, PR and HER2 status were validated by a central pathology review on 754 cases and Immunohistochemical detection of Ki67, EGFR, cytokeratins (CK) 5/6 and 14, was performed on tissue microarray (TMA).

Results: As of September 2014, the median follow-up was 36 months. The safety profile indicates that Docetaxel is more often associated to significant haemato logical toxicities whereas both neurotoxicities and haematological toxicities are reported in Ixabepilone arm. Log-Rank tests indicate no difference between two arms in terms of both DFS and OS (HR=1.2, 95%CI (0.864-1.728), p=0.256 and HR=1.8, 95%CI (0.751-1.855), p=0.473, respectively). Pathological analysis of the PACS08 collection showed that TNBC displayed significantly higher proliferative activity as shown by mitotic count and Ki67 index (p<0.001). As compared to ER+/PR-/HER2- subgroup, TNBC showed distinct characteristics, and displayed a so-called basal-like phenotype in 80%. Further efficacy analyses are ongoing in order to study whether chemotherapy may have better prognosis according to pathological characteristics including tumor lymphocytic infiltrate. These additional data will be available for the SABCS2015 meeting.

Conclusions: Our results indicate that Ixabepilone doesn't show higher efficiency compared to Docetaxel in adjuvant setting in poor prognosis early breast cancer. We have an unusual biological collection associated to our clinical data which will allow us to correlate efficacy data to breast cancer subgroups. Other several translational researches are still ongoing.
Title: Delayed initiation of adjuvant chemotherapy among breast cancer patients: A population-based study

Chavez-MacGregor M, Clark CA A, Lichetensztajn DY Y and Giordano SH H. The University of Texas MD Anderson Cancer Center and Cancer Prevention Institute of California.

Body: BACKGROUND: Adjuvant chemotherapy improves outcomes of breast cancer patients; however the optimal timing to initiation of chemotherapy remains unknown. No study has evaluated the relationship between time to chemotherapy (TTC) and outcome in a population-based study of patients treated with contemporary regimens according to tumor subtype. In this large study we identified the determinants associated with a delay in the initiation of chemotherapy and determined whether TTC is related to outcome.

METHODS: Breast cancer patients diagnosed with stage I-III breast cancer between 2005-2010 and treated with adjuvant chemotherapy were identified in the California Cancer Registry. TTC was defined as number of days between surgery and the day the first dose of adjuvant chemotherapy was administered. Delayed TTC was defined as >91 days. Logistic regression and Cox-proportional hazard modeling were used.

RESULTS: A total of 24,843 patients were included. Factors associated with delays in TTC included low socioeconomic status, reconstructive surgical procedure, non-private insurance and Hispanic or non-Hispanic black race/ethnicity. Compared to patients receiving chemotherapy within 31 days from surgery, there was no evidence of adverse outcome among those with TTC of 31-60 or 60-90 days. To the contrary, patients treated >91 days from surgery experienced statistically significant worse overall survival (OS) (HR=1.34; 95%CI 1.15-1.57) and worse breast cancer specific survival (BCSS) (HR=1.27; 95%CI 1.05-1.53). In a subgroup analysis according to breast cancer subtype, TTC >91 days had a statistically significant detrimental impact among patients with triple negative breast cancer (TNBC) in terms of both OS (HR=1.53; 95%CI 1.17-2.00) and BCSS (HR=1.53; 95%CI 1.17-2.07).

CONCLUSIONS: In this large cohort of breast cancer patients treated with contemporary regimens, a delaying in the initiation of adjuvant chemotherapy >91 days was associated with adverse outcomes. A delay in TTC is particularly detrimental among patients with TNBC. The majority of the determinants of delays in chemotherapy initiation are socio-demographic in nature. As medical providers we must make every effort to provide timely care to all our patients so they can receive the full benefit of our current treatments.
Title: Factors associated with delays in chemotherapy initiation among patients with breast cancer

Losk K, Vaz Duarte Luis I, Camuso K, Lloyd M, Kadish S, Hirshfield-Bartek J, Cutone L, Golshan M, Lin N and Bunnell C. Dana-Farber Cancer Institute, Boston, MA.

Body: Background: National guidelines endorse time-dependent quality metrics for breast cancer care. We examined factors associated with delays in chemotherapy initiation at an NCI designated comprehensive cancer center.

Methods: We identified 523 patients who received post-operative adjuvant chemotherapy between January 2011 and December 2013 at our center. We defined 28 days from last definitive surgery (LDS) to chemotherapy as the target timeframe, and unacceptable delay in chemotherapy initiation (UCD) as more than 42 days from LDS. Multivariate regression models were used to identify factors associated with UCD and the impact of Oncotype testing in HR+ patients.

Results: Median days between LDS and chemotherapy initiation was 34 (IQR 15), with 30% of patients starting within 28 days of LDS and 23% having UCD (Table 1). Tumor characteristics such as subtype and stage affected UCD; patients with HR+ or HER2+ tumors were more likely to be delayed compared to those with TNBC. Patients with stage I disease were more likely to be delayed as well as patients undergoing mastectomy or mastectomy with reconstruction. Patients whose pathology sign-out was more than 10 days post-operatively were more likely to be delayed. A higher proportion of UCD was found in HR+ patients (31%) who received an Oncotype recurrence score compared to those who did not (20%).

Table 1: Factors associated with delays in chemotherapy initiation

<table>
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<tr>
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</tr>
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</table>
Conclusions: This study provides insight into populations that may be at risk to experience delays in chemotherapy initiation, directing interventions to improve the timeliness of care.
**Title:** The oncologic effect of a gonadotropin releasing hormone (GnRH) agonist for ovarian protection during breast cancer chemotherapy

Kim HJ, Lee MH, Lee JE, Park SH, Lee ES, Kang Y-J, Lee JH, Shin HN, Kim SI, Im SA, Ahn SH, Lee KS, Sohn J, Han W and Nam SJ. Division of Breast and Endocrine, College of Medicine, University of Ulsan, Asan Medical Center; Seoul National University College of Medicine, Seoul; Division of Breast and Endocrine Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine; Yonsei University College of Medicine, Seodaemun-gu, Seoul, Korea; Cancer Biostatistics Branch, Research Institute for National Cancer Control and Evaluation, Research Institute and Hospital, National Cancer Center, Goyang, Korea; Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, Korea; Seoul National University College of Medicine; Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea and Division of Medical Oncology, Yonsei University College of Medicine.

**Body:** Background: Recently, chemotherapy with a GnRH agonist was reported to protect against ovarian failure. This study was aimed at determining the oncologic effect of a GnRH agonist concurrent with chemotherapy for breast cancer patients.

**Patients and Methods:** A total of 1189 patients aged 20 to 40 years with stage I to III breast cancer who received (neo or adjuvant) chemotherapy from five hospitals in Korea from 2002 to 2012 were reviewed. A gonadotropin releasing hormone (GnRH) agonist was given to 410 patients for ovarian protection during chemotherapy (GnRH agonist group), and 779 patients received chemotherapy without ovarian protection (Chemotherapy alone group). A matching strategy was used to create matched sets of two groups by age, stage, hormone receptor status, Her2/neu status, neo or adjuvant chemotherapy, and institute.

**Results:** Survival analysis using Cox regression showed that the GnRH agonist group had better distant metastatic-free survival (HR=0.65, 95%CI 0.44-0.97) outcomes but similar disease free survival (HR=0.78, 95% CI 0.57-1.08) compared with the chemotherapy alone group. The survival benefit was significant for hormone receptor positive, Her2/neu negative breast cancer on distant metastasis (HR=0.44, 95% CI 0.20-0.99) and disease free survival (HR0.47 95% CI 0.23-0.93).

**Conclusion:** Ovarian protection using a GnRH agonist can be safely considered for premenopausal breast cancer patients for whom chemotherapy is planned.
Title: Adjuvant platinum containing regimens significantly improve disease free survival in Chinese triple negative breast cancer patients

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Body: Background: It has been demonstrated that BRCA1/2 mutations predict good response to platinum in triple negative breast cancers (TNBC). However, in Chinese TNBC population whose BRCA1/2 mutation rate is only 3-7% in familial breast cancers compared to that of 20-40% in Caucasians, whether adjuvant platinum containing regimens still benefit these patients is of interest.

Methods: We retrospectively analyzed a consecutive cohort of TNBC patients diagnosed from June 2004 to June 2014 to evaluate the benefit of adjuvant platinum. Included patients were stage 1-3 TNBC patients who had curative surgeries followed by at least 4 cycles of adjuvant chemotherapy with or without radiotherapy. Adjuvant chemotherapy regimens were categorized into either platinum (either cisplatinum or carboplatinum) containing or non-platinum containing. Disease free survival (DFS) and overall survival (OS) were calculated accordingly and Kaplan-Meier survival analysis and multi-variable Cox regression analysis were performed. Clinicopathologic factors such as age, clinical stage, histological grades, and Ki-67 index were used as variables or stratifying factors in the above analyzes.

Results: Totally, 201 Chinese operable TNBC patients who had adjuvant chemotherapy were included. The median age was 48 years (range 22-70 years). Median follow-up was 49 months (range 12 to 120 months). Sixty patients were staged I, 109 staged II, and 32 staged III. Histological stage was I in 7, II in 65, and III in 129. Eighty-seven patients underwent adjuvant platinum containing regimens while 114 underwent adjuvant non-platinum containing regimens. In total, 39 DFS events were recorded with 5 in the platinum group and 34 in the non-platinum group. Kaplan-Meier survival analysis showed that platinum group had significantly better DFS than non-platinum group (Log Rank test, Chi-Square=8.873, P=0.003). Multiple variable Cox regression analysis showed that, platinum containing regimen was the only independent predicting factor for DFS and it decreased the risk of disease relapse by 73.8% (HR=0.262, 95% CI 0.106-0.677, P=0.006). The estimated 5-year survival rate was 94% in the platinum group VS. 69% in the non-platinum group. Only 12 OS events were recorded with 3 in the platinum group and 9 in the non-platinum group. Kaplan-Meier survival analysis showed that OS in the platinum group did not significantly differ from that in the non-platinum group (Log Rank test, Chi-Square=0.839, P=0.360), and Cox regression analysis failed to detect any independent predicting factors for OS.

Conclusions: Adjuvant platinum containing regimens significantly benefit Chinese TNBC patients in DFS but not in OS. Considering the critically low BRCA mutation rate in such patient population, the efficacy of platinum should be attributed to other hidden mechanisms and deserves further investigations. Future prospective randomized control trials are necessary before establishing the role of platinum for TNBC patients in the adjuvant settings.
Gluz O, Nitz U, Liedtke C, Christgen M, Sotlar K, Grischke EM M, Forstbauer H, Braun M, Warm M, Hackmann J, Uleer C, Aktas B, Schumacher C, Bangemann N, Lindner C, Kuemmel S, Clemens M, Potenberg J, Staib P, Kohls A, Pelz E, Kates RE E, Wuerstlein R, Kreipe HH H and Harbeck N. Westdeutsche Studiengruppe GmbH, Moenchengladbach, Germany; Ev. Hospital Bethesda, Breast Center Niederrhein, Moenchengladbach, Germany; University Clinics Schleswig-Holstein/Campus Luebeck, Women's Clinic; Medical School Hannover, Institute of Pathology; University of Munich (LMU), Institute of Pathology; University Clinics Tuebingen, Women's Clinic; Practice Network Troisdorf; Rotkreuz Clinics Munich; Clinics of Cologne - Hospital Holweide; Marien-Hospital Witten; Gynecologic Oncologic Practice Hildesheim; University Clinics Essen, Women's Clinic; St. Elisabeth Hospital Cologne; Charité Berlin, Clinic of Gynecology; Agaplesion Diakonie Clinic; Clinics Essen-Mitte, Breast Center; Mutterhaus der Borromäerinnen Trier; Ev. Waldkrankenhaus; St. Antonius Hospital, Clinic of Hematology and Oncology; Ev. Hospital Ludwigsfelde; Pathology Viersen; Palleos Healthcare Services, Statistics and Breast Center, University of Munich and CCCLMU.
Title: Early change in topoisomerase 1 (Top1) positive circulating tumor cells (CTCs) is associated with overall survival (OS) in patients with advanced breast cancer after treatment with etirinotecan pegol

Rugo HS S, Cortes J, Awada A, O'Shaughnessy J, Twelves C, Im S-A, Hannah AL L, Lu L, Sy S, Caygill K, Zajchowski D, Davis DW W, Hoch U and Perez EA A. University of California, San Francisco, San Francisco, CA; Vall D’Hebron University Hospital, Barcelona, Spain; Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium; Baylor Sammons Cancer Center, Texas Oncology, Dallas, TX; University of Leeds, Leeds, United Kingdom; Seoul National University College of Medicine, Seoul, Korea; Consultant, Sebastopol, CA; Nektar Therapeutics, San Francisco, CA; Oncology Consulting Services, San Francisco, CA; Apocell, Inc., Houston, TX and Mayo Clinic, Jacksonville, FL.

Body: Background: Etirinotecan pegol (EP) is a long-acting Top1 inhibitor providing sustained levels of active metabolite throughout the entire chemotherapy cycle. The phase 3 BEACON trial compared EP to treatment of physician's choice (TPC) in patients with advanced breast cancer, demonstrating a non-statistically significant 2.1 month difference in survival favoring EP in the intent to treat population. A novel aspect of the BEACON trial is to explore the utility of biomarkers measured in CTCs for predicting efficacy with EP. Pre- and post-treatment CTCs were isolated from blood of 77% of the 852 BEACON patients. Target-specific pharmacodynamic biomarkers for EP measured in CTCs were analyzed to identify patients most responsive to treatment with EP.

Methods: Donation of blood samples for CTC analysis was voluntary. Participating BEACON patients had serial (baseline, Cycle 2 Day 1 [C2D1], Cycle 4 Day 1 [C4D1], End of Treatment) 7.5-mL whole blood samples drawn in EDTA tubes and shipped within 96 hours ambient to ApoCell (Houston, TX) for processing. PBMCs were separated by Ficoll® gradient, and CTCs were isolated using ApoStream® technology. Isolated cells were deposited on three slides and stained for DAPI, CD45, cytokeratin markers, as well as Top1, Top2, Ki67, γH2AX, Rad51, ABCG2, and TUNEL. Biomarkers were quantified by iCys® laser scanning cytometer equipped with image analysis software, and correlated with OS using Cox multiple regression and Kaplan-Meier analyses.

Results: The CTC substudy yielded 611 pre-treatment, 519 C2D1, 268 C4D1, and 431 End of Treatment samples. Among the successfully processed blood samples, 98% had detectable CTCs, with a median of 63, 46, 51, and 57 CTCs/mL at baseline, C2D1, C4D1, and End of Treatment, respectively. Cox regression analyses of CTC number and percentage of Top1, Top2, Ki67, or TUNEL positive CTCs identified a correlation for post-treatment number of Top1-positive CTCs with OS in patients receiving EP. To assess the impact of Top1-positive CTCs, patients were classified as Top1-High (> median) or Top1-Low (≤ median) based on the percent of Top1-positive CTCs at baseline. Among the Top1-High patients at baseline, significantly improved OS (HR 0.54, p=0.007) was observed for those who converted to Top1-Low after their first treatment with EP (C2D1), but not TPC (HR 1.12, p=0.613). These results suggest that decreased number of Top1-positive CTCs may reflect EP target engagement with Top1, as these patients derived the most benefit from treatment.

Conclusions: CTC collection and analysis was successfully incorporated into the phase 3 BEACON study, with 77% patient participation. CTC detection rate using ApoStream® was high, permitting evaluation of biomarkers at baseline and post-treatment. Significantly improved OS was observed in patients who had a decreased number of Top1-positive CTCs following cycle 1 of EP.
Title: Genome wide association study (GWAS) of genetic variants associated with docetaxel toxicity in the ROSE/TRIO-012 trial

Damaraju S, Gorbunova V, Gelmon K, García-Saenz J, Morales-Murillo S, AbiGerges D, Canon J-L, Kiselev I, Cohen GL L, Jerusalem G, Thireau F, Fresco R, Houé V, Press MF F, Kumaran M and Mackey JR R. Cross Cancer Institute; University of Alberta, Edmonton, AB, Canada; N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russian Federation; British Columbia Cancer Agency, Vancouver, Canada; Hospital Clínico Universitario San Carlos, Madrid, Spain; Hospital Universitario Arnaú de Vilanova, Lleida, Spain; Middle East Institute of Health, Bsaim, Lebanon; Grand Hôpital de Charleroi, Charleroi, Belgium; Kursk Regional Oncology Dispensary, Kursk, Russian Federation; Mary Potter Oncology Center, Pretoria, South Africa; Centre Hospitalier Universitaire du Sart-Tilman, Liege, Belgium; Translational Reserach in Oncology (TRIO), Paris, France; Translational Reserach in Oncology (TRIO), Montevideo, Uruguay and University of Southern California, Los Angeles, CA.

Body: Background: Genetic predisposition to docetaxel (Doc) toxicity contributes to unacceptable toxicity and reduced dose intensity, and may influence disease outcomes. We previously reported variants associated with Doc toxicity in candidate gene single nucleotide polymorphism (SNP) associations in a breast cancer treatment setting (Damaraju et al. Eur J Cancer (suppl); Vol 8 (7), page 175, 2010) and the identified variants were confirmed in an independent validation study (Damaraju et al, J Clin Oncol Vol 33, Issue 15 suppl, 2015: 540). Others have reported candidate SNP (Breast Cancer Res Treat, 2011 SWOG 0221 study) and GWAS (Clin Cancer Res 2012 CALGB 40101 study) identified variants associated with paclitaxel mediated peripheral neuropathy. However, the overlap on the variants identified thus far between Doc and paclitaxel are limited, prompting a genome wide search to find variants contributing to Doc specific toxicity.

Methods: TRIO-012 is a double blinded, multinational trial that randomized 1,144 patients with advanced breast cancer to receive first-line Doc in combination with ramucirumab (RAM) or placebo (Mackey et al. J Clin Oncol Jan 10, 2015:141-148). Study subjects (n=719) in the Doc+RAM or Doc+placebo arm with available germline DNA are being genotyped; all subjects provided ethics-committee approved prospective consent for this genetic study. Genotyping are being performed with Affymetrix SNP 6.0 arrays. Genotype data will be filtered for deviations from Hardy Weinberg Equilibrium and minor allele frequency of >0.05. Doc-induced adverse events (AEs) are based on CTCAE (Common Terminology Criteria for Adverse Events v4.1) toxicity grades. Toxicities >2 scored for fatigue (n=96), myalgia (n=22), peripheral neuropathy (n=17) will be analysed as individual phenotypes in comparison with the no toxicity group (toxicity grades 0-1) and in a combined analysis of all Doc induced toxicities (0-1, n=599 vs. >2, n=120). Dominant genotypic model is assumed; Chi-square test, FDR and/or 10000 permutations were employed using SVS v8.3 and p<0.05 considered statistically significant. We will identify population stratification using EIGENSTRAT method and will correct the association statistics using the Eigenvectors along with age and BMI as covariates. Fine mapping of the identified loci will be attempted using imputation tools. We will interrogate the data for cumulative dose to toxicity and correlate SNPs identified with survival outcomes.

Results and conclusions: We expect to reconfirm the associations of loci reported in candidate SNP and previous GWAS studies; XKR4 (rs4737264) for peripheral neuropathy, CYP3A5*3 (rs776746) with fatigue, and FACND2 (rs7637888) with myalgia in addition to the potential novel variants distinct from paclitaxel AE GWAS studies. Fine mapping around these loci may help identify potential causal variants. Both candidate SNP and GWAS identified variants may aid in developing risk stratification models. The GWAS identified loci and the flanking genes will be interrogated using the ingenuity pathway analysis for insights in to the biological roles in the drug metabolism. We expect to complete the analysis by mid-November 2015.
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Title: Higher rate of severe toxicities in obese patients receiving dose-dense chemotherapy according to unadjusted body mass index – Results of the prospectively randomized GAIN study

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Body: Background: In routine clinical practice chemotherapy (CT) doses are frequently capped at a body surface area (BSA) of 2.0 m\(^2\) or adjusted to an ideal weight (i.e. [body length in cm - 10%] + 40% [current weight-ideal weight]) for obese patients (BMI>30 according to WHO) due to safety reasons. There are no data on CT dosing within intense dose-dense regimen for obese patients. Therefore, a retrospective analysis of the GAIN study population has been conducted.

Methods: Between August 2004 and July 2008 a total of 3023 patients were enrolled in the GAIN study, a randomized phase III adjuvant trial, comparing two types of dose-dense regimen. Patients were randomized to intense dose-dense ETC (Epirubicin 150 mg/m\(^2\), Paclitaxel 225 mg/m\(^2\), Cyclophosphamide 2500-2000 mg/m\(^2\), i.v. q15 for 3 cycles) or EC followed by T plus capecitabine (X) (EC-TX) (E 112.5 mg/m\(^2\) + C 600 mg/m\(^2\), i.v. q15 for 4 cycles followed by T 67.5 mg/m\(^2\) i.v. q8 for 10 weeks + X: 2000 mg/m\(^2\) p. o. day 1-14, q22 for 4 cycles). An adjustment of CT dose to an ideal weight for obese patients was implemented by a protocol amendment. Yet some patients received a dose adjustment by capping at 2.0 m\(^2\). We retrospectively evaluated a total of 543 patients with a BMI>30. Data on BSA and dose adjustment were collected from case report forms. Toxicities were compared between patients who received CT according to an unadjusted or adjusted BSA using the 2-sided exact test of Fisher. Disease-free survival (DFS) and overall survival (OS) were calculated using the Kaplan-Meier method and the log-rank test.

Results: Overall, 18.0% (n=543) of patients in the GAIN study were obese: 30.9% (n=168) of them received CT according to an unadjusted BSA. For the remainder BSA was adjusted to ideal weight or was capped at 2.0 m\(^2\) (69.1%; n=375). A total of 14.5% (n=24) of obese patients receiving full dose of chemotherapy vs 6.4% (n=24) of obese patients with an adjusted BSA experienced febrile neutropenia (p=0.005) and 9.6% (n=16) vs 2.9% (n=11) high grade thrombopenia (p=0.002). Overall, 16.7% (n=28) vs 10.1% (n=38) had a thromboembolic event (p=0.034), which was high grade in 12.5% (n=21) vs 6.4% (n=24), respectively (p=0.027) and 3.0% (n=5) vs 0.3% (n=1) experienced high grade hot flushes (p=0.012). The only significant differences in favor of the non-adjusted group were for dizziness (4.2% [n=7] vs 10.7% [n=40]; p=0.013), diarrhea (18.5% [n=31] vs 26.9% [n=101]; p=0.039) and an increase in serum creatinine (6.8% [n=11] vs 14.0% [n=52]; p=0.019). No differences in DFS and OS were observed between the two groups (5year DFS 81.9% [CI 74.9%-87.2%] vs 80.8% [76.3%-84.6%]; p=0.850; 5year OS 86.4% [79.9%-90.9%] vs 88.3% [84.4%-91.3%]; p=0.491).

Conclusion: This analysis of patients treated with a dose-dense regimen showed that obese patients who received CT according to their real BSA have a higher risk of severe toxicities, in particular of febrile neutropenia, high grade thrombopenia and high grade thromboembolic events. Therefore, a dose adjustment of intense dose-dense CT should be performed for obese patients to avoid life-threatening complications.
Body: Background: GAIN-2 compares the effectiveness and safety of a predefined intense dose-dense regimen (EnPC) vs. a dose-dense regimen with modification of single doses depending on individual hematological and non-hematological toxicities (dtEC-dtD) (NCT01690702). Moreover, the Trastuzumab substudy compares the subcutaneous administration of the drug to the abdominal wall vs. thigh.

Methods: The primary objective of the GAIN-2 trial is to compare the invasive disease-free survival (iDFS) in patients with high-risk primary breast cancer (luminal A ≥ 4 N+; luminal B N+; HER2+ and TNBC N0/N+). Patients are randomized between EnPC (epirubicin 150 mg/m² q2w x 3, nab-Paclitaxel 330 mg/m² q2w x 3, cyclophosphamide 2000 mg/m² q2w x 3) or dtEC-dtD (dd/tailored epirubicin/cyclophosphamide q2w x 4 followed by dd/tailored docetaxel q2w x 4) Two safety interim analyses after 200 (Noeding et al. Ann Oncol 2014) and 900 patients who have completed chemotherapy were planned. We present the results of the second safety analysis. In addition to the standard analyses for hematological and non-hematological toxicities, any affections of the cranial nerves as well as the rate of macula degenerations and anaphylactic reactions are of special interest.

Results: Between 09/2012 and 05/2015 a total of 1473 patients have been randomized (EnPC n=734; dtEC-dtD n=739). Among those, 84 patients have been included in the trastuzumab substudy. No safety data are currently available for the substudy. Median age was 52 years and median body-mass-index 26. In terms of hematological adverse events, the rate of febrile neutropenia grade 3-4 (12% vs. 8%) and thrombopenia grade 3-4 (12% vs. 5%) was significantly increased in the EnPC arm. As for non-hematological side effects, there were significantly more patients developing an increase in alkaline phosphatase (59% vs. 40%), ALAT (69% vs. 59%), peripheral sensory neuropathy (83% vs. 68%), arthralgia (63% vs. 49%), myalgia (48% vs. 41%) and bone pain (25% vs. 17%) in the EnPC arm, whereas nosebleed (10% vs. 25%), edema (13% vs. 26%) and hand-foot syndrome (12% vs. 28%) were more common in the dtEC-dtD arm. We observed two treatment related deaths, both in the dtEC-dtD arm (cause of death: acute respiratory distress syndrome and pneumonia). There were no differences between the treatment arms for the toxicities of special interest. In the EnPC arm, overall 30% of the patients required dose-reductions due to hematological toxicities compared with only 10% in the dtEC-dtD arm (p<0.001). The dose could be escalated to the maximum (epirubicin/cyclophosphamide 120/1200 mg/m² followed by docetaxel 100 mg/m²) in more than one third of the patients receiving dtEC-dtD. In 9% of women a reduction was required in the 4th cycle of docetaxel.

Conclusion: This interim safety analysis from a prospectively randomized trial investigating iddEnPC with predefined doses and a toxicity adapted idd/tailored strategy (dtEC-dtD) showed no additional or unexpected safety signals in the iddEnPC or dtEC-dtD arm. Therefore, no modifications in the conduction of the study are necessary and the study continues as expected.
Title: Does histological subtype play a role in treatment decision-making for hormone receptor positive metastatic breast cancer? A study of the Southeast Netherlands breast cancer consortium

Lobbezoo DJA JA, Truin W, Voogd AC C, Roumen RMH MH, Vreudingenhil G, Derksen MW, van den Berkmortel F, Smilde TJ J, van de Wouw AJ J, van Kampen RJW JW, van Riel JMGH MGH, Peters NAJB AJB, Peer PGM GM and Tjan-Heijnen VCG CG. Maastricht University Medical Center; Máxima Medical Center; Orbi-Atrium Heerlen; Jeroen Bosch Hospital; VieCuri Medical Center; Orbi-Atrium Sittard; Sint Elisabeth Hospital; St Jans Hospital and Radboud University Medical Center.

Body: Introduction
Breast cancer is a heterogeneous disease with distinct biological subtypes. Invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) are the two most frequent histological breast cancer subtypes. With this study, we aimed to provide insight into the role of histological subtype on the characteristics, choices with respect to systemic therapy in daily practice and outcome of patients with metastatic breast cancer.

Patients and methods
We analyzed 815 patients diagnosed with metastatic breast cancer in eight hospitals between 2007 and 2009. All hormone receptor (HR) positive patients with either IDC or (mixed) ILC were included. Patient and tumor characteristics, outcomes and treatment data were collected. Survival curves and time to first palliative systemic therapy (either chemotherapy or endocrine therapy) were estimated using the Kaplan-Meier method and compared using log-rank tests. To explore the association of palliative systemic therapy with the survival of patients with metastatic breast cancer a Cox proportional hazards model was performed with palliative chemotherapy and endocrine therapy as a time-dependent covariates.

Results
A total of 568 patients with HR-positive tumors were included; 437 with IDC and 131 with (mixed) ILC. Patients with ILC were older at diagnosis of primary breast cancer, had larger primary tumors and more node-positive disease compared with IDC. Median survival was not different between the subtypes (29 months for ILC and 25 months for IDC, \( P=0.53 \)).

One year after diagnosis of metastatic breast cancer, less patients with HR-positive ILC received chemotherapy (33% of patients with ILC and 47% of patients with IDC) and their time to first palliative chemotherapy was significantly longer compared with HR-positive IDC (\( P=0.001 \)). Time to first palliative endocrine therapy was significantly shorter for ILC compared with IDC (\( P=0.0001 \)).

In multivariable analysis for patients with ILC with palliative endocrine therapy and palliative chemotherapy as time-dependent covariates, palliative chemotherapy as first given systemic therapy was associated with an unfavorable outcome (hazard ratio 2.8, 95% CI 1.7-4.6, \( P<.0001 \)) compared to no palliative chemotherapy and treatment with palliative endocrine therapy as first given systemic therapy was associated with a favorable outcome (hazard ratio 0.4, 95% CI 0.2-0.8, \( P=0.005 \)). In multivariable analysis for patients with IDC, treatment with palliative chemotherapy as first given systemic therapy was also associated with unfavorable outcome (hazard ratio 2.1, 95% CI 1.6-2.7, \( P<.0001 \)), whereas treatment with palliative endocrine therapy as first given systemic therapy was not associated with outcome for patients with IDC (hazard ratio 0.9, 95% CI 0.6-1.2, \( P=0.4 \)).

Conclusion
There was no difference in survival of metastatic breast cancer patients with HR-positive ILC compared with those with IDC. This similar outcome was achieved with different treatment strategies, in which patients with ILC were more likely to receive endocrine therapy and less likely to receive chemotherapy.
Superiority of tandem high-dose chemotherapy (HDC) versus conventionally dosed chemotherapy (CDC) in patients with metastatic breast cancer (MBC): Long term follow-up of IBDIS: A prospective random assignment trial (PRT)

Crown J. Svuah, Dublin, Ireland.

Background
The partial chemo-sensitivity of MBC, together with pre-clinical models, provided a rationale for studies of extreme dose-escalation with autologous haematopoietic progenitor support (ASCT). Early studies of HDC as salvage following failure of CDC produced high rates of temporary response. Subsequent single arm studies in pts who were preselected for responsiveness to CDC yielded 10-20% durable remissions suggesting that HDC might cure some MBC pts. The likelihood of selection bias mandated randomised trials of this CDC induction-HDC consolidation approach. We hypothesised that an alternative strategy-accelerated multi-cycle HDC- might provide an optimal HDC strategy (Crown J, Norton L, Ann Oncol 1996).

Methods
In IBDIS, pts received a brief phase of CDC induction (doxorubicin/docetaxel-“AT”), followed by tandem cycles of HDC, or, further CDC (AT followed by CMF), as initial CRx for MBC. Hormone receptor-positive patients received endocrine therapy post-chemotherapy, and pts with localized metastases received consolidative radiotherapy.

Results: Accrual failed in the aftermath of the disclosure of research fraud involving a South African HDC study, and the reporting of negative PRCTs at ASCO 1999. Only 110 of a planned 264 patients were enrolled, but it was decided to maintain follow-up, which is now 15 years. HDC and CDC groups were well-balanced for prior adjuvant (25 v 25), prior anthracycline (8 v 10), positive receptor status (32 v 31). There were 8 treatment-related deaths (6-HDC, 2 CDC). Complete and overall response rates (CR/OR) were significantly superior for HDC v CDC (CR-29% v 6%, OR-71% v 44%). Event-free-survival at median 5 yrs were HDC 6 (11%) versus CDC 0 (p=.027). At fifteen years three HDC pts remain alive and free from relapse. All CDC are deceased. The hazard ratio for PFS is currently 0.59 (0.39-0.88) favouring HDC p=.009. The HR for overall survival is 0.72 (0.48-1.08)p=.11

Discussion:
Despite accrual failure, IBDIS was and remains a positive study for its primary endpoint of PFS. The 100% relapse and death rate for CDC on our study is typical of the published literature. Data from IBDIS are included in the Cochrane meta-analysis of HDC which showed an advantage for PFS. The contention that HDC produced superior activity to CDC appears to have been correct, however the magnitude of the benefit is smaller than was hoped by investigators in the field.

While our data support the hypothesis that there is a dose response relationship in the chemotherapy of MBC, the small benefit and high toxicity of HDC, preclude it being recommended as a standard treatment for any pts with MBC.
Adaptive therapy: Chemotherapy driven by evolutionary principles prolongs tumor control in preclinical breast cancer models

Enriquez-Navas PM M, Luddy K, Garcia L, Gillies RJ J and Gatenby RA A. Moffitt Cancer Center and Research Institute, Tampa, FL.

Background: Disseminated cancers are typically treated with the maximum tolerated dose to achieve the goal of killing as many tumor cells as possible. However, these therapies eventually fail due to emergence of resistant clones. Using mathematical models of Darwinian dynamics in cancer, we have predicted that adapting the chemotherapeutic dosing schedule to the tumor spatial variability and tumor microenvironment can retard the emergence of chemoresistance. Here, we present an evolutionary-guided treatment strategy (Adaptive Therapy, AT) designed to maintain stable chemosensitive populations while limiting the proliferation of chemoresistant clones by exploiting the fitness cost of the resistant phenotype. The efficacy of the AT has been tested in triple-negative and ER+ breast cancer preclinical models.

Methods: Four cohorts (composed by 12, 11, 13, and 10 mice, respectively) of nude mice were injected with MDA-MB-231 cells in the mammary fat pad. An additional cohort of 19 mice were injected in the same place with MCF7 cells. Control animals didn't receive any treatment. Mice under standard therapy received 20mg/kg of Paclitaxel twice per week for a total of 5 times. We defined two different Paclitaxel AT (Table 1).

AT algorithms

<table>
<thead>
<tr>
<th>AT</th>
<th>Initial Dose</th>
<th>If VT(n) ≤ 0.8 * VT(n-1) Dose decreased by 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT-1</td>
<td>20 mg/kg</td>
<td>If VT(n) &gt; 1.2 * VT(n-1) Dose increased by 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If the tumor volume is within 20% range,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>we will apply same dose as previous dose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If VT &lt; 150 mm³ -&gt; skip the dose</td>
</tr>
<tr>
<td>AT-2</td>
<td>15 mg/kg</td>
<td>If VT(n) &lt; 1.25 * VT(n-2) Dose = 15 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If VT(n) &gt; 1.25 * VT(n-2) Skip Dose</td>
</tr>
</tbody>
</table>

VT: MRI tumor volume.

When the tumors achieve ∼300 mm³ the therapy started. MRI T2 and DW acquisitions were performed on an Agilent ASR 310 7T scanner to determine volume and cellularity, respectively. Thereafter, dynamic contrast enhanced (DCE) MRI was preformed following bolus of 0.1 mmol/kg Magnevist to assess distribution of blood flow. Images were processed with in-house developed MATLAB scripts to obtain reliable information. At the end of the monitoring time, tumors were collected and processed for H&E, CD31, SMA, MDR-1, and ER expression. The slides were examined using the Aperio ScanScope XT microscope with a pixel-wise resolution.

Results and Discussion: Tumor growth was monitored using the MRI techniques. Among these three protocols (2 AT and one ST), both AT kept the tumor burden under control during more time than ST in the case of the triple-negative model. In the case of the ER+ model, ST kept the tumor control under control for an extended period than AT-2. However, in both models, the continuous and modulated dosage of Paclitaxel, AT-1, resulted in the most significant cancer control: AT-1 protocol was able to maintain tumors at a small size, with a lower percent of necrosis, and a higher tumor vascularity density. Also, in the case of the MDA-MB-231, it showed the lower expression of MDR1 system.

Conclusion: AT schema, especially AT-1, guided by MRI, can maintain a stable small tumor burden with prolonged progression-free survival compared to standard high dose therapy. Also, AT is able to maintain the lower necrotic volume, a
higher vessel density, and a lower expression of resistance mechanisms, which was correlated with tumor stabilization.
Title: Prognostic relevance of prior endocrine treatments in overall survival (OS) at the time of first line chemotherapy in ER[+]/HER2[-] advanced breast cancer (ABC) patients

Llombart A, de la Haba-Rodríguez JR R, Gligorov J, Aguirre E, Sampayo M and Cortes J. Hospital Arnau de Vilanova, Valencia, Spain; Hospital Universitario Reina Sofía, IMIBIC, Córdoba, Spain; APHP, Tenon Hospital, Paris, Spain; Medica Scientia Innovation Research, Barcelona, Spain; Scienco Klinico, Premià de Mar, Barcelona, Spain and Hospital Vall d'Hebron Institut d'Oncologia, Barcelona, Spain.

Body: Background: International consensus stresses the preference for endocrine therapies (ET) for the ER[+]/HER2[-] ABC population. However, in real-world, chemotherapy (CT) is an extended practice long before exhausting endocrine options (Swallow E, Curr Med Res Opin. 2014). There is little knowledge on the prognostic factors that drives OS among the ER[+]/HER2[-] population at the time of first line CT for ABC.

Methods: The Athena trial assessed safety of different first-line CT & bevacizumab regimens in 2,264 patients (pts) treated between 2006 and 2009 over 34 countries. A total of 1,492 ER/PgR[+]/HER2[-] pts were identified, as 585 TNBC pts (control arm). We adapted the ESMO 2012 guidelines of endocrine resistance to the data collected, considering 5 years the median duration of (neo)adjuvant ET. Endocrine resistance status (ERS) at the time of inclusion was measured by: (1) Progression on ET for Early stage (EEP) [on or within the first year of], and (2) Prior ET for ABC (AEP), whatever the duration or number of endocrine lines. Three ES were pre-defined: Low sensitive (LS): patients with both EEP and AEP criteria; moderately sensitive (MS): either EEP or AEP criteria, and Highly sensitive (HS): neither EEP nor AEP criteria. Other prognostic factors (PF) identified in a prior analysis (Llombart-Cussac A, Breast 2014) were incorporated in a multivariate Cox proportional-hazard model.

Results: Median age was 53 years (range 22-93); 5.2% ECOG ≥2 and 28.9% with prior analgesic treatment. ER/PgR positivity were 94.4%/76% respectively. Patients were previously exposed to anthracycline (53.5%) and taxanes (21.4%) for early stage BC. Prior endocrine therapy in (neo)adjuvant and metastatic settings were (65.3%) and (31.8%), respectively. Pts de novo metastatic represented 19.3%, and the median DFI in pts progressing from an early stage was 43.2 mo. Liver involvement was observed in 40.3% of pts and 20.5% presented ≥3 organs involved. The ES for the population was HS: 492 pts (33%), MS: 755 pts (50.6%) and LS: 245 pts (16.4%). After median follow-up of 22.6 months (range: 0.1 to 43.6) and 752 OS events (50.4% of pts), median OS for HS, MS, and LS groups were >40 mo. (median not achieved), 26.3 (95%CI: 24.5–28.4), 20.1 (95%CI: 17.8–23.9) and 18.3 (95%CI: 16.3–19.7) respectively. 3-years OS survival rates for HS, MS, and TNBC were 53.5%, 34.8%, 23.9% and 26.6%, respectively. Multivariate-adjusted hazard ratios of OS for HS vs MS were 2 (IC95%: 1.6–2.5) and 1.5 (IC95%: 1.2–1.8). The other PF related with OS were: ECOG≥2 or analgesics or corticosteroids 1.6 (IC95%: 1.4–1.8); liver mets or≥2 involved organs 1.4 (IC95%: 1.2–1.6) and adjuvant anthracyline and/or taxane 1.2 (IC95%: 1.1–1.4).

Conclusions: Nearly to 40% of ER[+]/HER2[-] ABC pts confronted to first line CT were precluded as highly sensitive to ET, achieving a median OS that doubles the less sensitive groups. Confirmatory studies with post-treatment information may be important to link this benefit to ET. However Endocrine Status may be useful to appropriately characterize or select patients in future first-line CT studies for HER2[-] pts.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-13-10

Title: Capecitabine in combination with bendamustine in pretreated women with HER2-negative metastatic breast cancer: Stage 1 results of a phase II trial (AGMT MBC-6)

Rinnerthaler G, Gampenrieder SP P, Fridrik M, Petzer A, Hubalek M, Petru E, Jäger T, Andel J, Balic M, Ulmer H, Mlineritsch B and Greil R. Salzburg Cancer Research Institute With Laboratory of Immunological and Molecular Cancer Research and Center for Clinical Cancer and Immunology Trials, Paracelsus Medical University Salzburg, Salzburg, Austria; General Hospital Linz, Linz, Austria; Barmherzige Schwestern Hospital Linz, Linz, Austria; Innbrucker Medical University, Innsbruck, Austria; Medical University Graz, Innsbruck, Austria; General Hospital Feldkirch, Feldkirch, Austria; County Hospital Steyr, Steyr, Austria; Division of Oncology, Medical University Graz, Graz, Austria and Medical University Innsbruck, Innsbruck, Austria.

Body: Background: Although there is no single accepted standard of care after failure of anthracycline and taxane therapy in HER2-negative metastatic breast cancer, capecitabine is a well-established treatment option. Bendamustine is a hybrid cytotoxic drug because of its structural similarity to alkylating agents and purine and it is generally well tolerated. Since bendamustine has already shown anticancer activity in breast cancer we evaluated the efficacy and tolerability of bendamustine in combination with capecitabine in 40 patients with advanced breast cancer after anthracycline and/or taxane pretreatment.

Patients and methods: MBC-6 is a non-randomized, multicenter, open-label, single-arm phase II study in patients with HER2-negative advanced breast cancer (ClinicalTrials.gov identifier: NCT01891227). All patients were pretreated with anthracyclines and/or taxans in the (neo-)adjuvant and/or metastatic setting and measurable disease according to RECIST 1.1. had to be present at baseline. Following a two-stage Green-Dahlberg design, 20 subjects were accrued and treated within stage 1 of the study. The trial was planned to enroll further 20 patients if there were at least four subjects (20%) with a complete (CR) or partial response (PR). Eligible patients received 1000 mg/m² capecitabine twice daily on days 1 to 14 in combination with 80 mg/m² bendamustine on day 1 and 8 of a 3-week cycle. After a maximum of eight cycles capecitabine was continued as single drug therapy until disease progression or unacceptable toxicity. The primary endpoint was overall response rate (ORR). Secondary endpoints were progression free survival (PFS), clinical benefit rate (CBR), safety profile and quality of life. Here we report the efficacy and safety analysis of stage 1 patients.

Results: From September 2013 to May 2015, 40 patients were recruited in eight Austrian centers. Median age of the stage 1 cohort was 59 years (range 29-77), 80% and 20% of patients had an ECOG performance score of 0 and 1, respectively. Thirty-three percent had triple-negative disease, 85% had had (neo-)adjuvant treatment and 65% patients were pretreated with at least one chemotherapy line for metastatic disease (15% one line, 50% two lines, 40% three lines). In stage 1, ORR was 50% with 9 confirmed PR and 1 confirmed CR, and ORR was comparable between hormone receptor-positive and triple-negative disease (54% vs. 43%). CBR was 55%. At data cut-off on 05/28/15 overall 15 of 20 patients had discontinued treatment: 10 patients (50%) due to progressive disease, 3 (15%) because of adverse events (AEs) and 2 patients on their own decision (10%). Five patients (25%) experienced at least one drug related non-hematological AE ≥ grade 3: 2 diarrhea, 2 fatigue, 3 respiratory or viral infections, 1 dyspnea, 1 thromboembolic event (each grade 3). One grade 4 hematological AE (neutropenia) was observed. One patient died as a result of restrictive cardiomyopathy, where a relationship to capecitabine cannot be excluded, but seems unlikely.

Conclusion: The combination of capecitabine and bendamustine has a moderate toxicity profile and the response data of the stage 1 are promising. Final study results are awaited in the first half of 2016.
Title: Phase II, randomized, parallel-cohort study of neoadjuvant buparlisib (BKM120) in combination with trastuzumab and paclitaxel in women with HER2-positive, PIK3CA mutant and PIK3CA wild-type primary breast cancer – NeoPHOEBE

Loibl S, de la Pena L, Nekljudova V, Zardavas D, Michiels S, Denkert C, Rezai M, Bermejo B, Lee S-C, Turri S, Urban P, Kümmel S, Lux M, Piccart M, von Minckwitz G, Baselga J and Loi S. German Breast Group, Neu-Isenburg, Germany; Sana Klinikum Offenbach, Offenbach, Germany; SOLTI Breast Cancer Research Group, Barcelona, Spain; Breast International Group, Brussels, Belgium; Institut Gustave Roussy, Villejuif, France; Pathology Charité, Berlin, Germany; Luisenkrankenhaus, Düsseldorf, Germany; Hospital Clinico Universitario de Valencia, Valencia, Germany; National University Cancer Institute Singapore, Singapore, Singapore; Novartis Pharma AG, Basel, Switzerland; University Hospital Erlangen, Erlangen, Germany; Kliniken Essen-Mitte, Essen, Germany; Institut Jules Bordet, Brussels, Germany; Memorial Sloan-Kettering Cancer Center, NY, USA; and Peter MacCallum Cancer Centre, Melbourne, Australia.

Body: Background: The PI3K/Akt/mTOR pathway is frequently activated in breast cancer (BC) and is important for the oncogenic function of HER2. Buparlisib is an oral pan-PI3K inhibitor that targets all 4 isoforms of class I PI3K (a, b, g, d). Clinical activity was observed with buparlisib in patients (pts) with advanced BC as a single agent, and in combination with endocrine and anti-HER2 treatment.

Methods: NeoPHOEBE (NCT01816594) is a phase II, randomized, double-blind, parallel-cohort study of neoadjuvant buparlisib/placebo plus trastuzumab and weekly paclitaxel in women with, untreated primary HER2-positive BC. Pts were stratified upfront into 2 independent cohorts according to PIK3CA mutation status. Other eligibility criteria: tumor size >2 cm; unilateral disease; available tumor tissue for central review of estrogen receptor (ER), HER2 status, and PIK3CA genotype; known PIK3CA mutation status; and ECOG PS ≤1. Pts in each cohort (PIK3CA mutant or wild-type [wt]) were randomized (1:1) to receive continuous daily buparlisib (100 mg) or placebo and weekly trastuzumab (4 mg/kg loading dose then 2 mg/kg) for 6 weeks, followed by continuous daily buparlisib (80 mg) or placebo with weekly trastuzumab (2 mg/kg) and weekly paclitaxel (80 mg/m²) for 12 weeks. Study treatment was followed by surgery. Stratification at randomization was based on PIK3CA (mutant vs wt) and ER (positive vs negative) status. The primary endpoint was pathologic complete response (pCR; ypT0) rate at time of surgery. The key secondary endpoint was objective response rate (ORR) at the end of week 6. Other secondary endpoints included pCR by other definitions, ORR prior to surgery, pCR and objective response by ER status, percent of pts with node-negative disease at surgery, rate of breast conserving surgery, and safety, as well as pCR by PTEN expression, Ki67 level, apoptosis rates, percentage of tumor infiltrating lymphocytes (TIL), and by phenotype of 50% TIL at baseline. The sample size is based on a minimax 2-stage randomized phase II design with a prospective control. This design allowed for early stopping if the desired efficacy was not observed after stage 1. Both cohorts were powered (80%) to detect a clinically meaningful increase in pCR of 18% at a one-sided significance level (α=0.15).

Results: Between 9/2013 and 10/2014 50 pts were randomized in 38 sites in 4 countries. Recruitment was suspended due to toxicity in 10/2014. Median age was 50 years, 42 pts had a PIK3CA wt and 8 a PIK3CA mutant tumor, 62% of pts presented with HER2+/ER+, 90% with ductal or ductal-lobular invasive, and 62% with G3 tumors; 86% had Ki67 >20%; 9 pts reported a serious adverse event including 3 pts with hepatotoxicity. Overall 14/50 (28 %) pts developed grade 3-4 liver enzyme elevation, of whom 9 (64%) recovered to at least grade1. Final safety and efficacy data on the primary endpoint (pCR) and biomarker data will be presented at the meeting.

Conclusions: The NeoPHOEBE study investigates for the first time the efficacy of adding a pan-PIK3 inhibitor to a taxane/trastuzumab-based neoadjuvant therapy in pts with primary HER2-positive BC with or without a PIK3CA mutation.
**Title:** Disease-free (DFS) and overall survival (OS) data from ACOSOG Z1041 (Alliance) a randomized neoadjuvant trial comparing FEC followed by paclitaxel plus trastuzumab (FEC → P+T) with paclitaxel plus trastuzumab followed by FEC plus trastuzumab (P+T → FEC+T) in HER2-positive operable breast cancer

Buzdar AU U, Suman VJ J, Meric-Bernstam F, Leitch AM M, Ellis MJ J, Boughey JC C, Unzeitig GW W, Royce ME E, Ewer MS S and Hunt KK K. MD Anderson Cancer Center, Houston, TX; Mayo Clinic, 201 W. Center St., Rochester, MN; MD Anderson Cancer Center, Houston, TX; University of Southwestern Medical Center, 2201 Inwood Road, Dallas, TX; Baylor College of Medicine, One Baylor Plaza, Houston, TX; Mayo Clinic, 200 First Street SW, Rochester, MN; Doctors Hospital of Laredo, 6801 Mcpherson Rd, Suite 106, Laredo, TX; University of New Mexico, Albuquerque, NM and MD Anderson Cancer Center, Houston, TX.

**Body:**

**Background**
ACOSOG Z1041 (Alliance) found pathological complete response rates in women with operable HER2-positive breast cancer were similar with FEC → P+T (Arm 1) and P+T → FEC+T (Arm 2) where treatment was administered as 5-FU 500 mg/m2, epirubicin 75 mg/m2 and cyclophosphamide 500 mg/m2 day 1 of a 21-day cycle x 4; paclitaxel 80 mg/m2 weekly x 12 and trastuzumab 4 mg/kg once then 2 mg/kg weekly x 11. We now report DFS and OS results with a median follow up of 4.4 years (range: 26 days to 6.2 years).

**Methods**
All patients who began study treatment were included in the analyses. Stratified log rank tests and stratified proportional hazard models were used to assess differences in DFS and OS from randomization between treatment arms.

**Results**
From September 15, 2007 to December 15, 2011, 282 women with HER2-positive breast cancer were enrolled. Two patients randomized to arm 1 withdrew consent prior to treatment and are excluded from these analyses. Patient and disease characteristics of the remaining 280 women (arm 1, n=138; arm 2, n=142) were similar between treatment arms. Recurrences and deaths are shown in the table. DFS were not found to differ with respect to treatment arm (stratified logrank p=0.6870; HR stratified (Arm2/Arm1)=0.88; 95%CI: 0.48-1.61). Also, OS was not found to differ with respect to treatment arm (stratified logrank p=0.8790; HR stratified (Arm2/Arm1)=1.07; 95%CI: 0.43-2.69).

**Conclusions**
Concurrent administration of trastuzumab with anthracyclines offers no additional benefit in terms of achieving pathologic complete response or improving survival and is not needed.

<table>
<thead>
<tr>
<th></th>
<th>FEC → P+T</th>
<th>P+T → FEC+T</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>138</td>
<td>142</td>
</tr>
<tr>
<td>Recurrences</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Deaths</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>

**Grant Support:** U10 CA76001

The study is registered with ClinicalTrials.gov, number NCT00513292.
Title: The evaluation of trebananib plus standard neoadjuvant therapy in high-risk breast cancer: Results from the I-SPY 2 trial

Albain KS S, Leyland-Jones B, Symmans F, Paoloni M, van ’t Veer L, DeMichele A, Buxton M, Hylton N, Yee D, Lyandres Clennell J, Yau C, Sanil A, I-SPY 2 Trial Investigators, Berry D and Esserman L. Loyola University, Chicago Stritch School of Medicine; Avera Medical Group; University of Texas, M.D. Anderson Cancer Center; QuantumLeap Healthcare Collaborative; University of California, San Francisco; University of Pennsylvania, Perelman School of Medicine; University of Minnesota, Masonic Cancer Center and Berry Consultants.

Body: Background: I-SPY 2 is a multicenter, phase 2 trial using adaptive randomization within biomarker subtypes to evaluate a series of novel agents/combinations when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer - investigational agent(I) +paclitaxel(T) qwk x 12 +/-trastuzumab(H), doxorubicin & cyclophosphamide(AC) q2-3 wk x 4 vs. T+/-H /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 experimental arm trebananib, an angiopoietin-1/2-neutralizing peptibody that inhibits interaction with the Tie2 receptor.

Methods: Women with tumors ≥2.5cm were eligible for screening. MP low/HR+/HER2- tumors were ineligible for randomization. MRI scans (baseline, 3 cycles after start of therapy, at completion of weekly T and prior to surgery) were used in a longitudinal statistical model to improve the efficiency of adaptive randomization. Trebananib was initially assigned to HER2- patients only; once additional safety data with H were obtained, it was also assigned to HER2+ patients (trebananib 15 mg/kg IV qwk +T +/-H). Analysis was intention to treat with patients who switched to non-protocol therapy counted as non-pCRs.

Results: Trebananib +/-H did not meet the criteria for graduation in any of the 10 signatures tested. When the maximum sample size was reached, accrual to trebananib +/-H ceased. Trebananib +/-H was assigned to XX patients, and there were XX concurrently randomized controls. We report probabilities of superiority for trebananib +/-H over control and Bayesian predictive probabilities of success in a neoadjuvant phase 3 trial equally randomized between trebananib +/-H and control, for each of the 10 biomarker signatures, using the final pathological response data from all patients (final pCR data available August 2015). There was a suggestion of activity in XX tumors. Safety was assessed in all patients (available August 2015).

Conclusion: I-SPY 2's adaptive randomization was successful in efficiently evaluating trebananib in the setting of neoadjuvant breast cancer. Although no subtype reached the efficacy threshold for graduation, the data suggest potential benefit in XX tumors that may be explored in further studies. Biomarker studies that may be predictive of response are in progress. The I-SPY 2 standing trial mechanism is effective in defining agents/combinations most likely to succeed in phase 3 biomarker-defined patient subsets.
Title: A randomized phase II neoadjuvant (NACT) study of sequential eribulin followed by FAC/FEC-regimen compared to sequential paclitaxel followed by FAC/FEC-regimen in patients (pts) with operable breast cancer not overexpressing HER-2


Body: Background: Neoadjuvant chemotherapy (NACT) is an integral component for locally advanced and large operable breast cancer. The sequence of taxanes followed by anthracyclines has been the standard of care for almost 20 years. Eribulin (E) is a synthetic analogue of halichondrin B with distinct mechanism of action as microtubule dynamics inhibitor. The FDA approved E in 11/2010 for the treatment of patients (pts) with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Research Hypothesis: Sequential administration of eribulin followed by FAC/FEC-regimen, would have greater pathologic complete response (pCR) rate than sequential administration of paclitaxel followed by FAC/FEC-regimen as primary systemic therapy for woman with operable breast cancer.

Methods: This is a phase II, randomized, single institution, open label study. Pts were randomized 1:1 to receive E (1.4 mg/m2 d1 and d8 q 21 days x 4) or paclitaxel (P) (80 mg/m2 weekly x12). Both arms received FAC/FEC regimen x 4 doses followed by surgery. Eligible pts were women age 18 or older, Karnosky PS 80 – 100, histologically confirmed invasive breast cancer, clinical T2-T3, N0-3, M0, HER2-negative. Baseline LVEF of > 50% and normal hematology, liver and kidney laboratory function tests. Primary endpoint was pathologic complete response (pCR/RCB-0) assessed by residual cancer burden (RCB). [Symmans F, 2007]. This protocol (2012-0167) IRB of The University of Texas, MD Anderson Cancer Center.

Results: A preplanned interim analysis aimed to validate trial assumption was conducted after treatment of 54 randomized pts. Between 8/2012 to 7/2014, 54 pts were randomized and 49 were evaluable for pCR(27 P arm and 22 E arm). Tumor response by RCB is shown in the table. pCR rates were 30% and 4.5% in the P and E arm, respectively

<table>
<thead>
<tr>
<th>Response</th>
<th>Paclitaxel - FAC/FEC Arm (N=27)</th>
<th>Eribulin - FAC/FEC Arm (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCB 0 (pCR)</td>
<td>8 (30%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>RCB I</td>
<td>6 (22.2%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>RCB II</td>
<td>9 (33%)</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>RCB III</td>
<td>4 (14.8%)</td>
<td>10 (45%)</td>
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</table>

53 pts were evaluable for toxicity. The combination was safe with mostly grade 1 and 2 toxicities in both arms. In the P arm grade 3 peripheral neuropathy and neutropenia was seen in 3% and 7%, respectively. In the E arm one patient died due to multiorgan failure during cycle 1. There was no other grade 3-5 toxicity. Biomarker analysis using CTCs by AdnaTest Breast were evaluated in 39 pts at baseline. 5/39 pts were positive for CTCs. 3 pts had transcripts for EpCAM, 1 for Muc-1 and another had both. 30 pts had an additional sample post therapy. 2 pts were positive for CTC at baseline as well as at follow up (FU) visit at 180 days. None of the samples showed CTC-EMT at baseline or at FU visits.

Conclusions: The interim analysis demonstrated that E arm lead to significantly lower pCR/RCB1 rate compared to P arm. Ongoing biomarker analyses include TIL, hot spot mutation analysis (HSMA) and molecular inversion probes (MIP) will be presented at the time of the meeting. Clinical trial information: NCT01593020.
Three distinct HER2 subtypes identified by BluePrint 80-gene functional subtyping predict treatment-specific response in the prospective neo-adjuvant NBRST registry

Whitworth P, Beitsch P, Baron P, Beatty J, Pellicane JV V, Murray MK K, Dul CL L, Mislowsky AM M, Nash CH H, Richards PD D, Lee LA A, Stork-Sloots L, de Snoo F, Untch S, Gittleman M, Akbari S and Rotkis MC C. Nashville Breast Center, Nashville, TN; Dallas Surgical Group, Dallas, TX; Breast & Melanoma Specialists of Charleston, Charleston, SC; The Breast Place, Charleston, SC; Virginia Breast Center, Bon Secours Cancer Institute, Richmond, VA; Akron General Hospita, Akron, OH; St. John Hospital & Medical Center, Detroit, MI; Coastal Carolina Breast Center, Murrells Inlet, SC; Northeast Georgia Medical Center, Gainesville GA, Gainesville, GA; Blue Ridge Cancer Care, Roanoke, VA; Comprehensive Cancer Center, Palm Springs, CA; Agenda Inc, Irvine, CA; Breast Care Specialists, Allentown, PA; Virginia Hospital Center, Arlington, VA and Northern Indiana Cancer Research Consortium, South Bend, IN.

Background
Ideally classification by subtype predicts treatment response and overall outcome. BluePrint 80-gene functional molecular subtype is based on mRNA expression (as is intrinsic subtype) associated with intact translation to protein (unlike intrinsic subtype). BluePrint (BP) classifies patients into Luminal, Her2 or Basal-type. Presently subtype is approximated using conventional immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) ("conventional subtype") or assigned by gene expression profiling. The main objective of the prospective neo-adjuvant NBRST study is to compare drug sensitivity as defined by pathological Complete Response (pCR), using 80-gene functional subtype vs. conventional IHC/FISH subtyping.

NBRST enrolled over 1,000 US patients between June 2011 and December 2014. In this analysis we present the results for IHC/FISH Her2-positive patients.

Methods
Here we report findings in the 260 NBRST patients who had IHC/FISH Her2+ breast cancer, according to ASCO CAP guidelines at the time of diagnosis. Treatment, including chemotherapy and HER2-targeted agents, was at the discretion of the physician adhering to NCCN approved or other peer-reviewed, established regimens over the course of the study. pCR was defined as T0/isN0. Fisher's exact test was used to compare pCR rates among IHC/FISH and functional subtypes and treatment groups.

Results
The 260 IHC/FISH Her2+ patients had median age 53 (range 23-81) and included T1-4, N0-3 tumors. Of 169 ER+/Her2+ tumors 49% were re-classified as BP Luminal, 43% as BP HER2, and 8% as BP Basal. The median ER% of ER+/Her2+/BP Luminal tumors was 93% (range 3-100), compared to 79% in ER+/Her2+/BP HER2 (range 1-91) and 8% in ER+/Her2+/BP Basal-type (range 2-99). The overall pCR rate in ER+/Her2+/BP Luminal was 17% (4% with chemo/trastuzumab; 39% chemo/trastuzumab/pertuzumab, p<0.0001) and statistically inferior (p<0.0001) to the 59% pCR rate in ER+/Her2+/BP HER2. Of 91 ER-/Her2+ tumors 74% were classified as BP HER2, 25% were re-classified BP Basal and <1% was BP Luminal. NCT pCR rates for ER-/Her2+/BP HER2 was 67% (64% with chemo/trastuzumab; 77% chemo/trastuzumab/pertuzumab, p=0.40) and significantly superior (p=0.026) to the 39% pCR rate in ER-/Her2+/BP Basal (p=0.026).

Conclusions
In the NBRST study, BP 80-gene functional subtype (based on mRNA expression and translation): 1. Re-classifies over half of all IHC/FISH ER+/Her2+ patients; 2. Predicts treatment response or resistance in Her2+ patients not segregated by conventional IHC/FISH classification and 3. Identifies ER+/Her2+ tumors that are sensitive to chemo/trastuzumab/pertuzumab but resistant to chemo/trastuzumab.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-14-06

Title: A phase II randomized study with eribulin/cyclophosphamide (ErC) and docetaxel/cyclophosphamide (TC) as neoadjuvant therapy in HER2-negative breast cancer- Final analysis of primary endpoint and correlative analysis results

Yardley DA A, Chandra P, Hart L, Wright GS S, Ward P, Mani A, Shastri M, Finney L, Guo S, DeBusk LM M, Hainsworth JD D and Burris III HA A. Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN; PathGroup, Brentwood, TN; Sarah Cannon Research Institute/Florida Cancer Specialists, Fort Myers, FL; Sarah Cannon Research Institute/Florida Cancer Specialists, New Port Richey, FL; Sarah Cannon Research Institute/Oncology Hematology Care, Inc, Cincinnati, OH; Memorial Cancer Institute, Hollywood, FL and Sarah Cannon Research Institute, Nashville, TN.

Body: Background: Eribulin mesylate (Er) is a non-taxane inhibitor of microtubule growth that results in G2-M cell cycle arrest, disruption of normal mitotic spindles and apoptosis. Er demonstrated an overall survival (OS) but not progression free survival (PFS) advantage in anthracycline and taxane refractory breast cancer pts. This OS rather than PFS benefit has been attributed to Er's potential to suppress new metastases through its effects on the epithelial mesenchymal transition (EMT) pathway, even in the absence of an effect on the primary tumor or established metastases. In this study ErC was compared to TC, a standard regimen for (neo) adjuvant treatment. A companion exploratory analysis examined the EMT markers E-cadherin and vimentin, as well as the endothelial marker CD-31 assessing tumor vasculature. Final assessments of the primary endpoint of pathological complete response (pCR) and results of the correlative studies will be presented.

Methods: Women with histologically confirmed invasive HER2-negative (IHC 0-1+ or FISH/SISH negative), cT1-3, cN0-2, M0 (pN3a disease allowed) adenocarcinoma of the breast were eligible. Following a 10 pt lead-in to confirm the safety/feasibility of ErC, pts were randomized 2:1. Arm 1, Er 1.4 mg/m$^2$ IV (Days 1 & 8) and C 600 mg/m$^2$ IV (Day 1); Arm 2, T 75 mg/m$^2$ IV and C 600 mg/m$^2$ IV on Day 1, both regimens administered q 21 days x 6 cycles followed by surgery. Tumor samples were collected at baseline and from residual breast cancer at the time of surgery. Samples were assayed for E-cadherin, vimentin, and CD-31 expression by immunohistochemistry.

Results: Enrollment was completed 4/2014 (76 pts); 10 pts in lead-in phase, 66 pts were randomized (Arm 1, 44; Arm 2, 22). In the randomized population, 77% had invasive ductal adenocarcinoma; median tumor size 3.1 cm (range, 0.4-10cm; 29.5% were T3); axillary nodes clinically positive in 52%. 34% of pts were triple negative (TN). 59 pts (89%) underwent surgery after receiving neoadjuvant chemotherapy (NAC) on study. pCR rates were 9% and 18% on the TC and ErC arms respectively. 4/7 pts with pCR on the ErC arm were TN. tumor samples were analyzed from 69 pts (including lead-in pts) for expression of the EMT biomarkers. Of these, 40 pts had paired pre- and post-treatment samples, and 29 pts had either a pre- or post-treatment sample (including 8 pre-treatment samples from pts who achieved pCR). In pre-treatment tumor specimens (61 samples), E-cadherin levels were modest-high in 80%, vimentin expression was seen in 39%, and CD-31 expression observed in 21% of the samples. Analysis of pre- and post-treatment paired specimens and differential effects according to treatment regimen will be presented.

Conclusion: The observed pCR rate of 18% with ErC in this HER2- pt population was comparable with other NAC regimens. Correlative evaluation of EMT markers and tumor vascular density with response is ongoing and will be presented.
Title: Association between quantitative values of estrogen receptor expression level and pathological complete response in human epidermal growth factor 2-negative breast cancer: Should the clinical definition of triple-negative breast cancer be redefined?

Fujii T, Kogawa T, Dong W, Moulder S, Litton JK K, Tripathy D, Lim B, Shen Y and Ueno NT T. The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX and National Cancer Center Hospital East, Kashiwa, Japan.

Body: Background: The American Society of Clinical Oncology/College of American Pathologists recommended that the cut-off for negative status of estrogen receptor (ER) should be <1% positively staining cells, although a 10% cut-off has often been used clinically. Prior studies reported that patients with ER ranging from 1% to 9% showed survival outcomes and molecular features similar to those of patients with ER positivity of <1%; however, those studies did not take into account patients' human epidermal growth factor 2 (HER2) status. This means we have yet to clarify the exact clinical definition of triple-negative breast cancer (TNBC) on the basis of response to preoperative chemotherapy. Previous studies reported that hormone receptor–positive tumors were less sensitive to systemic chemotherapies. On the basis of these facts, we hypothesized that in patients with HER2-negative breast cancer ER expression level as a continuous variable has an inverse linear association with pathological complete response (pCR) rate. Our primary objective was to determine whether a quantitative value of ER between 0% and 10% is predictive of pCR rate in HER2-negative patients treated with neoadjuvant chemotherapy. Secondary objective was to find the ideal cut-off value of ER expression.

Methods: We included newly diagnosed stage I-III HER2-negative breast cancer patients with available ER (0%≤ER<10%) who were treated with neoadjuvant systemic chemotherapy. ER status was determined by immunohistochemical (IHC) staining; HER2 status was determined by IHC and/or FISH. We used univariate and multivariate logistic regression models to determine the association between baseline variables and pCR. A backward stepwise method was used to select the covariates for the multivariate analysis. Recursive partitioning and regression tree method were used to identify the potential significant cut-off of ER.

Results: The analysis included 1155 patients with newly diagnosed HER2-negative invasive breast cancer. The univariate logistic regression analysis showed that ER as a continuous variable was not a statistically significant factor for predicting pCR (ER: OR=0.98, 95%CI: 0.9-1.07, P=0.68). In the multivariate analysis, ER status again was not a significant factor for predicting pCR (OR=0.97, 95%CI 0.9-1.06, P=0.55). ER as a categorical variable, there was no significant difference of the pCR rate between 0<ER<1 and 1≤ER<10 groups (OR=1.27, 95%CI: 0.62-2.62, P=0.52). Among ER> 0 (n=229), the recommended cut-off value of ER was 5.5. However, the odds ratio of pCR rate divided by this value of 5.5 was not significant (ER≤5 vs ER>5; OR 1.94 95%CI 0.54-6.95 P=0.31).

Conclusion: Evaluating ER (<10%) as a continuous variable showed no association with pCR rate, and no cut-off of ER was identified with which to stratify patients into groups more or less likely to achieve pCR. A potential meaningful cut-off ER value might exist between 10% and 100% in HER2-negative patients. We will explore whether a meaningful cut-off ER value exists that will change the pCR rate and possibly lead to redefining the clinical definition of TNBC.
Title: Prediction of pathological response (pCR) to neoadjuvant dose dense and intense cyclophosphamide and anthracycline in a prospective series of triple negative locally advanced breast cancers (TNLABC)


Body: Background: Stage II-III TNBC retains a poor outcome despite high chemosensitivity. Patients (pts) with pCR after neoadjuvant chemotherapy have a good prognosis whereas non-responding pts have a 25-40% risk of distant relapse at 5 years. pCR is thus a major goal in TNBC. We previously reported that TNLABC benefit the most of dose dense dose intense cyclophosphamide (C)-epirubicin (E) (S.Giacchetti; BJC, 2014)

Aim: To confirm these results prospectively and analyze the predictive factors of response to high dose chemotherapy in TNBC.

Patients and methods: From january 2009 to april 2015 non inflammatory TNLABC received high dose C (1200 mg/m2 d1 qw 2) with E (75 mg/m2/ d1 qw2) for 6 cycles. The pts had a breast biopsy with frozen tissue. We performed molecular studies: qRT-PCR for AR, FOXA1, PI3K and FASAY technic for p53 mutation. The percentage of stromal Tumor-infiltrating lymphocytes (TILs) was also evaluated by two independent pathologists and assessed as a continuous variable. A18F-FDG PET/CT was performed initially and after 2 courses of chemotherapy and the metabolic answer assessed as a variation of the tumor uptake (ΔSUVmax). We report here the pathological complete response (pCR) (absence of infiltrative carcinomas in the breast and in the lymph nodes) and the factors associated with pCR.

Results: The characteristics of the 74 pts are listed in table1. The median age is 48 years old, 48 pts (65.8%) are premenopausal and 79% did not have any family history of breast cancers. TIL was divided in 3 groups < 10 % (26 pts, 40 %); 10-50 % (30 pts, 46 %) > 50% (9 pts, 14 %). Pathological response was assessed in 66 pts, one pt progressed during chemotherapy and 6 pts did not undergo surgery yet. 28 pts were in pCR (42.4 %). With a median follow up of 25 months, 13 pts (17.8 %) progressed and 8 (11%) died.

Table 1: Patients characteristics and pCR according to tumor features and metabolic response

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of pts (%)</th>
<th>N of pts evaluated for pCR</th>
<th>pCR (%)</th>
<th>OR [IC 95%]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size T2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>36</td>
<td>35</td>
<td>19 (54)</td>
<td>1</td>
<td>0.34 [0.12 ; 0.96]</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>31</td>
<td>9 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodal status N0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>36</td>
<td>33</td>
<td>15 (46)</td>
<td>1</td>
<td>0.78 [0.29 ; 2.07]</td>
</tr>
<tr>
<td>N1/N2/N3</td>
<td>24/11/3</td>
<td>33</td>
<td>13 (39)</td>
<td></td>
<td></td>
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<tr>
<td>Histological grade: 2</td>
<td></td>
<td></td>
<td>0 (0)</td>
<td></td>
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<tr>
<td>3</td>
<td>6</td>
<td>6</td>
<td>28 (47)</td>
<td></td>
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<tr>
<td></td>
<td>67</td>
<td>60</td>
<td></td>
<td></td>
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<tr>
<td>TILs &lt;10 %</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>10-50 %</td>
<td>26 (40)</td>
<td>25</td>
<td>10 (40)</td>
<td>1</td>
<td>0.55 [0.17 ; 1.80]</td>
</tr>
<tr>
<td>≥ 50</td>
<td>30 (46)</td>
<td>26</td>
<td>7 (27)</td>
<td>1</td>
<td>5.25 [0.90 ; 30.62]</td>
</tr>
<tr>
<td></td>
<td>9 (14)</td>
<td>9</td>
<td>7 (78)</td>
<td></td>
<td></td>
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<tr>
<td>P53 Mutated</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>WT</td>
<td>54 (89)</td>
<td>51</td>
<td>21 (41)</td>
<td>1</td>
<td>2.14 [0.33; 13.96]</td>
</tr>
<tr>
<td></td>
<td>7 (12)</td>
<td>5</td>
<td>3 (60)</td>
<td></td>
<td></td>
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<tr>
<td>AR Negative Positive</td>
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<tr>
<td></td>
<td>43 (83)</td>
<td>43</td>
<td>18 (42)</td>
<td>1</td>
<td>1.74 [0.41 ; 7.38]</td>
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<tr>
<td></td>
<td>9 (17)</td>
<td>9</td>
<td>5 (56)</td>
<td></td>
<td></td>
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<tr>
<td>FOXA1 Negative Positive</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>40 (77)</td>
<td>40</td>
<td>15 (38)</td>
<td>1</td>
<td>3.33 [0.86 ; 12.99]</td>
</tr>
<tr>
<td></td>
<td>12 (23)</td>
<td>12</td>
<td>8 (60)</td>
<td></td>
<td></td>
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<tr>
<td>Molecular Apocrine TN</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>8 (17)</td>
<td>8</td>
<td>5 (63)</td>
<td>1</td>
<td>0.35 [0.07 ; 1.69]</td>
</tr>
<tr>
<td></td>
<td>38 (83)</td>
<td>38</td>
<td>14 (37)</td>
<td></td>
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</tbody>
</table>
Tumor size, tumor grade, percentage of TILs, the change in 18F-fluorodeoxyglucose tumor uptake ($\Delta$SUVmax) were significantly associated with pCR at univariate analysis. Only one factor remained significant at multivariate analysis, the $\Delta$SUVmax, OR: 0.04 [0.007-0.27], p = 0.0008.

**Conclusion:** In this prospective phase III trial we confirm the efficacy of a dose dense EC in TNBC. The metabolic response evaluated with 18 F-FDG PET/CT is a strong and reliable predictor of pCR and could allow an early change of treatment for the non responders. A clinical trial is planned to test this strategy.
Title: Long term survival of locally advanced breast cancers (LABC) treated with neoadjuvant treatment, results of a multicenter randomised phase II study (Remagus 02 trial)


Body: Background: The primary analysis of the REMAGUS-02 multicenter randomized phase II trial demonstrated that celecoxib did not improve pCR rates in pts with HER2-negative localized invasive breast cancer (BC), whereas trastuzumab increased pCR rates in HER2-positive ones [Pierga BCRT 2010]. We report here the long-term follow-up results of this trial for disease free survival (DFS) and overall survival (OS).

Patients and methods: From May 2004 to October 2007, 340 stage II-III BC patients were randomly assigned to receive 4 cycles (c) of epirubicin–cyclophosphamide q 3 w followed by 4 c of docetaxel q 3 w +/- trastuzumab in HER2 positive pts (120 pts) or +/- celecoxib in HER2 negative pts (n=220). From September 2005, all pts with HER2 positive BC received adjuvant T for a total of 18 c (n=106). Patients with hormone receptors (HR) positive tumor received adjuvant endocrine treatment according to menopausal status

Results: With a median follow up of nearly 8 years (94.4 months, 20-127m), 112 relapses and 75 deaths have been observed (median DFS and OS not reached). Eight years DFS and OS were respectively 63 % [57%-71%] and 75 % [70%-81%] in HER2 negative group; and 75% [67%-83%] and 82 % [74%-90%] in HER2 positive group. DFS was significantly higher in HER+ pts than in HER2-(HR: 0.64 [0.42-0.99], p=0.042), whereas OS did not differ significantly (HR: 0.67, [0.41-1.11], p=0.123).

In the overall population, progesterone receptor (PgR) positivity was associated with a better DFS (p=0.012) and OS (p<0.001) as compared to ER+/PgR- (DFS: HR=2.07 [1.27-3.39]; OS: HR=2.53 [1.3-4.92]) and ER-/PR-; DFS: HR=1.56 [0.98-2.46]; OS: HR: 3.34 [1.87 – 5.97]. In the ER-/PR- group, DFS reached a "plateau" after three years follow-up, while the annual risk of relapse remained constant in the ER+/PR- subgroup .

In the HER2- subgroup, no effect of neoadjuvant celocoxib was observed on survival, neither in intention to treat (ITT) nor in per protocol analyses. In the multivariate analysis clinical stage (T3/T4 versus T2, HR: 1.92 [1.209 - 3.05], p=0.006), PgR status (positive versus negative HR : 0.52, [0.32-0.84], p=0.007) and pCR (yes vs no, HR : 0.213 [0.066-0.687], p=0.01) were significant predictors of DFS.

In the HER2+ subgroup, neoadjuvant versus adjuvant trastuzumab was not significantly associated with DFS, neither in the ITT, nor in the per protocol analysis.

Conclusion: Celecoxib was not associated with pCR or survival benefit when added to conventional neoadjuvant CT in Her2-negative BC pts. Lack of PgR expression is a major prognostic factor for survival. Neoadjuvant versus adjuvant trastuzumab increased pCR rates but did not change significantly DFS and OS of HER2 positive BC pts.
**Title:** Phase II trial of neoadjuvant chemotherapy with carboplatin and nab-paclitaxel in patients with triple negative locally advanced and inflammatory breast cancer


**Body:** Background: Pathologic complete response (pCR) and residual cancer burden (RCB scores of 0 [pCR] or 1[near CR]) after neoadjuvant chemotherapy (NCT) may predict for improved survival (Symmans et al. J Clin Oncol 25:4414-22, 2007). We set out to test the pCR rate with an anthracycline-free regimen of carboplatin (carb) and nab-paclitaxel (nab) in patients (pts) with triple negative breast cancer (TNBC).

**Materials and Methods:** Forty-nine pts with stages II-III BC were to receive carb (AUC 6) on day 1 of a 28 day cycle, and nab 80 mg/m$^2$ weekly, for a total of 4 cycles. Core biopsies were performed prior to NCT. Blood procurement for circulating tumor cell (CTC) analysis using the CellSearch platform was carried out pre-treatment, mid-treatment, and at surgery. We set out to assess the predictive value of Mammaprint (poor vs. good), BluePrint (basal, vs. luminal, vs. HER2) molecular subtype as well as microarray RNA and miRNA profiling, for pCR. Responses were also dichotomized as complete or near complete response (Symmans RCB scores of 0-1) vs. suboptimal response (RCB score > 1).

**Results:** The median age was 53 (28-75). Pts presented with clinical stages II (63%) and III (37%). So far, 38 of the 49 pts accrued between 2/2012 and 6/2015, have undergone surgery, 68% of whom underwent modified radical mastectomy. The pCR rate (breast and lymph nodes in CR) was 53%, and RCB 0 and 1 were seen in 68% of pts. Toxicites included grade ¾ anemia (45%), thrombocytopenia (13%) and neutropenia (53%, 1 pt with neutropenic fever). Dose adjustments were needed in over 80% of pts. Grades 2 or 3 peripheral neuropathy were seen in 8% each, and grades 3-4 fatigue (13%), hypokalemia (3%), and hyponatremia (3%) were observed. The median number of CTCs (pre-NCT) observed in 7 CTC positive pts of the first 27 pts who completed surgery was 1 (0-7), and 2 of the 7 pts continued to have CTCs at the time of surgery (1 CTC each), while 2 pts without CTCs pre-NCT had CTCs (1 each) detected at surgery. The final pt enrolled is expected to complete surgery by 10/2015. Results of sequential CTC assessments, Mammaprint/Blueprint and RNA/miRNA analysis of pre- and post-treatment specimens and their correlation with pCR will be presented.

**Conclusion:** The non-anthracycline-containing regimen of carb and nab-paclitaxel induced a high pCR rate in TNBC, in preliminary analysis. Ongoing profiling may allow for future subset-specific modification of this regimen to increase pCR across all molecular subtypes of TNBC.
Title: nab-paclitaxel at a dose of 125 mg/m$^2$ weekly is more efficacious but less toxic than at 150 mg/m$^2$. Results from the neoadjuvant randomized GeparSepto study (GBG 69)

von Minckwitz G, Untch M, Jakisch C, Schneeweiss A, Conrad B, Aktas B, Denkert C, Eidtmann H, Weibringhaus H, Kümmel S, Hilfrich J, Warm M, Paepke S, Just M, Hanusch C, Hackmann J, Blohmer J-U, Clemens M, Costa SD, Gerber B, Nekljudova V and Loibl S. German Breast Group, Neu-Isenburg, Germany; Helios Klinikum Berlin-Buch, Berlin, Germany; Sana Klinikum Offenbach, Offenbach, Germany; University Hospital Heidelberg, Heidelberg, Germany; Elisabeth Krankenhaus, Kassel, Germany; University Hospital Essen, Essen, Germany; Charité-University of Berlin, Berlin, Germany; Universitätsklinikum Schleswig-Holstein, Kiel, Germany; St. Barbara Kliniken Heessen, Heessen, Germany; Kliniken Essen Mitte, Essen, Germany; Eilenriede Klinik Hannover, Hannover, Germany; Kliniken der Stadt Köln, Köln, Germany; Universitäts-Frauenklinik Rechts der Isar, München, München, Germany; Onkologie Praxis Bielefeld, Bielefeld, Germany; Rotkreuzklinikum München, München, Germany; Marienhospital Witten, Witten, Germany; Mutterhaus Trier, Trier, Germany; University Hospital Magdeburg, Magdeburg, Germany and University Hospital Rostock, Rostock, Germany.

Body: Background: We previously reported that nab-paclitaxel (nP) increases the pathological complete response (pCR, ypT0 ypN0) rate when it replaces solvent-based paclitaxel (P) as part of a sequential taxane followed by epirubicin/cyclophosphamide (EC) neoadjuvant chemotherapy for patients with early breast cancer (Untch et al. SABCS 2014). Here, we report efficacy and safety of patients being treated either with 150 mg/m$^2$ nab-paclitaxel (nP150) before an amendment or with 125 mg/m$^2$ nab-paclitaxel (nP125) thereafter in comparison to solvent-formulated paclitaxel at 80 mg/m$^2$ (P80).

Methods: In the GeparSepto study (NCT01583426), 1207 patients were randomized to either nP150 or P80 q1w for 12 weeks followed by 4 cycles of conventionally dosed EC (E: 90mg/m$^2$; C: 600 mg/m$^2$) q3w. The primary objective of the study was to compare the pCR rate (pCR, ypT0 ypN0). Patients with untreated, histologically confirmed uni- or bilateral, cT2- cT4d carcinoma, and no clinically relevant cardiovascular and other co-morbidities were included. Patients with HER2+ tumors received trastuzumab (loading dose 8mg/kg; 6 mg/kg) plus pertuzumab (loading dose 840 mg; 420 mg) q3w concomitantly to all chemotherapy cycles. After a safety analysis showed a higher rate of dose reductions and treatment discontinuations with nP150 compared to P80, weekly dose of nP was reduced to 125 mg/m$^2$.

Results: nP was given for the majority of cycles at a dose of 150 mg/m$^2$ to 179 patients and at a dose of 125 mg/m$^2$ to 426 patients. Treatment characteristics were fairly balanced between these two sequential cohorts as well as compared to 601 patients receiving P80 except for HER2 status (HER2-positive: nP150 22%, nP125 37% and P80 33%) and Ki67 (<20%: nP150 60%, nP125 73% and P80 69%). Taxane treatment was discontinued in 16% (nP150), 11% (nP125) and 6% (P80) of patients, respectively. Median dose per cycle (based on relative total dose intensity (RTDI)) was 129 mg/m$^2$ with nP150, 119 mg/m$^2$ with nP125 and 78 mg/m$^2$ with P80, respectively. Peripheral sensory neuropathy (PNP) grade 3/4 (NCI-CTCAE v4.0) was observed in 15% with nP150, 8% with nP125 and 3% with P80, respectively. pCR was 32% with nP150, 41% with nP125 and 29% with P80 in all patients and 46% with nP150, 49% with nP125 and 26% with P80 in 277 patients with triple-negative breast cancer, respectively.

Conclusions: Risk-benefit ratio of nP125 was improved over nP150 with better drug adherence and RTDI, lower frequency of PNP but a higher pCR rate. It should therefore be considered as the preferred schedule when nP is used as neoadjuvant treatment for primary breast cancer.

The trial was financially supported by Celgene and Roche.

Body: Pathological evaluation of response after NAC is a controversial issue. M&P has been for years the most widely employed score. Recently RCB index, a new system including axillary evaluation, seems to improve prognostic discrimination.

PURPOSE: The aim of this study was to validate the RCB index as a method to define prognosis in a real-life cohort of EBC patients treated with NAC and compare it to M&P system.

METHODS: We performed a retrospective analysis of our database. Patients with stage I-III considered candidate for NAC between January 2003 and August 2011 were included. RCB and M&P were calculated as previously published. Hormone receptor expression (RH), and Her2 were assessed following international guidelines. The Harell c-index and Roc curves were used to compare the prognostic value of RCB and M&P. Clinical, therapeutic and pathological data were obtained from medical records. A correlation with disease-free survival (DFS) and overall survival (OS) was done using the Kaplan-Meier method and Cox regression model.

RESULTS: 333 patients were included in this study. Distribution of breast cancer subtypes was: luminal 50.9%, Her2+ 31% and triple negative 18.1%. Mean tumour size was 42.3 mm. The majority of the patients had histological grade II-III tumours (87.3%), with N stage 0-1 (97.3%). 87.4% received anthracycline and taxane-based NAC, associated to trastuzumab in her 2+ patients. Pathological complete response was 14% for the global population, being 30% for TN and 21.3% Her2+ subtypes. With a median follow-up of 56.3 months, DFS and OS at 5 years were as follows: RCB-0 93.6% and 97.9%, RCB-I 84.2% and 98.2%, RCB-II 79.1% and 89.5%, RCB-III 38.3% and 63.6%. Harell c-index value of RCB was statistically superior to M&P in both DFS (0.80 vs 0.68, p= 0.001) and OS (0.87 vs 0.69, p<0.001). This superiority value of RCB was consistent among all breast cancer subtypes.

CONCLUSION: RCB index is as more accurate prognostic score to predict DFS and OS compared to M&P.
Title: Residual proliferative cancer burden (RPCB) is superior to RCB index as prognostic tool in early breast cancer patients (EBC) treated with neoadjuvant chemotherapy (NAC)


Body: BACKGROUND: Many different scales have been developed in order to assess response to NAC. Apart from Miller and Payne and RCB systems, recently the addiction of post-NAC pathological Ki 67 (yp Ki67) to RCB, called RPCB system, has been considered as a more accurate prognostic tool. The aim of this study is to assess the prognosis value of RPCB in a routine practice cohort and to compare it to RCB index and ypKi67.

METHODS: We performed a retrospective analysis of our database. Patients with stage I-III considered candidate for NAC from July 2008 and August 2011 were included. RPCB and RCB were calculated as previously published. Hormone receptor expression (HR), ypKi 67 and Her2 were assessed following international guidelines. The Harell c-index were used to compare the prognostic value of RPCB, RCB and ypKi67. Clinical, therapeutic and pathological data were obtained from medical records. A correlation with disease-free survival (DFS) and overall survival (OS) was done using the Kaplan-Meier method and Cox regression model.

RESULTS: From our database including 333 EBC patients treated with NAC 184 had data to calculate RPCB, RCB and ypKi 67, of whom 51.6% were HR+Her2- tumours, 21.7% HR+Her2+, 8.2% HR- Her2+ and 18.5% triple negative. Mean tumour size was 37.5 mm (25-45). The majority of the patients had histological grade II-III tumours (84.2%) and N stage 0-1 (96.7%). 67.4% of the patients received anthracycline and taxane-based NAC, associated to trastuzumab in Her2+ patients (26.1%). Pathological complete response by subtypes were 6.3%, 17.5%, 60% and 26.5%, respectively. With a median follow-up of 49.9 months, DFS and OS at 36 months were 85.2% and 95.1%. In the multivariate analysis all three systems were prognostic for DFS (RPCB p<0.001; RCB p=0.001; ypKi 67 p= 0.002) and OS (RPCB p<0.001; RCB p=0.011; ypKi 67 p= 0.037). Comparison of Harell c-index for DFS between RCPB and RCB showed a trend of RPCB towards a more accurate prognostic power (0.89 vs 0.81, p=0.061) that was significant when comparing RCPB vs ypKi67 and RPCB vs ypKi67 (0.89 vs 0.77, p=0.010). However no statistically differences were found in terms of OS (RPCB vs RCB 0.85 vs 0.82, p=0.357 and RPCB vs ypKi67 0.85 vs 0.72, p= 0.088).

CONCLUSION: RPCB, RCB and ypKi67 are prognostic for both DFS and OS in EBC patients treated with NAC. RPCB is a more accurate prognostic tool than ypKi67 and showed a trend towards superiority compared to RCB.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-14-14

Title: Impact of body mass index (BMI) and neo-adjuvant chemotherapy (NAC) dosing on pathologic complete response (pCR) in operable breast cancer (bc)

Raman R, Mott SL L, Schroeder MC C and Thomas A. University of Iowa, Iowa City, IA.

Body: Introduction:
pCR following NAC is associated with improved long term outcomes. Though obesity is associated with chemo-resistance, its impact on pCR is less clear, likely because most studies were unable to account for NAC dose adjustments. An association between taxane dose reduction and BMI has previously been shown in European populations. However, most patients (pts) with increased BMI had doses capped at BSA of 2. We studied the impact of BMI on NAC dosing when treatment is based on actual weight and whether dosing adjustments preferentially impact pCR rate.

Methods: Pts prospectively enrolled in the University of Iowa Breast Molecular Epidemiologic Resource from 2010-14 with invasive bc who received at least one cycle of NAC were eligible. Pts were stratified by BMI category: normal (BMI ≤25) or overweight-obese (BMI >25). Planned total dose was calculated based on both dosing and number of cycles. Dose reduction was defined as any decrease in total intended dose. pCR was defined as no residual invasive disease in breast and lymph nodes. To investigate the relationship between BMI, dose reductions, and pCR, chi-square tests and logistic regression models were used.

Results: 87 pts were eligible. 22 (26%), 25 (29%) and 51 (59%) of pts had HER2 positive, triple negative and hormone receptor positive bc (HER- or +), respectively. 62 (71%) pts had Stage I-II bc. All pts received a combination of taxanes with other agents (Cytoxan, 5FU, Carboplatin, Gemcitabine) with or without an anthracycline (Adriamycin or Epirubicin) or anti HER 2 therapy (Pertuzumab, Trastuzumab or Lapatinib) and were initially dosed based upon actual body weight. Taxanes were planned at treatment initiation in all pts. Anthracyclines were given to 71 (82%) pts. pCR was achieved in 28 (32%) pts. Association between BMI and NAC dosing as shown in -

Table 1: Association between NAC dosing and BMI category

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Overweight-Obese</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>36</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Taxane dose reduction</td>
<td>30(83%)</td>
<td>6(17%)</td>
<td>29(57%)</td>
</tr>
<tr>
<td>Non taxane dose reduction</td>
<td>28(78%)</td>
<td>8(22%)</td>
<td>40(78%)</td>
</tr>
</tbody>
</table>

Relative to normal weight pts, overweight-obese pts were more likely not to achieve a pCR (OR 2.09, CI 0.84-5.21, p=0.11) and have residual disease in the breast alone (OR 2.92, CI 1.18-7.24, p=0.02). Overweight-obese pts with taxane reductions, relative to overweight-obese pts without taxane reductions, were at elevated odds of not achieving a pCR (OR 2.03, CI 0.53-7.73).

Table 2: Impact of taxane dose reduction on pCR by BMI category

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Overweight-Obese</th>
</tr>
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<tr>
<td></td>
<td>No pCR</td>
<td>pCR</td>
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<tr>
<td>Taxane reduction</td>
<td>No</td>
<td>19(63%)</td>
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<tr>
<td></td>
<td>Yes</td>
<td>2(33%)</td>
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</table>

Interaction between BMI and taxane dose reduction on pCR trended towards significance (p=0.10), a trend not seen for non-taxane drugs. The most common adverse event resulting in taxane dose reduction was neuropathy (10/28 pts).
Conclusion: Overweight-obese women experienced significantly higher rates of taxane dose reductions during NAC for bc with initial full-weight dosing. BMI status may modify the effects of taxane dose reduction on the likelihood of not achieving a pCR. Further investigation of this outcome in a larger cohort is warranted.
Title: A randomized phase III trial of neoadjuvant sequential chemotherapy with 4 cycles of Adriamycin plus cyclophosphamide followed by 4 cycles of Docetaxel (AC4-D4) versus shorter 3 cycles of FEC followed by 3 cycles of Docetaxel (FEC3-D3) in node-positive breast cancer (Neo-Shorter): First report of efficacy & toxicity profile


Body: Background: The addition of a taxane to anthracycline-based chemotherapy provided an improved outcome in neoadjuvant setting. Two neoadjuvant chemotherapy with 4 cycles of AC followed by 4 cycles of docetaxel (AC4-D4) and 3 cycles of FEC followed by 3 cycles of docetaxel (FEC3-D3) are widely used. Short duration of chemotherapy, 6 cycles rather than 8 cycles might be an attractive approach.

Methods: This is a randomized, single-center, prospective, parallel group, comparative phase III trial (NCT02001506). Patients (pts) with breast cancer of clinically stage II or III, or sized > 1.5 cm with histologically proven lymph-node involvement were included. Pts were stratified according to hormone receptor and HER2 expression status and randomized to AC4-D4 and 3 cycles of FEC3-D3 treatment. The primary endpoint was pathological complete response, defined as the absence of invasive disease in the breast and axillary lymph nodes, analyzed by intention to treat.

Results: At the time of submission, a total of 207 pts were enrolled; 1 pt failed screening; 25 pts dropped out (5 pts in AC4-D4 arm and 2 pts in FEC3-D3 arm discontinue treatment due to progressive disease); 39 pts are still receiving neoadjuvant chemotherapy; 142 pts, who received surgery, were included for this analysis. In AC4-D4 arm, among 64 pts, 57 pts achieved clinical response (6 complete response [CR] and 51 partial response [PR]) and among them 9 pts achieved pathologic complete response [pCR]. In FEC3-D3 arm, among 78 pts, 66 pts achieved clinical response (7 CR and 59 PR) and among them 11 pts achieved pCR. Addition of docetaxel increased clinical response in both arms. The most common adverse event was febrile neutropenia. Without prophylactic G-CSF, grade ≥3 febrile neutropenia (FN) occurred 23/661 cycles (3.5%) in AC4-D4 arm and 23/552 cycles (4.2%) in FEC3-D3 arm, respectively. Grade 3 and 4 toxicities other than FN were reported at expected levels in both groups. Sixty-one severe adverse events were reported; 33 (including 23 FN) in AC4-D4 arm and 28 (including 23 FN) in FEC3-D3 arm.

Conclusion: Compared to AC4-D4, shorter duration of FEC3-D3 neoadjuvant chemotherapy showed similar efficacy of pCR rate of 14.0% (versus 14.1% in AC4-D4 arm). The most common and important adverse event was febrile neutropenia in both arms. Updated study findings will be provided.
Body: Background A trastuzumab/carboplatin/docetaxel regimen is an established alternative for the more commonly used and toxic anthracycline/cyclophosphamide containing regimen in HER2-positive breast cancer. Weekly paclitaxel, however, may be more effective and better tolerable than three-weekly docetaxel.

Aim To assess the efficacy and safety of an anthracycline/cyclophosphamide-free neoadjuvant treatment regimen with weekly paclitaxel, carboplatin and trastuzumab in HER2-positive breast cancer.

Patients and methods The TRAIN-study is a multicenter phase II trial which was developed during the 9th ECCO/AACR/ASCO Workshop on "Methods in Clinical Cancer Research" in Flims, Switzerland. Patients with stage II or III HER2-positive breast cancer were eligible. Treatment consisted of weekly administrations of paclitaxel ([P], 70mg/m2), trastuzumab ([T], 2mg/kg, loading dose 4mg/kg), and carboplatin ([C], AUC = 3mg/ml/minute) for a total of 24 weeks. In cycles 7, 8, 15, 16, 23 and 24 only trastuzumab was administered. The primary endpoint was pathologic complete response (pCR), defined as no residual invasive tumor cells in the breast and axilla. Event-free survival was evaluated as a secondary endpoint. In addition, we report efficacy results in an additional cohort of patients treated with the same regimen after study closure. Toxicity data were only recorded for the study population.

Results Baseline characteristics of 109 study patients and 72 additional patients were similar. The pCR rate in the study population was 43% (95% confidence interval [CI] 33-53%) and in all evaluable patients combined 47% (95% CI 39-54%). The median follow-up was 41 months (interquartile range [IQR] 20-53). The 3-year event-free survival estimate was 89% (95% CI 84-95%). Patients who achieved a pCR had a better prognosis than patients who did not (hazard ratio [HR] 0.33; 95% CI 0.11-1.00, p<0.05).

In the study population the most common grade 3-4 adverse events were neutropenia (67%) and thrombocytopenia (43%). Febrile neutropenia occurred in less than five percent of patients. During the neoadjuvant treatment period no symptomatic left ventricular systolic dysfunction was observed. Dose reductions were implemented in 56% of study patients and at least one chemotherapy dose was skipped in 67% of patients.

Conclusion Weekly paclitaxel, trastuzumab, and carboplatin is a highly effective neoadjuvant regimen in HER2-positive breast cancer with manageable toxicity. In the currently running randomized TRAIN-2 study this regimen will be directly compared to an anthracycline/cyclophosphamide containing neoadjuvant regimen in the setting of dual HER2-blockade with pertuzumab (clinicaltrials.gov NCT01996267).
Title: Pathologic complete response rate with doxorubicin and cyclophosphamide followed by weekly paclitaxel with trastuzumab and pertuzumab in patients with HER2-positive early stage breast cancer: A single institution experience


Body: Background:
Trastuzumab and pertuzumab (HP) with standard chemotherapy is approved for use in the neoadjuvant setting. We performed a retrospective analysis of patients (pts) treated with dose-dense doxorubicin and cyclophosphamide (AC) → paclitaxel, trastuzumab, pertuzumab (THP) in the neoadjuvant setting. Here we report the pathologic complete response (pCR) rate.

Methods:
We abstracted medical records of patients who were treated with pertuzumab-based therapy in the neoadjuvant setting from September 1, 2013 to March 1, 2015. Charts were analyzed for pt demographics, stage of breast cancer, pathology reports, surgical data, and information on systemic therapy.

Results:
Charts from 66 pts were reviewed; 60 pts were evaluable for pCR defined as absence of invasive disease in the breast, and 6 were not (3-no anthracycline, 1-incomplete chart, 1-no surgery yet, 1-metastatic). Median age was 47 years (range 28-68 years). Of 60 pts, 52 (86%) had operable breast cancer (T1-3, N0-1, M0) of which 7 had clinical stage I disease (T1N0); 7 (12%) had locally advanced disease (T2-3, N2-3, M0 or T4a-c, any N, M0), and 1 (2%) had inflammatory breast cancer (T4d, any N, M0). 49 (82%) and 11 (18%) had hormone receptor (HR)-positive and negative diseases, respectively. All patients had HER2-positive breast cancer defined as immunohistochemistry (IHC) 3+ and/or fluorescent in-situ hybridization (FISH) of > 2.0. 30 pts (50%) underwent mastectomy and lumpectomy, respectively. Out of 60 evaluable pts, 41 (68%) had pCR; 32/49 (65%) with HR-positive and 9/11 (82%) with HR-negative diseases had pCR, respectively. Overall 58/60 (97%) pts completed neoadjuvant therapy; 2 did not (1 developed Steven Johnson Syndrome after one cycle of AC and 1 developed pneumonitis after third weekly dose of T with HP).

Conclusions:
At our single center experience the pCR rate of dose dense AC→THP is high at 68 %. These data are similar to results seen in the TRYPHAENA study, and we await the results from the BERENICE trial evaluating pCR as a secondary endpoint.

Patient Demographics

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<td>&gt;55</td>
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<td>49 (82%)</td>
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<tr>
<td>HR- Her2+</td>
<td>11 (18%)</td>
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<td>--------------------------</td>
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</tr>
<tr>
<td>IHC positive</td>
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<td>FISH positive</td>
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<td><strong>Median tumor size</strong></td>
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<td><strong>Stage</strong></td>
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<td>Operable (T1-2, N0-1, M0)</td>
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<td>Operable Stage II/III</td>
<td>45 (74%)</td>
</tr>
<tr>
<td>Locally advanced (T2-3, N2-3, M0 or T4a-c, any N, M0))</td>
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</tr>
<tr>
<td>Inflammatory (T4d, any N, M0)</td>
<td>1 (2%)</td>
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<tr>
<td><strong>Type of surgery</strong></td>
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<tr>
<td>Lumpectomy</td>
<td>30 (50%)</td>
</tr>
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<td>Mastectomy</td>
<td>30 (50%)</td>
</tr>
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</table>
Title: Postmastectomy radiotherapy improves the outcomes of stage III breast cancer patients with negative lymph nodes after neoadjuvant chemotherapy

He M, Li J, Ni X-J, Chen S, Jiang Y-Z, Di G-H and Shao Z-M. Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Purpose: The aim of this study is to evaluate the role of postmastectomy radiotherapy (PMRT) in clinical stage II and III breast cancer patients who achieved negative node status (pN0) after neoadjuvant chemotherapy (NAC).

Material and methods: We retrospectively analyzed the outcomes of 143 patients with pN0 after NAC and mastectomy at Fudan University Shanghai Cancer Center. In total, 103 (72%) patients received PMRT, and 40 (28%) patients did not. Univariate and multivariate survival analyses were performed to evaluate the effect of PMRT on locoregional recurrence-free survival (LRRFS) and overall survival (OS) of the two groups.

Results: There were no differences between the two groups with respect to age, nuclear grade, estrogen receptor (ER) status, HER2/neu receptor status, lymphovascular space invasion (LVSI) status or pathological tumor size. However, a significantly higher proportion of patients in the irradiated group (64%) had clinical lymph node involvement than in the nonirradiated group (45%). After a median follow-up time of 49 months, 10 locoregional recurrence events occurred. For the entire cohort of patients, use of radiation therapy improved the 5-year LRRFS rate (94.5% vs. 80.2%; P = 0.032) but not the 5-year OS rate (92.2% vs. 88.7%; P = 0.617). In the subset of patients who presented with clinically stage II disease, the 5-year LRRFS and 5-year OS did not differ significantly between the PMRT and no-PMRT group (96.3% vs. 91.3%; P = 0.190 and 96.2% vs. 91.3%; P = 0.199, respectively). For patients with stage III disease at diagnosis, a trend was seen toward better local regional control with PMRT (the 5-year LRRFS rate was 92.7% vs. 64.2%; P = 0.063), although the benefit from radiation with respect to OS was not significant (5-year OS rate was 88.1% vs. 85.2%; P = 0.657). On multivariate Cox regression analyses, the clinical tumor size (hazard ratio [HR], 3.27; 95% confidence interval [CI], 1.05-10.18; P = 0.041), pathologic breast tumor response (HR, 1.82; 95% CI, 1.11-3.77; P = 0.046) and delivery of radiation therapy (HR, 1.27; 95% CI, 1.08-9.25; P = 0.047) were independent predictors of locoregional recurrence.

Conclusions: For patients who achieved pN0 after NAC, PMRT seemed to provide a clinical benefit for breast cancer patients with stage III disease. Omission of PMRT in patients with stage II disease did not increase the risk of locoregional recurrence and death.
Clinical and morphological results of neoadjuvant treatment with tumor necrosis factor-alpha-thymosin-alpha1 (TNF-T) of triple-negative locally advanced breast cancer patients


Background. There is a few data on tumor necrosis factor drugs used in neoadjuvant treatment in locally advanced breast cancer (LABC) patients but there is a clinical need to increase the efficiency of standard therapy and to gain complete regression which is of great importance for survival. The aim of the study was to evaluate the efficacy of recombinant hybrid protein of tumor necrosis factor-alpha-thymosin-alpha1 (TNF-T) in neoadjuvant treatment of triple-negative LABC (TN LABC).

Methods. Eligibility criteria included TN LABC of IIB-IIIB stages, ECOG ≤ 1, adequate liver, kidney and bone marrow function, no brain metastases. Recombinant hybrid protein of TNF-T 200000 IU was used peritumorally (injected around the tumor) on D1-5 (30 min before cytostatics injection), combined with standard FAC or PA regimens.

Results. 52 women were recruited between April 2012 – October 2013 (mean age 53.3±1.1 years). Group A (20 pts) received recombinant hybrid protein of TNF-T combined to PA (11) and FAC (9) up to 6 courses. Group B (32pts) received standard PA (18) and FAC (14). Tumor response (TR) in Group A was 80% and in Group B 71.9% (p<0.05), including CR 15% and 6.25% correspondingly (p<0.05). After neoadjuvant chemotherapy quantity of CD3+CD8+ cells in group A (PA) was significantly higher than in group B (PA) (31.5±2.8% and 21.7±2.25% correspondingly, p<0.05). Content of B-lymphocytes (CD20) decreased during the treatment in group B (from 15.5±0.53% to 13.7±0.55% (FAC) and from 16.7±0.97% to 12.7±1.0% (PA) correspondingly, p<0.05). CD20 level in group A after the treatment PA+TNF-T was 15.0±1.0%, after FAC+TNF-T was 15.4±1.4% which were different significantly from those in group B (p<0.05). Common toxicities in Group A were: neutropenia Gr1 – 15.9% courses, nausea and vomiting Gr2– 27.2%, polynueropathy Gr1/2 – 9%. TNF-T-specified toxicities were: hyperthermia Gr1 – 45.4%; local reaction (aula, pain) Gr1 lasted for 24-48 hrs – 90.9%. Group B: neutropenia Gr 1/2 – 21.2%, nausea and vomiting Gr2– 32.5%, polynueropathy Gr1/2 – 10.6%, diarrhea Gr1 – 4.5. All patients in Group A were operated successfully, no postoperative complications connected with recombinant hybrid protein of TNF-T use were observed. By the time of abstract submission OS in Group A was 28.4±1.1mos, in Group B was 26.1±1.4mos; EFS in Group A was 26.9±1.3mos, in Group B was 22.9±1.2mos (p<0.05).

Conclusions. TNF-T injected peritumorally is well-tolerated and allows to enhance an antitumor effect of cytostatics (pCR) which is of great importance for survival in TN LABC. The proposed method of the locally application of recombinant hybrid protein of TNF-T has mainly immunostimulant effect on CD3+CD8+ in patients with PA regimen. Recombinant TNF-T with PA and FAC has protected B-cell component of the immune system from immunosuppressive effect as well.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P1-14-20

**Title:** HER2 and estrogen receptor status drive decisions regarding the use of neoadjuvant chemotherapy


**Body: Background:** Neoadjuvant chemotherapy (NC) is widely utilized to facilitate breast-conserving surgery and is considered equivalent to adjuvant chemotherapy with regard to recurrence-free and overall survival. However, controversy exists about specific indications for NC and its effects on other outcomes, particularly related to surgery in the breast and axilla. Limited data are available about specific recommendations of breast cancer-focused clinical investigators (CI).

**Methods:** A 139-item survey was sent in May 2015 to 94 US-based surgical (S) and medical oncology (MO) CI from a proprietary database to assess their usual practice patterns. A modest honorarium was provided.

**Results:** The assessment was completed by 59 CI (28 S and 31 MO; 63% of those sent the survey). Most (86%) recommend NC to patients with estrogen receptor-negative/HER2-negative (ER-/HER2-) and HER2-positive (HER2+) tumors larger than 2 cm or with biopsy-proven axillary node involvement. For such patients with HER2+ tumors, MO routinely (98%) use pertuzumab (P) with trastuzumab (T) combined with NC — either paclitaxel (14%), docetaxel (7%), docetaxel/carboplatin (55%) or a taxane and doxorubicin (24%). For patients with these higher-risk HER2+ tumors not receiving NC, 52% of MO use P in the adjuvant setting, either during chemotherapy or continuing for a year. There was more heterogeneity and less use of NC in patients with ER-positive (ER+)/HER2- tumors, and 48% of MO use genomic profiling (generally the 21-gene Recurrence Score® to facilitate this decision. Globally, for the 36 defined scenarios that were evaluated, S were somewhat more likely to recommend NC (70%) than MO (63%). In terms of axillary management, S typically employ post-NC sentinel node biopsy (SNB; 82%) and only proceed to axillary dissection if the SNB is positive.

**Conclusions:** S and MO CI consider ER and HER2 (with tumor size and axillary node status) essential factors in NC decisions. In 2013, P (in combination with NC/T) became the first agent to receive FDA approval in the neoadjuvant setting in patients with HER2+ tumors, and it is now commonly used in both the adjuvant and neoadjuvant settings. The algorithms generated by this assessment will be helpful in educating community-based physicians, and as a result an online point-of-care tool to facilitate these decisions is being developed and evaluated.

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<th>Tumor size (cm)</th>
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<th>ER+/HER2+</th>
<th>ER-/HER2-**</th>
<th>ER+/HER2-</th>
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<td>2.1-3.0</td>
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<td>Yes: 92 No: 5</td>
<td>Yes: 88 No: 5</td>
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<td></td>
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<td>Yes: 98 No: 2</td>
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* Remaining responses = no preference between neoadjuvant and adjuvant chemotherapy; ** BRCA-negative; G = genomic assay ordered
Title: Predictive factors of pathologic complete response of HER2-positive breast cancer after preoperative chemotherapy with trastuzumab: Development of a specific predictor

Jankowski C, Guiu S, Cortet M, Hudry D, Arnould L, Charon-Barra C, Desmoulins I, Rouzier R, Fumoleau P, Coudert B, Reyal F and Coutant C. Georges Francois Leclerc Cancer Center, Dijon, France; Institut Régional du Cancer, Montpellier, France; Georges Francois Leclerc Cancer Center, Dijon, France; Georges Francois Leclerc Cancer Center, Dijon, France; Georges Francois Leclerc Cancer Center, Dijon, France; Georges Francois Leclerc Cancer Center, Dijon, France; Georges Francois Leclerc Cancer Center, Dijon, France; Institut Curie, Paris, France; Georges Francois Leclerc Cancer Center, Dijon, France; Georges Francois Leclerc Cancer Center, Dijon, France; Institut Curie, Paris, France and Georges Francois Leclerc Cancer Center, Dijon, France.

Body: Purpose: The aim of this study was to assess the M.D Anderson Cancer Center / Gustave Roussy Institute (MDACC/IGR) nomogram in predicting pathologic complete response (pCR) to preoperative chemotherapy in a cohort of HER2 tumors treated with preoperative chemotherapy with trastuzumab. Then, we combine clinical and pathological variable associated with pCR into a new nomogram specific of HER2 tumors treated by preoperative chemotherapy with trastuzumab.

Methods: Data from 270 patients treated with preoperative chemotherapy with trastuzumab at Curie Institute and at Georges Francois Leclerc Cancer Center were used to assess MDACC/IGR nomogram and then to develop a nomogram for pCR based on multivariate logistic regression. Model performance was quantified with respect to calibration and discrimination.

Results: The IGR/MDACC was not accurate to predicting pCR in HER2 tumors treated by preoperative chemotherapy with trastuzumab with poor discrimination (AUC=0.54, IC [0.51-0.58]) and poor calibration (p=0.01). After uni and multivariate analysis, the new pCR nomogram was based on T stage (TNM), hormonal receptor status, and ki67(%). The model had a good discrimination 0.74 (IC95% : [0.70-0.79]) and a good calibration (p=0.93).

Conclusion: To our knowledge, this is the first nomogram to predict pCR in HER2 tumors treated by preoperative chemotherapy with trastuzumab. To ensure exportability, the model need to be evaluate with a external validation set.
**Title:** Neo-adjuvant chemotherapy for the treatment of breast cancer exterts a selection pressure toward luminal phenotype


**Body:** The effect of anthracycline and taxane based chemotherapy on biological features of residual disease after neo-adjuvant therapies in breast cancer patients is poorly described.

**PATIENTS AND METHODS:** We collected information through the institutional clinical database on all consecutive breast cancer patients treated with neo-adjuvant chemotherapy at INT, Milan, Italy, between January 2010 and March 2015. We selected patients who did not achieve pathological complete response at final surgery. All patients had a pathological evaluation including the shrinking pattern (scattered or concentric); ER, PgR, HER2 and Ki-67 expression were evaluated both at diagnostic biopsy and at final surgery. McNemar's test was used to compare paired proportions.

**RESULTS:** We identified a total of 325 patients. Median age was 51 yrs (range: 23 - 85 yrs). 304 (93%) pts received anthracycline and taxane containing chemotherapy for a median number of 6 (range 2-18) cycles. Radical modified mastectomy was performed in 68% of cases. Scattered residual disease was diagnosed in 112 (34%) cases. HER2 over-expression in diagnostic biopsy was significantly associated to scattered response (OR 1.94, CI 1.13 – 3.36, p= 0.017). 11/220 pts (5%) with ER-positive diagnostic biopsy had ER-negative residual tumor; 9/54 pts (17%) with initial ER-negative tumors became ER-positive. 34/183 (19%) pts with initial positive PgR at diagnostic biopsy had PgR-negative residual tumor; whereas, 17/86 pts (20%) with negative PgR became positive. The HER2 expression changed from positive to negative in 9/49 (18%) cases and from negative to positive in 7/190 (4%) cases. The Ki-67 expression changed from > or =20% to <20% in 63/175 (36%) cases and vice-versa in 14/54 (26%) cases. Compared to diagnostic biopsy, the rate of PgR-positive tumors decreased from 68 to 62% (p= 0.024) and the rate of Ki67<20% tumors increased from 24 to 45% (p=<0.001) in surgical specimen. Subtype changes at surgery occurred in 37/245 (15%) of cases, i.e. none in triple negative, 8/20 (10%) in HER2 positive, and 29/202 (14%) in luminal tumors. 73% of cases that changed after treatment showed a trend towards luminal differentiation. There was no significant correlation between pre- and post-treatment biological characteristics and the type of tumor shrinkage.

**CONCLUSION:** Anthracycline and taxane-based neo-adjuvant chemotherapy induces loss of PgR and Ki-67 in breast cancer. These changes are independent of the pattern of tumor shrinkage. The subtype switching toward more luminal phenotype suggest an endocrine effect of chemotherapy and paves the way to possible combinatorial approach of chemo- and hormone-therapy.
Title: Adjuvant trastuzumab +/- anthracycline and cardiotoxicity in a community cohort of 962 HER2+ breast cancers from 2005-2011: Comparison of incidence by risk factors and by diagnostic codes vs clinical chart review

Fehrenbacher L, Capra A, Krishnaswami A, Quesenberry C and Habel L. Kaiser Permanente Medical Center Vallejo, Vallejo, CA; Division of Research, Kaiser Permanente, Northern California, Oakland, CA and Kaiser Permanente Medical Center, Santa Theresa, San Jose, CA.

Body: Background: Prospective clinical trials using clinical criteria and observational studies using diagnostic codes from electronic health records have reported seemingly contradictory cardiotoxicity risk for adjuvant trastuzumab (T). Accurate estimates of individualized patient specific cardiotoxicity risk are essential for treatment decisions in early HER2+ breast cancer (BC).

Methods: 1,109 consecutive non-metastatic HER2+ invasive BC's diagnosed 5/1/2005 to 12/31/2011 at Kaiser Permanente Northern California receiving adjuvant T were reviewed for symptomatic congestive heart failure (SxCHF), baseline and post-T cardiac ejection fraction (EF), anthracycline (A) use, and CHF risk factors (RF) including age, race, hypertension (HTN), diabetes (DM), obesity, smoking. Records of patients with CHF ICD9 codes or an EF drop to <50% were reviewed by a RN, a cardiologist, and an oncologist. Primary outcomes were SxCHF (NYHA Class II or higher), EF fall to <50%, and <38 weeks of T (75% of prescribed).

Results: Median age of 962 eligible patients was 54 years (range 24-95). 305 (31.7%) were > 60 years old. During a median follow-up of 4.1 years, 4.6% of patients had CHF ICD9 codes, but only 2.5% had SxCHF or cardiac death confirmed by clinical review. At 1 year, cumulative incidence of an EF fall to <50% was 5.7% with T alone and 17.7% with T+ A. A total of 15.3% finished <38 weeks of T, 9.4% with T alone and 20.6% with T+A. The overall 2- and 5-year cumulative incidence of SxCHF/cardiac death was low: 1.3% and 2.2% with T alone and 2.7% and 3.1% with T + A. These rates were lower than reports based solely on diagnostic or billing codes, and varied substantially by RF (see table). The rates were similar to those predicted by the NSABP B-31 Cardiac Risk Score. Clinical heart failure based on diagnostic codes was not confirmed at chart review 48% of the time. Results from multivariable analyses will be presented.

Predictors of Trastuzumab Cardiotoxicity

<table>
<thead>
<tr>
<th>Predictors</th>
<th>N</th>
<th>Fall EF to&lt;50%</th>
<th>Symptomatic CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-year</td>
<td>2-year</td>
</tr>
<tr>
<td>All</td>
<td>962</td>
<td>12.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Anthracycline</td>
<td>505</td>
<td>17.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Non-anthracycline</td>
<td>457</td>
<td>5.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Age&lt;60</td>
<td>657</td>
<td>11.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Age 60-69</td>
<td>222</td>
<td>12.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Age 70-79</td>
<td>71</td>
<td>12.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Baseline EF 50-55%</td>
<td>92</td>
<td>30.7</td>
<td>8.8</td>
</tr>
<tr>
<td>HTN dx</td>
<td>392</td>
<td>12.6</td>
<td>3.4</td>
</tr>
<tr>
<td>BMI 30+</td>
<td>315</td>
<td>16.3</td>
<td>2.6</td>
</tr>
<tr>
<td>DM Dx</td>
<td>103</td>
<td>15.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Smoking (ever)</td>
<td>333</td>
<td>14.2</td>
<td>3.7</td>
</tr>
<tr>
<td>HTN and BMI 30+</td>
<td>182</td>
<td>15.5</td>
<td>3.9</td>
</tr>
<tr>
<td>HTN, BMI 30+, DM</td>
<td>50</td>
<td>16.0</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Conclusions: Risk of clinically confirmed CHF/cardiac death was substantially lower than risk based on ICD codes alone. Risk was consistent with prior clinical trials and differed substantially by age, baseline EF, use of A, and other CHF risk factors. Greatest risk was with age of 70+, borderline baseline EF, and comorbidities known to increase CHF risk. Quite low risk (1.1% at 5 years) was seen in patients under 60 years old.
Title: Gene expression profiling of doxorubicin cardiotoxicity in peripheral blood cells of breast cancer patients

Todorova VK K, Siegel ER R, Makhoul I, Marquette M, Wei JY Y and Klimberg VS. University of Arkansas for Medical Sciences, Little Rock, AR.

Body: **Background:** Doxorubicin (DOX), a widely used anti-cancer drug for treatment of breast cancer is known for its cardiotoxicity. DOX cardiotoxicity is cumulative-dose-dependent and begins with the first dose of chemotherapy. To date, no biomarker for early presymptomatic detection of DOX cardiotoxicity has been validated. Our previous data indicated that peripheral blood mononuclear cells (PBMCs) can be used as a surrogate tissue for identification of biomarkers for DOX cardiotoxicity. The aim of this study was to analyze PBMC gene expression induced by a single dose of DOX-based chemotherapy in breast cancer patients and correlated the data with DOX-induced cardiotoxicity.

**Materials and Methods.** Blood samples of 33 women treated for breast cancer with DOX-based chemotherapy were collected before the start and after the first cycle of chemotherapy. Total RNA was isolated from PBMC and whole-genome gene expression was performed using Illumina HumanHT-12 v4 Expression BeadChip array. Gene expression data were log2 – transformed and gene transcripts with average log2-intensities > 7 were considered to be expressed. The group-specific means were analyzed via repeated-measures with ANOVA for expression changes after DOX. Genes with p-value<0.05 were considered differentially expressed. Cardiac function was assessed before and after the completion of chemotherapy by echocardiogram and/or multigated acquisition scan. An absolute decrease of left ventricle ejection fraction >10% or <55% was considered abnormal. Differentially expressed genes (DEG) of patients who developed abnormal LVEF decrease were compared with DEG of patients who did not.

**Results.** A single dose of DOX-based chemotherapy resulted in 235 DEG in PBMC (P<0.05, FDR<0.05), mapped to cell death, oxygen transport and iron ion binding. Further analysis identified 87 DEG in the PBMC of eight (n=8) women who developed abnormal decline in LVEF from the baseline in comparison with women who did not (n=25). Most of the 87 DEG encode proteins secreted by activated neutrophils, such as alpha-defensins, arginase, cathepsin G, elastase, haptoglobin. The functional analysis of the 87 DEG showed enrichment for inflammatory response, immune response, cell death and peptidase activity.

**Discussion.** The results from this study indicated that elevated neutrophil-associated transcripts in the early stages of DOX-based chemotherapy were independent of the neutrophil count. These data suggest an association between the neutrophils activation after a single dose of DOX-based chemotherapy and later impairment of cardiac function. The early PBMC transcriptome signature can be used in the future development of biomarkers for DOX-associated cardiotoxicity.
**Title:** Prevention of letrozole–induced bone loss using risedronate in postmenopausal women with hormone receptor positive breast cancer: A multicenter randomized clinical trial

Kadoya T, Masumoto N, Shigematsu H, Emi A, Kajitani K, Kobayashi Y, Funakoshi M, Kawabuchi Y, Ohara M, Matsuura K, Noma M, Sasada T and Okada M. Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan; Hiroshima City Asa Citizens Hospital, Hiroshima, Japan; Hiroshima General Hospital, Hatsukaichi, Japan; Hiroshima Prefectural Hospital, Hiroshima, Japan and Onomichi General Hospital, Onomichi, Japan.

**Body**

**Background**
Prevention of letrozole–induced bone loss using oral risedronate has not been proved in the Japanese women. The aim of this study was to assess the effect of risedronate 17.5mg/week on bone mineral density (BMD) in postmenopausal, early breast cancer patients scheduled to receive adjuvant letrozole.

**Patients and Methods**
Postmenopausal women with hormone receptor–positive early breast cancer were assigned to one of two strata according to their baseline BMD T-score as being at low and high risk of osteoporosis. Patients with low risk (-2.5 ≤ T score) were randomly assigned to letrozole and risedronate (L+R) or to letrozole alone (L). Patients with high risk (-2.5 > T score) received letrozole and risedronate (L+R). Letrozole was given at a dosage of 2.5 mg/day while oral risedronate was given at 17.5mg/week. The primary end point was the change in lumbar spine (LS) BMD at 12 months. The secondary end points included change in total hip (HP) BMD and bone turnover markers.

**Results**
In the low risk group (N=103), treatment with L+R resulted in a significant increase in BMD at LS and at HP compared to treatment with L only at 12 months (1.8% vs -2.2%, P < 0.001, and -0.3% vs -2.9%, P = 0.001, respectively). In the L+R group, significant decreases in bone turnover makers, NTX and PINP, were recognized compared with L only at 12months (-11.1% vs. 27.5%, P<0.001, -42.3% vs. 15.2%, P<0.001, respectively). In the high risk group (N=28), treatment with L+R resulted in a significant increase in BMD at LS and prevention of decrease in BMD at HP (3.6%; 95%CI, 1.8% to 5.3%, p=0.003, 0.3%; 95%CI, -1.3% to 1.8%, p=0.47, respectively).

**Estimated Percentage Change From Baseline to 6 and 12 Months in Lumbar Spine and Total Hip BMD**

<table>
<thead>
<tr>
<th>BMD area</th>
<th>Risk Group</th>
<th>Treatment</th>
<th>From Baseline to 6 Months</th>
<th>From Baseline to 12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Change in BMD (%)</td>
<td>95% CI P</td>
<td>Change in BMD (%)</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>Low risk</td>
<td>L+R</td>
<td>1.7 (-1.3 to 4.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>-1.6 (-4.3 to 1.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>L+R</td>
<td>1.8 (0.4 to 3.2)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>-2.2 (-5.4 to 1.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total hip</td>
<td>Low risk</td>
<td>L+R</td>
<td>-0.2 (-2.7 to 2.3)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>-2.2 (-5.4 to 1.0)</td>
<td>-2.9 (-7.2 to 1.4)</td>
</tr>
<tr>
<td></td>
<td>High risk</td>
<td>L+R</td>
<td>0.1 (-1.3 to 1.6)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

BMD: bone mineral density, L: Letrozole, R: risedronate

Four patients (14.3%) improved from osteoporotic region to the osteopenic region with L+R treatment. Letrozole and risedronate were well tolerable and there was no serious adverse event including osteonecrosis of jaw.

**Conclusions**
At 12 months, 17.5mg/week risedronate therapy prevented bone loss in postmenopausal women with breast cancer who were receiving adjuvant letrozole, of which results were compatible with previous findings of western populations.
Title: Irrversible chemotherapy-induced alopecia in breast cancer patient

Kim S, Park HS, Kim JY, Nam S, Kim GM, Sohn JH and Kim SI. Yonsei University College of Medicine, Seoul, Republic of Korea and Yonsei University College of Medicine, Seoul, Republic of Korea.

Body: Introduction
Patients with breast cancer who received chemotherapy have distressing side effects such as mucositis, alopecia, gastritis, and BM suppression. Chemotherapy-induced alopecia (CIA) is one of considerable psychological events in self-esteem in patients with breast cancer, but the possibility of irreversible alopecia is often overlooked by physician.

We investigated clinical characteristics of CIA and prevalence of irreversible severe hair loss in patient with breast cancer who received chemotherapy.

Methods
We conducted a survey to collect demographic information about CIA with 150 breast cancer patients who had passed at least 6 months since their last day of chemotherapy from February 2015 to May 2015 in Yonsei Cancer Center. We obtained clinical information as age, elapsed time from end of chemotherapy, chemotherapy regimen, and other adjuvant therapy using their electrical medical records. We compared irreversible CIA characters between anthracycline and cyclophosphamide (AC) and taxane based regimen groups. The severe alopecia was defined as the hair density loss over 50% compared to the hair density before chemotherapy.

Results
The mean age at chemotherapy was 48 years old (±17.3) and the mean elapsed time after chemotherapy was 37 months (±9.5) in total patients.

Remnant alopecia was reported in 71 patients (47.3%). Wig or hat were used in 39 patients (26.0%).

The mean satisfaction score with a five-point scale was 4 in patients without alopecia or hair character change and 2.2 in patients with irreversible alopecia (p<0.001). The severe irreversible hair loss was complained by the 12 (8.2%) patients.

AC and taxane based chemotherapy were carried out in 65 and 85 patients, respectively. In AC group, remnant alopecia was shown in 18 patients (27.7%), and more than a half of patients in taxane group, 53 patients (62.4%), showed remnant alopecia (p<0.001). While only five patients (7.8%) in AC group suffered for severe hair loss, 26 patients (31.3%) in taxane group were affected by severe hair loss (p=0.001). The mean satisfaction level of hair status in patients in taxane group was 2.5 as compared to 3.6 in those in AC group (p<0.001).

Conclusion
Contrary to general expectation, About a half of breast cancer patients who received chemotherapy complained of irreversible hair loss even though at least 6 months has elapsed since the end of chemotherapy. In particular, patients with taxane based chemotherapy had more irreversible and severe alopecia than those with AC chemotherapy.
Title: Predictors of chemotherapy-induced peripheral neuropathy among breast cancer patients treated with taxanes

Candelario N, Wongrakpanich S and Morginstin M. Internal Medicine, Einstein Medical Center, Philadelphia, PA and Division of Hematology and Oncology, Einstein Medical Center, Philadelphia, PA.

Body: Background
Breast cancer is one of the most common types of cancer. Taxanes are approved in various treatment algorithms for both early and metastatic breast cancer. Chemotherapy-induced peripheral neuropathy (CIPN) is the most common dose-limiting side effect of the taxanes. This side effect is debilitating and could alter the treatment and patients' quality of life. This study aims to assess the predictors in the development and severity of CIPN.

Methods
A retrospective chart review of 229 stage I to IV breast cancer patients was done to identify if age, BMI, race, smoking history, alcohol use, diabetes, chronic kidney disease, estrogen and progesterone receptor and HER2 status, type and dose of taxane (Docetaxel or Paclitaxel) were predictors in the development and severity of CIPN. Severity of peripheral neuropathy was graded from 1 to 4 based on the common terminology of adverse events for peripheral neuropathy. Pearson Chi-square and T-test were done to see if there was a statistical difference in the development and severity of peripheral neuropathy among the above predictors. Odds ratio was computed using a logistic regression analysis for the predictors that showed statistical difference from the initial analysis.

Results: Among the 229 patients in this study, 158 patients (69%) developed neuropathy with 90 patients (57%) having grade 1 neuropathy and 25 (15.8%) suffering from grade 3 neuropathy. Majority of the subjects included in this study were African American (75.1%). Age, BMI, race, smoking, alcohol use, chronic kidney disease and diabetes did not show any statistical significance as predictors of development and severity of CIPN (p > 0.05, 95% CI). There was a significant difference in the development of neuropathy in terms of estrogen and progesterone receptor status (p=0.014). On logistic regression analysis, patients with ER+/PR- and ER+/PR+ had lower odds of developing neuropathy with OR 0.36 (p= 0.006, 95% CI 0.17-0.75) and 0.44 (p=0.026, 95% CI 0.21-0.91) respectively. HER2 positivity was associated with higher chances of neuropathy (OR 2.11, p=0.028, 95% CI 1.09-4.11). Paclitaxel was associated with higher chances of neuropathy compared to docetaxel (OR 2.89, p= 0.02, 95% CI 1.49-5.59). Dose of paclitaxel did not show any difference in the occurrence of CIPN. Those treated with paclitaxel had more severe neuropathy (p= 0.04).

Logistic Regression Analysis for the Development of CIPN

<table>
<thead>
<tr>
<th>Estrogen/Progesterone Receptor Status</th>
<th>Confidence interval</th>
<th>Odds Ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-/PR-</td>
<td>-</td>
<td>-</td>
<td>Ref</td>
</tr>
<tr>
<td>ER+/PR-</td>
<td>0.17-0.75</td>
<td>0.36</td>
<td>0.006</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td>0.21-0.91</td>
<td>0.44</td>
<td>0.026</td>
</tr>
<tr>
<td>HER2 Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>-</td>
<td>Ref</td>
</tr>
<tr>
<td>Positive</td>
<td>1.09-4.11</td>
<td>2.11</td>
<td>0.028</td>
</tr>
<tr>
<td>Taxane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docetaxel</td>
<td>-</td>
<td>-</td>
<td>Ref</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1.49-5.59</td>
<td>2.89</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CIPN: Chemotherapy Induced Peripheral Neuropathy; ER: Estrogen Receptor; PR: Progesterone Receptor
Conclusion: Breast cancer patients with ER+ and PR+ have lower chances of developing CIPN. HER2 positivity increases the odds of developing neuropathy. Paclitaxel is more neurotoxic than docetaxel though a dose-dependent risk was not seen in this study. Age, race, body mass index, smoking, alcohol use, diabetes and chronic kidney disease were not predictors in the development and severity of chemotherapy induced peripheral neuropathy.
Title: Evaluation of miracle mouthwash (MMW) plus hydrocortisone versus prednisolone mouth rinses as prophylaxis for everolimus-associated stomatitis: Preliminary results of a randomized phase II study

Jones VL L, Jensen LL L, McIntyre KJ J, Oommen SP P, Patt DA A, Cortas TE E, Harris RP P, Wilks ST T, Fox P and O'Shaughnessy JA A. US Oncology Research, Inc., The Woodlands, TX; Yakima Valley Memorial Hospital/North Star Lodge, Yakima, WA; Rocky Mountain Cancer Centers, LLP, Boulder, CO; Texas Oncology - Dallas Presbyterian Hospital, Dallas, TX; Texas Oncology - Fort Worth 12th Ave., Fort Worth, TX; Texas Oncology - Austin Central, Austin, TX; Arizona Oncology Associates, PC - HAL, Phoenix, AZ; Broome Oncology, LLC, Johnson City, NY; Cancer Care Centers of South Texas, San Antonio, TX; McKesson Specialty Health, Inc., The Woodlands, TX and Texas Oncology - Baylor Charles A. Sammons Cancer Center, Dallas, TX.

Body: Background: Oral stomatitis is a frequent adverse event (AE) associated with mTOR-inhibitor therapy, and can impact treatment adherence. In the BOLERO-2 trial in patients (pts) with hormone receptor-positive (HR+) metastatic breast cancer (MBC) treated with exemestane (EXE) plus everolimus (EVE), the incidence of all-grade stomatitis or related AEs was 67%, with 24% and 8% of pts developing Grade (G) 2 and G3 stomatitis or related AEs, respectively (Perez et al ASCO 2013 Abst 7029). In BOLERO-2, 24% of pts required EVE dose reduction for stomatitis (Rugo et al Ann Oncol 2014;25:808). The current study evaluated 2 different steroid-based mouth rinses for the prevention or amelioration of oral stomatitis in pts with MBC treated with EVE.

Methods: This prospective randomized phase II study enrolled postmenopausal pts (planned accrual=100) with HR+ MBC within the US Oncology Network who were initiating therapy with an aromatase inhibitor plus EVE (10 mg/day). Eligible pts were randomized, blinded, 1:1 to prophylactic treatment with 1 of 2 oral rinses (ARM 1: MMW 480 ml recipe: 320 mL oral Benadryl, 2 g Tetracycline, 80 mg Hydrocortisone, 40 mL Nystatin suspension, water; or ARM 2: Prednisolone 15mg/5mL oral solution). Pts were instructed to swish/expectorate 10 ml of the assigned rinse 4 x daily starting with Day 1 of EVE treatment, for a total of 12 wks. The primary objective was to determine the incidence of G≥2 stomatitis during the first 12 wks of treatment. Secondary objectives included assessment of AEs (all grades), determination of the percentage of pts requiring dose interruption and/or dose reduction of EVE or discontinuation of therapy due to toxicity, and evaluation of the impact of the oral rinses on the duration and severity of stomatitis.

Results: As of April 16, 2015, a total of 48 pts have been randomized and 47 pts have received treatment, with a mean time on mouth rinse of 68 days (range 2-84 days). Median age was 65 yrs (range 31-82 yrs). Twelve patients developed an oral AE and the incidence of all-grade stomatitis was 25% (n=12/48). The incidence of G1 stomatitis was 17% (8/48), G2 stomatitis was 8% (4/48) and there were no G3 events. The 4 G2 stomatitis AEs occurred within the first 30 days of treatment. One pt (1/48; 2%) required EVE dose delay. One pt developed oral candidiasis while on the steroid mouth rinse and no pts have stopped the steroid mouth rinse therapy due to mouth rinse-related toxicity.

Conclusion: These preliminary data are the first from a prospective trial to provide evidence of a reduced incidence of mTOR-associated stomatitis with prophylactic use of a steroid mouth rinse. The 25% incidence of all-grade and 8%/0% incidence of G2/3 stomatitis compare favorably with the 67% and 24%/8% incidence of all-grade and G2/3 stomatitis, respectively, in BOLERO-2. These preliminary data also demonstrated the safety and tolerability of these 2 steroid mouth rinses. The incidence of stomatitis on each study arm will be available when accrual is completed. The prophylactic use of steroid mouth rinses substantially decreases the incidence of G2/3 stomatitis and the need for EVE dose interruption/reduction.
The neuroprotective aminopropyl carbazole, P7C3-A20, prevents paclitaxel-induced pain

LoCoco PM M, Rissing AL L, Mooberry SL L, Berg KA A and Clarke WP P. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Paclitaxel (PTX), a microtubule-targeting anticancer agent, produces a debilitating peripheral neuropathy that is accompanied by neuropathic pain. Currently, there are only marginally effective therapeutic interventions available. Consequently, patients are forced to reduce or discontinue life-saving chemotherapy to cope with the pain. Recently, a newly identified agent, P7C3-A20, was found to be protective in several models of neurodegeneration, including Parkinson's disease and traumatic brain injury. Given that PTX triggers progressive degeneration of peripheral afferent neurons, this study was performed to evaluate the potential neuroprotective efficacy of P7C3-A20 in rodent models of PTX-induced peripheral neuropathy. P7C3-A20 (10 mg/kg) or vehicle (Cremophor EL/DMSO/5% Dextrose; 1:1:3) was administered intraperitoneally (i.p.) to Sprague-Dawley rats (250-300 g) everyday over a 28-day experimental paradigm. Following two days of treatment with P7C3-A20 or vehicle, rats also received 3 injections of PTX (11.7 mg/kg, i.p.) or vehicle (Cremophor EL/DMSO/5% Dextrose; i.p.) administered every other day. Treatment with P7C3-A20 did not alter body weights or leukocyte counts in control or PTX-treated rats. PTX treatment increased sensitivity to mechanical and cold stimulation (allodynia) of the hindpaw, evidence of peripheral neuropathy. Notably, P7C3-A20 treatment prevented the development of allodynia in response to PTX. Immunohistochemical analysis of paw biopsies indicated that P7C3-A20 prevented PTX-mediated degeneration of terminal nerve endings. Collectively, these data suggest that P7C3-A30 prevented the neurotoxic effects of PTX on peripheral sensory neurons. A xenograft tumor model of triple negative breast cancer was then used to determine whether P7C3-A20 diminished the antitumoral efficacy of PTX. MDA-MB-231 tumors were bilaterally implanted into flanks of female athymic nude mice and allowed to grow for 3 weeks prior to treatment. Using the same treatment protocol as the rats, tumor-bearing mice received daily treatment with P7C3-A20 (20 mg/kg, i.p.) or vehicle for 16 days. P7C3-A20 did not alter PTX-mediated inhibition of tumor growth. Furthermore, P7C3-A20 prevented PTX-induced mechanical allodynia in the mice. Taken together, this work indicates that P7C3-A20 prevents PTX-induced neuropathic damage without diminishing its antitumoral efficacy. P7C3-A20 may be an exciting new candidate to prevent peripheral neuropathy in patients undergoing cancer treatment with PTX.
Body: Introduction: About 5-10% of newly diagnosed breast cancers present with de novo metastatic disease. Clinicians are increasingly faced with the dilemma of how to manage a primary tumor that may produce physical and emotional discomfort in the setting of stable distant disease. Lack of outcome data for primary local therapy (PLT) in locally advanced metastatic breast cancer (LAMBC) makes patient counseling difficult. We conducted a population-based analysis of morbidity of PLT among older women with LAMBC. Methods: Patients with de novo LAMBC (T4M1) diagnosed between 2005 and 2009 were identified in the Surveillance, Epidemiology, and End Results (SEER)-Medicare database. Rates of treatment-related complications seen within 1 year of diagnosis and overall 1-year survival were analyzed. Complications were identified using ICD 9 codes (table 1). Results: Among 5,111 patients with LAMBC most did not have PLT (N=3699, 72%). PLT included surgery (N=656, 13%), radiation (N=542, 11%), and both surgery and radiation (N=214, 4%). The most common surgeries were modified radical mastectomy (N=558, 53%) and total mastectomy (N=207, 20%). Complication rates differed significantly by type of PLT, with highest rates seen in patients who had surgery plus radiation.

<table>
<thead>
<tr>
<th>Patient characteristics and outcomes, by PLT</th>
<th>Surgery (N=761)</th>
<th>Surgery and Radiation (N=284)</th>
<th>Radiation (N=472)</th>
<th>Neither (N=3594)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER Positive</td>
<td>440 (67%)</td>
<td>151 (71%)</td>
<td>373 (69%)</td>
<td>2348 (64%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER Negative</td>
<td>177 (27%)</td>
<td>51 (24%)</td>
<td>108 (20%)</td>
<td>740 (20%)</td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>47 (7%)</td>
<td>17 (8%)</td>
<td>41 (8%)</td>
<td>276 (8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Grade II</td>
<td>234 (36%)</td>
<td>75 (35%)</td>
<td>163 (30%)</td>
<td>993 (27%)</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>315 (48%)</td>
<td>99 (46%)</td>
<td>178 (33%)</td>
<td>1150 (31%)</td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity index, p&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>465 (71%)</td>
<td>127 (59%)</td>
<td>336 (62%)</td>
<td>2649 (72%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>93 (14%)</td>
<td>53 (25%)</td>
<td>123 (23%)</td>
<td>495 (13%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48 (7%)</td>
<td>23 (11%)</td>
<td>53 (10%)</td>
<td>269 (7%)</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>50 (8%)</td>
<td>11 (5%)</td>
<td>30 (6%)</td>
<td>286 (8%)</td>
<td></td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>4 (0.5%)</td>
<td>2 (0.7%)</td>
<td>7 (1.5%)</td>
<td>24 (0.7%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>32 (4.2%)</td>
<td>17 (6%)</td>
<td>12 (2.5%)</td>
<td>66 (1.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Brachial plexopathy</td>
<td>11 (1.5%)</td>
<td>12 (4.2%)</td>
<td>17 (3.6%)</td>
<td>30 (0.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wound dehiscence</td>
<td>7 (0.9%)</td>
<td>9 (3.2%)</td>
<td>2 (0.4%)</td>
<td>8 (0.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cancer-related pain</td>
<td>30 (4%)</td>
<td>38 (13%)</td>
<td>60 (13%)</td>
<td>131 (4%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphedema</td>
<td>18 (2.4%)</td>
<td>25 (8.8%)</td>
<td>8 (1.7%)</td>
<td>17 (0.5%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>All complications</td>
<td>122 (16%)</td>
<td>99 (34.9%)</td>
<td>103 (21.8%)</td>
<td>349 (9.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1-year survival</td>
<td>74%</td>
<td>87%</td>
<td>69%</td>
<td>51%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

. Patients who did not have PLT had a complication rate of 9.8%. Cancer-related pain was the most frequent complication, with
patients who had surgery and radiation and radiation alone having higher rates of it (13%) vs. surgery only and no intervention (4% each, p<0.0001). One-year survival was higher in patients who had surgery and radiation (87%), surgery only (74%), and radiation only (69%) vs. no intervention (51%) (p<0.0001). **Conclusions:** Few LAMBC patients who did not undergo PLT had local complications, suggesting a low burden of discomfort from untreated local disease. Women who had both surgery and radiation were found to have the highest complication rates and the highest 1-year overall survival. These results should be interpreted with caution, as patients having PLT are likely to be a highly selected group. Prospective data to inform these patients' management are required.
Title: Efficacy of hyaluronidase for treatment of chronic lymphedema

Kim W and Kim W. Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea and Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea.

Body: Current management of lymphedema is based on complex decongestive therapy (CDT), including manual lymph drainage, low-stretch bandaging, exercises and skin care. CDT may be insufficient for achieving efficacious volume reduction in chronic status of secondary lymphedema. Concentration of hyaluronic acid (HA) in the tissue of lymphedematous limb is 8 times higher, and use of hyaluronidase may improve lymphatic flow by reducing HA concentration, thus reducing edema volume. However, its efficacy for treatment of secondary lymphedema has not been studied sufficiently due to impurities such as other animal derived protein ingredients, which may cause allergic reactions, and even anaphylactic shock. The objective is to study the effects of Hirax®, highly purified solutional hyaluronidase, for treatment of chronic secondary lymphedema.

100 secondary lymphedema patients in maintenance phase without change in limb volume after CDT were recruited and randomized into two groups, G1 (CPT+injection, n=50) and G1 (CPT, n=50). Selection of injection sites were based on the findings of lymphscintigraphy and circumferential difference. Injection was performed twice, day 1 and day 4 (72hours from initial injection). Limb volume was measured and compared using the Casley-Smith truncated formula. Intragroup and intergroup difference in volume of affected limb (VLΔ), percent excess volume (PEVΔ) and percent reduction of excess volume (PREVΔ) at pre-, post 1 and post 2 were compared using t-test.

The VLΔ between the two groups revealed significant change at both post 1 and post 2 (p = 0.014, p = 0.004) The PEV difference (PEVΔ) within each group revealed significant change in G2 between post 1 and post 2 (p <0.001). The PEVΔ between the two groups revealed significant difference at both post 1 and post 2 (p <0.001, p <0.001). The PREV difference between the two groups also revealed greater PREV in G1 and significant differences at post 1 and post 2 (p <0.001, p <0.001).

We found highly purified solutional hyaluronidase, Hirax® injection in chronic secondary lymphedema patients in maintenance phase to be a useful adjunct for reducing lymphedema volume. Further studies with larger number patients as well as long term follow up evaluation on effectiveness and safety is needed in the future.
Title: Quantitative versus semi-quantitative assessments of radiation-induced pulmonary fibrosis post adjuvant breast radiotherapy

Pramana A, Browne L, Cox H, Saba A, Pham K, Trakis S, Crawford K, Hall M, Batchelor N, Lim J and Graham P. St George Cancer Care Centre, Sydney, NSW, Australia; St George Hospital, Sydney, NSW, Australia and The University of New South Wales, Sydney, NSW, Australia.

Objective
To evaluate the quantitative versus semi-quantitative assessments of radiation induced pulmonary fibrosis (RIPF) post adjuvant breast radiotherapy (RT).

Methods
High resolution computed tomography (HRCT) assessed lung physical density changes (CTD) and physician identified HRCT visual grading scores (CTS) were analysed at the minimum of 12 months post RT at one institution. The treated side in-portal lung regions for CTD and CTS assessments were: central-axis (CA) + regions 5cm superior & inferior to CA and the corresponding mid anterolateral region respectively. Respiratory motion was accounted for by subtracting the untreated side lung density from the treated side. Mean lung densities correspond to each voxels were automatically calculated by Pinnacle software (Phillips, Eindhoven, The-Netherlands). Grading of CTS was according to the RTOG/EORTC (grade 0, 1, 2, and 3 defined as none, slight, patchy, and dense HRCT appearance respectively) and analysed by a radiologist (JL) and re-checked a radiation oncologist (PG).

Results
Total numbers of 403 patients were analysed. A substantial association was verified between CTD and CTS assessment. An increase of $\sim 0.01$ g/ml (95% CI 0.003-0.02) in CTD with each CTS score increase of 1 was observed (Table-1a). The RIPF can be categorised quantitatively into three groups of CTS 0 vs. 1-2 vs. 3 based on the mean CTD (Table-1b).

Table-1a. Correlation between CTD and CTS method

<table>
<thead>
<tr>
<th>CTS</th>
<th>Treated side mean CTD - Left</th>
<th>Treated side mean CTD - Right</th>
<th>Mean Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.055 (119)</td>
<td>-0.011 (136)</td>
<td>0.020 (255)</td>
</tr>
<tr>
<td>1</td>
<td>0.065 (60)</td>
<td>0.005 (56)</td>
<td>0.036 (116)</td>
</tr>
<tr>
<td>2</td>
<td>0.083 (15)</td>
<td>0.012 (13)</td>
<td>0.050 (28)</td>
</tr>
<tr>
<td>3</td>
<td>0.108 (4)</td>
<td>0</td>
<td>0.108 (4)</td>
</tr>
<tr>
<td>Total</td>
<td>0.061 (198)</td>
<td>-0.005 (205)</td>
<td>0.028 (403)</td>
</tr>
</tbody>
</table>

Table-1b. Grouping of CTS based on CTD method

<table>
<thead>
<tr>
<th>CTS</th>
<th>Treated side mean CTD - Left</th>
<th>Treated side mean CTD - Right</th>
<th>Mean Total</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.055 (119)</td>
<td>-0.011 (136)</td>
<td>0.020 (255)</td>
<td>0.012-0.027</td>
</tr>
<tr>
<td>1-2</td>
<td>0.069 (75)</td>
<td>0.006 (69)</td>
<td>0.039 (144)</td>
<td>0.029-0.048</td>
</tr>
<tr>
<td>3</td>
<td>0.108 (4)</td>
<td>0</td>
<td>0.108 (4)</td>
<td>0.079-0.137</td>
</tr>
</tbody>
</table>

A cut off CTD of 0.089 g/ml exemplified the best compromise between sensitivity (100%) and specificity (88.2%) for dense HRCT appearance. However, there was no good compromise of CTD cut off for slight and patchy HRCT score possibly due to intra observer variation and the scale of the CTD measure (small increase in CTD may not be detected visually by the observer). Multivariable analysis revealed increasing age, current smoker, V20 $\geq 10\%$ (the volume of lung that was covered by the 20Gy
isodose line), central lung distance $\geq 2\text{cm}$ (the distance between posterior RT tangents and the chest wall), combined endocrine & chemotherapy, and treated side mean CTD to be significantly associated with development of grade $\geq 1\text{RIPF}$.

Conclusions
There was a good correlation between quantitative (CTD) and semi-quantitative (CTS) assessment of RIPF post adjuvant breast RT. The CTD method could be advantageous for both routine clinical practice and future clinical trials that require more detailed quantification of dense RIPF.
Title: First-line chemotherapy for breast cancer patients by site of care (SOC): Treatment patterns, cost and quality indicators


Body: Background: Previous studies found differences in treatment patterns and costs by SOC for first-line chemotherapy treatment for both early stage and metastatic breast cancer (esBC and mBC) in commercial populations. This study extends the research to a predominantly Medicare population comparing chemotherapy treatment patterns, cost and quality of care in physician office (PO) and hospital outpatient (HO) centers.

Methods: First-line chemotherapy or biologic therapy for esBC and mBC patients was compared by SOC. Patients initiating infusion therapy in 2008–2012 were identified in Humana medical claims data. First-line length of therapy (LOT) in days and number of infusions (NI) were calculated. SOC cohort (HO vs PO) was based on where the patient received ≥90% of their infusions. Total healthcare costs based on medical and pharmacy claims were assessed. Differences in quality indicators, use of infusions or hospitalizations 30 days prior to death were evaluated. SOC differences were assessed using \( \chi^2 \), T-tests and Wilcoxon Rank Sum (Wil) tests. P-values are for \( \chi^2 \) and Wil tests. Cost-related results are from generalized linear models adjusted for age, sex, comorbidity and geographic region. LOT and NI are presented as median (IQR).

Results: A total of 2,784 esBC patients (73% PO and 27% HO) and 1,602 mBC patients (64% PO and 36% HO) were identified. Most patients (67%) were Medicare beneficiaries. Mean comorbidity index was similar by SOC for esBC patients (PO 4.2, HO 4.1, \( p=0.3308 \)) but higher in HO for mBC patients (PO 7.5, HO 7.9, \( p=0.0003 \)). LOT in days for esBC was greater in the PO for anthracycline-based therapy, PO 64(43-72), HO 47(43-64), \( p=0.0420 \) and taxane-based therapy, PO 64(64-106), HO 64(64-76), \( p=0.0005 \). NI for esBC was greater in the PO for patients on biologic and cytotoxic therapy, PO 21(17-29), HO 18(16-25), \( p=0.038 \) and taxane-based therapy PO 4(4-6), HO 4(4-4), \( p=0.0005 \).

No difference in LOT by SOC was seen for mBC patients; however, patients on taxane-based therapy had a greater NI at the PO 6(4-12) vs HO 5.5(4-9), \( p=0.0225 \).

Total healthcare costs were higher in the HO vs PO setting for esBC and mBC patients. Costs were 22% higher in the HO $51,191 vs PO $41,943, \( p<0.0001 \) for esBC patients and 17% higher in the HO $58,105 vs PO $49,591, \( p<0.0001 \) for mBC patients.

Conclusion: Differences by site of care, particularly in healthcare costs, were found in a mostly Medicare population of esBC and mBC patients. Patients in the HO setting had shorter length of therapy and fewer infusions, but had higher total healthcare costs than those in the PO setting. Quality indicators, infusions and hospitalizations prior to death were similar by site of care. Future research will focus on other quality indicators and patient satisfaction.
Body: Background: Fulvestrant (FUL), an estrogen receptor (ER) antagonist, is an effective treatment for patients (pts) with hormone receptor-positive (HR+) breast cancer (BC) whose disease has progressed or recurred during previous anti-estrogen therapy. The androgen receptor (AR), expressed in the majority of HR+ BC, may contribute to resistance to hormonal therapy. Enzalutamide (ENZA) is a potent inhibitor of AR signaling. Preclinical models with ER+/AR+ BC cell lines showed synergistic inhibitory effects for ENZA combined with FUL on tumor cell growth. ENZA is a potent CYP3A4 inducer, and in vitro studies show that CYP3A4 is the only CYP enzyme involved in the oxidative metabolism of FUL. In this phase 1 trial (NCT01597193), we evaluated the potential for ENZA to affect FUL pharmacokinetics (PK), as well as the safety and tolerability of the combination of ENZA with FUL.

Methods: Postmenopausal pts with HR+/AR+ advanced BC were enrolled; any number of prior therapies were permissible. Tumor tissue was analyzed centrally for AR expression; pts who had ≥10% tumor cells with nuclear AR staining were eligible. All pts received at least 3 doses of FUL (500 mg intramuscularly on days 1, 15, and 29 and once monthly thereafter) to ensure steady-state concentrations prior to initiating ENZA 160 mg/day orally. The combination of ENZA with FUL was given until disease progression. PK and hormone sampling occurred on day 1 prior to ENZA initiation and on days 29 and 57. All pts were monitored for safety and response to treatment.

Results: As of 01May2015, 11 pts were enrolled; PK data are available for 8 of 11 pts, and 6 pts remain on study. Median age was 59 years; median ECOG performance status was 1. Two pts previously received FUL as a prior therapy for advanced BC; 4 pts received no prior therapy for advanced BC. The median duration of exposure to the combination was 16.6 weeks (range 4.0-42.3); the median duration of exposure to FUL (including at least 3 preloading doses) was 24.4 weeks (range 11.7-67.3). Common (>2 pts) ENZA-related adverse events (AEs) included fatigue (n=6), nausea (n=5), cognitive disorder (n=4) and diarrhea (n=3). Cognitive changes Grade 1/2 were reported in 4 pts based on the cognitive function assessment questionnaire. Two pts reported unrelated serious AEs (erosive gastritis, urinary tract infection, iron deficiency anemia, and dehydration). Four pts had AEs ≥ Grade 3: hypertension, anemia, hyperglycemia, urinary tract infection, asthenia, erosive gastritis, dehydration, and iron deficiency anemia; only asthenia and hypertension were considered treatment-related. Circulating levels of estradiol and estrone were within the expected range. Trough plasma concentrations of FUL (C_{min}) were similar for FUL alone and FUL combined with ENZA (C_{min}=13.7 ± 2.8 and 12.5 ± 1.8 ng/mL, respectively).

Conclusions: The safety profile for the combination of daily ENZA with FUL appears to be consistent with the published data for ENZA and FUL monotherapies. ENZA with FUL achieves similar plasma exposure to FUL alone, indicating no PK drug interaction.
2015 San Antonio Breast Cancer Symposium

Publication Number: P1-16-06

Title: Luteolin inhibits progestin-dependent VEGF induction, stem-cell like characteristics, and tumor progression of human breast cancer cells

Hyder SM M, Cook MT T, Besch-Williford C and Liang Y. University of Missouri, Columbia, MO and IDEXX BioResearch, Columbia, MO.

Body: Clinical trials and epidemiological evidence show that combined estrogen (E) and progestin (P) hormone replacement therapy (HRT) increases the risk of breast cancer in postmenopausal women, whereas HRT containing E alone does not. Tumor progression is dependent on angiogenesis, which provides nutrients vital to the developing cancer. We previously showed, both in vitro and in vivo, that natural and synthetic P (including the widely used progestin Medroxyprogesterone acetate, MPA), increase production of vascular endothelial growth factor (VEGF), a potent angiogenic factor, in human breast cancer cells (Cancer Res., 1998, 58:392). This effect is blocked by the anti-progestin RU-486, suggesting involvement of progesterone receptors in the process (Int J Cancer, 2001, 92:469). Evidence from our laboratory using in vivo breast cancer models suggests that P accelerates the development of tumors from latent tumorigenic cells. This leads to the formation of palpable tumors and tumor metastasis, processes that may be attributed to increased production of VEGF (Cancer Res., 2007, 67:9929; Menopause, 2010, 17:1040). RU-486 blocks P-dependent VEGF production and thereby reduces tumor growth; however, the anti-progestin has severe side-effects averting its long-term use. Recently, we have studied less toxic naturally-occurring compounds for their ability to antagonize P-dependent VEGF induction and block tumor progression. In this study, we tested the effects of luteolin, a flavonoid commonly found in fruits and vegetables, on proliferation of BT-474 and T47-D breast cancer cells and their P-dependent production of VEGF. Luteolin treatment (25-100 µM) for 24-48 h reduced in vitro tumor cell viability and induced apoptosis. Interestingly, treatment with a lower concentration of luteolin (10 µM) blocked the production of P-dependent VEGF, indicating that VEGF suppression precedes luteolin-mediated loss of cell viability. Furthermore, luteolin (20 mg/kg, i.p.) suppressed the growth of MPA-dependent T47-D human xenograft tumors in nude mice. Immunohistochemical analysis showed that luteolin reduced P-induced VEGF in tumor sections (p<0.05). These findings strongly suggest that the flavonoid disrupts tumor progression by blocking P-dependent angiogenesis and preventing tumor cell proliferation. Furthermore, luteolin blocked the MPA-induced acquisition of stem cell like properties by breast cancer cells; CD44 expression, ALDH activity and mammosphere formation were all reduced by the flavonoid. We contend therefore that luteolin is a compound with valuable therapeutic properties. Its ability to reduce levels of VEGF, coupled with its capacity to interfere with the acquisition of stem-cell like properties by breast cancer cells, make luteolin a compound with significant clinical potential in the battle against P-dependent human breast cancer.

Supported by a COR award from the College of Veterinary Medicine and in part by funds from generous donors to the Ellis Fischel Cancer Center, University of Missouri.
Title: Pharmacogenomics-pharmacokinetics study of selective estrogen-receptor modulators with intra-patient dose-escalation for Japanese breast cancer patients

Ohno S, Ishiguro H, Yamamoto Y, Takao S, Sato N, Fujisawa T, Kadoya T, Kuroi K, Bando H, Teramura Y, Iwata H, Tanaka S and Toi M. The Cancer Institute Hospital Of JFCR, Tokyo, Japan; Graduate School of Medicine Kyoto University, Kyoto, Japan; Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; Hyogo Cancer Center, Akashi, Hyogo, Japan; Niigata Cancer Center Hospital, Niigata, Japan; Gunma Prefectural Cancer Center, Ohta, Gunma, Japan; Hiroshima University Hospital, Hiroshima, Japan; Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; University of Tsukuba, Tsukuba, Ibaraki, Japan; Hikone Municipal Hospital, Hikone, Shiga, Japan; Aichi Cancer Center Hospital, Nagoya, Aichi, Japan and Graduate School of Medicine and Public Health, Kyoto University, Kyoto, Japan.

Body: Background
The association between CYP2D6 polymorphisms and efficacy of tamoxifen (TAM) is inconclusive, partly due to inaccurate prediction of active metabolite, endoxifen exposure by solely CYP2D6 genotype. Moreover, TAM dose escalation is not effective for poor metabolizers. Since the contribution of CYP2D6 to toremifene (TOR) activation is small, TOR might be a good alternative to TAM for poor metabolizers.

Methods
Patients who maintained good compliance with TAM or TOR without regular use of strong CYP2D6 inhibitors were enrolled in a screening study. The pharmacokinetics (PK) of TAM or TOR and the pharmacogenomics (PGx) of metabolizing enzymes and transporters were assessed. Associations between TAM and TOR PK and PGx, other CYP inhibitor use, and smoking status were examined by regression analysis. An intra-patient dose escalation study was conducted for patients showing low endoxifen levels during TAM treatment (n = 14). TAM was switched to 40 mg of TOR, and then increased to 120 mg for ≥24 weeks with periodic PK sampling. Total TAM or TOR activity was calculated as the sum of the concentration of each active metabolite adjusted by their respective in vitro activity.

Results
CYP2D6 genotype was the major determinant for TAM activity (p < 0.01) for Japanese breast cancer patients. Current smoking status (p = 0.07) and CYP2C19 (p = 0.07), but not CYP2D6 genotype (p = 0.61), showed marginally significant effects on TOR activity. TOR activity sufficiently increased with dose escalation from 40 mg to 120 mg, even among the poor TAM metabolizers, and was maintained for ≥24 weeks.

Total Activity of TOR (ng/mL)

<table>
<thead>
<tr>
<th></th>
<th>Screening (n=271)</th>
<th>Intra-patient dose escalation (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg)</td>
<td></td>
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</tr>
<tr>
<td>Mean (ng/mL)</td>
<td>4168.5</td>
<td>3218.1</td>
</tr>
<tr>
<td>Median (ng/mL)</td>
<td>3865.4</td>
<td>3020.3</td>
</tr>
<tr>
<td>SD</td>
<td>1446.2</td>
<td>1207.0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.04</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*(Sample taken at ≥24 weeks)*

Conclusion
Since the contribution of CYP2D6 to TOR metabolism is relatively small, TOR is a valid alternative to TAM, especially in patients predicted to be poor TAM metabolizers. Further clinical trials are warranted to validate this concept.
Title: Inhibition of β-catenin pathway to overcome endocrine resistance in breast cancer

Won HS, Lee KE, Lee KM, Nam EM, Mun Y-C, Seong C-M and Lee SN. Uijeongbu St. Mary’s Hospital, The Catholic University of Korea, Gyeongg-do, Korea and School of Medicine, Ewha Womans University, Seoul, Korea.

Body: Background: It is well known that Wnt/β-catenin signaling pathway is involved in hormone receptor negative breast cancer, especially triple-negative breast cancer. In the case of hormone resistant breast cancer cells, they rarely showed hormone receptor expression. So we aimed to investigate whether the β-catenin pathway becomes activated in endocrine-resistant breast cancer cells and inhibition of β-catenin pathway can overcome endocrine resistance.

Methods: We have established an MCF-7-derived tamoxifen-resistant cell line (TamR) by long-term culture of MCF-7 cells with gradually increasing 4-hydroxytamoxifen concentration till 3µM. The levels of protein expression and mRNA transcripts were determined using western blot analysis and real-time quantitative PCR. The transcriptional activity of β-catenin was measured using luciferase activity assay. We used ICG-001 as inhibitor of β-catenin transcription activity.

Results: The expression of estrogen receptor was significantly decreased in TamR cells. On the other hand, the expressions of HER2 and EGFR were increased in TamR cells than in control cells. The active (uncomplexed form) β-catenin level was increased in TamR cells, and also showed a significantly increased β-catenin transcriptional activity. The ICG-001, small-molecular inhibitor of Wnt/β-catenin pathway, treatment significantly reduced β-catenin transcriptional activity in TamR cells. The ICG-001 reduced cell viability to TamR cells which showed resistance to tamoxifen by 65.3%, and also inhibited target gene cyclin D1 expression. The combination of ICG-001 and mTOR inhibitor, rapamycin reduced cell viability of TamR cells by 81.7% and there was an additive effect of two drugs as a combination index of 1.022.

Conclusions: The β-catenin pathway is activated in endocrine-resistant breast cancer cells and inhibition of that pathway would be a new therapeutic strategy which overcomes endocrine resistance in breast cancer.
Impact of statin use on cancer recurrence and mortality in patients with breast cancer: A systematic review and meta-analysis

Manthravadi S, Shrestha A and Madhusudhana S.  Internal Medicine, University of Missouri-Kansas City, Kansas City, MO and Hematology and Oncology, University of Missouri-Kansas City, Kansas City, MO.

Background:
Statins have been described as having an association with a decreased risk of breast cancer. They have also shown antineoplastic properties in preclinical studies. Statins inhibit the enzyme ‘HMG CoA reductase’ and the expression of this enzyme in cancer cells has been implicated as an adverse prognostic factor in patients with breast cancer.

Methods:
We performed a systemic review of literature through April 2015 and utilized PubMed and Embase to identify studies that described an association between statin use and survival in breast cancer. Studies which did not report a comparison of survival using Kaplan-Meier curves were excluded. Summary hazard ratio (HR) with 95% confidence intervals (CI) was estimated using the random effects model, and heterogeneity was measured using the inconsistency index ($I^2$).

Results:
After reviewing 637 abstracts, 12 studies which included a total of 87951 patients were identified and data was extracted. 8 studies provided a summary statistic for the association of statins with recurrence-free survival (RFS) in patients with breast cancer and were included in a meta-analysis. Statin use was associated with improved RFS (N= 29729 patients, HR 0.66; 95% CI 0.53- 0.84) with moderate heterogeneity ($I^2$ = 48%). Furthermore, this survival benefit appeared to be confined to use of lipophilic statins (3 studies, HR 0.72; 95% CI 0.59- 0.89) as hydrophilic statin use was not associated with improvement in RFS (3 studies, HR 0.80; 95% CI 0.44- 1.46). A meta-analysis of 5 studies showed no significant association between the use of statins on breast cancer-specific survival (CSS) (N= 60686 patients, HR 0.71; 95% CI 0.49- 1.03, $I^2$ = 84%). We also found no association between statin use and overall survival (OS) (5 studies and 22283 patients, HR 0.83; 95% CI 0.58- 1.19, $I^2$ = 62%).

Conclusions:
Statin use, or more specifically, lipophilic statin use is associated with an improved recurrence-free survival for patients with breast cancer. However, there was no effect of statin use on either cancer-specific survival or overall survival. These benefits need to be assessed in a prospective randomized cohort and the choice of statin, dose and biomarkers that may predict the efficacy of these drugs will need to be identified.

Characteristics of included studies

<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Country</th>
<th>Impact on RFS</th>
<th>Impact on CSS</th>
<th>Impact on OS</th>
<th>Sample Size</th>
<th>Median follow-up (in years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desai</td>
<td>2015</td>
<td>USA</td>
<td>NA</td>
<td>0.59 [0.32, 1.06]</td>
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<td>7,883</td>
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<td>Cardwell</td>
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<td>UK</td>
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<td>0.84 [0.72, 0.97]</td>
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<td>Murtola</td>
<td>2014</td>
<td>Finland</td>
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<td>0.46 [0.38, 0.55]</td>
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</tr>
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<td>Boudreau</td>
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<td>NA</td>
<td>4,216</td>
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<tr>
<td>Sendur</td>
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<td>Turkey</td>
<td>P 0.004</td>
<td>P 0.005</td>
<td>P = 0.005</td>
<td>1,172</td>
<td>4</td>
</tr>
<tr>
<td>Brewer</td>
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<td>USA</td>
<td>0.49 [0.28-0.84]</td>
<td>0.85 [0.46, 1.57]</td>
<td>0.80 [0.43, 1.49]</td>
<td>724</td>
<td>NA</td>
</tr>
<tr>
<td>Nickels</td>
<td>2013</td>
<td>Germany</td>
<td>0.83 [0.54,1.24]</td>
<td>0.89 [0.52, 1.49]</td>
<td>1.21 [0.87, 1.69]</td>
<td>3,085*</td>
<td>5.3</td>
</tr>
<tr>
<td>Zeichner</td>
<td>2013</td>
<td>USA</td>
<td>1.42 [0.42, 4.81]</td>
<td>NA</td>
<td>1.5 [0.07, 32.02]</td>
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<td>Chae</td>
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<td>USA</td>
<td>0.40 [0.24, 0.67]</td>
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<td>NA</td>
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</tr>
<tr>
<td>Ceacareanu</td>
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<td>0.23 [0.08, 0.66]</td>
<td>294</td>
<td>2.5</td>
</tr>
<tr>
<td>Ahern</td>
<td>2011</td>
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<td>18,769</td>
<td>6.8</td>
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<tr>
<td>Kwan</td>
<td>2008</td>
<td>USA</td>
<td>0.67 [0.39, 1.13]</td>
<td>NA</td>
<td>NA</td>
<td>1,811</td>
<td>5</td>
</tr>
</tbody>
</table>

* = sample size for RFS: 2912, Abbreviations: RFS: recurrence-free survival, CSS: cancer-specific survival, OS: overall survival (expressed in hazard ratio and 95% confidence intervals)
Title: Statin use and breast cancer incidence in the Nurses' health study

Ahern TP P, Tamimi RM M, Chen WY Y, Garber JE E, Eliassen AH and Borgquist S. University of Vermont; Brigham and Women's Hospital; Dana-Farber Cancer Institute and Lund University.

Body: Background
Statin drugs lower cholesterol and prevent cardiovascular disease. Laboratory and epidemiologic evidence suggests that statins may also have anti-cancer properties. Published associations between statin use and breast cancer incidence are heterogeneous. Few studies have comprehensively addressed confounding by lifestyle and reproductive factors, or detection bias due to potentially higher screening rates among medication users. The most recent study on this topic reported an increased risk of invasive breast cancer among statin users.

Methods
To improve upon the present evidence base we studied the association between statin use and incident breast cancer among 79,518 postmenopausal women in the Nurses' Health Study cohort. We followed these women from 2000 (the year statin exposure was first ascertained prospectively) until the first of breast cancer diagnosis, death from any cause, or the end of follow-up in 2012. We defined statin exposure as current or former use (both factored with duration) or never use. We fit Cox regression models to estimate associations, encoding statin use and covariates as time-dependent variables updated every two years. We evaluated confounding by adiposity, reproductive history, menopausal hormone therapy, family history of breast cancer, history of benign breast disease and diabetes, alcohol consumption, physical activity, and use of co-medications. We also measured associations among those cohort members who underwent screening mammograms every two years.

Results
Over 823,086 person-years of follow-up, 3,055 cases of invasive breast cancer were diagnosed (1,078 of which were among statin users). Compared with non-users, statin users were somewhat older, had a higher mean BMI, were more likely to be users of aspirin, beta blockers, calcium channel blockers, digoxin, and ACE inhibitors, had a higher prevalence of diabetes, and were more likely to undergo mammographic screening. Current users of any statin had a similar rate of breast cancer incidence as never users (for current users of ≥8 years' duration, HR_{adj}=1.1, 95% CI: 0.91, 1.3). Analyses of specific statin exposures among new initiators of therapy in 2004 returned similarly null associations (for current use of hydrophilic statins, HR_{adj}=1.0, 95% CI: 0.82, 1.3; for current use of lipophilic statins, HR_{adj}=1.1, 95% CI: 0.95, 1.3). Associations did not vary substantially by duration of statin use or according to breast cancer subtypes defined by histology (invasive ductal vs. invasive lobular disease) or estrogen receptor status. Statin use was not associated with incident breast carcinoma in situ. These results were similar in analyses restricted to women who underwent regular screening mammograms.

Conclusions
Our results indicate that cholesterol-lowering statin therapy neither increases nor decreases breast cancer incidence rate in postmenopausal women. Considering the latest report indicated an increased breast cancer risk among statin users, our neutral findings should reassure physicians that statin therapy for the prevention of cardiovascular disease is safe with respect to breast cancer risk.
Title: Cholesterol, cholesterol lowering medication use, and breast cancer outcomes in the BIG 1-98 study


Body: Background:
Cholesterol lowering medications (CLM)—statins in particular—may exert anti-cancer effects. A proposed mechanism involves attenuated signaling through the estrogen receptor by the cholesterol metabolite, 27-hydroxycholesterol, which correlates with systemic total cholesterol. Assessment of cholesterol levels and use of cholesterol-lowering medications among breast cancer patients receiving endocrine treatment may enrich our understanding of factors affecting endocrine therapy effectiveness and the role of statins and cholesterol in cancer survival.

Aim:
To investigate the prognostic effect of baseline cholesterol level and baseline CLM use, and to study the effect on outcome of concurrent use of CLM and endocrine therapy in the BIG 1-98 study.

Design and Methods:
The BIG 1-98 study enrolled 8,010 postmenopausal women with early-stage, estrogen receptor-positive invasive breast cancer from 1998-2003. Participants were randomized to five years of tamoxifen, letrozole, or their sequence. Cholesterol levels and use of CLM were assessed at baseline and every six months up to 5.5 years. Multivariable prognostic models of baseline cholesterol or baseline CLM use were adjusted for patient- and tumor characteristics and treatment regimen. Prognostic analyses of baseline cholesterol were restricted to women not taking CLM at baseline. Marginal structural modeling was used to investigate the relationship between initiation of CLM during endocrine therapy and outcome. Median follow-up was approximately 8 years. Endpoints were: disease-free survival (DFS), breast cancer-free interval (BCFI), and distant recurrence-free interval (DRFI).

Results:
Among 7,963 women who received at least one dose of endocrine therapy, 637 reported use of CLM at baseline, including statins (n=490) and non-statins (n=147). Compared with non-users, women on CLM at baseline were older, more often had a history of diabetes, were more likely to use hormone replacement therapy, and were more often diagnosed with smaller tumors or node-negative disease. During follow-up, 2,005 DFS-events, 1,303 BCFI-events and 1,004 DRFI-events were reported. Among non-users of CLM at baseline, baseline cholesterol levels showed a significant U-shaped association with BCFI and DRFI when grouped according to the quartiles of the distribution; however, these results were not maintained using cardiovascular risk-based cholesterol cut points. Prognostic models also suggested that use of any CLM at baseline was associated with better DFS compared with non-use (HRadj=0.82, 95% CI: 0.68, 0.99); similar, but non-significant associations were seen for BCFI (HRadj=0.83, 95% CI: 0.65, 1.06) and DRFI (HRadj=0.81, 95% CI: 0.61, 1.09). Results from the marginal structural modeling showed no differences in outcomes for women who initiated CLM during endocrine therapy compared with those who did not.

Conclusions:
In the BIG 1-98 study investigating the use of tamoxifen, letrozole or their sequence as endocrine therapy in the adjuvant setting, use of cholesterol-lowering therapy at baseline was associated with beneficial tumor characteristics. The effects on clinical outcome need further investigation.
Title: Association of aspirin and clinical outcomes in patients with invasive breast cancer

Li YR R, Steel L, Carrigan E and Tchou J. Medical Scientist Training Program, Perelman School of Medicine; University of Pennsylvania, Philadelphia, PA; Cell and Molecular Biology Graduate Program; Perelman School of Medicine; University of Pennsylvania, Philadelphia, PA; Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA and Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Body: Long-term low-dose aspirin use has been observed to reduce the risk of colorectal, breast and other cancers. The most prominent effect has been in colorectal cancer, in which large-scale meta-analyses have shown that there is an approximately 20% relative risk reduction in participants who took aspirin for four or more years. The role of long-term NSAID use in breast cancer risk is less clear although preliminary observational case-control studies suggest an association between aspirin use and reduced incidence of hormone receptor-positive breast cancers though no clear evidence exists to support a clear mortality benefit among patients with a history of prior NSAID use as opposed to those who do not.

To investigate whether a history of aspirin use is associated with improved clinical outcome in breast cancer, we examined the pattern of aspirin use, cancer pathology and overall survival of over 1000 patients diagnosed with and treated for invasive breast cancer at our institution, for whom long-term follow up was available. A history of aspirin use for at least a period of 30 days prior to breast cancer diagnosis was reported in nearly 14% of individuals. Aspirin use was associated with being older than the age of 50 at diagnosis (79.8% vs 66.5%; Fisher's Exact Test (P < 3.2x10^-3) and being of African American race (49.1% vs 28.7%; P < 3.4x10^-2), when compared to those who have not used aspirin.

Aspirin use correlated with prognostic factors that are known to be associated with poor outcomes. They include axilla node positive disease (44.5% vs 27.0%, p< 0.032), evidence of lymphovascular invasion (24.7% vs 15.4%, p< 0.049), Her2-neu positive disease (<0.0083). In contrast to prior retrospective case-control studies, no significant association between aspirin use and hormone receptor positive disease was noted for either ER (p=0.19) or PR(+) receptor status (p=0.12). Finally, we examined if aspirin use prior to breast cancer diagnosis has any impact on disease outcome. Over a median follow up of 60.0 months, univariate analysis using cox proportional hazard modeling demonstrated that the use of low-dose aspirin prior to the diagnosis of breast cancer was associated with an increased all-cause mortality when compared to patients without aspirin use prior to cancer diagnosis (HR=3.084, 95% CI=1.961 to 4.848). On multivariate analysis, we found that recent history of aspirin use was significantly associated with a worse overall survival (HR 2.65; 95%CI 1.37 - 5.12, P < 3.77 x 10^-3), when controlled for other prognostic factors including receptor status, tumor size, tumor grade, number of positive regional lymph nodes, positive margins, as well as race and age at diagnosis.

This is the first study to report on the association of aspirin use with breast cancer outcomes in a large patient cohort treated at a single institution. Although aspirin in breast and cancers has been associated with reduced cancer incidence, a history of aspirin use prior to breast cancer diagnosis does not appear to be protective or associated with improve clinical outcomes or survival among breast cancer patients. Ongoing efforts are examining the mechanism underlying this association.
Body: **Background:** Current breast cancer prevention agents have substantial side effects and do not prevent estrogen receptor negative (ER-) breast cancer. Aspirin is a promising breast cancer prevention therapy; it is cheap, safe, well tolerated, with strong biologic and epidemiologic evidence for a prevention effect on both ER- and ER+ breast cancers. However, clinical trials to date have failed to corroborate a prevention effect; these results are potentially related to study design (dose, duration of therapy and followup, population treated). We sought to evaluate the effect of aspirin on mammographic density, as breast density is a well-accepted, modifiable risk factor for both estrogen receptor positive (ER+) and ER- breast cancer. **Methods:** Electronic medical records from the University of Pennsylvania were retrospectively evaluated for women from a core set of 36 primary care/ObGyn practices. Individuals were selected if they had both undergone routine screening mammography during 2012-2013 and had an ambulatory visit within the year prior with a confirmed list of medication use. We selected the medication record closest to the screening exam. Logistic regression was performed to test for associations between clinically-recorded BIRADS breast density and aspirin use, after adjusting for the additional risk factors of age, body mass index (BMI) and ethnicity. **Results:** We identified 26000 women who fit the above criteria, of whom 19.7% reported current aspirin use and 41% were African American. Mean age was 57.3 (standard deviation [sd], 10.9) and mean BMI was 28.9 (sd, 7.3) kg/m² for the entire cohort. Aspirin users were significantly older and had higher BMI (see Table). There was an independent, inverse association between aspirin use and mammographic density ($P_{\text{trend}} < 0.001$). Compared to women with extremely dense breasts, women with fatty (OR=1.73, CI: 1.33-2.25) or scattered fibroglandular (OR=1.50; CI: 1.17-1.92) breasts were more likely to be aspirin users. A dose-response pattern was observed, as there was a lower likelihood of having extremely or heterogeneously dense breasts with increasing aspirin dose (OR=0.62, CI: 0.50-0.76 for >300 mg; OR=0.84, CI=0.77-0.91 for <=300 mg; compared to non-users as reference group). The association between aspirin use and density was more pronounced for women <60 and for African American women ($P<0.01$). **Conclusion:** We demonstrate an independent association between aspirin use and lower mammographic density in a large, diverse screening cohort. Our results suggest that this association is stronger for younger and African American women: two groups at greater risk for ER- breast cancer. Future evaluation of this cohort will examine duration of aspirin use, and evaluate an automated measure of breast density. These results and others highlight the potential value and need for a randomized, controlled trial of aspirin as a preventive agent for breast cancer.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aspirin Non-Users</th>
<th>Aspirin Users</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>55.3 (10.2)</td>
<td>65.3 (9.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>28.5 (7.2)</td>
<td>30.4 (7.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Breast density, no. (%)</td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>BIRADS 1</td>
<td>2006 (9.6)</td>
<td>861 (16.9)</td>
<td>1.73 (1.33 - 2.25)</td>
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<tr>
<td>BIRADS 2</td>
<td>9346 (44.7)</td>
<td>2859 (55.9)</td>
<td>1.50 (1.17 - 1.92)</td>
</tr>
<tr>
<td>BIRADS 3</td>
<td>8480 (40.6)</td>
<td>1312 (25.7)</td>
<td>1.22 (0.95 - 1.56)</td>
</tr>
<tr>
<td>BIRADS 4</td>
<td>1057 (5.1)</td>
<td>79 (1.6)</td>
<td>1.00 (Reference)</td>
</tr>
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</table>
Mammographic breast density as a predictor of hormone receptor positive breast cancer recurrence: A single centre longitudinal analysis

Redfern AD D, Martin HL L, Stone J, Davidson JA A, Yap F and Chung K. Fiona Stanley Hospital, Perth, WA, Australia; University of Western Australia, Perth, WA, Australia; Royal Perth Hospital, Perth, WA, Australia and Sir Charles Gairdner Hospital, Perth, WA, Australia.

Body: Mammographic breast density as a predictor of breast cancer recurrence: a single centre longitudinal analysis of women with hormone receptor positive breast cancer Background Mammographic breast density has been associated with risk of development of breast cancer. To date clinical studies examining the use of tamoxifen and fall in mammographic breast density during this treatment have shown reduction in mammographic breast density examining change in mammographic breast density between baseline and a single follow-up mammogram to be predictive for disease recurrence.

Aim
To examine serial change in mammographic breast density over years to describe the changes which occur with use of aromatase inhibitors and tamoxifen, as well as changes following cessation of this treatment and to determine whether changes observed correlate with outcome.

Method
Eligible patients were identified from the Royal Perth Hospital breast unit database between January 1994-December 2011. Patient data was prospectively collected through the breast unit database. Additional data regarding endocrine therapy, adherence, weight, height and concomitant medications were obtained from case note review. Recurrence data was obtained from the hospital medical records system, as well as the breast unit database. Mammograms were obtained and mammographic breast density readings undertaken by a single reader using Cumulus. Percentage breast densities were obtained and statistical analysis undertaken to investigate changes in mammographic density on endocrine therapy, at switch of therapy, and cessation of therapy and correlation with disease free and overall survival.

Results
1942 eligible patients were identified. 417 were premenopausal at time of diagnosis, 148 perimenopausal, 1328 postmenopausal and the remainder unknown status. 12 declined adjuvant endocrine therapy, 520 received both at least 1 aromatase inhibitor and tamoxifen during follow-up, 1189 tamoxifen only, 56 tamoxifen plus goserelin, and the remainder either aromatase inhibitor only or aromatase inhibitor with ovarian suppression. Over 10,000 mammograms were obtained for analysis. Currently results are available from 4301 mammograms from 689 patients. Mean density change between baseline scan and subsequent imaging after between 11-24 months of patient-reported endocrine adherence was -6.0%, with mean reduction of -11% in patients who were premenopausal at baseline and -4.5% in those who were postmenopausal at baseline. Kaplan Meier analysis showed late separation of overall survival curves favouring those with reduction in mammographic breast density however there was no statistically significant difference in the curves Conclusion Reduction in mammographic breast density was greatest in those who were premenopausal at baseline. Further multivariate analysis and assessment of the additional mammograms in this data set is required to assess the association between mammographic breast density and outcome in this cohort.
**Title:** Mammographic density and SNPs add to Tyrer-Cuzick and Gail model breast cancer risk in a UK screening cohort

Evans DG, Astley SM M, Brentnall A, Howell A and Cuzick J. University of Manchester, Manchester, United Kingdom and Queen Mary University London, London, United Kingdom.

**Body:**

**Background:** The predicting risk of cancer at screening study (PROCAS) in Manchester UK is a prospective study of breast cancer risk estimation. This article considers whether mammographic density and SNPs in PROCAS may help refine breast cancer risk estimation using the Gail (BCRAT) and Tyrer-Cuzick (TC, or IBIS) models, based on incident and prevalent breast cancers identified between two three-yearly screening rounds.

**Methods:** Mammographic density was measured at entry as a percentage using the average visual assessment from two trained readers. Tyrer-Cuzick and Gail risks were based on a questionnaire completed at the same time. The contribution of density to risk models was assessed after adjustment for age and body mass index (BMI) using odds ratios (ORs) and profile likelihood confidence intervals (CIs). A secondary analysis compared cancer pathology characteristics using a two-sided Wilcoxon test. Eighteen breast cancer risk variants (SNP18) were assessed with a polygenic risk score (PRS) alongside TC and density in a subset of 8870 women with 341 prospective cancers.

**Results:** Analysis included 50628 women of routine screening age (47-73 yrs), recruited between Aug 2009 and Jul 2014. 697 had a breast cancer diagnosed after enrolment. Median follow-up was 3.2 years. Visually assessed percentage breast density (inter-quartile range odds ratio (IQR OR) 1.48 (95%CI 1.34-1.63)) was a slightly stronger univariate risk factor than TC (IQR OR 1.36 (1.25-1.48)) or Gail (IQR OR 1.22 (1.12-1.33)). It continued to add information after allowing for TC (IQR OR 1.47 (1.33-1.62)) or Gail (IQR OR 1.45 (1.32-1.60)). 36472/50628 (72%) women had less than 3.5% 10-yr risk from the TC model and breast density combined. Women with dense breasts were more likely to have a higher stage breast cancer (P <.001). SNP18 showed a 2 fold relative breast cancer risk between the top and bottom quintile. Using a combined analysis of SNP18, TC and density there was a 5.6 fold risk between those identified at NICE defined high risk compared to low risk.

**Conclusion:** Breast density and SNPs when combined with the TC or Gail risk model identify a larger number of high risk women at screening, and it is associated with higher stage of disease. Approximately 70% of women are identified with a combined TC and density risk assessment of less than 3.5% 10-yr risk (average or less than average risk), for whom three-yearly screening might be effective. SNP18 adds further precision and addition of further newly identified SNPs is likely to add greater discrimination.
Factors affecting uptake and adherence to breast cancer chemoprevention: A systematic review and meta-analysis


Chemoprevention is a risk reduction option for women who have increased risk of breast cancer. Selective Estrogen Receptor Modulators (SERMs) have been extensively tested, and alternative agents are being evaluated. Long-term adherence to chemoprevention is critical to obtaining the drug's full benefit. We systematically reviewed articles reporting uptake rates and adherence among healthy adult women, who were prescribed medication to prevent primary breast cancer. We also extracted data on the clinical, socio-demographic and psychological predictors of uptake and adherence.

Searches were performed in PubMed, CINAHL, EMBASE, and PsychInfo, yielding 3851 unique articles. Title, abstract and full text screening left 53 articles that met inclusion criteria, and a further 4 studies were identified from reference lists, giving a total of 57. The mean quality score using the Mixed Methods Appraisal Tool was 3 out of 4.

Thirty-one articles reported uptake, of which 14 tested predictors, and 23 reported adherence of which 11 tested predictors. Seven studies reported qualitative data. Most studies (50) involved SERMs, but 5 tested Aromatase Inhibitors, 1 tested Aspirin, 1 tested a statin. Twenty studies included data from a clinical setting, 35 reported trial data, and 2 reported both.

Twenty-four studies reporting 26 instances of uptake in 21,423 women were included in a meta-analysis. The pooled uptake estimate was 16.3% (95% CI, 13.6-19.0), with high heterogeneity (I^2=98.9%, p<0.0001). Uptake was unaffected by study location or agent, but was significantly higher in trials (25.2% [95% CI, 18.3-32.2]) than in clinical settings (8.7% [95% CI, 6.8-10.9]). Factors associated with higher uptake in two or more studies included having an abnormal biopsy, a physician recommendation, higher objective risk, fewer side-effect or trial-related concerns, and older age. Heterogeneity in data collection prevented a meta-analysis of adherence. Data suggested adequate day-to-day adherence among women who initiated treatment, with 5/6 studies reporting ≥80% of medications being taken appropriately. Persistence over 3-12 months was also high, with 5/7 studies reporting that ≥80% women were still taking chemoprevention. Long-term persistence was lower, with only 1/10 studies reporting a persistence of ≥80% by 5-years. Factors associated with lower adherence or persistence included allocation to Tamoxifen (vs. placebo or Raloxifene), depression, smoking, and older age. Objective and subjective risk was a theme in all qualitative studies, although other topics involved in decision-making included concerns about medications (6/7), low knowledge (3/7), lack of information (2/7), and trial-related issues (2/7).

Chemoprevention uptake for the prevention of breast cancer is low, and long-term adherence is often insufficient for the full preventive effect. Uptake rates were higher in trials than in clinical settings, suggesting further work should focus on implementing chemoprevention within routine patient care. Further research is warranted to identify factors amenable to modification and to improve informed decision-making surrounding chemoprevention.
Body: Background
The FACE trial was designed to evaluate the efficacy and safety of adjuvant letrozole (LET) versus anastrozole (ANA) in postmenopausal patients with hormone receptor-positive (HR+), node-positive breast cancer.

Materials and methods
In this phase 3b, open-label, multicenter trial, postmenopausal women with HR+ and lymph node positive breast cancer were randomized 1:1 to receive either LET (2.5 mg) or ANA (1 mg) daily for 5 years in the adjuvant setting. Randomization was stratified by the number of lymph nodes (1-3 versus 4+) and HER2 status (positive versus negative). Patients with stage IIA, IIB or IIAI invasive cancer were eligible. Treatment continued for 5 years or until disease recurrence. The primary endpoint was disease-free survival (DFS) at 5 years. Key secondary endpoints were safety and overall survival (OS).

Results
Between Dec 2005 and Mar 2008, 4170 patients were randomized to receive LET (n = 2076) or ANA (n = 2094). Baseline characteristics were generally balanced between the two arms. Median age was 62 years; 71.4% of pts had 1-3+ lymph nodes, and 8.7% of cancers were HER2+. Median duration of exposure was 60 months in both arms. With 709 of the protocol-planned 959 DFS events, 5-yr estimated DFS rate was 84.9% for LET vs. 82.9% for ANA (HR = 0.93 [95% CI: 0.80 – 1.07]; p = 0.3150). 5-yr estimated OS rate was 89.9% for LET vs. 89.2% for ANA (HR = 0.98 [95% CI: 0.82 – 1.17]; p = 0.7916). Primary reasons for treatment discontinuation in the LET versus ANA arms were AEs (15.1% vs. 14.3%) and disease progression (9.5% vs. 10.4%). Safety profiles were similar between treatment arms. The most common adverse events (AEs) in the LET versus ANA arms were arthralgia (48.2% vs. 47.9%), hot flushes (32.5% vs. 32.3%) and fatigue (16.8% vs. 16.6%). Suspected drug-related grade 3/4 AEs were reported in 9.5% of patients in the LET arm versus 8.1% of patients in the ANA arm; suspected drug-related AEs leading to discontinuation were reported in 14.0% vs. 12.9% of patients in LET vs. ANA arms, respectively. Preplanned and exploratory subgroup analyses will be presented.

Conclusions
Treatment with LET did not demonstrate DFS efficacy difference over ANA in postmenopausal patients with HR+, node-positive breast cancer. (Funded by Novartis; ClinicalTrials.gov number NCT00248170).
Title: NEO-EXCEL phase III neoadjuvant trial of pre-operative exemestane or letrozole +/- celecoxib in the treatment of ER positive postmenopausal early breast cancer

Body: COX2 has been implicated in breast tumorigenesis, tumour proliferation & invasion. The role of COX2 in carcinogenesis is thought to be related to its abilities to increase production of prostaglandins, convert pro-carcinogens to carcinogens, inhibit apoptosis, promote angiogenesis, modulate inflammation & immune function & increase tumour cell invasiveness. COX2 inhibition may synergise with aromatase inhibition in controlling endocrine responsive breast cancer. The COX2 product prostaglandin E2 (PGE2) & cytokines such as interleukin-6 (IL6) can up regulate aromatase expression suggesting that aromatase inhibition may be more effective in combination with a COX2 inhibitor. There may be additional COX2 mediated anticancer activity. The hypothesis addressed is that activity of aromatase inhibitors(AI) as neoadjuvant endocrine therapy for early breast cancer may be enhanced by the addition of a COX2 inhibitor.

TRIAL OBJECTIVES
To determine whether the activity of AIs as neo-adjuvant endocrine therapy for ER positive breast cancer in postmenopausal women may be enhanced by the addition of the selective COX2 inhibitor celecoxib.

TRIAL DESIGN
Prospective phase III multicentre randomised trial. Patients were randomised to receive 16 weeks of exemestane 25 mg daily or letrozole 2.5 mg daily (open label) and celecoxib 400 mg twice daily or matched placebo (double blinded). Translational research tumour samples were collected before, during & after therapy.

KEY ELIGIBILITY CRITERIA
Post menopausal, ER positive, invasive cancer, 2cms or greater with calipers & visible on USS.

PRIMARY OUTCOME MEASURE
Objective clinical response to neoadjuvant treatment by RECIST criteria.

RESULTS
Primary Outcome; Response to treatment has been calculated for 266 patients (Table 1). Response rate was 73% in the celecoxib arm & 55% in the placebo arm (p=0.0022). The response rates 4 arm comparison are shown in Table 2. After adjustment for AI effect the significant difference in response rates remained (p=0.0023); the difference in response rates was greater in the exemestane treated group (29%) compared to the letrozole group (7%) although heterogeneity between AI arms was statistically non-significant (p=0.06).

Secondary outcome; There was an USS response rate of 42% v 37% for celecoxib & placebo arms respectively (p=0.2513)

Table 1 Primary Outcome Results: response rates

<table>
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<th>OUTCOME</th>
<th>PLACEBO N (%)</th>
<th>CELECOXIB N (%)</th>
<th>TOTAL N (%)</th>
<th>X²statistic</th>
<th>P-value</th>
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<td>RESPONSE</td>
<td>73(55)</td>
<td>97(73%)</td>
<td>170 (64%)</td>
<td>9.3882</td>
<td>0.0022</td>
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Table 2: Response Rates 4 Arm Comparison

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<tr>
<th></th>
<th>EXEMESTANE PLACEBO n(%)</th>
<th>CELECOXIB n(%)</th>
<th>TOTAL n(%)</th>
<th>LETROZOLE PLACEBO n(%)</th>
<th>CELECOXIB n(%)</th>
<th>TOTAL n(%)</th>
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<td>RESPONSE</td>
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<tr>
<td>RESPONSE</td>
<td>33 (49)</td>
<td>52 (78)</td>
<td>85 (63)</td>
<td>40 (61)</td>
<td>45 (68)</td>
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<tr>
<td>NO RESPONSE</td>
<td>34 (51)</td>
<td>15 (22)</td>
<td>49 (37)</td>
<td>26 (39)</td>
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<td>134</td>
<td>66</td>
<td>66</td>
<td>132</td>
</tr>
</tbody>
</table>

CONCLUSION
The addition of the COX2 inhibitor celecoxib to an AI significantly & substantially increased the clinical response from 55% to 73%. Effect on tumour size assessed with USS is less marked with a non-significant increase in responses from 37% to 42%.
This work was supported by CRUK: CRUK/06/005 and Pfizer.
Title: Final results of a first-in-human phase I study of the tamoxifen (TAM) metabolite, Z-Endoxifen hydrochloride (Z-Endx) in women with aromatase inhibitor (AI) refractory metastatic breast cancer (MBC) (NCT01327781)


Body: Background: AI's are more effective than TAM in ER+ breast cancer. In AI refractory MBC, the response rate to TAM is 0% (Osborne 2011). Z-Endx is an active metabolite of TAM and among TAM treated women in the adjuvant and metastatic settings, reduced CYP2D6 metabolism and low Endx concentrations (Css <20 nM) have been associated with increased likelihood of disease recurrence. Preclinical studies have demonstrated greater Z-Endx exposure and anti-tumor activity with oral Z-Endx compared to equivalent doses of oral TAM (Reid 2014)

Methods: We conducted a phase I trial to determine the maximum-tolerated dose (MTD) and evaluate the toxicities, clinical activity, and pharmacokinetics (PK) of Z-Endx in patients (pts) with ER+, AI refractory MBC. Unlimited prior endocrine regimens were allowed. An accelerated titration schedule was applied (2 pts/dose level) until moderate toxicity or DLT, followed by a 3+3 design and then to expansion cohorts (40, 80, and 100 mg/day). Z-Endx was administered orally once daily (28 day cycle). Eye exams were performed at baseline, and end of cycles 2 and 6. PK was performed during cycle 1 and prior to subsequent cycles. For pts in the expansion cohorts, tumor biopsies were obtained at baseline for DNA sequencing (Foundation Medicine). Plasma cholesterol levels were obtained at baseline and after 1 cycle.

Results: From March 2011 to Dec 2014, 41 pts (38 evaluable), median age 60, received Z-Endx once daily encompassing 7 dose levels (20-160 mg/daily). The median number of prior hormonal regimens was 2 and 3 for the dose escalation and expansion cohorts, respectively. Dose escalation was stopped at 160 mg/day given MTD not reached and attainment of mean Endx Css of 3.6 uM. Cycle 1 DLT (PE) was observed in one patient (60 mg). No eye toxicity was observed. PK demonstrated mean Endx Css of > 1 uM at all dose levels ≥ 40 mg/day. Antitumor activity was observed at multiple dose levels including 3 confirmed partial responses and an additional 7 with stable disease for ≥6 cycles. Of these 10 pts, 9 had prior progression on both AI and fulvestrant and 3 additionally on TAM. After 1 cycle, total and LDL cholesterol decreased > 20 points in 54% and 40% of pts, respectively. Tumor sequencing in the expansion cohorts (n=14) did not identify ESR1 mutations; however, ESR1 amplification was identified in 1 pt with prolonged stable disease (>200 days). Of 6 pts with rapid progression (≤2 cycles), 4/6 had either CCND1 amplification (n=1) or at least one of the following activating mutations: ERBB2 L755S (n=1), AKT1 E17K (n=1), MTOR E1799K (n=1).

Conclusions: The direct administration of Z-END provides substantial drug exposure, acceptable toxicity, and “proof of principle” antitumor activity in endocrine resistant MBC. While the MTD was not determined, the goal of achieving Endx Css concentrations of > 1 uM was achieved. Tumor sequencing identified pts with predicted and confirmed endocrine resistance. A randomized phase II comparing endoxifen (80 mg/day) with TAM in AI refractory MBC was recently activated (NCT02311933). Supported in part by CA 133049, CA186686, CA15083, CA116201, and CA15083.
Title: ERα phosphorylation at pS294: A biomarker of ligand or mutational (Y537S, D538G) activation, and a receptor target for CDK2 inhibition

Benz CC C, Scott GK K, Chu D, Kaur R, Muthurajah M, Rothschild D, Frazier K and Park BH H. Buck Institute for Research on Aging, Novato, CA and The Johns Hopkins University School of Medicine, Baltimore, MD.

Body: Background: Certain ERα phosphorylation (p) sites are essential for ERα transcriptional activity; and with development of ERα p-specific antibodies, some of these sites predict endocrine responsiveness. Unlike other ERα p-sites, pS294 has been shown to be induced by ligand activation and not by cross-talking growth factor signals. With development of a new rabbit monoclonal, pS294 induction was found to be dependent on a cyclin-dependent kinase (CDK). This study aimed to identify the specific CDK mediating induction of pS294, determine if ligand-independent ERα activating mutations (Y537S, D538G) also induce pS294, and learn if specific CDK inhibitors might enhance endocrine therapeutic efficacy by suppressing pS294.

Methods: MCF7 cells, untreated (stripped media) or stimulated by estradiol (E2, 10nM) or growth factor (EGF, 5nM), were treated with either CDK-specific knockdown siRNAs or small molecule CDK inhibitors (with indicated specificities): Roscovitine (pan-CDKs); Dinaciclib (CDK1, CDK2, CDK5, CDK9); Palbociclib (CDK4, CDK6); JNJ7706621 (CDK1, CDK2); BMS265246 (CDK1, CDK2); and SNS032 (CDK2, CDK7, CDK9). Whole cell, nuclear or cytosolic lysates were either Western blotted (for ERα or specific CDKs) or first immunoprecipitated (total ERα, pS294-ERα) and then immunoblotted. RT-PCR of cellular RNA quantified pS294-ERα induced transcripts (EGF3, AREG, CXCL12 vs. GAPDH) potentially inhibited by CDK inhibitors. MCF7 overexpressing ERα activating mutations (Y537S, D538G) were produced by either transient transfection or knock-in; knock-in clones were inoculated into immunocompromised mice to assess ligand-independent xenograft tumor growth in vivo, while transfected cells and tumors were assessed for ligand-independent ERα phosphorylation.

Results: CDK2 was determined to be the primary kinase mediating ligand-dependent induction of pS294-ERα, with co-precipitation of cyclins A/E confirming the expected mechanism of CDK2 recruitment to chromatin-bound pS294-ERα. Knock-in MCF7 cells expressing either Y537S or D538G ERα rapidly formed tumors in vivo without E2 supplementation; tumors and transiently transfected cells overexpressing mutated ERα showed pS294 >> pS118 expression, with constitutive pS294 suppressed by Dinaciclib but not by Palbociclib. CDK1/2 inhibitors (Dinaciclib, BMS265246) but not a CDK4/6 inhibitor (Palbociclib) cooperated with tamoxifen (4-HT) to induce apoptosis in wildtype MCF7.

Conclusion: CDK2 is the primary mediator of pS294 induced by either ligand stimulation or ligand-independent mutational activation of ERα. While the CDK4/6 inhibitor Palbociclib is a recently approved adjunct to endocrine therapy, it enhances cytostatic growth arrest without affecting ERα phosphorylation or receptor induced gene expression. In contrast, CDK2 inhibitors like Dinaciclib should be explored for their ability to enhance ER-positive breast cancer cell death in combination with antiestrogens and for their ability to prevent the emergence of constitutively active ERα mutations by suppressing pS294 induction, essential for ERα mediated gene transactivation and breast tumor growth.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD2-05

Title: Evaluating the role of recurrent ESR1-CCDC170 in breast cancer endocrine resistance

Hu Y, Veeraraghavan J, Wang X, Tan Y, Kim J, Schiff R and Wang X-S. Lester ans Sue Smith Breast Center, Baylor College of Medicine, Houston, TX; Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX and Baylor College of Medicine, Houston, TX.

Body: Background
Recurrent gene fusions resulting from chromosome translocations are critical genetic aberrations causing cancer. In our previous study, we identified recurrent rearrangements between ESR1 and its neighbor, CCDC170, in 6-8% of luminal B tumors. Luminal B subtype is a more aggressive ER+ breast cancer, with a higher risk of early relapse after endocrine therapy. These rearrangements enable the expression of N-terminally truncated CCDC170 (∆CCDC170) under ESR1 promoter. Consistent with the behavior of luminal B tumors, ectopic ∆CCDC170 expression in ER+ breast cancer cells, led to markedly increased cell motility, invasion, anchorage-independent growth, and reduced endocrine sensitivity in vitro, as well as enhanced xenograft growth in vivo. In the present study, we studied the role of ESR1-CCDC170 in breast cancer endocrine resistance in vivo and explored the potential mechanism.

Methods
To study endocrine resistance in vivo, we transplanted T47D cells stably overexpressing (OE) control (empty) construct or 2 ∆CCDC170 fusion variants (E2-E7 and E2-E10) bilaterally to 4-6 week old female athymic nude mice (supplemented with 17β-estradiol pellets). The tumor growth was monitored biweekly and tumor volume was measured by the formula 1/2(length × width²). When the tumors reach 150–200 mm³, mice were randomly allocated to vehicle or tamoxifen (tam) treatment groups. For ERE luciferase assay, cells were co-transfected with ERE luciferase reporter (ERE-TK-Luc) and pCMV β-galactosidase. The luciferase levels were measured and normalized to β-gal activity. For immunoblot analysis, T47D OE cells were estrogen-deprived, serum-starved, and treated with vehicle, estrogen (E2) or tam. Reverse Phase Protein Array (RPPA) analysis was performed using ~200 validated antibodies against an array of key signaling molecules in cancer.

Results
Our in vivo endocrine sensitivity study showed that, while T47D vector control tumors mostly regressed after tam treatment, the regression of E2-E7 tumors was significantly slower. Moreover, E2-E10 tumors continued to grow despite tam treatment. These observations suggest that ∆CCDC170 may render the T47D xenografts less sensitive to tam in vivo. Kaplan–Meier analysis revealed a significantly worse progression-free survival (defined by tumor doubling time) for E2-E7 (p<0.01) and E2-E10 (p<0.001) tumors treated with tam compared to control tumors. ∆CCDC170 expression in T47D cells enhanced the ER transcriptional activity in the presence of E2 but not tam, suggesting that the fusion-mediated endocrine-sensitivity changes is unlikely due to restoration of classic ER activity. Immunoblot analysis of T47D OE cells revealed hyperactive growth factor signaling even after serum withdrawal, which was not significantly affected by tam treatment. Preliminary RPPA analysis revealed upregulation of key signaling molecules in T47D cells expressing ∆CCDC170, such as Her3, AMPK, Akt, Erk, c-Myc, and Src-3.

Conclusion
These data suggest a potential role of ESR1-CCDC170 in mediating breast cancer endocrine resistance, presumably due to hyperactive growth factor signaling endowed by this fusion. Further studies are required to elucidate the role of endogenous ESR1-CCDC170 in breast cancer endocrine resistance, and discover the precise engaged mechanisms.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD2-06

Title: Inhibition of 3-phosphoinositide dependent protein kinase 1 (PDK1) synergizes with CDK4/6 inhibitors against ER-positive breast cancer


Body: Background: Dysregulation in cell cycle checkpoints is common in cancer. Small molecule inhibitors that target the CDK4/6/cyclinD1 pathway of the cell cycle are in clinical development. Recently the combination of the CDK4/6 inhibitor palbociclib and the aromatase inhibitor letrozole was approved for the treatment of post-menopausal women with ER+/HER2-advanced breast cancer. However, not all patients benefit from CDK4/6 inhibitors and a significant fraction of them eventually progress on these agents, underscoring the need to develop potent therapeutic strategies to circumvent drug resistance.

Methods: We performed a high-throughput RNA interference (RNAi) kinome screen targeting 720 kinases to identify targetable molecules whose inhibition, in combination with the CDK4/6 inhibitor LEE011 (ribociclib), induced synthetic lethality in MCF7 ER+ breast cancer cells. PDK1 RNAi oligonucleotides and the PDK1 inhibitor GSK2334470 in combination with each of the CDK4/6 inhibitors, palbociclib and LEE011, were tested against ER+ breast cancer cells. In vivo anti-tumor efficacy of LEE011 and GSK2334470 was assessed in ovariectomized athymic nude mice bearing MCF7 xenografts.

Results: A siRNA kinome screen identified PDK1 as the top RNA whose downregulation sensitized MCF7 cells to CDK4/6 inhibitors. This was confirmed with independent siRNAs in ER+ MCF7, T47D, HCC1428 and HCC1500 breast cancer cells. Pharmacological inhibition of PDK1 with the ATP-competitive, small molecule inhibitor GSK2334470 in combination with each of the CDK4/6 inhibitors, LEE011 and palbociclib, synergistically inhibited proliferation and increased apoptosis of MCF7 and T47D cells (combination index 0.19-0.89). LEE011-resistant MCF7 and T47D cells were generated by chronic treatment with doses of LEE011 up to 1 µM. Drug-resistant cells displayed increased levels of PDK1, phosphorylated Rb, and phosphorylated S6 ribosomal protein (pS6), an effector of the PDK1 substrate p70S6K, compared to parental drug-sensitive cells. Inhibition of PDK1 with siRNA or GSK2334470 re-sensitized the LEE011-resistant cells to the CDK4/6 inhibitors. Genetic (RNAi) and pharmacological inhibition of PDK1 (with GSK2334470) abrogated pS6 levels whereas inhibition of AKT with the small molecule inhibitor MK2206 did not affect pS6 levels, suggesting PDK1 can induce resistance to CDK4/6 inhibitors via p70S6K/pS6 signaling in an AKT-independent manner. The effects observed in cell lines in culture were recapitulated in vivo using MCF7 xenografts established in ovariectomized nude mice in the absence of estrogen supplementation. Treatment with GSK2334470 and LEE011 induced tumor regressions (8/8 tumors by RECIST criteria) more potently than either drug alone.

Conclusions: These data support a critical role of PDK1 in mediating acquired resistance to CDK4/6 inhibitors in ER+ breast cancer cells. Co-targeting of the PDK1 and CDK4/6 pathways may overcome resistance to CDK4/6 inhibitors and is worthy of further translational and clinical investigation in patients with ER+ breast cancer.
Title: Insulin receptor substrate (IRS) targeting by the tyrophostin NT157 inhibits breast cancer cell growth

Yee D, Temiz NA A, Levitzki A and Yang Y. Pharmacology, University of Minnesota, Minneapolis, MN; Masonic Cancer Center, University of Minnesota, Minneapolis, MN and The Hebrew University of Jerusalem, Jerusalem, Israel.

Body: Insulin and insulin-like growth factor (IGF) signaling systems regulate the malignant phenotype. However, targeting of the type I IGF receptor (IGF-IR) has shown little activity in clinical trials. One potential reason for these disappointing results is that activation of the closely related insulin receptor (InR) could compensate for IGF-IR blockade. Since both receptors phosphorylate the insulin receptor substrates (IRS), perhaps a better strategy would involving targeting of this key post-receptor protein. Two IRS proteins are expressed in breast cancer cells. IRS-1 is regulated by estradiol in breast cancer cell lines, while IRS-2 is the predominant IRS species in hormone receptor negative cells. NT157, a small-molecule tyrphostin, binds IGF-1R but does not affect receptor autophosphorylation. Instead, it downregulates IRS proteins in several model systems. In primary breast cancers, IRS-1 was positively correlated to ERα expression in the TCGA database. In ERα+ and basal-like breast cancer cell lines NT157 treatment suppressed IRS protein expression in a dose dependent manner. Short term exposure to NT157 treatment did not affect IGF-I, IGF-II, and insulin induced activation of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), but longer exposure resulted in inhibition of these signaling pathways. NT157 resulted in serine phosphorylation of IRS proteins and was dependent on MAPK activation. Serine phosphorylation resulted in disassociation between IRS proteins and their receptors resulting in IRS degradation. NT157 decreased S phase fraction, monolayer, and anchorage independent growth after IGF/insulin treatment in ERα+ breast cancer cells. NT157 downregulation of IRS protein expression also sensitized ERα+ breast cancer cells to rapamycin. Moreover, NT157 inhibited the growth of tamoxifen resistant ERα+ breast cancer cells. In the basal-like breast cancer cells (MDA-MB-231), NT157 repressed the proliferation (G2/M abrogation) and migration through downregulation of IRS1/2 protein. Given that both IGF-IR and InR play a role in cancer biology, targeting of IRS adapter proteins could be a more effective inhibitory strategy compared to receptor-targeting approaches.
Body: Invasive lobular carcinoma (ILC) is the eighth most frequently diagnosed cancer in any organ, and accounts for 8-11% of breast cancer. This histological subtype is characterized by loss of E-cadherin, and favorable prognostic factors, such as low Ki67 and high rates of ER/PR-positive tumors. Only recently is the lobular subtype gaining recognition as a distinct disease, displaying a unique growth pattern, unique molecular changes in addition to loss of E-cadherin, and evidence for late recurrences and reduced response to targeted endocrine therapy. It is widely accepted that a late age at first full term birth (FFTB) increases a woman's risk for breast cancer. Interestingly, several published epidemiological studies have shown that the increased risk after a late age at FFTB is preferential for the lobular subtype of breast cancer compared to the ductal subtype. We therefore hypothesized that pregnancy hormones like prolactin play an integral role in the development and progression of ILC. Interrogation of the Cancer Genome Atlas (TCGA) data revealed a high expression of milk protein genes as well as prolactin signaling molecules, specifically Stat5a and Stat5b in lobular carcinomas compared to ductal carcinomas. We developed a lactation score including 7 milk protein genes and found that in the TCGA data set ILC tumors have a significantly higher lactation score than IDC tumors. Additionally, we found that ILC cell lines express increased prolactin receptor mRNA and protein levels compared to IDC cell lines. Prolactin treatment in ILC and IDC cells reveals divergent signaling pathways - prolactin stimulates ERK activation in IDC but not ILC cells. We are currently further delineating the prolactin signaling pathways, and resulting phenotypes, comparing ILC and IDC cells. We expect these experiments to move the field forward by establishing a relationship between prolactin and lobular carcinoma.
Title: Next generation sequencing of circulating tumor DNA to predict recurrence in triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy

Chen Y-H, Hancock BA A, Solzak JP P, Miller KD D and Radovich M. Indiana University School of Medicine and Simon Cancer Center, Indianapolis, IN.

Body: Background: Incorporation of next-generation sequencing to detect plasma-derived tumor DNA (ptDNA) is emerging as a popular method for tumor genotyping and for monitoring therapeutic response. The vast majority of studies so far have focused on detecting ptDNA from patients with metastatic disease. Herein, we tested whether ptDNA could be used as a biomarker to predict relapse in triple-negative breast cancer (TNBC) patients with residual disease after neoadjuvant chemotherapy and surgery.

Methods: BRE09-146 was a Phase II clinical trial that randomized TNBC patients with residual disease after neoadjuvant chemotherapy to Cisplatin or Cisplatin+Rucaparib. From the combination arm, 1ml of plasma was collected at four predefined time points post-surgery. In total, 39 patients with matched tumor, blood, and plasma were analyzed. Extracted DNA underwent library preparation and amplification using the Ion Ampliseq Oncomine Research Panel which consists of 134 cancer genes that are well-known to be mutated in cancer. Samples were then sequenced on an Ion Proton next-generation sequencer to at least 2500X coverage followed by bioinformatic analyses using the Torrent Variant Caller.

Results: We first detected high-quality somatic mutations in primary tumors. TP53 mutations were the most prevalent (70%) followed by AKT1 (8%). Somatic mutation frequencies in our trial were congruent with publically-available mutation data of TNBCs from The Cancer Genome Atlas. Using these somatic mutations, we then analyzed the plasma-sequencing data to detect the same mutations in the circulation. Out of 39 patients, 14 patients had a clinical relapse (median follow-up for disease free survival = 24 months). Of the 14 patients, we were able to detect somatic ptDNA in 4 patients (3 TP53 mutations, 1 AKT mutation). Notably, all 4 patients had a rapid recurrence (0.3, 4.0, 5.3, and 8.9 months). ptDNA-sequencing was unable to detect distant recurrence. The combination of a paucity of ptDNA molecules in the circulation of patients who have no evidence of disease along with a limited amount of plasma available per patient are potential factors for the inability to detect distant recurrence.

Conclusions: Next-generation ptDNA-sequencing of triple-negative breast cancer patients after neoadjuvant chemotherapy and surgery can detect rapid-recurrence but sensitivity to detect distant recurrence is limited. Studies to increase sensitivity by incorporating mutation calling from ptRNA along with ptDNA are currently underway.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-01-02

Title: Capturing intra-tumor genetic heterogeneity in cell-free plasma DNA from patients with oligometastatic breast cancer

Ng CKY K Y, Bidard F-C, Piscuoglio S, Lim RS S, Pierga J-Y, Cottu P, Vincent-Salomon A, Viale A, Norton L, Sigal B, Weigelt B and Reis-Filho JS S. Memorial Sloan Kettering Cancer Center, NY, NY; Institut Curie, Paris, France; Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, NY, NY and Memorial Sloan Kettering Cancer Center, NY, NY.

Body: Background: The analysis of cell-free tumor DNA (ctDNA) from plasma has been heralded as a non-invasive technique for disease monitoring and as a means to overcome the challenges posed by intra-tumor genetic heterogeneity. ctDNA levels have been shown to correlate with tumor burden in breast cancer patients. Hence, we sought to define whether massively parallel sequencing of cell-free plasma DNA would capture the entire repertoire of somatic mutations present in the primary tumors and/or metastases from patients with oligometastatic breast cancer.

Methods: Frozen diagnostic biopsies from primary tumors and their distant metastases were obtained from five prospectively accrued treatment-naïve patients with stage IV breast cancer at presentation (1 estrogen receptor (ER)+/HER2+, 2 ER+/HER2-, 2 ER-/HER2+). A second, independent formalin-fixed paraffin-embedded (FFPE) diagnostic biopsy was obtained from the primary tumor and metastasis from 4 patients. Plasma samples were obtained from all patients. DNA samples from microdissected frozen tumors and peripheral blood, as well as plasma from one patient, were subjected to high-depth whole exome sequencing. DNA samples from all biopsies (frozen/FFPE), plasma and peripheral blood were subjected to targeted capture massively parallel sequencing, with baits for all somatic mutations detected by whole exome sequencing and all exons of the 100 genes most frequently mutated in breast cancer. Driver mutations were defined by state-of-the-art bioinformatic methods and literature search.

Results: We identified and confirmed a median of 54 (range 25-75) and 53 (range 26-85) non-synonymous mutations in the primary tumors and metastases from the 5 cases analyzed, respectively. By sequencing the plasma DNA to a median depth of 248x (range 92-431x), state-of-the-art mutation callers revealed 0-4 mutations (0%-8% of mutations) per patient, and direct interrogation of the sequencing data, based on prior knowledge of the mutations present in the lesions, resulted in the identification of 2-18 mutations (3%-38% of mutations) per patient. Of the bona fide driver mutations, 2/3 TP53 mutations, 0/1 PIK3CA hotspot mutation, 0/1 BRCA2 frameshift mutation, 0/1 GATA3 frameshift mutation and 0/1 ERBB3 activating mutation were captured in the plasma DNA. A SMAD4 pathogenic mutation and a TCF7L2 truncating mutation were found in two diagnostic biopsies of metastatic lesions but not in two biopsies of the primary tumors in one patient each. Whilst the SMAD4 mutation was detected in the plasma DNA from the respective patient, the TCF7L2 mutation was not. Of the 62 mutations restricted to the primary tumors (0-42 per patient) and 74 restricted to the metastatic tumors (1-41 per patient), 4 and 7, respectively, were captured in the plasma DNA.

Conclusions: Massively parallel sequencing assessment of plasma DNA allows for the identification of mutations found in primary tumors and/or their metastases, however, only a subset of these could be detected at up to 431x depth. These observations suggest that current approaches for whole exome or targeted massively parallel sequencing may not be sufficient to capture the genetic heterogeneity of breast cancers in patients with oligometastatic disease.
Table 1: Cohort 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days between tissue biopsy and blood draw</th>
<th>Sequencing FFPE tumor tissue</th>
<th>ddPCR plasma for ESR1 Y537S*</th>
<th>ddPCR plasma for ESR1 Y537N*</th>
<th>ddPCR plasma for ESR1 D538G*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>186</td>
<td>ESR1 Y537S</td>
<td>Y537S (0.87%)</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
<td>2</td>
<td>344</td>
<td>ESR1 Y537S</td>
<td>Y537S (1.69%)</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>3</td>
<td>275</td>
<td>ESR1 D538G</td>
<td>WT</td>
<td>WT</td>
<td>D538G (1.55%)</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>ESR1 Y537S</td>
<td>Y537S (0.63%)</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>ESR1 D538G</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.03%)</td>
</tr>
<tr>
<td>6</td>
<td>165</td>
<td>ESR1 D538G</td>
<td>WT</td>
<td>WT</td>
<td>D538G (4.23%)</td>
</tr>
<tr>
<td>7</td>
<td>88</td>
<td>ESR1 D538G</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>ESR1 Y537N</td>
<td>WT</td>
<td>Y537N (0.68%)</td>
<td>WT</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
<td>10</td>
<td>145</td>
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<td>WT</td>
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<tr>
<td>11</td>
<td>270</td>
<td>WT</td>
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</table>

*Percentage reflects the fractional abundance of mutant ESR1 to the total ESR1 DNA

Table 2: Cohort 2

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<tr>
<th>Patient</th>
<th>Days between tissue biopsy and blood draw</th>
<th>Sequencing FFPE tumor tissue</th>
<th>ddPCR plasma for ESR1 Y537S*</th>
<th>ddPCR plasma for ESR1 Y537N*</th>
<th>ddPCR plasma for ESR1 D538G*</th>
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<tr>
<td>ER-positive</td>
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<tr>
<td>12</td>
<td>-</td>
<td>n/a</td>
<td>Y537S (0.47%)</td>
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<tr>
<td>13</td>
<td>0</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>n/a</td>
<td>Y537S (5.02%)</td>
<td>WT</td>
<td>D538G (2.62%)</td>
</tr>
<tr>
<td>15</td>
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<td>WT</td>
<td>WT</td>
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<tr>
<td>16</td>
<td>0</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
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<td>WT</td>
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<td>WT</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>D538G (0.01%)</td>
</tr>
<tr>
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<td>-</td>
<td>n/a</td>
<td>WT</td>
<td>Y537N (0.06%)</td>
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</tr>
<tr>
<td>ER-negative</td>
<td></td>
<td></td>
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<td>WT</td>
<td>WT</td>
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</table>

*Percentage reflects the fractional abundance of mutant ESR1 to the total ESR1 DNA*
Title: A method for comprehensive genomic analysis of cell free DNA

Parpart-Li S, Angiuoli SV V, Chesnick B, Galens K, Jones S, Kadan M, Kann L, Lytle K, Murphy D, Nesselbush M, Phallen J, Riley D, Shukla M, Zhang T, Husain H, Velculescu V, Diaz, Jr LA A and Sausen M. Personal Genome Diagnostics, Baltimore, MD; The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD and Moores Cancer Center, University of California San Diego, San Diego, CA.

Body: Circulating tumor DNA (ctDNA) is released from tumor tissue into the blood, carries tumor specific genetic alterations, and can be analyzed through noninvasive "liquid biopsy" approaches to identify genetic alterations in cancer patients. Liquid biopsies offer a considerable advantage as they may eliminate the need for invasive tissue biopsies and allow for the detection of alterations in multiple metastatic lesions throughout the course of therapy. However, the fraction of ctDNA obtained from a blood sample is often very low (<1.0%) and can be difficult to detect. Additionally, most methods to evaluate circulating tumor DNA (ctDNA) interrogate single hot spot mutations or few genetic alterations. The next generation of ctDNA assays must interrogate multiple gene regions from a single sample with high precision and accuracy and need to evaluate all forms of actionable genomic alterations including point mutations, amplifications, and translocations. To address these issues, we have developed a ctDNA approach called PlasmaSelect to detect somatic sequence mutations, amplifications and translocations at low allele frequencies in the circulation of cancer patients. Utilizing digital genomic approaches, PlasmaSelect achieves high sensitivity and specificity while interrogating >250,000 nucleotides spanning 63 well-established cancer genes. In addition to sequence mutations in the entire coding region of 18 genes and the exons of 40 genes that are frequently mutated in cancer, PlasmaSelect also performs a comprehensive genomic analysis of amplifications in 57 genes and translocations in 10 genes significant in cancer tumorigenesis. To evaluate the PlasmaSelect approach, we performed dilution series using tumor-derived DNA, containing well-characterized somatic mutations, in the presence of wild-type DNA. PlasmaSelect was able to detect genetic alterations with high specificity and a lower level of detection of 0.10% for sequence mutations and translocations, as well as a focal amplification of ERBB2 with a lower level of detection of 0.20%. We evaluated the clinical utility of PlasmaSelect for detection of genetic alterations in the plasma and matched tissue biopsy specimens from late stage cancer patients. These analyses demonstrated high concordance between the somatic sequence mutations, amplifications, and translocations identified in the tumor sample and those identified directly in the plasma, including alterations in both driver genes as well as those related to acquired resistance to targeted therapies. PlasmaSelect provides a non-invasive platform to enable liquid biopsy detection of clinically relevant genetic alterations across a large number of genomic loci.
Title: Sensitive blood-based monitoring of breast cancer

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Body: Breast cancer is the leading cause of cancer in women worldwide. Although there have been significant advances in the clinical management of breast cancer over the past few decades, women continue to die from this disease. Current methods for detection and monitoring of breast cancer progression, metastasis, and late recurrences lack sensitivity and have not yet been proven to significantly extend overall survival. Current research is focused on identifying novel monitoring methods, with a particular focus on blood-based tumour markers. Monitoring of circulating tumor cells improves detection, but the methods are complex and not applicable to routine practice. A new more sensitive method based on detection of circulating cell free tumor DNA has shown to be more sensitive for women known to have late stage disease. This is a great advance but has not been shown to be a valuable prospective monitoring tool. Tumor derived exosomes (TEX) represent an alternative blood-based method. These are small membrane vesicles that are secreted into the blood and harbor a molecular signature that are directly representative of their ‘parent cell’. Emerging evidence suggests that TEX are a veritable ‘treasure chest’ from which an abundance of stable biomarkers can be isolated and applied to disease monitoring. We hypothesized that: (1) TEX harbor patient-specific DNA mutations similar to those of the patient’s tumor; and (2) determining the number and genomic profile of TEX has the capacity for earlier identification of recurrent or progressive disease. To investigate this in a pilot study, TEX were isolated from the plasma of breast cancer patients (n=11) by ultracentrifugation. Western blotting and electron microscopy were performed to validate TEX isolation. DNA was extracted from TEX, patient-matched breast tumor tissue and buccal swabs. Next generation sequencing was performed using the Ion Torrent platform and the Cancer Hotspot Panel v2 (Life Technologies). Despite the small cohort size, there was a statistically significant association (p=0.028) between exosome protein yield and clinical disease stage. TEX protein levels were higher among patients with axillary lymph node involvement (mean 4.8µg per µl of plasma) compared to those with localized disease (mean 3.5µg per µl of plasma). In keeping with other studies the TEX contained low levels of double-stranded DNA that spanned the majority of the genome. Validation of the variants detected in the TEX is currently underway to confirm whether exosomal DNA contains the same genetic variants present in the primary neoplastic tissue. The early detection of breast cancer recurrence and progression represents an unmet challenge that needs to be overcome. This study demonstrates that with continued exploration, TEX may represent a novel approach for disease monitoring that has the potential to improve clinical management and survival.
Differential proteomics identifies complement factor H proteolytic species as early breast cancer biomarkers

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Background
Breast cancer is the most common cancer among female population worldwide. We have been particularly interested in whether proteolytic species in human blood plasma can be biomarkers for detection of early breast cancer. Thus, a new differential proteomics approach has been implemented to evaluate this hypothesis.

Methods
We first collected plasma samples from 6 patients before and after neoadjuvant chemotherapy. These pairs of samples were then subjected to a modified two-dimensional differential gel electrophoresis (modified 2-D DIGE), comprising fluorescent dye labeling, macroporous reverse phase (mRP) HPLC and reducing/non-reducing SDS-PAGE. The difference protein species were analyzed with LC-MS/MS. Cleavage site-specific antibodies have been produced to perform a large-scale examination of total 379 plasma samples. These samples include 75, 74, 46, 48, and 48 patients at stage 0, 1, 2, 3 and 4, respectively. Also, there are 29 samples from normal individuals and 59 from other cancers/diseases to serve as control.

Results
A group of proteolytic species in some breast cancer patients were found by modified 2-D DIGE. Notably, these species disappeared from the plasma after the diseased tissue was surgically removed. A pair of complement factor H (CFH) derivatives were identified using LC-MS/MS analyses. Through a series of examination, we concluded that proteolytic removal of Arg-341 is likely the molecular mechanism that leads to these findings. According to these data, we have generated antibodies that can specifically recognize these proteolytic products.

We used antibodies to tested over 350 clinical samples and found these biomarkers can be specifically detected in plasma of breast cancer patients. It is quite encouraging that positive detection is shown in 15% of stage 0 pts (11/75) and 20% of stage 1 pts (15/74). Other 20 patients (21–22%) with stage 2 and 3 are found positive. 13 out of 48 patients (27%) with metastatic tumor have also been detected. Surprisingly, the signal did not observed in normal individuals or patients with other diseases, strongly suggesting that these biomarkers are highly specific to breast cancer.

Conclusion
Our initial results show the utility of this novel strategy in detection of cancer-specific proteolysis. About 20% of early breast cancer patients including stage 0 disease can be detected through these biomarkers. The promise of these proteolytic species as early cancer biomarkers and use of the cleavage site-specific antibodies are particularly remarkable, since early breast cancer detection can be applied to establish higher cure rates and thus lead to better prognosis for patients.

Keyword: Breast cancer, Early cancer biomarker, Proteolytic processing.
Title: Neutrophil lymphocyte ratio and breast cancer: A future prognostic indicator for outcome after surgery?

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Body: There is a growing body of literature highlighting the prognostic use of pre-operatively comparing certain white blood cell differentials, namely neutrophils and lymphocytes, with outcomes in many different types of solid tumour cancers. It has been postulated that a raised neutrophil to lymphocyte ratio (NLR) may reflect a greater tumour burden on the patient, and therefore lead to poorer outcomes. There is some evidence that a raised pre-operative NLR may help to predict poorer outcomes following surgery for breast cancer.

This observational retrospective proof of concept study aims to assess the association between pre-operative NLR and breast cancer outcomes over a three year period of follow-up from a cohort of 147 patients. The results of this study revealed that the cohort of patients with a raised pre-operative NLR was associated with a clinically significant increased rate of local recurrence of their breast cancer following surgery with curative intent (with or without adjuvant chemotherapy and radiotherapy) compared to those that had a low or normal NLR (20.4% ± 4.9% vs 5.1% ± 4.9% respectively, CI 95%; p=0.004). In relation to intra-operative sentinel lymph node biopsy, the analysis also showed that the incidence of a raised pre-operative NLR was not related to whether there was nodal involvement or not at the time of surgery (33% vs 33%, p=1). This suggests that a raised pre-operative NLR may be an independent prognostic indicator for breast cancer outcomes.

This study provides a template for a prospective observational study which is needed to corroborate this result and to account for confounding factors such as concomitant chronic disease. Additionally, a larger cohort sample size with a follow-up of five years is necessary to assess the relationship between pre-operative NLR and mortality due to the latter's relatively low incidence in breast cancer. However, this study further supports that consideration of the pre-operatively calculated NLR may provide an early prognostic indicator for breast cancer outcomes and warrants further investigation.
Title: Adaptive dynamic artificial poly-ligand targeting: Aptamer-based profiling of liquid biopsies to improve the accuracy of breast cancer diagnoses in women with dense breast tissue


Body: Introduction:
Breast cancer screening relies upon mammography, but for women with dense breast tissue this method is often uninformative. Routine screening identifies suspicious breast lesions in some women, but the pain and risk associated with follow-up biopsies along with the poor accuracy of traditional histopathology urgently call for improved approaches to breast cancer screening. This is especially important for those high-risk patients for whom mammography is of limited value. We describe a non-invasive liquid biopsy method of profiling plasma exosome preps designed to improve the accuracy and safety of breast cancer screening for women with dense breast tissue.

Results:
We incubated plasma samples (300 microliters per sample) from breast cancer patients (n=60) and a control cohort (n=60) with a high-complexity DNA aptamer library using a modified SELEX scheme, termed “adaptive dynamic artificial poly-ligand targeting (ADAPTTM)”. Differentially bound (cancer vs. non-cancer) aptamers were recovered from precipitated exosomes and were identified by deep sequencing. Two thousand aptamer sequences were resynthesized and used to probe a larger set of 500 plasma samples from a patient cohort (n=206) and a control cohort comprised of self-reported healthy volunteers (n=117) and patients whose biopsies led to a diagnosis of non-cancer (n=177). We employed several statistical models to build a cancer/non-cancer predictor, including a Random Generalized Linear Model (RGLM) and a Random Forest Model (RFM). Both models yielded an equivalent classification performance with areas under the receiver-operator characteristic curve (ROC AUC) of 0.7. Testing the prediction performance by 100 Out-of-Bag permutations or by pre-filtered (read cutoff and estimated sample size) cross-validation (CV) resulted in ROC AUC values of 0.66 and 0.62, respectively. When samples were randomly assigned to groups, the aptamers were no longer able to distinguish the groups (ROC AUC = 0.54), indicating that the underlying information driving the model is truly specific to cancer. Importantly, incorporation of BIRAD results as a clinical covariate did not influence model performance, signifying that predictions by ADAPTTM were independent of breast tissue density.

Conclusions:
We have identified a set of 2000 DNA aptamers that distinguish women with breast cancer from women without breast cancer. Our liquid biopsy approach requires only 300 microliters of plasma and is amenable to high-throughput processing. By employing a number of statistical approaches including rigorous cross-validation, we consistently achieve cross validation ROC AUC values approaching 0.7. The performance of the predictor was not affected by BIRAD scores, supporting its potential utility in difficult cases where imaging is insufficient, such as in women with dense breast tissue. Further optimization of the aptamer library and testing on additional samples should improve performance. Upon complete validation, an ADAPTTM – derived breast cancer test may serve as a vital diagnostic adjunct that can be easily incorporated into standard clinical practice.
**Title:** In primary breast cancer patients with HER2-negative tumors, HER2-positive circulating tumor cells with stem cell character predict worse outcome

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**Body:** Introduction: The detection of disseminated (DTCs) and circulating (CTCs) tumor cells in patients with early stage breast cancer is a well described independent prognostic factor associated with increased risk of disease recurrence and disease-related death. We recently demonstrated that CTCs in blood of primary breast cancer patients rather than DTCs were significantly associated with reduced progression free survival (p=0.02). Using comprehensive molecular characterization, we here demonstrate that the negative prognostic impact was predominantly related to HER2-positive CTCs with stem cell character from patients with HER2-negative primary tumors.

Patients and Methods: 2 x 5 ml blood from 482 primary breast cancer patients with first diagnosis between 2006 and 2010 were analyzed for CTCs with the AdnaTest BreastCancer (QIAGEN Hannover GmbH, Germany) for the detection of EpCAM, MUC-1, HER-2, and beta-Actin transcripts. The recovered c-DNA was now additionally tested for the expression of the estrogen (ER) and progesterone receptor (PR) (single-plex RT-PCR) and stem cell like CTCs (slCTCs) applying the AdnaTest TumorStemCell (single-plex RT-PCR for ALDH1) and the AdnaTest EMT (multiplex RT-PCR for TWIST, AKT2, PI3K). The analysis of PCR products was performed by capillary electrophoresis on the Agilent Bioanalyzer 2100.

Results: CTCs were detected in 103/482 (21%) of the patients expressing EpCAM (27%), MUC-1 (26%), HER-2 (75%), ER (14%) and PR (8%), respectively. Notably, in 49/103 (48%) of the CTC-positive patients, HER2 was the only marker expressed. slCTCs could be analyzed in 72/103 CTC-positive patients. At least one of the EMT markers was expressed in 56/72 patients (78%), ALDH1 was present in 32/72 patients (44%) and 31/72 (44%) were positive for both, ALDH1 and EMT markers, respectively. Comparisons of expression profiles on CTCs with those on the primary tumor were only performed in CTC-positive patients. Primary tumors and CTCs displayed a concordant HER2, ER and PR status in 37% (p=0.81), 24% (p=0.257) and 27% (p=0.876) of cases, respectively. Most interestingly, in 60/75 (80%) patients with HER2-positive CTCs, primary tumors were HER2-negative. In contrast, the percentage of patients with ER- and PR-positive CTCs but negative ER/PR primary tumors was 29% and 25%, respectively. When the presence of HER2-positive CTCs was correlated with the presence of slCTCs (ALDH1-and/or EMT-positive), the concordance was 83% (p=0.0019). In detail, the concordances were HER2+ vs ALDH1+ (54%, p=0.037), HER2+ vs EMT+ (85%, p=0.0002) and HER2+ vs ALDH1+/EMT+ (80% p=0.00053), respectively.

Conclusion: Our results provide evidence that a) the negative prognostic impact of CTCs in our patient cohort is related to HER2-positive CTCs with EMT and tumor stem cell characteristics which indicate therapy resistant tumor cell populations and, therefore, an inferior prognosis and b) “secondary” adjuvant treatment with HER2-targeting agents, alone or in combination, may probably be effective to eliminate these cells and thus, lead to an overall decreased relapse rate. This study further confirms that the molecular characterization of CTCs might help to stratify patients for individual treatment options.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-02-02

Title: Predictors for discordance in HER2 phenotype between primary tumor and circulating tumor cells in women with metastatic breast cancer

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Body: Aim: The DETECT study program evaluates whether treatment efficacy in women with metastatic breast cancer (MBC) is increased by taking into account the molecular characteristics of circulating tumor cells (CTCs). Here, we present data on both prevalence and HER2 phenotype of CTCs in patients with HER2 negative MBC screened within the DETECT program. The aim of this study is to evaluate the rate of discordance in HER2 phenotype between primary tumor and CTCs and to analyze whether primary tumor and/or patient characteristics can predict discordance in HER2-status.

Methods: The number of CTCs in 7.5 ml of peripheral blood (using the FDA-cleared CellSearch® System; Janssen Diagnostics, LLC) and their HER2 status were evaluated. Patients were defined as having a positive HER2-status on CTCs if at least 1 CTC with a strong (+++) immunocytochemical HER2 staining intensity was found. To assess which factors predict discordance of HER2 phenotype between primary tumor and CTCs, we used a multivariate binary logistic regression model with backward selection procedure. Patient and primary tumor characteristics included as independent factors were patient age, time since primary diagnosis, tumor stage, nodal stage, grading, histological type, and hormone receptor status (HRS).

Results: 1123 women with HER2-negative MBC were screened for CTCs. Based on a cutoff of ≥ 1 CTC, 711 (63.3%) of 1123 screened patients were positive for CTCs, while 412 (36.7%) of the 1123 screened patients were categorized as CTC positive if a cutoff of ≥ 5 CTCs was used.

At least one HER2-positive CTC was found in 134 of the 711 HER2-negative MBC patients with one or more CTCs (median 2 HER2-positive CTCs, range 1 – 80), indicating a discordance between primary tumor and CTCs with regard to HER2-status in 18.8% of patients. If the analysis was restricted to the 412 patients with 5 or more CTCs, at least one HER2-positive CTC was found in 121 patients, resulting in 29.4% discordance rate.

A multivariate logistic regression with discordance in HER2 phenotype (yes/no) as binary response variable, including number of CTCs (1 – 4 CTCs vs. 5 or more CTCs) to account for the difference in discordance rate observed at different cutoff values for CTC positivity, showed that histological type (lobular vs. ductal, odds ratio OR 2.66, 95% confidence interval CI 1.62 – 4.37, p < 0.001), HRS (positive vs. negative, OR 2.89, 95% CI 1.16 – 7.19, p = 0.022) and CTC number (5 or more CTCs vs. 1 – 4 CTCs, OR 7.57, 95% CI 3.93 – 14.89, p < 0.001) significantly predicted discordance in HER2 phenotype between primary tumor and CTCs.

Conclusion: Our data revealed discordance in HER2 status between primary tumor and CTCs in 19% to 29% of patients with HER2 negative MBC. Discordance in HER2 status was predicted by histological type and HRS of the primary tumor, as well as by the number of CTCs detected. Individualized breast cancer treatment based on CTC phenotype is currently investigated in Phase III trials and not part of clinical routine yet. However, the knowledge of factors associated with discordance in HER2 status may be incorporated in today's clinical practice by guiding the decision process for performing a biopsy to characterize a metastatic relapse.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-02-03

**Title:** HER2 expression on circulating tumor cells before adjuvant chemotherapy and during follow-up in patients with HER2-negative early breast cancer

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**Body:**

**Background:** Detection of circulating tumor cells (CTCs) before adjuvant chemotherapy as well as during follow-up is related to poor prognosis in early breast cancer. The presence of HER2-positive CTCs has been also associated with significantly decreased disease-free and overall survival. We hereby assessed the HER2 status of CTCs in HER2-negative patients with early breast cancer, before the initiation of adjuvant chemotherapy and during follow-up.

**Methods:** Double staining immunofluorescent experiments using pancytokeratin (A45-B/B3) and HER2 antibodies were performed in peripheral blood mononuclear cells (PBMCs) of patients. Patients were evaluated before adjuvant therapy (n=103) and during follow-up (n=290) at intervals ranging from 6 months to 20 years post-chemotherapy.

**Results:** CTCs were detected in 28 (27.2%) out of 103 patients pre-chemotherapy and in 94 (32.4%) out of 290 during follow-up. The mean CTC count detected at \( \leq 2 \) years of follow-up was not significantly different compared to baseline [3.4 CTCs (range 1-13) vs 2.9 (range 1-12), respectively]. However, this value showed a significant decrease from 2-5 to \( \geq 10 \) years of follow-up (4.2, range 1-11 vs. 1.8, range 1-4). Among CTC-positive patients, CTCs expressing HER2 were detected in 21 (75%) and in 73 (77.7%) patients pre-chemotherapy and during follow-up, respectively. The mean proportion of HER2-positive CTCs per patient was 73.5% pre-chemotherapy and 67% at follow-up. This proportion showed a significant increase at follow-up time points \( \leq 2 \), 2-5 and 5-10 years [52% (p=0.0027), 65.6% (p=0.0476) and 69% (p=0.0440), respectively] compared to \( \geq 10 \) years (89.2%).

**Conclusions:** These data demonstrate that CTCs persist during follow-up in a significant percentage of early breast cancer patients. Similarly, HER2 positive CTCs are detected in a significant proportion of patients both at baseline and during follow-up. Mean CTC count appears to decrease after 10 years, whereas the population of HER2 positive CTCs seems to increase during follow-up. These results suggest that targeting HER2-positive CTCs may have implications for the prevention of late relapses.
**Title:** Distinct clinical and biological values of subpopulations of circulating tumor cells (CTCs) in primary breast cancer

Mego M, Jurisova S, Karaba M, Minarik G, Benca J, Sedlackova T, Manasova D, Malejcikova M, Sieberova G, Macuch J, Gronesova P, Sufliarsky J, Pindak D, Cristofanilli M, Reuben JN and Mardiak J.  Faculty of Medicine, Comenius University, Bratislava, Slovakia (Slovak Republic);  National Cancer Institute, Bratislava, Slovakia, Bratislava, Slovakia (Slovak Republic); Cancer Research Institute, Bratislava, Slovakia (Slovak Republic); Thomas Jefferson University-Kimmel Cancer Center, Philadelphia, PA and University of Texas, MD Anderson Cancer Center, Houston, TX.

**Body:** Background: CTCs represent a heterogeneous population of cells with different phenotypes and biological values. Epithelial to mesenchymal transition (EMT) gives rise to cells with stem cell-like properties with increased resistance to chemotherapy that may be under detected by currently approved assays. The aim of this study was to characterize CTCs based on the expression of epithelial and mesenchymal markers in primary breast cancer (BC) and to correlate them with patients/tumor characteristics.

**Methods:** This prospective translational study included 422 patients with primary BC enrolled from March 2012 to February 2015. Blood for CTC detection was drawn before surgery (422 patients), before 1st cycle (95 patients) and before 2nd cycle (53 patients) of adjuvant therapy. Isolated peripheral blood mononuclear cells (PBMC) were depleted of cells of hematopoietic origin (CD45+) using RosetteSep kit (StemCell Technologies) negative selection with anti-CD45 antibody. RNA extracted from CD45-depleted (CD45-) PBMC was interrogated for expression of EMT-inducing transcription factors (TWIST1, SNAIL1, SLUG, ZEB1) and epithelial (CK19) gene transcripts by quantitative reverse transcription-PCR. Expressions of gene transcripts in CD45-PBMC from patients were compared to those of CD45- PBMC of 60 healthy donors.

**Results:** Totally, CTCs were detected in 116/422 (27.5%) patients before surgery, in 21/95 (22.1%) patients after surgery and before 1st cycle and in 19/53 (35.8%) of patients before 2nd cycle of adjuvant therapy. Before surgery, CTCs exhibited only epithelial markers in 38 (9.0%) patients, only EMT markers in 68 (16.1%) of patients, while in 10 (2.4%) patients CTCs with both epithelial and EMT markers were detected. Epithelial CTCs were more often detected before surgery compared to after surgery (11.4% vs. 2.1%; p = 0.003), while mesenchymal CTCs were more often detected after the 1st cycle of chemotherapy as opposed to detection before surgery (30.2% vs. 18.2%; p = 0.05). Patients with N2-3 disease had more often detectable CTCs compared to patients with N0-1 disease (41.4% vs. 24.9%, p = 0.01) and this was mainly driven by mesenchymal CTCs (31.0% for N2-3 vs. 16.0% for N0-1; p = 0.007). Similarly, patients that lacked p53 expression (wild type TP53) in primary tumor had more often CTCs with EMT phenotype opposite to patients with p53 expression (p = 0.02). Presence of epithelial CTCs was significantly associated with lower absolute lymphocyte (p = 0.02) and neutrophil (p = 0.02) counts in peripheral blood.

**Conclusions:** Our results support the concept of CTCs phenotypic heterogeneity in breast cancer patients. These results support the role of EMT in cancer pathogenesis and suggest that CTCs with EMT phenotype are involved in tumor dissemination while their increase after chemotherapy might be a mechanism of treatment resistance. Moreover, these data suggest inverse relationship between immune cells and epithelial CTCs which stress the role of immune cells in tumor dissemination.
Circulating tumor cells in triple-negative and non-triple negative breast cancer patients show different genetic profiles

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Background: Triple negative breast cancer (TNBC) is known for its aggressive behavior, poor prognosis and still remains as a difficult disease since treatment options are limited. Despite some success in PARP inhibition in BRCA gene mutation patients or platinating agents that may offer superior outcomes in a subset of TNBC patients (pts), currently, there are no targeted therapies for TNBC available. Specific biomarkers are urgently needed for developing effective treatments to predict which patients will respond to the given therapy. In this regard, circulating tumor cells (CTCs) are discussed to be an ideal surrogate marker for individualized treatment options. Since TNBC is closely related to epithelial-mesenchymal transition (EMT), a stem cell phenotype and, in addition, androgen receptor (AR) expression has been detected in up to a third of TNBC pts, we here established a multi-marker gene panel for the characterization of CTCs in TNBC pts and compared these findings with CTC characteristics in non-TNBC pts.

Methods: 2x5 ml blood of 30 TNBC pts before and/or after neoadjuvant therapy and 30 non- TNBC pts (E+/PR+: n=23; ER+/PR-: n=4; HER2+; n=1; HER2+/ER+: n=1; HER2+/ER+/PR+: n=1) before 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 (androgen 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: In general, the distribution of the markers across all patients was highly variable. However, different expression patterns were found when CTCs of TNBC pts were compared with those of non-TNBC pts. In TNBC pts, SRC1 was the gene that was predominantly expressed, followed by c-Kit, HER3, BRCA1 and AURKA expression, before as well as after therapy. Interestingly, AKT2, EGFR, ERCC1 and PARP1 expression could not be detected at any time point studied. In addition, ALK, AR, c-Met, HER2 and KRT5 were only detected before but not after therapy. All other genes were expressed below 15%. In contrast, in non-TNBC pts, AKT2 was the gene that was predominantly expressed, followed by c-MET, HER3 and PI3K whereas c-KIT, ERCC1, mTOR and NOTCH1 were never found. All other genes were expressed below 10%.

Conclusion: We successfully established a gene panel for the detection of the heterogeneous CTC population and demonstrated that CTCs in TNBC pts and non-TNBC pts show different genetic profiles. Although these data have to be confirmed in a bigger patient cohort, the knowledge about the individual target gene expression profile might efficiently help to predict a personalized targeted therapy for these pts in the future.
Impact of apoptotic circulating tumor cells in metastatic breast cancer

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Background
Circulating tumor cells (CTC) are a heterogeneous cell population and an independent negative prognostic factor for progression-free (PFS) and overall survival (OS) in patients with metastatic breast cancer (MBC). The study aimed to prospectively assess CTC status for the subtypes apoptotic CTC (aCTC) and intact CTC (iCTC) at baseline (CTCBL) and after one cycle of a new line of systemic therapy (CTC1C). Changes from CTCBL to CTC1C (CTC kinetics, CTCKIN) were evaluated for their utility in predicting response, progression-free (PFS) and overall survival (OS).

Methods
423 MBC patients were included in a prospective trial prior to a new regimen of treatment. Intact and apoptotic CTC were analyzed at baseline (CTCBL) and after one cycle of systemic therapy (CTC1C) using CellSearch™ (Veridex) and morphologic criteria. Samples with ≥5 CTC/7.5ml blood were regarded as positive. Therapy response was assessed using the RECIST-criteria on three-monthly radiological controls. CTCKIN were characterized by ≥25% change from CTCBL to CTC1C to differentiate stable, increased and decreased CTC kinetic.

Results
35% of patients were iCTCBL-positive and 28% aCTCBL-positive at baseline (CTCBL). PFS and OS differ significantly between the iCTCBL-positive and the iCTCBL-negative group (PFS 4.5 vs. 8.0; OS 12.5 vs. 27.2 (months)). Positive aCTC in conjunction with positive iCTCBL at baseline has worst prognostic impact (PFS 6.3; OS 8.7).

Regarding the CTCKIN (BL to 1C), aCTC-decrease (≥25%) is a positive prognostic factor compared to aCTC-stable and aCTC-increase (PFS 7.6 vs. 3.7; 3.3 and OS 21.0 vs. 4.8; 5.7). Decreasing aCTCKIN shows favorable prognostic impact versus decreasing iCTCKIN (PFS 7.6 vs. 5.9 and OS 21.0 vs 16.4).

Conclusion
Elevated aCTC levels at baseline have an unfavorable prognostic impact on both OS and PFS in conjunction with elevated iCTC. Additionally, the decrease of aCTC is a relevant prognostic value for systemic therapy response. aCTCKIN allows better differentiation for therapy response in patients with positive CTC-status at baseline. Differentiated enumeration of intact and apoptotic CTC should be considered in clinical application.
Title: Collection, high-resolution imaging, and single cell isolation of circulating tumor cells from patient derived xenograft models using the AccuCyte® – CyteFinder® system

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Body: Background: Patient-derived xenograft (PDX) models of human tumors offer many advantages over traditional cell line xenograft models and other mouse models of cancer. A PDX model may be used to randomize a given patient's tumor to multiple treatment regimens in order to predict treatment responses. When PDX models are grouped, they represent a clinical trial “cohort” for testing new therapies and identifying biomarkers of response. One such biomarker is circulating tumor cells (CTCs), which provide a window of the metastatic process. CTCs have been reported in several PDX models, further supporting their clinical relevance. Thus, PDX models may also be used to study the utility of CTC analysis to inform treatment decisions. However, most current CTC technologies intended for use with human samples cannot be used with the small blood volume from mice. The objective of our study was to adapt the AccuCyte® – CyteFinder® (AC-CF) system to detect CTCs from low volumes of mouse blood, and apply this method for the analysis of CTCs in a PDX model, including individual cell retrieval for molecular analysis. Methods: The AC-CF PDX process was modified to include a red blood cell lysis step instead of the density-based separation for the removal of red blood cells. The isolated cells were spread onto microscope slides using a stabilization solution, stained by multi-color immunofluorescence, and visualized by the CF high-resolution multi-channel fluorescence scanner. Automated image analysis identified CTCs, which was followed by single cell retrieval. For optimization of the assay, BT474 breast cancer cells were spiked into blood from a tumor-free control mouse (approx. 500 cells in 250 µl). Slides with BT474 cells were used to test sensitivity by using antibodies against human cytokeratins (pan-CK), epithelial cell adhesion molecule (EpCAM), and erbB family growth factor receptors (EGFR and HER2) to detect the spiked-in cells. Assay specificity was tested by using antibodies specific for the mouse isofrom of CD45. The antibody panel was tested on blood samples from 6 mice carrying small (300-400 mm3) tumors of the breast cancer PDX model (BCM-4888) previously published to have CTCs. Results: BT474 were identified by their large nuclei, positive staining with human specific antibodies against pan-CK, EpCAM, and EGFR/HER2 markers, and negative staining for mouse CD45. BT474 were detected in approximately the same amount as were spiked in. CTCs were identified in the blood of all 6 PDX mice tested. We found 1-6 CTCs per 330 µl of blood, and clusters of CTCs were also identified in 4 mice. Overall, these findings agree with published data on this PDX model. Single CTCs will be isolated using the CytePicker® retrieval module for single cell sequencing to confirm the human origin of these cells. These results along with ongoing work on additional PDX models will be presented at the meeting. Conclusion: The modified AC-CF process is a simple and sensitive method of analyzing small volumes of blood for CTC detection and isolation, features that are critical for the longitudinal analysis of CTCs in PDX models of cancer.
Title: Initial circulating tumor cell count and venous thrombosis-free interval in the course of metastatic breast cancer

Institut Curie, Paris, France.

Body: Background: Circulating tumor cells (CTC) count is a major prognostic factor in metastatic breast cancer. It has been also reported to be associated with venous thrombosis in short-term studies on advanced metastatic breast cancer patients.

Methods: We assessed whether an early CTC detection (CellSearch®), before the start of the first line chemotherapy, impacts Thrombosis-Free Interval during the whole course of metastatic breast cancer. Electronic medical files of all patients included in the large prospective IC 2006-04 CTC detection study (NCT00898014, Pierga et al, Ann Oncol 2012) and treated at Institut Curie (Paris, France) were manually and automatically searched for thrombosis events in the course of their metastatic breast cancer. Superficial venous thromboses were not taken into account. Thrombosis-Free Interval (TFI) was defined as the first event occurring between venous or arterial thrombosis.

Results: In the 142 patients studied, with a median follow-up of 64 months [25-81 months], venous (deep venous thrombosis, pulmonary embolism, catheter thrombosis and portal vein thrombosis) and arterial thrombosis occurred in 21.8% and 4.2% of patients, respectively. Twenty-four (30%) of the 80 patients with ≥1 CTC/7.5ml of blood before the start of first line chemotherapy experienced at least one thrombotic events in the course of their disease, while only 10 (16%) of the 62 CTC-negative patients experienced a similar event (p=0.06). Baseline positive CTC was associated with a higher risk of experiencing a venous or arterial thrombosis (TFI: HR=2.6 [1.2-5.5], p=0.009). The median TFI was 64 months in CTC-positive patients and not reached in CTC-negative patients. Among the other patients characteristics tested at baseline, Performance Status (PS=1 vs PS=0: HR=0.85 [0.4-1.9], p=NS; PS=2 or 3 vs PS=0: HR=3.7 [1.5-8.7], p=0.003) and LDH levels at baseline (LDH>UNL vs LDH<UNL HR=2.4 [1.2-4.9], p=0.012) were also associated with TFI.

Conclusions: Metastatic breast cancer patients with ≥1 CTC before the start of chemotherapy are at higher risk of venous thrombosis; the clinical relevance of thrombo-prophylaxis should be investigated in this population.
Title: Obesity associated factors are inversely associated with circulating tumor cells in metastatic breast cancer

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Body: Background: Elevated levels of circulating tumor cells (CTCs) are associated with adverse outcomes in metastatic breast cancer (BC). However, relationships between CTCs and various patient-related factors that may impact outcome remain undefined. Consequently, associations of CTC counts with obesity and metabolic factors were evaluated in order to gain insight into potential interactions between patient physiology and disease burden. We hypothesized that obesity and associated metabolic factors would be associated with higher CTC counts.

Methods: Non-diabetic women with metastatic BC beginning a new line of treatment due to progressive disease were recruited from four Ontario cancer hospitals between February 2013 and April 2015. Patients provided blood for CTC analysis, which was completed within 72 hours of collection using the Janssen CellSearch platform. Fasting serum was also collected for assessment of metabolic factors including glucose (mmol/L), insulin (pmol/L), leptin (ng/mL) and adiponectin (ng/mL). Associations of CTC counts with these factors, as well as anthropometric measurements (height (cm), weight (kg), BMI (kg/m2)) were evaluated using Pearson correlation coefficients after transforming the variables involved to normality. For CTC counts, the log transformation with half integer correction was used.

Results: 96 patients with a median age of 60.5 years completed the study. Most were post-menopausal (87, 90.6%) and exhibited grade II/III tumors (75, 78.1%). The majority of patients had hormone receptor positive disease (83, 86.5%), but 16.7% (16) were HER2 positive and 10.4% (10) were triple negative. The number of CTCs observed ranged from 0 to 1238 (median 2, geometric mean 3.63). No CTCs were detected in 29 patients (30.2%), whereas 25 patients (26 %) exhibited counts of 1 to 4 CTCs and 42 (43.8%) had 5 or more CTCs. CTCs were not significantly associated with tumor characteristics including ER/PgR, HER2, grade, stage (T/N) or lymphovascular invasion. The number of CTCs inversely correlated with BMI (r=-0.26, p=0.01), leptin (r=-0.29, p=0.004), and leptin-adiponectin ratio (r=-0.3, p=0.004). A similar trend that approached significance was noted for body weight (r=-0.19, p=0.07), insulin (r=-0.19, p=0.06) and homeostatic model assessment (HOMA, an estimate of insulin resistance, r=-0.2, p=0.055). Conversely, adiponectin (r=0.18, p=0.07) and height (r=0.18, p=0.07) were positively associated with CTC counts in correlations that neared significance. No associations were observed for age (r=0.09, p=0.4) or glucose (r=-0.09, p=0.4).

Conclusions: Obesity associated metabolic factors including weight, BMI, insulin, HOMA and leptin were inversely associated (and adiponectin and height positively associated) with CTC counts. These patterns are consistent with weight loss and/or cachexia in women with elevated CTC counts who have higher disease burden. Additional analyses are underway to further characterize these associations and include assessment of serum albumin, free fatty acids, creatine kinase and hepcidin.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-02-10

Title: Circulating cells from the tumor microenvironment as liquid biopsy biomarkers alongside circulating tumor cells in metastatic breast cancer

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Body: Background: Metastasis is a multistep process that involves the shedding of tumor cells in the peripheral circulation. These Circulating Tumor Cells (CTCs) have prognostic implications in patients with metastatic breast cancer (MBC). Cancer Associated Fibroblasts (CAFs) are a major component of the breast tumor microenvironment. The reciprocal signaling between tumor cells and its microenvironment promotes carcinogenesis, invasion, and metastasis. Studies in mouse models have shown that metastatic cells can bring their own stromal components from the primary site to the site of metastasis, and that these cotraveling stromal cells provide an early growth advantage to the accompanying metastatic cancer cells. CAFs have not been identified in the peripheral circulation. Using a microfilter capture technique, we discovered non-tumor, non-immune cells in the blood of metastatic patients and identified these cells as circulating CAFs (cCAFs). The purpose of this study is to demonstrate the presence of cCAFs as a biomarker of metastasis simultaneously with CTCs in patients with MBC.

Materials and Methods: We identified 20 patients with MBC (Metastatic/MET Group) and 10 patients with cured breast cancer (Ductal carcinoma in situ or Stage I post definitive treatment with >5 years of disease free survival i.e. Localized/LOC Group). A total of 7.5 ml of peripheral blood was obtained from each patient. The enumeration of CTCs and cCAFs was carried out by the microfilter capture technique. Identification of these cells was done by a triple immunofluorescence staining for pan-CK (cytokeratin), FAP (Fibroblast Activated Protein) and CD45. cCAFs were identified as CK-, FAP+, CD45- cells and CTCs as CK+, CD45- cells. Identification and confirmation of cCAF was also carried out in parallel samples by a simultaneous FAP/α-Smooth Muscle Actin staining.

Results: cCAFs were detected in 17/20 (85%) MET patients but in only 2/10 (20%) LOC patients. CTCs were detected in 20/20 (100%) MET patients and in 8/10 (80%) LOC patients. The counts of CTCs and cCAFs in MET group ranged between 1-98 (median 13.5) and 0-117 (median 4), respectively. The counts of CTCs and cCAFs in the LOC group ranged between 1-14 (median 6) and 0-2 (median 0), respectively. For patients with exhibited cCAFs, 2/10 LOC and 5/17 MET patients had cCAFs counts of 2 or less. Although the sample size was small, patients exhibiting cCAFs (odds ratio=22.67, 95% CI: 3.14-163.63, p=0.002) were more likely to be in MET group than LOC group.

Conclusion: This is the first demonstration that CAFs, the predominant mesenchymal cell in the breast tumor microenvironment, are shed into the circulation and can be identified and enumerated as cCAFs in MBC patients along with CTCs. There was a clear difference in the numbers of CTCs and cCAFs levels between the MET and the LOC groups suggesting that CTCs and cCAFs are associated with advanced stage disease. While most patients, both in the LOC and MET group, exhibited CTCs, very few LOC patients exhibited cCAFs. We suggest that cCAFs could independently or along with CTCs serve as liquid biopsy biomarkers of metastasis. Validation of these findings in a larger cohort of patients will be presented during the meeting.
 Publication Number: P2-02-11

Title: Detection of activating estrogen receptor 1 (ESR1) mutation on single circulating tumor cells from metastatic breast cancer patients

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Body: Background: 65% of primary breast cancers express the estrogen receptor α (ERα) and the mainstay of treatment are therapies that result in selective estrogen receptor modulation (SERM) of estrogen deprivation (aromatase inhibitors, AIs). Even thought endocrine therapy resulted in reduced recurrence and mortality, a significant portion of patients relapse with a metastatic disease and subsequently progress while of therapy for advanced disease (endocrine resistance). Recent evidence showed that activating hot spot mutation in the ligand binding domain of the ERα are acquired on treatment (frequency of 20%) and can drive resistance to endocrine therapy. Circulating tumor cells (CTCs) provide a non-invasive accessible source of tumor material and the molecular profiling of these rare cells might lead to insight on disease progression and therapeutic strategies. These features suggest that the detection of ESR1 mutation on single CTC may be a useful biomarker for therapy guidance.

Purpose: Investigate the incidence and heterogeneity of ESR1 mutational status within single CTCs isolated from individual metastatic breast cancer patients (mBCs), combining the FDA approved CellSearch® system for enumeration of CTCs with the DEPArrayTM technologies.

Methods: CTCs were enriched and enumerate by CellSearch® in 7.5 ml blood samples collected from 21 mBCs according to standard protocol. Each CTC-enriched sample with at least 20 CTCs was recovered from Veridex cartridge and loaded into the DEPArrayTM A300K chip, since the DEPArrayTM analyzed only the 66% of the sample volume loaded, according to the manufacturer's instructions. The chip scanning was performed by automated fluorescence microscope. The loaded cells were recovered as single cell and subdivided in three different group: Cytokeratin (CK) positive (Dapi+, CK+, ER-, CD45-); ER positive (Dapi+, ER+, CK+, CD45-); White Blood cells (WBCs) (Dapi+, CD45+, CK-, ER-). Single CTCs and WBCs were then submitted to whole genome amplification (WGA) using the Single Cell WGA kit (Yikon Genomics) according the manufacturer's instructions. Detection of target 14 ESR1 hot spot mutations was performed on ABI PRISM® 3700 genetic analyzer by target Sanger sequencing.

Results: 3 out of 21 mBCs with ≥20 CTCs were sorted and a total of 65 cells were recovered. WGA and ESR1 mutational status were performed on a total of 25 cells (respectively 11 ER+, 6 CK+ and 8 WBCs). In 1 of the 3 patients, that failed 2 lines of chemotherapy and previous single agent endocrine therapy, molecular heterogeneity was detected among its ER+ cells. 4 of 5 ER+ cells were heterozygote for the Y537S while one cell was homozygous, maybe due to a loss of heterozygosity. Y537S is one of the most common mutations that leads to a ligand independent ER transcriptional activity that does not respond to endocrine manipulation. No mutations were reported in all the CK+ and WBC cells analyzed.

Conclusions: This study demonstrates the feasibility of a non-invasive approach based on liquid biopsy in mBCs. Evaluation of ER status and early identification of ESR1 mutation in ER+ CTCs might allow to predict effect of the endocrine therapies and switching to other treatments before the emergence of metastatic disease.
Title: Association of inflammatory and tumor markers with circulating tumor cells in metastatic breast cancer

Lohmann AE, Chang M, Dowling RJO JO, Ennis M, Amir E, Elser C, Brezden-Masley C, Vandenberg T, Lee E, Fazae K, Stambolic V and Goodwin PJ J. Mount Sinai Hospital, Toronto, ON, Canada; Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; Applied Statistician, Markham, ON, Canada; St. Michael's Hospital, Toronto, ON, Canada and London Regional Cancer Program, London, ON, Canada.

Background: Circulating tumor cells (CTCs) are associated with prognosis in metastatic breast cancer (BC). We evaluated the association of inflammatory/tumor markers and CTCs in women with progressing metastatic breast cancer prior to commencing a new line of systemic therapy.

Methods: From February 2013 to April 2015, 96 patients with metastatic BC about to start a new treatment (due to progression), without current diabetes or use of anti-inflammatory agents, were recruited from four Ontario cancer hospitals. Women provided fasting blood for inflammatory and tumor markers and CTC measurement; CTCs were assayed within 72 hours of collection using CellSearch. Blood was frozen at -80C until assays were performed in a single batch (C-reactive protein (CRP), IL-6, PAI-1, Ca15-3, Ca125, VEGF, TNFa). Associations of CTCs with blood factors were evaluated using Pearson correlation coefficients after transforming the variables to normality. For CTCs the transformation log(x+0.5) was used. Associations with categorical variables were tested using one-way analysis of variance. P values <0.05 were significant.

Results: Median age of patients was 60.5 years, 87 (90.6%) were post-menopausal, 83 (86.5%) had hormone receptor positive BC, 16 (16.7%) HER2 positive BC, 10 (10.4%) triple negative; 75 (78.1%) grade II/III. At the time of CTC measurement, bone, lung, liver and brain metastases were present in 79%, 44%, 40% and 6% of patients respectively, with 54%, 37%, 35% and 3% having progression at these sites respectively. PAI-1 and CA15-3 exceeded the limit of the assay in 11 and 5 cases respectively (the upper limit of the assay was used in the analysis). 33.4% of patients were starting first line therapy, 25% second line and 16.7% third line. CTC counts (per 7.5cc) ranged from 0 to 1238 (median 2, geometric mean 3.63); none were detected in 29 (30.2%) patients, 1 to 4 in 25 (26%) and 5 or more in 42 (43.8%) patients. CTCs were not associated with age, estrogen receptor, progesterone receptor, HER2, line of treatment, lymph-vascular invasion or tumor grade. Compared to metastatic disease at other sites, CTCs were higher in the presence of bone (p=0.027) and liver metastases (p=0.002) and with progressing bone (p=0.018) and liver (p=0.012) metastases. CTCs were significantly associated with CRP (R =0.25, p=0.014), IL-6 (R=0.31, p=0.002), PAI-1 (R=0.31, p=0.002), Ca15-3 (R=0.44, p=<0.0001) and Ca 125 (R=0.21, p=0.04) but not with VEGF and TNFa (R = 0.11, p= 0.29 and R = 0.16, p=0.11, respectively).

Conclusion: CTCs were associated with bone and liver metastases and with higher levels of inflammatory and tumor markers, potentially reflecting tumor burden. Additional inflammatory marker assays are underway. Future studies are warranted to confirm these findings.
Title: EpCAM-independent enrichment approach for isolation of circulating tumor cells (CTCs) in breast cancer - What can be found in the EpCAM-depleted fraction?

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Body: Circulating tumor cells (CTCs) are the potential precursors of metastatic disease. Many assays have been established for the enumeration of CTCs. However, major limitations include the reliance on the expression of the cell surface marker epithelial cell adhesion molecule (EpCAM). These approaches may not detect CTCs that either express no/low levels of EpCAM or undergo epithelial-to-mesenchymal transition (EMT). We present an enrichment strategy combining different antibodies specific for surface proteins and extracellular matrix (ECM) components to capture EpCAMneg cell lines and EpCAMneg CTCs from EpCAM-depleted breast cancer blood samples. Expression of proteins (Trop2, CD49f, cMet, CK8, CD44, ADAM8, CD146, TEM8, CD47) was verified by immunofluorescence on EpCAM-positive (e.g. MCF7, SKBR3) and -negative (MDA-MB-231) breast cancer cell lines; antibodies and ECM proteins (e.g. hyaluronic acid (HA), collagen I, laminin) were further spotted in a single- and multi-arrayed format onto glass slides (Schott, NEXTERION® AL) and coupled to immunomagnetic beads (Dynabeads/Adembeads). Tumor cell adhesion of EpCAMpos/neg cell lines was visualized by Coomassie/MitoTracker; EpCAMneg CTCs enriched via functionalized Adem-/Dynabeads were identified by immunofluorescence staining for anti-pan-Cytokeratin(CK)-FITC/anti-CD45 AF647/DAPI and quantified manually by microscopy. Regarding cell lines, marginal binding of EpCAMneg MDA-MB-231 cells to EpCAM-antibodies could be observed. Efficient adhesion/capturing of EpCAMneg cells could be achieved via HA and immobilized antibodies against CD49f and Trop2. By analyzing 29 EpCAM-depleted fractions from 25 metastatic breast cancer patients, we were able to identify EpCAMneg CTCs in 69% of the samples [range 1-24] applying Trop2, CD49f, cMet, CK8 and/or HA magnetic enrichment. Accessorily, EpCAMneg dual-positive (CKpos/CD45pos) cells could be traced in 28 out of 29 samples [range 1-480]. Herein, we demonstrate an enhanced enrichment strategy to optimize capturing of EpCAMneg CTCs by targeting various cell surface antigens with antibody mixtures and ECM components. Thereby, potential relevant CTCs can be gathered and subjected to subsequent molecular analysis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-02-14

Title: Detection and characterization of CTCs isolated by ScreenCell®-Filtration in metastatic breast cancer


Body: Background: Circulating Tumor cells (CTCs) detection has prognostic and predictive implications in patients with metastatic breast cancer (MBC). Genomic and phenotypic analysis of CTCs hold enormous promise as blood-based molecular characterization and monitoring disease progression and treatment benefit with a strong potential to be translated into more individualized targeted treatments. FDA-approved CellSearch™ detection allows only enumeration of CTCs expressing EpCAM without molecular characterization. CTCs represent very heterogeneous populations of tumorigenic cancer cells and some subpopulations have undergone epithelial-Mesenchymal transition (EMT), which is associated metastasis process and an unfavourable outcome. EpCAM-based enrichment technique has failed to detect EMT subpopulations due to the decreased expression or loss of epithelial markers. Non-EpCAM-based approaches are needed for identifying EMT CTCs. The ScreenCell® devices are single-use and low-cost innovative devices that use a filter for enrichment-free isolation of CTCs by a two-steps combining size-based separation and staining using different markers. The DEPArray™ system is the ideal downstream isolation system to collect single or pooled CTCs for molecular and genetic analysis. In this study, we evaluated the feasibility of achieving CTCs detection/ enumeration using ScreenCell® filtration followed by single cell isolation with the DEPArray™ in MBC patients.

Methods: The first part of the study consisted in evaluating CTCs detection/enumeration in 30 patients with stage III and stage IV breast cancer. 3 mL of whole blood in an EDTA or Transfix tubes was collected and processed on the ScreenCell® Cyto device following the instructions of the supplier. CTCs were stained with cytokeratin (CK-8, 18, and 19), leukocyte antigen (CD45), and a nuclear dye (DAPI) and counted under fluorescence microscope. CTCs were identified as positive staining for CK and DAPI and negative staining for CD45 (CK+/DAPI+CD45-). In the second part, After enrichment, CTCs were stained with CK, CD45, and DAPI and sorted with DEPArray™ Platform (Silicon Biosystems, Inc). Single CTCs were collected and the DNA of each single CTCs was amplified with Ampli1™ WGA kit, and the genome integrity index (GII) was assessed by Ampli1™ QC kit (Silicon Biosystems, Inc). Library was constructed and whole exome sequencing (WES) of DNA mutations was conducted.

Results: Twenty patient samples had CTCs detected (66.7%), the number of CTCs was 1 to 347 per 3.0 ml of whole blood. CTC-clusters were detected in 7 patient samples (23.3%). Single CTCs were collected on DEPArray™ platform after enrichment with ScreenCell filtration. GII was confirmed with the presence of short, medium, and long DNA fragments (3 to 4 PCR bands) in the WGA library by PCR-based assay. All collected CTCs showed high GII as measured by Ampli1™ QC kit (GII ≥ 3) for WES of DNA mutations. The data analysis of WES results is under processing.

Conclusions: ScreenCell® filtration is simple and effective devices to isolate CTCs and identify CTC-clusters. Isolation of single cells for molecular analysis using the combination of ScreenCell® filtration and DEPArray™ Platform is feasible for genetic characterization of CTCs.
Title: Discovery of putative circulating tumor cells through somatic mutation profile of epithelial cell adhesion molecule positive single cells from blood of metastatic breast cancer patients

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Body: Background: Circulating tumor cell (CTC) enumeration provides prognostic information for chemotherapy in metastatic breast cancer. However, due to its rarity and heterogeneity, it is difficult to distinguish true CTCs from normal blood cells and perform genomic analysis on them for use in therapeutic strategies. The main application of most currently available CTC detection systems consists of an enumeration of putative CTCs without further analysis. The aim of this study was to evaluate the feasibility of single cell picking and target sequencing of epithelial cell adhesion molecule (EpCAM)-positive cells for detecting CTCs.

Methods: Whole blood sampled from metastatic breast cancer patients who were newly diagnosed with metastasis or who had disease progression during palliative treatment were used for this study. After applying IsoFlux Circulating Tumor Cell Enrichment Kit (Fluxion, South San Francisco, CA, USA), single CTC candidates were picked from a pool of EpCAM-positive cells. Genomic DNA from the picked cells was whole genome amplified and target sequencing was performed using Ion AmpliSeq™ Cancer Hotspot Panel (Life Technologies, Carlsbad, CA, USA). Target sequencing reads were mapped to human genome reference (hg19) using BWA-MEM (0.7.10). Single nucleotide variants (SNVs) were annotated using dbSNP, Variome Data 0.2, and COSMIC databases.

Results: A total of 172 EpCAM-positive cells were selected according to size and EpCAM status from whole blood of 11 patients. The remaining cells were grouped into a pooled sample for each patient. The mean read depth of the target genes was 13455×. A mean 7.82 mutations as determined by SNVs listed in the COSMIC database but not in dbSNP and Variome Data 0.2 were detected in each patient. Cells with multiple mutated genes, or those with a mutated gene repeatedly observed in another cell from the same patient were judged to be putative CTCs. At least 2 putative CTCs were detected in 7 patients while no CTCs were detected in 2 patients. Mutated genes observed in the putative CTCs were ABL1, AKT1, APC, CDH1, CDKN2A, ERBB2, FGFR3, HRAS, IDH1, JAK2, KDR, NPM1, RB1, RET, SMARCB1, STK11, and TP53.

Conclusions: Potential CTCs were successfully identified by single cell picking and target sequencing of EpCAM-positive cells from whole blood of metastatic breast cancer patients. Unique mutations not detected in other single cells and pooled samples can be used to distinguish putative CTCs from normal cells. Genomic profiling of corresponding primary tumor and metastatic site biopsy is warranted to verify the CTCs and investigate their role in disease progression.
**Title:** The proliferation index of circulating tumor cells (CTCs) is not influenced by the administration of adjuvant chemotherapy in early breast cancer (BC) and seems to reflect Ki67 expression of the primary tumor

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**Body:** Background: The assessment of Ki67 in the primary tumor represents a prognostic marker with potential predictive implications in early BC. We evaluated Ki67 expression in CTCs from patients with early BC and assessed the effect of adjuvant chemotherapy as well as Ki67 expression in the primary tumor.

Methods: Ki67 was evaluated in CTCs of 97 patients with early BC pre- and post- adjuvant chemotherapy by the use of immunofluorescence analysis. Cytospins of peripheral blood mononuclear cells were double stained with A45-B/B3 cytokeratin mouse antibody and a Ki67 rabbit antibody. Ki67 staining of the primary tumor was also performed for 13 CTC-positive patients. A proliferation index (PI) in CTCs was considered as the ratio of Ki67-positive CTCs/total CTCs. A PI of up to 14% was defined as 'low' whereas a PI above 14% was defined as 'high'.

Results: CTCs were detected in 26 (26.8%) patients before and/or after chemotherapy. Seven (27%) of 26 CTC-positive and 8 (11%) of 74 CTC-negative patients relapsed (p = 0.047). Ki67-positive CTCs were identified in 20 (76.9%) of 26 patients, whereas in 1 (3.9%) and 5 (19.2%) patients, exclusively Ki67-positive and Ki67-negative CTCs, respectively, were detected. Seven (33%) of 21 Ki67-positive patients relapsed in contrast to none among the exclusively Ki67-negative patients (p = 0.13). A total of 154 and 161 CTCs were detected pre- and post-chemotherapy, respectively; the PI in CTCs was 56% and 55%, respectively. In 8 patients with detectable CTCs at both time points, the PI was 65% and 49% pre- and post-chemotherapy, respectively. In 5 (62.5%) out of 8 patients, the PI remained high, in 2 (25%) increased and in 1 patient no Ki67–positive CTCs were detected post-chemotherapy. Seventeen patients were CTC-positive at baseline [HER2 positive, n=5; triple negative, n=1; hormone receptor positive, n=11]. A concordance in Ki67 staining between the primary tumor and CTCs was recorded in 10 (77%) out of 13 patients. Moreover, in 2 (67%) of 3 patients with exclusively Ki67-negative CTCs, low Ki67 expression was also observed in the primary tumor. Interestingly, 2 out of 5 patients with HER2 positive primary relapsed and both had high PI in their CTCs, whereas 2 out the 3 HER2 positive patients that did not relapse had low CTC proliferation index. Similarly, the triple negative patient had low PI in her CTCs and has not relapsed after 4 years of follow up.

Conclusions: Adjuvant chemotherapy fails to decrease the proliferation index in CTCs. Ki67 expression in CTCs seems to reflect Ki67 expression in the primary tumor and could be predictive of patient outcome.
T-DM1 in HER2-negative metastatic breast cancer patients with HER2-amplified circulating tumor cells: Current status of the CirCe T-DM1 phase II trial

Bidard F-C, Romieu G, Jacot W, Cottu P, Dieras V, Lerebours F, Servent V, Luporsi E, Lortholary A, Tubiana-Mathieu N, Espie M, Bollet M, Bourgeois H, Renaud N, Pelissier S, Armanet S, Baeten K and Pierga J-Y. Institut Curie, Paris, France; Institut du Cancer de Montpellier, Montpellier, France; Centre Oscar Lambret, Lille, France; Institut de Cancerologie de Lorraine, Vandoeuvre Les Nancy, France; Centre Catherine de Sienne, Nantes, France; Limoges University Hospital, Limoges, France; Saint Louis University Hospital, Paris, France; Institut de Cancerologie Hartmann, Levallois-Perret, France; Clinique Victor Hugo, Le Mans, France and Janssen Diagnostics, Beerse, Belgium.

Body: **Background:** Liquid biopsy can reassess key therapeutic targets in metastatic breast cancer. Several studies showed that a low albeit significant rate of metastatic breast cancer initially considered as HER2-negative can be reclassified as HER2-positive by systematic biopsy procedures. We report here the current status of the CirCe T-DM1 trial [NCT01975142] which aims to demonstrate the clinical utility of HER2 status reassessment on circulating tumor cells (CTCs).

**Methods:** The first step of the trial consists in CTC count and HER2/CEP17 FISH on detected CTCs (CellSearch, Janssen Diagnostics) in patients (pts) with measurable disease progressing after the second line of chemotherapy. Pts with amplified CTCs (HER2/CEP17 ratio equal or higher than 2.2) are eligible to the treatment step of the study in two distinct cohorts: low CTC count (1 or 2 HER2-amplified CTCs) and high CTC count (3 and more HER2-amplified CTCs). In the treatment step has a Simon's two stage design, the anti-HER2 antibody-drug conjugate T-DM1 being administered until tumor progression. The primary objective of the trial is the confirmed response rate (RECIST). This trial is supported by Roche.

**Results:** CirCe T-DM1 has been initiated in 10 centers in France. As of June 2015, 105 metastatic breast cancers pts considered as HER2-negative were screened. 29 pts (27%) had no CTC detected, 68 pts (65%) had at least 1 CTC detected with no HER2 amplification, and 8 pts (8%) exhibited HER2-amplified CTCs. Among the 8 pts, 1 pt had 5 HER2-amplified CTC, 2 pts had 2 HER2-amplified CTC and 5 pts had 1 HER2-amplified CTC. HER2/CEP17 ratios among HER2-amplified CTCs ranged from 2.5 to 7. Five of the 8 pts were treated by T-DM1. One objective confirmed partial tumor response has been observed (20%).

**Conclusion:** The accrual is ongoing; the first efficacy assessment will occur after having treated 14 pts. This innovative trial highlights the promise and the complexity of liquid biopsy-based programs in the era of precision medicine: scarcity of the target, reliability and reproducibility of the target assessment, major efficacy when the target is matched to the appropriate drug.
Targeted next-generation sequencing reveals heterogeneity of single CTCs in metastatic breast cancer


Background: Cell-free DNA (cfDNA) and circulating tumour cells (CTCs) are under investigation as a "liquid biopsy" for real-time monitoring of patients with cancer. We aimed to compare single CTCs with matched cfDNA by targeted NGS in metastatic breast cancer (MBC).

Methods: CTCs were enriched and enumerated by CellSearch® from 7.5 ml of blood in 2 patients with MBC. For the first patient, five single CTCs and 5 lymphocytes were isolated for the CellSearch® cassette by DEParray™ and DNA was extracted and amplified using the Ampli1 WGA kit (Silicon Biosystems). For the second patient DNA was extracted from the total pool of 3808 CTCs. Targeted NGS was performed with 2 amplicon panels (Ampli1 and a custom mutation panel (1)) using the Ion PGM™ platform.

Results: The 5 single CTCs showed mutational heterogeneity. Two CTCs had both a PIK3CA p.H1047R and an ESR1 p.E380Q mutation present and 2 other CTCs had a TP53 p.P72R mutation. A number of novel variants were also identified that were heterogeneous between the 5 CTCs but not seen in the matched lymphocytes. All mutations found across the 5 CTCs were detected in the paired cfDNA sample with no additional mutations unique to cfDNA. In the second patient with an extremely high CTC count, an ESR1 p.D538G mutation was detected in the CTC pool and matched cfDNA, but the cfDNA also had a novel variant in exon 7 of FGFR1.

Conclusion: These data confirm the molecular heterogeneity of single CTCs and suggest cfDNA as a suitable biomarker for the genetic landscape of CTCs in MBC. Analysis of other samples is ongoing.

Title: Somatic genetic profiling of circulating tumor cells (CTC) in metastatic breast cancer (MBC) patients


Body: Introduction: Somatic mutations, including those in TP53, PIK3CA, and estrogen receptor alpha (ESR1), are key to the biology of cancer and response to therapy. Recently, somatic cancer-associated mutations have been identified in circulating cell free plasma tumor DNA (ptDNA). Less is known about the mutation profile of DNA extracted from CTC (CTC-DNA). Since CTC-DNA provides mutational information of single cells, we hypothesize CTC-DNA will complement ptDNA to give greater insight into tumor heterogeneity.

Methods: Patients with ER positive MBC who were enrolled in the Mi CTC-ONCOSEQ, a companion trial to Mi-ONCOSEQ (the Michigan Oncology Sequencing Program), and who had ≥5CTC/7.5 ml whole blood were included. CTC were enriched from white blood cells (WBC) with CellSearch® (CXC kit). CTC and WBC were then purified using DEPArray™. DNA from individual CTC and WBC was isolated and subjected to whole genomic amplification (Ampli1™ WGA). Genetic analysis was performed on individual CTC, pooled CTC and pooled WBC DNA by multiplexed PCR based targeted next generation sequencing (NGS) using the Oncomine Comprehensive Panel (targeting ~130 onco- and tumor suppressor genes) and the Ion Torrent Proton. All patients had exome sequencing performed on research biopsies of metastases using an Illumina HiSeq 2500 platform.

Results: This pilot study was conducted using high quality DNA from two patients assessed to date. Both patients had lobular carcinoma and as expected harbored somatic, deleterious CDH1 (E-cadherin) mutations (frameshift and non-sense) in both research biopsy and CTC-DNA. These data supported our approach. Patient #1 was TP53 wild type in her research biopsy, but multiple CTC harbored somatic TP53 frame-shift mutations (Table). Patient #2 harbored an ESR1 Y537S mutation in her research biopsy. However, only 4 of 7 CTC also harbored this somatic, heterozygous mutation.

Prioritized mutations in CTC

<table>
<thead>
<tr>
<th>Pt#</th>
<th>Cell Type (CTC vs WBC), number</th>
<th>Gene</th>
<th>Mutation</th>
<th>Variant fraction (expected 1=homozygous; 0.5=heterozygous)</th>
<th>Found in research biopsy?</th>
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<tr>
<td>1</td>
<td>CTC_A2</td>
<td>CDH1</td>
<td>p.I584fs</td>
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<tr>
<td></td>
<td>CTC_A4</td>
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</table>

* pool of all CTC

**Conclusions:** We demonstrate the ability to purify CTC, isolate, and amplify DNA of suitable quality for genetic analysis using a comprehensive targeted sequencing panel. Both known and novel alterations were identified in comparison to research biopsy specimens. This approach allows single cell analysis demonstrating heterogeneity of mutational status in different single cells. Studies of CTC-ESR1 and other genetic abnormalities in patients with known tissue mutations who participated in Mi CTC-ONCOSEQ are now underway.
Title: Enumeration of heterogeneous circulating tumor cells (CTCs) in metastatic breast cancer patients based on size and deformability

Tozuka K, Nagai SE E, Inoue K, Komatsu K, Matsumoto H, Hayashi Y, Kurozumi S and Suganuma M. Division of Breast Surgery, Saitama Cancer Center, Ina, Kita-adachi-gun, Saitama, Japan; Division of Breast Oncology, Saitama Cancer Center, Ina, Kita-adachi-gun, Saitama, Japan; Division of Breast Surgery, 2 Division of Breast Research Institute for Clinical Oncology, Saitama Cancer Center, Ina, Kita-adachi-gun, Saitama, Japan and Graduate School of Science and Engineering, Saitama University, Shimo-okubo, Sakura-ku, Saitama, Japan.

Body: Background:
The detection of circulating tumor cells (CTCs) in peripheral blood is an independent predictor of the efficacy of systemic therapy and a prognostic marker for patients with metastatic breast cancer. One of the leading techniques to detect CTCs uses immune-magnetic separation followed by immunocytochemistry. A microdevice can capture and enumerate CTCs using distinctive physiological difference (size and deformability) between cancer cells and blood cells. This microdevice thus obtains a larger CTC yield than that of affinity based separation which enriches the samples from a particular subgroup of cells based on biomarker (EpCAM) used. In this study, we investigated CTCs in peripheral blood from metastatic breast cancer patients using this microdevice.

Patients and methods:
We examined blood samples of 9 patients with heavily treated locally recurrent or metastatic breast cancer. Informed consent from these patients was obtained before blood extraction. Blood samples were taken into sodium EDTA tubes after discarding the first 1 ml of blood samples. Two ml whole blood were subjected to the microdevice (Clear cell system), and CTCs were trapped in the microwells: Trapped cells were analyzed by immunocytochemistry with monoclonal antibodies specific for leukocytes (CD45) and epithelial cells (CK8/18), along with 4,2-diamidino-2-phenylindole dihydrochloride (DAPI) for nuclei. CK8/18-positive, DAPI-positive and CD45-negative cells were defined as CTCs. Three patients were examined using both this microdevice and affinity-based separation with EpCAM, to compare the yield of CTCs.

Results:
Of the 9 patients: 7 had ER-positive primary tumors, and 6 had PgR-positive ones, HER2 overexpression was detected in 2 primary tumors. CTCs were detected in 8 patients. The single patient in whom CTCs were not detected suffered from local recurrence (axillary lymph node metastasis) only, with no distant metastases. We were also unable to detect CTCs using EpCAM affinity method for this patient. The number of detected CTCs in the other patients ranged from 19/2ml to 156/2ml (mean 90/2ml), and the sizes of CTCs varied from 5 to 16 µm. CK8/18-negative and DAPI positive were detected in most patients, and these cells tended to be larger than CK8/18-positive cells, suggesting that epithelial-mesenchymal transition (EMT) might occur in CTCs. The total number of CTCs detected by the microdevice from 2 patients was larger than that of CTCs detected by EpCAM affinity method (107/2ml vs 1/7.5ml, and 19/2ml vs 39/7.5ml).

Conclusion:
CTCs detected by this microdevice varied in regard to the size of trapped cells and characteristics examined by immunocytochemistry, suggesting the heterogeneity of CTCs. Further research on this heterogeneity is vital in order to develop personalized treatment for patients with metastatic breast cancer.
Title: Longitudinal analysis of circulating tumor cells and cell free tumor DNA by next generation sequencing in triple negative breast cancer


Body: As the practice of genetically profiling patient tumors is considered for making clinical treatment decisions, recent methodologies for screening of genomic aberrations in circulating tumor cells (CTCs) and cell-free plasma DNA (cfDNA) may provide non-invasive tools for such applications. Genomic analysis of DNA from CTCs and plasma can also provide useful insight into tumor heterogeneity and thus disease progression by revealing sub-populations of tumor cells that evolve during treatment, have novel drug-resistant genotypes, or carry alternate cancer driver mutations not identified by the sequencing of primary tumors.

Comprehensive evaluation of DNA isolated from CTCs and cfDNA from a breast cancer patient by whole exome sequencing was performed to better understand the role of liquid biopsies in investigating the etiology of tumor progression. The patient was diagnosed with metastatic triple negative breast cancer (TNBC) six years after remission from estrogen receptor (ER-3+), progesterone receptor (PR-1+), human epidermal receptor growth factor 2 negative (Her2-), grade 3 intra-ductal carcinoma of the right breast. Metastatic lesions were found in the spine, pelvis and sacrum and bone-marrow. The patient was enrolled in the Intensive Trial of OMics in Cancer clinical Trial (ITOMIC-001; ClinicalTrials.gov ID NCT01957514) and initially received weekly cisplatin infusions followed by additional targeted therapy.

Peripheral blood was obtained during regular clinic visits over the 272 days the patient was enrolled in the clinical trial. CTCs were identified and enumerated from each blood draw using the AccuCyte® -CyteFinder® (AC/CF) system (RareCyte, Seattle WA). Multiple CTCs along with white blood cells (WBCs) were picked from various time points throughout the treatment regimen. The selected CTCs and WBCs were whole genome amplified and whole exome sequencing was performed to identify tumor specific variants. A comparative analysis with variants present in genomic DNA isolated from the bone-marrow metastasis tissue biopsy samples and cfDNA revealed the evolution of tumor-specific variants during therapy. Each CTC had somatic alterations in genes associated with therapies in current use or those in the clinical trials setting. Sequencing analysis of cfDNA provided similar information on potential therapeutic approaches. The monitoring of disease over time through genomic analysis of CTCs and cfDNA can identify novel sub-populations related to disease progression for the tailoring of cancer treatment regimens. Further analysis is being performed to better understand the evolution of the genomic heterogeneity among CTCs at the same time point and across different time points and therefore better understand the etiology of progression of metastatic breast cancer in this patient.
Title: Differentially methylated miRNA methylomes of normal breast tissue from ER negative and ER positive breast cancer mimic their respective tumor phenotypes

Worsham MJ J, Chen KM Mei, Chitale D, Stephen JK K and Divine G. Henry Ford Health System, Detroit, MI.

Body: Background: The unique structure and function of normal tissues is known to be regulated by epigenetic mechanisms. Understanding how normal cells in their respective tumor milieus might affect their susceptibility to become not only malignant but acquire breast cancer (BC) subtype-specific phenotypes, may determine tumor clinical behavior outcomes. The goal was to compare genome wide methylation profiles of non-coding miRNAs of breast cancer tissue and normal breast epithelium, respectively, from ER negative and ER positive tumors, and assess their miRNA methylomes in the context of tumor ER phenotypes as ER negative vs ER positive.

Methods: Breast cancer tissue from 79 patients (40 ER-positive and 39 ER-negative) and normal tissue from 39 of these patients (19 ER-negative and 20-ER-positive) were assayed using the Illumina 450K bead array. A sub analysis focused on 2249 miRNA CpGs assigned to 615 unique miRNAs. M-values were computed as a logit function \([(\log (\beta/ (1-\beta)))]\) of the methylation beta values. T-tests were used to compare the means of the M-values for the ER-positive and ER-negative groups. The t-test p-values were used to generate adaptive FDR (aFDR) levels and aFDRs of 0.05 or lower were considered to be statistically significant (Tier 1). Tier 1 CpGs were subsequently filtered to select only those with a mean beta ratio between ER-positive and ER-negative of under 0.5 or over 2.0 (Tier 2). The Tier 2 CpGs were further filtered to select only those with a mean beta difference of 0.2 or more (Tier 3).

Results: In the tumor cohort, 1224/2249 (54%) CpGs were differentially methylated between ER negative and ER positive BC at Tier 1 (aFDR 0.05 or lower). Of the 1224, 963 (78.7%) were hypermethylated, and 1035 (84.6%) were associated with the promoter region. The 1224, 24 and 2 CpGs were associated with 379, 22 and 2 genes for Tiers 1, 2 and 3, respectively. When the same analysis was performed on normal tissue only (19 ER-negative and 20-ER-positive) 76 of the 2249 CpGs had significant aFDR values and none of those met the Tier 2 or Tier 3 criteria. Seventy-one of the 76 (93.4%) where hypermethylated, and 65 (85.5%) were associated with the promoter region. The 76 significant Tier 1 (aFDR) differentially methylated CpGs were associated with 48 genes of which 43 were common to tumor Tier 1 differentially methylated miRNA genes, 10 were common to tumor Tier 2 genes, and 5 were restricted to normal tissue only.

Conclusions Normal epithelial tissues demonstrated similar differential methylation directionality as their respective tumor counterparts (although to a lesser extent), favoring promoter region localization. Accordingly, the recognition of normal breast tissue-specific epigenetic propensities that align with their tumor phenotypes, suggest the possibility of progression markers specific for estrogen receptor status as well as markers not associated with progression. This provides insights into our view of possible links between epigenetic programming, progression continuums, and how hormonal receptor subtypes may be determined. Support: Komen Foundation: KG110218.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-03-02

Title: Modulatory action of melatonin and miR-17 on ROCK-1 in breast cancer metastasis model

Borin TF Ferraz, Pongeluppi RI Inacio, Gelaleti GB Bottaro, Leonel C, Moschetta MG Gobbe, Ferreira LC Carvalho and Zuccari DAPdC Aparecida Pires de Campos. Faculdade de Medicina de Sao Jose do Rio Preto - FAMERP, Sao Jose do Rio Preto, Sao Paulo, Brazil; Universidade Estadual de Sao Paulo - UNESP, Sao Jose do Rio Preto, Sao Paulo, Brazil and Laboratorio de Investigação Molecular do Cancer - LIMC, Sao Jose do Rio Preto, Sao Paulo, Brazil.

Body: Breast cancer is the most common cancer in women is often associated with high morbidity and mortality rates, with great financial impact on health system. Besides, the disease is characterized by the high rates of metastasis, which worsen significantly the prognosis. This process is associated with two regulatory molecules, microRNAs (miR), specially miR-17, and ROCK-1, which overexpression has been associated to tumor growth and metastasis. In contrast, melatonin has shown oncostatic and anti-metastatic properties by reducing the cell ability to migrate and invade the tissue, besides the inhibition of cell proliferation. The aim of this study was to investigate the effect of melatonin to modulate miR-17 and ROCK-1, a possible candidate gene to miR-17 target in metastatic breast cancer cell line, MDA-MB-231. To determine the effect of melatonin to modulate miR-17 and ROCK-1, MDA-MB-231 cells were treated with melatonin and anti-miR-17-5p. ROCK-1 and miR-17 gene expression were accessed by real time PCR and ROCK-1 protein expression verified by immunocytochemistry and western blotting. Migration and invasion assay was performed to verify the action of melatonin and anti-miR-17-5p to inhibit these processes. In the in vivo study, was developed pulmonary metastasis model followed for six weeks, the tumor induction was continued for 4 weeks, and treatment with anti-miR-17-5p inhibitor for two more weeks. At the end of treatment, animals were euthanized, the lungs removed and used for analysis of miR-17-5p and Let-7c (positive control) and ROCK-1 gene expression. ROCK-1 protein was analyzed by immunohistochemistry. MiR17-5p inhibition managed directly modulate gene and protein expression of ROCK-1 in MDA-MB-231 cells, as well, the gene expression of MYC. In additional, the migration and invasion were decreased after melatonin and anti-miR-17-5p treatment. To validate the findings of the study in vitro with miR-17, was used for lung metastasis model in athymic nude mice. According to our findings, normal animals without metastasis (negative control) had lower levels of miR-17 compared to animals with metastasis and without treatment (positive control). In contrast, animals with metastasis who received anti-miR17-5p treatment, interestingly also had low miR-17 levels compared to positive control animals. Furthermore, it was observed fewer metastases and a reduction in ROCK-1 protein expression in these treated animals compared to positive control animals. Our results demonstrated that miR-17-5p inhibition can modulate ROCK-1 gene and protein in MDA-MB-231 cells and decreasing the number of lung metastases in treated animals. Furthermore, melatonin can act as a synergic mechanism to decrease migration and invasion on metastasis processes mediated by ROCK-1.
MicroRNA-497 targets SMAD7 and has a tumour suppressive effect and its prognostic significance in breast cancer

Liu J, Zhang S, Hu Y, Zhang M, Li C, Liu Y, Zhang X and Zhang J. China Tianjin Breast Cancer Prevention, Treatment and Research Center, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China.

Background: Latest studies have shown that SMAD 7 played a major role in breast cancer during tumour development and progression. In the current study, we aimed to examine the relationship between microRNA-497 (miR-497) and SMAD 7 and its function in MDA-MB-231 breast cancer cells and MCF-7 breast cancer cells. And to investigate the clinicopathologic and prognostic significance of miR-497 expression in human breast cancer.

Methods: Quantitative polymerase chain reaction was used to measure the expression levels of miR-497 in 40 breast cancer specimens and adjacent normal breast tissues. MTT assays, colony formation assays and cell cycle assays were used to explore the potential function of miR-497 in MDA-MB-231 breast cancer cells and MCF-7 breast cancer cells. Dual-luciferase reporter assays were performed to analyze the regulation of putative target of miR-497, and western blot assays were used to validate the dual-luciferase results. Also, miR-497 expression was detected in another 240 breast cancer tissues and its correlations with clinicopathologic features of patients were analyzed. Kaplan-Meier analyses were used to assess survival of patients.

Results: The results showed that MiR-497 was downregulated in breast cancer tissues compared with normal tissues (P <0.05). Dual-luciferase reporter assays manifestations that SMAD 7 is the target of miR-497. Quantitative polymerase chain reaction and Western blot assays validated that overexpression of miR-497 can reduce SMAD 7 protein levels, miR-497 can inhibit cellular growth and cause a G1 arrest. Of 240 BC patients, 132 (55%) were placed in the high miR-497 group and 108 (45%) were placed in the low-miR-497 group. By statistical analyses, The miR-497 expression levels were significantly lower in HER2-positive and base-like patients than luminal subtype patients (p=0.036). Moreover, patients with high miR-497 expression had better 5-year disease-free and overall survival compared with the low miR-497 group (P = 0.0027 and 0.0032, respectively). In the TNBC subtype with high miR-497 expression had better 5-year DFS and OS compared with the low miR-497 group (P = 0.0491 and 0.035). In the HER-2 + subtype with high miR-497 expression had better 5-year DFS and OS compared with the low miR-497 group (P = 0.0142 and P=0.024).

Conclusions: In summary, MiR-497 act as a tumor suppressor gene in breast cancer. Overexpression of miR-497 inhibited cell proliferation and G1 cell cycle arrest were observed. Downregulation of miR-497 was correlated with breast cancer progression and has negative correlation with SMAD7, The above results indicated that miR-497 might be a potential molecular biomarker for predicting the prognosis of patients and miR-497 could be considered an ideal therapeutic target for the HER-2 positive and TNBC breast cancer.
Title: Down-regulation of Bcl2-related ovarian killer (BOK) by miR-296-5p protects breast cancer cells from paclitaxel-induced apoptosis


Body: Accumulating evidence shows that miRNAs play a role in drug resistance. Despite these observations, little is known about the identities of the miRNAs involved in drug resistance and their downstream targets. In the present study, we identified miR-296-5p for which a tumor suppressive role has been previously described, as a miRNA that is involved in paclitaxel drug resistance in triple negative breast cancer (TNBC) cells. Enforced expression of miR-296-5p suppressed cell growth, migration, and invasion in MDA-MB-231 breast cancer cells. Using a microarray approach, we identified BCL2-related Ovarian Killer (BOK), a pro-apoptotic gene as a target of miR-296-5p. BOK levels were validated BOK levels in miR-296-5p transfected MDA-MB-231 and MDA-MB-468 cells using real-time PCR and Western blot. Our results demonstrated that over-expression of miR-296-5p down-regulated BOK expression in TNBC cells. Transfection of miR-296-5p significantly suppressed luciferase reporters containing wild-type BOK-3′-UTR constructs. In contrast, mutant BOK-3′-UTR constructs were unaffected by ectopic miR-296-5p. Furthermore, BOK expression was induced in the presence of paclitaxel, but ectopic miR-296-5p significantly suppressed BOK induction by paclitaxel treatment compared to the control cells. These data provide new insights on the role of miRNAs in drug resistance and suggests that therapeutic strategies against miR-296-5p may be warranted.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-03-05

Title: MiRNA expression in breast cancer varies with lymph node metastasis and other clinicopathologic features


Body: Background: Breast carcinoma is the most common malignant tumor in females, lymph node status is one of the most important prognostic factors in patients with breast cancer. MiRNAs have been shown to have important role in oncogenesis, invasion, and metastasis via epigenetic posttranscriptional gene regulation. However lymphatic metastasis related miRNAs of breast cancer has not been well documented. The aim of this study was to identify and evaluate miRNAs related to breast cancer lymph node metastasis, and explore the clinical significance of the differential expressed miRNAs in breast cancer patients.

Methods: The expression of miRNAs in primary breast cancer patients with lymph node metastasis and that without lymph node metastases was compared by miRNA microarray. We further validated the miRNAs (miR-185-5p, miR-339-5p, miR-542-5p, miR-3923) between lymph node (n=31) and non-lymph node (n=42) group using real-time reverse transcriptase polymerase chain reaction. Furthermore, the relationship between miRNA expression and clinical pathological features was analyzed.

Results: The miRNA microarray initially identified that 8 miRNAs (miR-206, miR-3923, miR-181a, miR-92a, miR-421, miR-339-5p, miR-3196, and miR-29b) were down-regulated in lymph node metastasis group, whereas 5 (miR-542-5p, miR-200a, miR-564, miR-451, miR-30c, miR-200b, miR-191-3p, miR-142-5p, and miR-185-5p) were up-regulated in lymph node group when compared with those in non-lymph node group. In the validation cohort, the expression levels of miR-185-5p and miR-542-5p were significantly higher expressed in lymph node group (P=0.002, and P=0.000, respectively), the expression levels of miR-339-5p and miR-3923 were significantly lower expressed in lymph node group (P=0.000, and P=0.000, respectively).

Conclusions: Our results indicated the potential role of miR-185-5p, miR-542-5p, miR-339-5p and miR-3923 in predicting metastasis to the lymph node and prognosis in breast cancers.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-03-06

Title: Dysregulation of miR-34a-SIRT1 axis reduced breast cancer stemness

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Body: Recent studies show that enforced expression of miR-34a results in elimination of cancer stem cells (CSCs) in many malignant tumors. Sirtuin-1 (SIRT1) being confirmed as a direct target of miR-34a was reported to be involved in regulation of growth and survival of leukemia stem cells (LSCs). In this study, we aim to understand regulatory mechanism of miR-34a-SIRT1 axis in breast cancer stem cells (BCSCs). Lower endogenous level of miR-34a and higher level of Sirtuin1 (SIRT1) gene were identified in CD44+/CD24- BCSCs than breast cancer cells. Either ectopic expression of miR-34a or silenced SIRT1 in MCF-7 cells inhibited cellular proliferation, and led to cell apoptosis. Overexpression of miR-34a also suppressed expression of ALDH1, BMI1 and Nanog, and decreased capacity of mammosphere formation significantly. Studies in vivo showed that stable expression of miR-34a reduced tumor burden significantly in nude mice xenografts. Taken together, our results showed that miR-34a inhibit the proliferative potential of BCSCs in vitro and in vivo, at least partially through downregulating SIRT1. miR-34a-SIRT1 axis may play an important role in self-renewal and stemness maintenance of BCSCs. This study may provide a novel BCSCs specific therapeutic strategy to improve breast cancer treatments.
Title: Rhythmic time oscillations of microRNAs in human breast epithelial normal and cancer cell lines

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Body: BACKGROUND: Diverse molecular mechanisms are being reported in human breast cancer (BC), which can affect the biochemical functions throughout malignant cells development. The microRNAs (miRNAs) are an emerging class of modulators of gene expression with relevant roles in several biological processes, as oncogenic, tumor-suppressive, and metastatic-influencing in BC cells. Recently, a few reports have implied the possible pattern of expression (time oscillation) of miRNAs in time that may be related to molecular changes in mammalian cells. These findings suggest a biological connection among normal and cancer cells, and rhythmic regulation of some miRNAs, but such connection has not yet been studied. In this study, we aimed to identify and compare the rhythmic expression of miRNAs in human breast epithelial normal and cancer cell lines.

METHODS: We used cell culture to explore three cell lines, one breast epithelial normal (MCF10A) and two cancer (MCF-7 and MDA-MB-231) cell lines under standard growth conditions in vitro. The cells were synchronized by serum shock (50% horse serum for 2 h), and we collected sample cells (triplicate) for intervals of 4 hours during 48 hours. Collected cells at 12h to 40h (8 time-points) were genome-wide analyzed of miRNA expression using high-throughput Agilent Human miRNA microarray of 2006 human miRNAs. Analysis for identification of rhythmic miRNAs was developed by cosine analysis in R software.

RESULTS: We observed diverse oscillation patterns (minimum 6 patterns, i.e. cosine or sine oscillation) of miRNAs in cell lines. Each cell line shows approximately 85 miRNAs with rhythmic oscillation. These also showed distinct phases between cell lines, which could suggest as part of molecular changes in breast normal and cancer cell lines.

CONCLUSIONS: Our results suggest that miRNAs may present rhythmic oscillation in the regulation of molecular changes of human breast normal and cancer cells.
miR-621 suppresses the metastatic cascade in breast cancer patients

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Body: Purpose: In previous study, we had shown that miR-621 could sensitize breast cancer to chemotherapy by suppressing FBXO11 and enhancing p53 activity. In this study, we aimed to define miR-621 prognosis value in breast cancer patients, and to explore the potential molecular mechanisms.

Experimental Design: 70 patients with stages II and III breast cancer were included as validation set. The correlation between miR-621 expression level and prognosis in breast cancer patients was confirmed. In parallel, in vitro and in vivo analyses were carried out to determine the potential mechanisms of miRNA-dependent prognosis.

Results: We validated that lower than higher miR-621 expression level was markedly associated with poor metastasis-free survival in breast cancer patients (P=0.03). In breast cancer cell lines, ectopic overexpression of miR-621 inhibited proliferation, migration, invasion, and metastasis both in vitro and in vivo. The potential miR-621-target genes were determined by TargetScan and miRNA CLIP-seq database. Among those, PAK7 was verified as one of the direct targets of miR-621 in breast cancer cells, whose expression level positively associated with recurrence and metastasis in breast cancer patients. The molecular mechanisms by which miR-621/PAK7 axis regulates recurrence and metastasis may involve regulation of epithelial-mesenchymal transition (EMT) in breast cancer cells.

Conclusions: Our study revealed a strong correlation between miR-621 expression and metastasis in breast cancers. High level of miR-621 was negatively associated with poor metastasis-free survival. The increased metastasis-free survival may be mediated by down-regulation of PAK7 gene, which leads to reduced EMT in breast cancer cells. Therefore, miR-621 may represent a therapeutic target for early metastasis breast cancer.
The role of Jmjd1a in mammary gland development and breast tumor growth

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Body: Histone modification alters chromatin architecture and thereby influences gene transcription. Histone methylation status is reversible and counter-regulated by methyltransferases and demethylases. Jmjd1a (also known as KDM3A, TSGA, JMJD1, JHDM2A and JHMD2A) is a histone demethylase. It belongs to JmjC domain-containing protein family and could specifically remove di- and mono-methyl residues from di or mono-methylated histone H3K9 (H3K9me2/me1). Recent studies showed that Jmjd1a plays an important role in embryonic stem cell self-renewal, spermatogenesis, regulation of metabolic gene expression and body weight, sex determination, tumor angiogenesis, and macrophage infiltration. However, its role in mammary gland (MG) development, breast carcinogenesis and breast cancer progression hasn’t been systemically investigated. In this study, we found that Jmjd1a is expressed in mouse luminal epithelial cells. Genetic disruption of the Jmjd1a gene significantly slowed down MG development as indicated by retarded MG elongation and decreased ductal density in virgin mice observed at the ages of 4, 6 and 8 weeks. In agreement with the retarded MG development, the expression of Ki67 and cyclinD1 in epithelial cells of MGs from Jmjd1a knockout (KO) mice dramatically reduced compared with that from wild type (WT) mice. H3K9me1 and H3K9me2 levels in the epithelial cells of KO MGs are much higher than that in WT MGs. To assess the role of Jmjd1a in breast cancer progression, we crossbred Tg(Jmjd1a-/-) mice with MMTV-TVA(RCAS-PyMT) mice and obtained Tg(Jmjd1a-/-)×MMTV-TVA(RCAS-PyMT) mice. Infection of the TVA-expressing MG epithelial cells with the RCAS-PyMT virus induced mammary tumors in these mice and MMTV-TVA(RCAS-PyMT) control mice. We found that KO of Jmjd1a slightly accelerated mammary tumor initiation but significantly decreased tumor growth. Ki67 and cyclinD1 expression statistically reduced in KO tumors versus WT tumors. At the molecular level, Jmjd1a expression positively correlated with cyclin D1 expression in mammary epithelial cells and mammary tumors. Knockdown of Jmjd1a in MCF-7 cells significantly reduced cyclin D1 expression, while ectopic expression of Jmjd1a in MCF-7 cells increased cyclin D1 expression. ChIP assay revealed that Jmjd1a is associated with a promoter region of cyclin D1. Co-expression of c-Myc and Jmjd1a boosted the activity of the cyclin D1 reporter. In conclusion, our study indicated that Jmjd1a plays an important role in promoting mammary gland development and breast tumor growth by up-regulating cyclin D1 expression. Targeting Jmjd1a may inhibit breast cancer progression.
Title: Immunomodulatory effects of entinostat on PD-L1 and MHC class I and II in different subtypes of breast cancer


Body: Background: Targeting immune checkpoint programmed death receptor 1 (PD-1)/PD-L1 pathway has shown promising clinical activity with some preliminary association of clinical benefit with PD-L1 expression on tumors. Recent preclinical and clinical studies highlight the beneficial immunomodulatory potential of epigenetic therapy. Entinostat is a class I specific histone deacetylase inhibitor (HDACi). A promising preclinical study showed that entinostat in combination with immune checkpoint blockade agent can eradicate modestly immunogenic breast tumors in mice via reduction in immunosuppressive myeloid-derived suppressor cells. In this study, we investigated the effects of entinostat on expression of immune-related genes in breast cancer cells to further explore the potential mechanism of its combined activity.

Method: Gene expression was assessed on Nanostring platform using the nCounter GX Human ImmunologyV2 panel comprised of 594 immune-related and 15 reference genes. Gene expression was normalized to the internal positive controls and reference genes using nSolver2.0 software. Hormone receptor-positive (HR+) breast cancer (MCF-7 and T47D) and triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and Hs578T) were used for the analysis. Gene expression analysis was performed on control and after 24-hour treatment of entinostat at clinically relevant 125 and 500 nM concentrations.

Results: Overall, a greater number of immune-related genes were induced > 2 fold with entinostat at 125 and 500 nM in TNBC compared to HR+: 77 and 118 genes in MDA-MB-231, 80 and 147 genes in Hs578T, 20 and 64 genes in MCF-7, and 73 and 72 genes in T47D, respectively. In particular, MHC class I (HLA-A, HLA-B, HLA-C) and II (HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DQB2, HLA-DRA, and HLA-DRB1) genes were induced by entinostat in a dose dependent manner (range 1.5-22.44 fold). These inductions were observed in both HR+ and TNBC cell lines. Interestingly, we found higher baseline expression and a several fold increase in PD-L1 expression in TNBC. PD-L1 mRNA expression increased by 1.74 and 2.14 fold in MDA-MB-231 and 3 and 9.6 fold in Hs578T with 125 and 500 nM treatment, respectively. Corresponding increase in PD-L1 protein expression after entinostat treatment was also observed. In contrast, there appeared to be no significant changes in PD-L1 expression after entinostat treatment in MCF-7 and T47D.

Furthermore, we also identified 21 genes that were differentially induced by entinostat in TNBC but not in HR+. These genes include PTPN22, ARG2, CISH, IL17A, ICAM2, KIR3DL1, CXCR3, TLR2, CFD, CCR5, IL13, LILRA3, IL8, TNFRSF9, DPP4, MR1, SELPLG, PTGS2, IL1B, CD3D, and MBL2. No significant change in PDL2 expression was observed in any of the cell lines.

Conclusion: Our data suggest that entinostat induces immune-related genes involved in antigen presentation in both ER+ and TNBC cells, potentially increasing the immunogenicity of these tumors. Given the significant induction of PD-L1 expression with entinostat in TNBC, our preclinical data provides support for further investigation of entinostat in combination with anti-PD1 or anti-PD-L1 in this subtype of breast cancer.
Title: SUMO wrestles breast cancer: SUMO posttranslational modification directs breast cancer cell epigenome

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Body: Epigenetic reprogramming allows breast cancer (BCa) cells to bypass normal growth checkpoints and acquire an aggressive morphology. Function of critical epigenetic proteins is directed by small ubiquitin-like modifiers (SUMO) posttranslational modification (PTM). Components of the SUMO system are dysregulated in BCa. Our objective is to delineate how changes to SUMO-PTM affect epigenetic programs that coordinate BCa pathology. We report that chromatin-bound SUMO protease SENP7L is upregulated in metastatic tamoxifen-resistant (TamR) and triple-negative BCa. SENP7L facilitates chromatin remodeling that potentiates the growth and invasiveness of these BCa subtypes. SENP7L interacts with and deSUMOylates heterochromatin protein 1 alpha (HP1-alpha/CBX5) to mediate these responses. Canonical models suggest that HP1-alpha reads the histone mark to localize predominantly at constitutive heterochromatin. Our studies challenge the old paradigm as we show that SUMOylated HP1-alpha is enriched outside heterochromatin at euchromatin, including E2F-responsive and mesenchymal gene loci. Recruitment of SUMOylated HP1-alpha supports de novo heterochromatin formation at these loci. However, SUMOylated HP1-alpha is lost in aggressive BCa due to SENP7L induction. Consistently, SENP7L upregulation prompts chromatin de-condensation and gene transcription. Nucleic acid and chemical targeting of SENP7L reduces self-renewal/differentiation properties of TamR mammospheres as well as tumor volume and secondary tumor formation of TamR cells. Hence, SENP7L inhibitors can serve as promising tools to modulate epigenetic abnormalities in TamR BCa.
Title: Single cell sequencing analysis of formalin-fixed paraffin-embedded ductal carcinomas in situ and invasive breast cancers reveals clonal selection in the progression from in situ to invasive disease


Body: Background: Ductal carcinoma in situ (DCIS) is considered to be a precursor of invasive breast cancer (IBC), and is found synchronously in over 45% of patients with invasive disease. Whether the progression from DCIS to IBC results in clonal shifts and the genomic imbalances that may drive this process remain to be elucidated. Single-cell sequencing constitutes a powerful approach to address these questions as it enables the phylogenetic reconstruction of subpopulations of cancer cells at single cell resolution. To date, single-cell sequencing approaches remain limited to fresh/frozen samples, precluding the use of archival formalin-fixed and paraffin-embedded (FFPE) samples, the largest source of tumor material in pathology departments. Here, we describe the development of a methodology for single-cell copy number (CN) profiling of single nuclei derived from FFPE tumor samples and subsequently employed this approach to define whether DCIS displays intra-lesion genetic heterogeneity and if clonal shifts are observed in the progression from DCIS to IBC.

Methods: DCIS and IBC areas were independently microdissected from archival FFPE samples. Microdissected tissue fragments were subsequently reverse-crosslinked, and their extracellular matrix was digested. Intact individual diploid nuclei from cells in G1 were FACS-sorted into individual wells of 96-well plates. DNA was extracted from each cell and subsequently repaired and amplified. Sequencing libraries were prepared using standard protocols followed by whole genome sequencing on a HiSeq 2000. Single-cell sequencing data were analyzed to define the CN profiles for each cell, to infer the clonal composition of each DCIS and IBC, and to trace the genomic events that occurred during the progression from in situ to invasive disease.

Results: We performed single-cell sequencing of 192 cells derived from two synchronous FFPE DCIS and IBCs. One pair of DCIS and IBC was diploid and the other was tetraploid. Principal component analysis discriminated neoplastic from normal cells and resulted in the identification of clonal cancer cell populations. Genome-wide CN profiling of single cells from DCIS and IBC components demonstrated the extent of intra-tumor genetic heterogeneity present in these samples. In addition, founder CN events present in these lesions, including TP53 and RB1 losses and FGFR1/BRF2 and CCND1 amplifications, were already detected in the DCIS samples. Phylogenetic analysis based on CN profiles from single cells revealed that the invasive cancer likely stemmed from minor subclones from the DCIS and provided evidence of branched evolution in the progression from DCIS to IBC.

Conclusions: We developed a robust procedure to perform single-cell massively parallel sequencing of individual nuclei isolated from FFPE samples. This approach revealed that the progression from DCIS to IBC results in clonal shifts, suggesting that this biological phenomenon may constitute an evolutionary bottleneck.
Title: Divergent patterns of copy number changes in primary breast carcinomas versus synchronous lymph node metastases


Body: Background: Breast cancer is the most common malignancy among women, and although early detection and new treatment regimens have improved the survival, metastatic spread to lymph nodes is indicative of a more aggressive disease. Molecular analyses of primary tumors versus synchronous lymph nodes have shown variation in gene expression and in phenotype. Further, dissimilarities in genomic changes have been found, but the studied cases are few and often based on selected markers only.

Methods: In this study, we have analyzed paired primary tumors and lymph node metastases from 35 breast cancer patients with high-resolution genomic SNP array analyzes (SNP6 arrays, Affymetrix). The ASCAT algorithm (Allele specific copynumber analysis of tumors) was used to estimate both DNA ploidy level, tumor cell percentage as well as allele specific genome wide copy number alterations.

Results: Of the 35 sample pairs, 20 had high tumor cell percentage and high quality data from both samples. Seven of these showed similar alterations in the primary tumor and in the metastasis. In 13 cases a divergent profile was observed either in the direction of fewer alterations in the lymph node metastasis or fewer alterations in the primary tumor. The estimated ploidy value also varied between the primary and lymph node for several of the patients.

Conclusion: Lymph node metastases can have more, less or equal number of genomic copy number alterations compared to the corresponding primary breast carcinoma. This indicates that the metastatic process can occur at different time points during tumor evolution. A more detailed characterization of genomic alterations in relation to clinical and pathological characteristics will be presented.
Whole genome transcriptome analysis of sequential breast to brain metastasis uncovers new signalling pathways and druggable targets

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Body: The occurrence of brain metastasis (BM) in breast cancer (BC) is currently on the rise across all molecular subtypes with 10-30% reported incidence. The need to uncover the mechanisms underlying this clinically devastating complication is apparent, and in the current study we sought to identify BC cell mediators of BM.

In our cohort of metastatic patients (n=196) we found that BM developed in 13% of the cases. Despite the previous reports of negative ER status being a risk factor for BM, the ER+ve patients accounted for 42% of all diagnosed BM. To elucidate the gene alterations required for successful colonisation of the brain we undertook RNA sequencing (RNA-seq) of sequential breast to brain metastasis of known receptor status (n=7). This study presents the first whole transcriptome next-generation RNA-seq analysis of resected BM and their matching primary breast tumours.

We identified 500 differentially expressed genes (DEGs) (< 0.05, fcThreshold >±1.5), accounting for those that were both upregulated and downregulated in BM compared to the primary. Analysis of protein-coding genes identified collective ER-specific metastatic pathways. Additionally, common functional pathways altered included ECM, cell adhesion and neuronal differentiation. Our analysis of the BM transcriptomic landscape and verification in cell line models that preferentially metastasise to the brain has unravelled a complex network of driver genes, cooperating with stromal derived factors, responsible for the organ-specific behaviour of the metastatic cells. Genes such as ANTRX1, THBS2, FAP, VCAN and TIMP2 were found to be part of the invasion and migration network that drives the extravasation of the BM cells. Furthermore, an EMT stemness signalling network driven by ANTRX1 and WNT pathway driven RUNX was prominent in the cells acquiring the ability to migrate to the brain. Additional work is being carried out on uncovering the adaptations that re-activate the dormant brain metastatic cells and the contribution of the neuronal niche in the facilitating the colonisation by the MBC.

This study highlights the requirement of unique gene sets for the invasion, migration and colonisation to the brain and that functional characterisation of the DEGs will enable the identification of novel molecular targets for prevention and treatment of breast cancer BM.
Title: The development of the metastatic site pattern during time in different subtypes of breast cancer

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Body: Introduction:
The most common metastatic sites in breast cancer (BC) are bone, lung and liver, but metastases to CNS, other visceral organs, skin and lymph nodes are often seen. Depending on the BC subtypes there are data on different patterns of the metastatic sites. However, little is known about the risk for development of new metastatic sites during the course of the disease. Possibly, the development of new sites of the disease is signs of novel intrinsic capabilities of the metastases that might be explained by new mutations. Primary aim of this study was to examine the metastatic pattern and development of new metastatic sites in different BC subgroups.

Materials and Methods:
This is a population based cohort study covering patients in the Uppsala County treated for metastatic BC (MBC) from 2009 to 2014. The information was collected from the real-time treatment registry and medical records. The distant sites were classified as bone, lung/pleura, soft tissue, other visceral and CNS and the timing was defined in steps starting with M1 when patient first was defined as having MBC. The BC subtypes were defined as luminal A, luminal B, HER2-positive (HER2) and triple negative (TN) based on immunohistochemical analyses. The metastatic patterns at death were compared with the patterns at M1.

Results:
Totally 391 patients with MBC with a median age of 65 years were included. Median disease-free survival was 51 months. The development of metastases in new sites occurred in up to three distinguished steps, with median lag times of 22, 13 and 14 months, respectively. The median survival from M1 was 69, 37, 64 and 17 months for the luminal A, luminal B, HER2 and TN subtypes.

The frequencies of metastases in in first (M1) and subsequent sites (next) in each subtype

<table>
<thead>
<tr>
<th></th>
<th>Bone</th>
<th>Lung/pleura</th>
<th>Soft tissue</th>
<th>Liver</th>
<th>Other visceral</th>
<th>CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>74/4</td>
<td>31/21</td>
<td>22/10</td>
<td>20/28</td>
<td>16/15</td>
<td>1/4</td>
</tr>
<tr>
<td>Luminal B</td>
<td>66/11</td>
<td>44/14</td>
<td>38/3</td>
<td>27/32</td>
<td>16/5</td>
<td>4/10</td>
</tr>
<tr>
<td>HER2</td>
<td>46/15</td>
<td>36/20</td>
<td>42/17</td>
<td>19/28</td>
<td>10/13</td>
<td>19/31</td>
</tr>
<tr>
<td>TN</td>
<td>27/7</td>
<td>46/17</td>
<td>51/6</td>
<td>29/0</td>
<td>17/11</td>
<td>17/17</td>
</tr>
</tbody>
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The sums of the figures represent the risk for metastases in a certain site at death.

Conclusion:
The pattern of metastatic sites at M1 in different BC subtypes does not represent the pattern for development of new metastatic sites during the course of the disease. We observed a significantly different risk for developing new metastatic sites depending on subtypes, most obvious regarding liver metastases. TNBC seemed to have less ability to develop new metastatic sites.
Expression profiling of in vivo DCIS progression models identified BCL9 as a molecular driver of invasive progression

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Introduction: There are an estimated 60,000 new cases of ductal carcinoma in situ (DCIS) each year. At present, it is not clear why some DCIS remain non-invasive for decades while others become invasive. A lack of understanding in DCIS pathobiology has led to overtreatment of more than half of DCIS patients. To identify factors that promote DCIS invasion, we have profiled the temporal molecular changes during DCIS transition to invasive ductal carcinoma (IDC) using two in vivo models, MIND (mouse-intraductal) and DCIS/IDC tandem lesions. These studies led to the identification of B cell lymphoma-9 as a potential molecular driver of early invasion. BCL9 is a newly found co-activator of Wnt-stimulated β-catenin-mediated transcription. BCL9 has been shown to promote progression of multiple myeloma and colon carcinoma. However its role in breast cancer progression had not been recognized.

Methods: Microarray and RNA sequencing were utilized to characterize the sequential and temporal changes in mRNA expression during DCIS invasive transition. BCL9 shRNA knockdown was performed to assess the role of BCL9 in in vivo invasion, EMT and canonical Wnt signaling. Immunofluorescence of 28 patient DCIS samples was used to assess a correlation between the expression of BCL9 and biomarkers of high risk DCIS. TCGA data was analyzed to assess the status of BCL9 gene alterations in 959 human breast cancers.

Results: Analysis of BCL9, by RNA and protein showed BCL9 up-regulation to be associated with DCIS transition to IDC. Analysis of patient DCIS revealed a significant correlation between high nuclear BCL9 and pathologic characteristics associated with DCIS recurrence: ER and PR negative, high nuclear grade, and high HER2. In vivo silencing of BCL9 resulted in the inhibition of DCIS invasion and reversal of epithelial-mesenchymal transition (EMT). Analysis of the TCGA data showed BCL9 gene to be altered in 26% of breast cancers. This is a significant alteration when compared to ERBB2 (19%) and ESR1 (8%). A significantly higher proportion of basal like invasive breast cancers showed BCL9 amplification.

Conclusion: BCL9 is a molecular driver of DCIS invasive progression and may predispose to the development of basal like invasive breast cancers. As such, BCL9 has the potential to serve as a biomarker of high risk DCIS and as a therapeutic target for prevention of IDC.
Clinical utility of systematic biopsy of first metastatic event in breast cancer: Results from a prospective multicenter trial


BACKGROUND: Cumulative evidence for phenotypic and molecular heterogeneity between primary breast cancer (BC) site and matched metastasis (mets) has been obtained in retrospective studies. Current expert consensus suggests performing biopsies of mets, but clinical utility and cost are unknown. The primary objective of the ESOPE study (NCT01956552) was to compare the phenotype and genotype of the primary tumor (PT) with those of matched mets at time of first distant relapse, before the start of any treatment, in order to optimize the treatment of mets

PATIENTS and METHODS: Between Nov. 2010 and Sept. 2013, we conducted a prospective multicenter study on BC patients (pts) with diagnosis of first mets. All pts were to have available Formalin-Fixed Paraffin-Embedded (FFPE) PT sample and mets accessible to either percutaneous or surgical sampling. All tissue samples were centrally analyzed with immunohistochemistry (ER, PgR, HER2, and Ki67) and FISH when indicated. Frozen samples were stored for further analyses. We recorded intended therapeutic decision before and after biopsy.

RESULTS: Of 93 pts included, 89 were eligible for biopsy. Median age was 57 years (28-81); median interval between PT and mets was 42 months (0-211), including 14 pts with novo metastatic breast cancer. Mets biopsy was performed in 85 pts (96%, refusal n=2, not feasible n=2). Toxicity was limited to only 1 grade 1 hemorrhage. Sampled sites were liver (44%), lung (16%), bone (13%), lymph node (13%), skin/muscle/chest wall (9%), ovary/peritoneum (4%), and adrenal gland (1%). PT was not available in 4 pts; mets biopsy was non contributive in 6 pts but led to a diagnosis of second primary cancer in 3 pts. In 72 pts with matched PT and mets, PT were luminal A (n=11), luminal B (n=33), triple negative (n=13), HER2 (n=13), non-evaluable (n=2). Mets were luminal A (n=6), luminal B (n=30), triple negative (n=16), HER2 (n=14), non-evaluable (n=6). Discrepancy rates were: ER 18% [kappa for concordance =0.6, CI 95% (0.42-0.77)], PgR: 39% [kappa=0.19, CI 95% (0.01-0.39)], Her2: 4% [kappa=0.86, CI 95% (0.7-1)], Ki67: 25% [kappa=0.19, CI 95% (-0.09; 0.49)]. The most frequent discrepancy rate was observed in pts with lum A PT, as only 3/10 developed Lum A mets. HER2 and triple negative were the most stable subtypes (12/13 and 12/12 respectively). Most importantly, mets biopsy led to a change in therapeutic decision in 25 pts (independent evaluation by 2 oncologists). Additional comparative targeted NGS analyses are ongoing on a first subset of 54 FFPE paired samples, and parallel whole exome sequencing is planned on 38 paired samples with available constitutional DNA.

CONCLUSION: Comparative analysis of breast cancer PT and first mets is routinely feasible, with very low morbidity and a significant impact for patients' management: 29% had a second cancer diagnosis or were proposed a therapeutic change. Furthermore, this study will provide additional data on quality and quantity of tissue available for molecular analysis, and ultimately in terms of cost-efficacy.
Title: RAGE-ligand signaling drives breast cancer metastasis through affecting cells of the tumor and microenvironment

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Body: Breast cancer is most common malignant state in women, with 20% of these patients developing metastasis during the course of their disease. Further understanding is needed of the process and mechanisms of metastasis. Our lab and others have been shown that Receptor of Advanced-Glycation End-products (RAGE) plays a role in tumorigenesis and metastasis. RAGE is highly expressed in various cancers including breast cancer and its protein levels correlate with poor patient outcome in breast cancer and other cancers. Activation of RAGE results in increased proliferation, migration and invasion of cancer cells. Further studies in mice have shown it may be a therapeutic target to reduce tumor growth and the resulting metastasis. Further understanding is needed of the role of RAGE in driving metastasis through affecting cells of both the tumor and tumor stroma to design novel therapeutics. Using the breast cancer cell model (MDA-MB-231) and its organotropist sister cells lines selected in vivo for increased metastasis to lung (4175) and bone (1833), we tested the role of RAGE in driving tumor metastasis in vitro and in vivo with xenograft mouse models. To test the role of RAGE in the tumor microenvironment we used the AT-3 syngeneic breast cancer cell model in C57BL6 wild-type and RAGE knockout mice. We demonstrated that the highly metastatic variant of 231 cells (4175 and 1833) have increased expression level of RAGE compared to MDA-MB-231 parental cells. Ectopic over-expression of RAGE in parental 231 cells led to increased migratory and invasive properties compared to vector control cells, without affecting cell proliferation or viability. RAGE knockdown by shRNA in 4175 and 231 parental cells showed decreased cell invasion in transwell assays compared to control scramble shRNA. To validate our data in vivo, we performed mammary fat pad injection of 4175 cells (RAGE and scr shRNA) in NOD SCID gamma mice. Tumor growth and weight was impaired in RAGE gene knockdown 4175 cells compared to scramble (scr) controls. Analysis of lung and liver tissue retrieved from mice revealed RAGE knockdown in 4175 cells prevented metastasis compared to 4175 scr control cells. To test the role of RAGE on non-tumor cells of the breast stroma we next performed syngeneic studies with AT-3 cells (MMTV-PyMT spontaneous BC cell model), by injection into the mammary fat pad of wild-type and RAGE knockout C57BL6 immunocompetent mice. RAGE knockout mice (RAGE -/-) displayed striking impairment of tumor cell growth compared to wild-type (RAGE +/-) mice. We are currently testing whether novel RAGE inhibitors impact breast cancer progression and metastasis.

These data highlight RAGE drives breast cancer progression and metastasis through affecting both tumor cell intrinsic and non-tumor cell microenvironment effects. Future studies will demonstrate the potential of RAGE inhibition as a novel therapeutic approach for preventing and treating metastatic disease in breast and other cancers.
Title: p53 deficiency enhances metastatic potential of triple negative breast cancer by promoting growth in primary and metastatic sites

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Body: TP53 is one of the most commonly mutated genes in human cancer. Mutation or loss of TP53 can occur during the early stages of oncogenesis or as a later event as tumors progress to more aggressive forms. We used a patient-derived xenograft (PDX) model of triple negative breast cancer (TNBC) to investigate how p53 deficiency contributes to metastasis. The PDX line was generated by engrafting the primary breast tumor of a patient with TP53 wild-type metastatic TNBC into the humanized mammary fat pad of NOD/SCID mice. Isogenic lines differing only in TP53 status were generated from this model by silencing p53. Here we demonstrate that the p53-proficient and -deficient tumors metastasized from the mouse mammary gland and colonized the lung, liver, bone, brain, and lymph nodes with similar frequency. p53-deficient tumors grew faster in both the primary and metastatic sites as a result of increased mitosis and decreased apoptosis. Circulating tumor cells (CTCs) are currently being evaluated in p53-proficient and –deficient PDX models. Gene expression profiling identified candidate genes with potential clinical relevance that may be responsible for increased tumor growth upon p53 loss in late stage tumors. This model is also being used to identify genes that promote breast cancer metastasis. To enrich for metastasis, metastatic subpopulations of tumor cells were isolated from lungs and serially passaged in vivo. This serial passaging enriched for metastasis to multiple secondary sites. A transcriptional signature of putative metastasis genes was generated and is being functionalized with high throughput screening in vivo.
Title: Vascular mimicry in metastatic breast cancer patients: Molecular insight to vascular mimicry using in vitro and in vivo models


Body: Background: Vascular Mimicry (VM) is an endothelium-independent matrix-embedded, blood-perfusion phenomenon exhibited by highly plastic and aggressive tumor cells in patients with solid tumors including breast. VM is characterized by PAS⁺ de novo formations of micro-vascular networks which are essentially CD31⁻ and CD34⁻. VM is a micro-circulatory phenomenon which perfuses rapidly growing tumors, transporting fluid from leaky vessels and/or connects with the constituent endothelial-lined vasculature. One of the hallmarks of metastasis in BC is the heterogeneity observed between primary tumors and metastases (& among metastases) (Marino et al., 2013). Recently a model of BC heterogeneity revealed VM as a driver of metastasis which in turn has been associated with disease heterogeneity (Wagenblast et al., 2015). Aim: Since BC is an aggressive and heterogeneous disease and VM is associated with the aggressiveness / poor outcome in BC, we sought to understand the functional relationship of VM with metastasis. Method: Tumors from our BC patients and TMAbs were stained for CD31/PAS and CD34/PAS to identify VM. Genomic and proteomic data from these patients were obtained from re-biopsied (after consultation) samples (IHC for ER, PR, and HER2; FFPE samples for genomic [Foundation Medicine] and proteomic [Theralink] analyses).

Result: VM was identified in metastatic tumors from ER+ and TNBC patients as CD31⁻/PAS⁺ and CD34⁻/PAS⁺ structures in contrast to the CD31⁺/PAS⁺ and CD34⁺/PAS⁺ angiogenic compartment of the individual tumor(s). Metastatic tumors exhibiting VM were characterized by pathological features like metaplastic lesions, positive lympho-vascular invasion and were found to be poorly differentiated. Predominant genetic alterations in these patients included (1) PI3K-mTOR pathway genes, (2) p53 (R273P, D281V), (3) BRCA1 E1683* (for TNBC), (4) MYC amplification, (5) aurora kinase, (6) CCND1 & CDK4 amplifications and (7) loss of CDH1 exons1-3. Amplifications of cell surface TKIs/ligands included EGFR, FGFR and several FGFs. Proteomic data indicated an overexpression/activation of HER family (HER1/HER2/HER3), mTOR activation (p~S2448), MEK1/2 activation (p~S217-221) and JAK2 activation. In order study VM, we standardized VM formation both in 2D and 3D configurations in multiple BC cell lines which exhibited VM at differing times. The earliest response was observed around 2-3 hours in BT20, Hs578t and MDA-MB231. By 24 hours BT474, BT474HerR, SUM149, DKAT, MDA-MB231BR, Hs578t and MDA-MB468 cells demonstrated 2D VM. The BT20 cell line showed the most pronounced 3D-VM at 24 hours while MDA-MB468 was least sensitive to VM. Typical cord formation in HUVEC cells stained with hematoxylin and PAS were used for comparison. Using 2D and 3D models of VM we demonstrated the involvement of PI3K and Wnt-beta-catenin pathways in VM. Considering the involvement of VM in mediating the aggressive/metastatic nature of TNBC, we also tested VM in in vivo xenograft models using brain metastatic specific MDA-MB231BR cells, results of which will be presented in the meeting. Significance: To our knowledge this is the first report to identify genetic alterations and proteomic changes associated with VM in metastatic BC.
Abstract Withdrawn
Title: Sphingosine-1-phosphate signaling promotes metastatic niches and lung metastasis in obesity-related breast cancer

Body: INTRODUCTION: The link between obesity and elevated breast cancer mortality is well known, however, the underlying mechanisms are poorly understood. Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid mediator produced by sphingosine kinases (SphKs) that plays critical roles in inflammation and cancer progression. Previously, we found that obesity increases levels of S1P not only in breast tumors, but also in the lung. "Metastatic niches" are specialized microenvironments in distant organs primed by factors from cancer cells. We hypothesized that S1P secreted from the primary tumor could promote formation of a "metastatic niche" in the lung, which assists circulating cancer cells to form metastatic lesions. Further, HFD-induced obesity increases S1P secretion from the primary tumor, which could promote the formation of "metastatic niches" in the lung and lung metastasis. The aim of this study is to test these hypotheses.

METHODS: A mouse model utilizing tail vein injection of E0771 syngeneic breast cancer cells was used. Prior to tail vein injections of naive E0771 cells, 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). Histological analysis, RT-qPCR, and western blot were used.

RESULTS: The lungs after K1-CM treatment demonstrated much more infiltration of macrophages with greater IL-6 secretion than lungs from CT-CM mice in areas without metastasis. Furthermore, SphK1, S1P receptor 1 and IL-6 expression were all significantly higher in the lungs of mice treated with K1-CM than with CT-CM, suggesting that S1P secreted from the primary tumor promotes formation of a metastatic niche in the lung. Next, mice were fed with HFD or ND for 12 weeks before treatment with SphK1-CM, and lungs were examined 7 days after intravenous injection of E0771 cells. Histological analysis demonstrated that there were significantly more lung metastases in mice on HFD than in mice on ND. Importantly, treatment with FTY720, a functional antagonist of S1P receptor 1, significantly reduced the lung metastases in HFD fed animals. Immunofluorescent staining revealed higher expression of IL-6 and greater number of F4/80 positive macrophages in mice fed with HFD compared with mice fed with ND, whereas FTY720 dramatically suppressed both IL-6 and macrophage infiltration in the lung of HFD-fed mice. HFD-induced obesity also increased pERK, pAKT, pStat3, and pp65 in the lung, and FTY720 suppressed these signaling pathways.

CONCLUSION: Our results suggest that S1P plays a role in the formation of "metastatic niches" in the lung and lung metastasis of breast cancer, and obesity promotes this process. S1P will be a promising target for treatment of breast cancer metastasis, especially in condition with obesity. This work was supported by the Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research Grant Number 15H05676 and 15K15471 for M.N and 15H04927 for W.T. M.N. is supported by the Uehara Memorial Foundation, Nakayama Cancer Research Institute, and Tsukada Medical Foundation. K.T. is supported by NIH/NCI grant R01CA160688 and Susan G. Komen Investigator Initiated Research Grant IIR12222224.
Title: Epithelial paradox; clinical significance of co-expression of E-cadherin and vimentin in invasive breast cancer

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Body: Background: E-cadherin and vimentin are now regarded as major and conventional canonical markers of epithelial-mesenchymal transition (EMT). It is commonly assumed E-cadherin is uniformly lost during the process of EMT. We previously reported that the elevated expression of vimentin contributes to the aggressive phenotype in invasive breast cancer. On the other hand, the role of E-cadherin in breast cancer biology might be unclear and more complex. Although, cell cohesion during breast cancer invasion is often overlooked, accumulating evidences indicate breast tumor cells are typically cohesive and often display membrane-localized E-cadherin in both the primary tumor and distant metastases, termed collective invasion. Multiple mechanisms have emerged to address how epithelial breast tumors invade.

Aims: The aim of this study is to reveal the clinical importance of the expression of E-cadherin and vimentin in breast cancer.

Methods: The E-cadherin and vimentin protein expression were evaluated by immunohistochemistry (IHC) in 177 invasive breast cancer samples. Among these, E-cadherin and vimentin expression were evaluated in the set of primary breast cancer and metastatic lymph nodes in 65 cases.

Results: The positive vimentin expression was highly correlated with poor disease-free survival (DFS) and overall survival (OS) (p=0.019 and p=0.0044), however, the E-cadherin expression alone did not correlate with prognosis. Interestingly, Both E-cadherin and vimentin positive tumor had the worst DFS and OS among all breast cancer (p=0.03 and p=0.0089). Vimentin expression was highly correlated between primary tumors and metastatic lymph nodes. However, E-cadherin expression levels were significantly elevated in metastatic lymph nodes (p=0.0017), Co-expression of E-cadherin and vimentin in the metastatic lymph nodes also showed worst DFS and OS (p=0.12 and p=0.027). Conclusions: Co-expression of E-cadherin and vimentin seems to be associated with the most aggressive phenotype and poorest prognosis in breast cancer, and positive E-cadherin expression may not always play roles for tumor suppression.
Title: A novel mutagenesis screen identifies SHARPIN as a breast cancer metastasis gene that predicts survival of breast cancer patients

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Body: Breast cancer (BC) is a heterogeneous disease and the second leading cause of malignancy among women in the U.S. Metastasis of the primary tumor results in poor prognosis and increased mortality and the molecular mechanisms by which metastatic tumors occur are not well understood. Identifying the genes that drive the metastatic process could provide targets for improved therapy and biomarkers to improve outcomes for BC patients. Here, we utilized a replication-incompetent gammaretroviral vector (γRV) to perform a forward insertional mutagenesis screen to identify genes involved in BC metastasis. In this approach, BC cells mutagenized with a γRV were xenotransplanted into the mammary fat pad of immunodeficient mice, and primary tumors and metastases were allowed to develop. Metastatic lesions were collected and analyzed for proviral integration sites to identify vector integration sites and nearby candidate metastasis genes. The γRV has a bacterial origin of replication and kanamycin resistance gene that allows for rescue in bacteria and rapid identification of vector integration sites. Using this approach, we identified the previously described metastasis gene WWTR1, and several other novel candidate metastasis genes including SHARPIN. SHARPIN was then independently validated as a BC metastasis gene in vivo using RNAi. Analysis of patient data showed that SHARPIN expression predicts metastasis-free survival after adjuvant chemotherapy (p < 0.005, Concordance Index = 55.3, Risk Groups Hazard Ratio = 1.87). Our replication-incompetent γRV approach is efficient and has broad potential to identify genes involved in oncogenic processes for BC and other cancers.

Keywords
Insertional mutagenesis screen, Gammaretroviral vector (γRV), Metastasis, Inducible shRNA, Prognostic biomarker.
Increased emphasis on breast cancer screening has led to a dramatic surge in diagnosis of pre-cancerous ductal carcinoma in situ (DCIS) over the past 30 years. Unfortunately, diagnosis of late stage invasive and metastatic disease has not proportionally declined, suggesting significant over diagnosis and over treatment of innocuous DCIS. Due to a lack of biomarkers, the heterogeneity of DCIS lesions, and an insufficient understanding of mechanisms of invasive progression, there is currently no clinically relevant method of predicting which DCIS lesions will advance to invasive disease. As a result, all DCIS patients undergo an aggressive treatment regimen to prevent disease progression. Therefore, there is a critical need to determine the underlying mechanisms driving breast cancer progression to better inform patient treatment options and nominate novel therapeutic entry points for treatment of invasive disease.

Recently, long noncoding RNAs (lncRNAs) have gained attention as critical regulators of epigenetic states and gene expression. Although the study of lncRNAs is in its infancy, they are being uncovered as pivotal regulators of development and tumorigenesis, thus they are a rich source of potential drivers of breast cancer progression. We propose that lncRNAs functionally drive breast cancer invasion and their expression can discriminate between innocuous and potentially invasive DCIS.

Using biopsies from women that exhibit tandem DCIS and invasive breast cancer lesions, we have identified a cohort of long noncoding RNAs (lncRNAs) that are enriched in the invasive biopsy. From this cohort we have identified the lncRNA BHLHE40-AS1 as increasing in a step-wise manner in a breast cancer progression series that escalates from normal, non-transformed cells to highly invasive disease. Preliminary evidence suggests that BHLHE40-AS1 expression regulates invasive potential in vitro. Using a GeneChip® Human Transcriptome 2.0 Array (HTA) we have identified several targets previously associated with driving breast cancer invasion as being potentially regulated by BHLHE40-AS1. Future directions will focus on determining the effect of BHLHE40-AS1 expression on tumor cell invasion in an orthotopic xenograft model, mechanistically elaborating its function, and determining the utility of BHLHE40-AS1 as a clinically relevant biomarker of invasive breast cancer.
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Title: NOS2 & COX2 activation of TLR4 & EGFR signalling causes poor outcome in oestrogen receptor-negative breast cancer via pro-survival signals and immune polarisation

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Body: Background
We seek to further elucidate mechanisms by which inflammatory mediators promote estrogen receptor (ER)-negative breast cancer progression and poor survival. We previously reported association between inducible nitric oxide synthase (NOS2) and poor outcome in ER-negative tumours. In tumours aberrant NOS2 induction facilitates tissue remodelling and stimulates neovascularisation. Also involved in inflammation and wound healing is cyclooxygenase-2 (COX2). We demonstrated that COX2 is associated with Akt activation and poor outcome in ER-negative tumours. We hypothesise that co-expression COX2 with NOS2 in ER-negative tumours amplifies effects of NOS2 on poor outcome, via EGFR and TLR4 signalling loop activation, and polarization of the tumour immune-compartment to pro-tumorigenic M2 phenotype.

Methodology
We determined the association of NOS2 and COX2 co-expression on breast cancer specific survival in ER-negative and triple negative breast tumours (N=102), via immunohistochemistry and cox regression statistical analysis. To explore the mechanism of NOS2 induction of COX2 through transactivation of EGFR, NO donors in combination with EGFR inhibitors were used to determine if NO exposure results in amplified EGFR and PGE2 pro-survival and pro-metastatic signalling in triple negative breast cancer cell lines. Finally, we explored the ability of NO to modify the ability of triple negative breast cancer secretome to induce polarisation of macrophages to a pro-tumorigenic M2 phenotype.

Results
Co-expression of NOS2 and COX2 in triple negative breast cancer results in poor outcome, via activation of pro-survival signalling and modification of the immune compartment to a pro-tumorigenic M2 associated phenotype. NO induces activation of growth factor signalling pathways and secretion of M2 promoting cytokines that induce THP1 macrophage polarization to an M2 phenotype.
Enhanced invasion and migration into the surrounding tissues are hallmarks of the malignancy of tumor cells. To successfully metastasize, a cancer cell has to detach from the primary tumor, invade into surrounding tissues, and intravasate into blood or lymphatic vessels. These processes are composed of complex mechanisms involving tumor recognition, degradation of extracellular matrix (ECM) proteins and migration into tissue. Triple negative (TN) breast cancers are defined by a lack of expression of estrogen, progesterone, and HER2 receptors. It is widely recognized that TN breast cancers have a poorer prognosis than any other subtype of breast cancer. Given the lack of effective targeted therapies for TN breast cancer patients, understanding of the mechanisms of migration and invasion of these tumors will provide insight into developing novel approaches to lower the mortality from TN breast cancer.

Previous studies demonstrated that NEDD9 plays a key role facilitating progression and metastasis of various tumor cells including breast. We previously demonstrated that NEDD9 plays a critical role in promoting migration and growth of MDA-MB-231. In order to further characterize the mechanisms of NEDD9-mediated cancer migration and growth, we established stable cell lines expressing NEDD9 using HCC38 as a parental cell line which expresses low level of endogenous NEDD9. Microarray studies demonstrated that enzymes (CHST11, CHST15, and CSGALNACT1) involved in biosynthesis of chondroitin sulfate (CS) but not heparan sulfate (HS) were markedly upregulated in HCC38(NEDD9) compared to control HCC38(Vector) cells. These results suggest that NEDD9 regulates specific structures of tumor-associated glycans such as chondroitin sulfate. Core proteins of CD44 and Serglycin were markedly upregulated in HCC38(NEDD9) cells compared to HCC38(Vector) cells, while those of Syndecan-1, Syndecan-2, and Versican were downregulated in HCC38(NEDD9). Immunofluorescence studies using specific antibody, GD3G7, confirmed the enhanced expression of CS-E subunit in HCC38(NEDD9). Immunoprecipitation and western blotting analysis demonstrated that CS-E was attached to Serglycin and CD44 core proteins. We demonstrated that removing CS by chondroitinase ABC significantly inhibited anchorage-independent growth of HCC38(NEDD9) in methylcellulose. Importantly, the fact that GD3G7 significantly inhibited colony formation of HCC38(NEDD9) cells suggest that CS-E subunit plays a key role in this process. Furthermore, treatment of HCC38(NEDD9) cells with chondroitinase ABC or GD3G7 significantly inhibited mammosphere formation. Exogenous addition of CS-E enhanced colony formation and mammosphere formation of HCC38 parental and HCC38(Vector) cells. These results suggest that NEDD9 regulates the synthesis and expression of tumor associated glycocalyx structures including CS-E, which plays a key role in promoting and regulating breast cancer progression metastasis and possibly stem cell phenotypes.

The opinion and assertions contained herein are the private views of the authors and are not to be construed as official or as representing the views of the Department of the Army or the Department of Defense.
Title: ARGX-111 depletes MET-expressing circulating tumor cells via enhanced ADCC, resulting in inhibition of metastasis


Body: Several lines of experimental evidence suggest that Hepatocyte Growth Factor (HGF) and its receptor MET play an important role in breast cancer progression and drug resistance. To date, targeted MET inhibitors in clinical development have primarily shown cytostatic rather than cytotoxic effects. Development of a cytotoxic MET inhibitor would serve to complement standard breast cancer therapy, especially when administered in the adjuvant/neo-adjuvant setting.

We have developed ARGX-111, a human antibody antagonist of MET function. ARGX-111 blocks both HGF-dependent and -independent signaling, down-regulates tumor cell surface expression of MET and kills MET-overexpressing cells by enhanced antibody-dependent cellular cytotoxicity (ADCC).

ARGX-111 was shown to be more efficacious than an ADCC-inactive control antibody in both HGF-dependent and -independent tumor xenograft models. ADCC reporter assays confirmed the cytotoxic effects of ARGX-111 in patient-derived primary tumor specimens, including MET-expressing breast cancer stem-like cells. In an orthotopic mouse model of metastatic mammary carcinoma (MDA-MB-231), adjuvant or neo-adjuvant treatment with ARGX-111 was significantly more effective in depleting circulating tumor cells (CTCs) and suppressing the development of bone and lung metastases than the ADCC-inactive control. Taken together, these results provide a rationale for clinical investigation of ARGX-111 in the early breast cancer setting. An ongoing Phase 1 study (NCT02055066) is examining the effects of ARGX-111 on CTCs, alongside the assessment of its safety and efficacy.
Title: Mesofluidic platform for high throughput screening of inhibitors of metastasis

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Body: A fundamental limitation in the development of new therapies to prevent metastatic cancer is a lack of in vitro systems that can accurately recapitulate the steps of cancer cell metastasis. Currently, most assays for examining the steps of metastasis fail to incorporate the biophysical forces experienced by tumor cells due to blood flow, or are low throughput and thereby not amenable to drug screens or high throughput experimentation.

We have developed a novel high throughput mesofluidic platform for assaying cell adhesion under flow in a 96-well format. This device functions like a cone and plate viscometer in each well by inducing shear stress on cells cultured in a standard 96-well plate. We validated the fluid flow and alignment of the device and studied the adhesion of cultured leukocytic monocytes (THP-1 cells) and multiple cancer cell lines (MDA-MB-231 and MCF-7 breast cancer cell lines) to purified extracellular matrix molecules (ECM), endothelial cells and immobilized platelets. Assays were carried out under flow (0.5 dynes/cm² shear stress) and static conditions.

Our studies show that adhesion assays performed under flow yield markedly different results from static adhesion assays. Treatment of breast cancer cells with a small library of integrin inhibitors demonstrated that these compounds had minimal effect on cancer cell adhesion to endothelial cells or immobilized platelets under static conditions, whereas under shear conditions many of these compounds significantly reduced adhesion of cancer cells. As well, this experiment elucidated integrins important for breast cancer adhesion to endothelial cells and platelets.

A static adhesion assay of breast cancer cells to various types of ECM showed greater adhesion of the less aggressive MCF-7 cell line in comparison to the more aggressive MDA-MB-231 cell line. In contrast, flow incorporating assays showed increased adhesion of the more aggressive MDA-MB-231 breast cancer cell line. Specifically, the shear assay saw a significant increase in adhesion for multiple ECM as well as an increase in the strength of adhesion to laminin.

Finally, we performed a high throughput screening experiment using a kinase inhibitor library of 80 compounds and found that the shear based assay yielded notably different results from a similar screen under static conditions for breast cancer cell adhesion to endothelial cells, immune cell adhesion to endothelial cells and breast cancer cell adhesion to platelets. This shear experiment yielded seven "hits", many of which match targets of drugs in clinical trials.

In conclusion, our studies show that adhesion assays performed under flow yield markedly different results from static adhesion assays, and are better at identifying both aggressive cancer cells lines and known pathways for circulating cancer and immune cell adhesion. Thus, this high-throughput screening platform may enable the development of novel compounds to inhibit cancer metastasis and facilitate the study of the systems level behavior of cancer-endothelium adhesion.
Body: Breast cancer (BrCa) mortality continues to result predominately from distant metastases that can emerge years after successful treatment of the primary disease. Metastatic resistance to agents that eradicate the primary mass is likely due to protection from the metastatic microenvironment and the quiescent state of dormant BrCa cells. Advancements for the treatment of metastatic tumors have been made, but significant progress has been hampered by the lack of relevant model systems, particularly for dormancy. We address this gap with an innovative all-human 3D liver microphysiological system (MPS). The liver is both a major site for BrCa metastasis (and other solid tumors) and the primary site of drug metabolism and limiting toxicities, an important consideration in evaluating cancer therapy efficacy and availability.

Primary hepatocytes and non-parenchymal cells (NPC) from human liver resections were seeded into the MPS. Following tissue formation on day 3, tagged BrCa cells were seeded and allowed a minimum of 4 days to integrate into the tissue before interventions were initiated. On day 7, chemotherapy treatment of micrometastases was initiated for 72h. Cultures were allowed 3 days to recover before the MPS was challenged with inflammatory factors (LPS/EGF) for 48h. BrCa cells were then re-treated with chemotherapy (either the same or alternate therapy) on day 21 for 72h. Hepatocyte function and injury were measured by urea, AST, ALT, A1AT, fibrinogen and CYP P450 assays. BC proliferation was monitored by quantification, Ki67 staining, and EdU incorporation. Communication networks within the metastatic microenvironment during different stages of metastatic BrCa progression were identified using Luminex assays (55 analytes).

The metastatically aggressive MDA-MB-231 BrCa cells demonstrated growth attenuation after 12d of culture in a subpopulation of cells (Ki67-/EdU-). Treatment of BrCa cells with doxorubicin for 72h eradicates the cycling cells, leaving behind a dormant cell population (Ki67-/EdU-) that can be subsequently stimulated to cycle by addition of inflammatory stimuli. A second dose of doxorubicin or cisplatin reduced the BrCa load but did not eradicate the BrCa. Luminex analysis of culture supernatants identified signaling molecules potentially involved in metastatic progression. In addition, we present the use of adjuvant therapy in the MPS to prevent this outgrowth of the dormant tumor cells.

In parallel, we have piloted hydrogel scaffolds that better support tissue formation and produce signals consistent with a healthier liver physiology. Hydrogels enhanced MDA-MB-231 cell entry into dormancy, resulting in reduced efficacy of doxorubicin treatment with greater persistence of tumor load.

The MPS provides a mechanism to close the gap in understanding metastatic dormancy. We demonstrate spontaneous dormancy for the first time in an all-human system and mimicked the dormancy and outgrowth observed in patients. Namely, that dormant BrCa are resistant to chemotherapy and can be stimulated to reemerge following an inflammatory insult. The completion of these studies will provide insights into the tumor biology of metastatic seeding, dormancy, and re-emergence and provide an accessible tool for testing therapeutics against metastatic BrCa in a metabolically competent system capable of evaluating dose-limiting toxicity.
Title: SSBP1 suppresses TGF-β-driven epithelial-mesenchymal transition and metastasis by regulating mitochondrial retrograde signaling in triple-negative breast cancer

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Body: Background: Triple-negative breast cancer (TNBC) is a highly aggressive tumor subtype associated with poor prognosis. The onset of metastasis in organs such as the lung, bone and brain is a major cause of mortality in TNBC patients. Thus, identification of novel targets for the treatment of triple-negative breast cancer is urgent for improved outcomes in patients with this disease.

Methods and Results: In this study, by performing quantitative proteomic analyses (iTRAQ) using highly metastatic and parental breast cancer cell lines, we found that single-strand DNA-binding protein 1 (SSBP1) was significantly down-regulated in highly metastatic breast cancer cells. Moreover, SSBP1 down-regulation was found to promote TNBC cell metastasis in vitro and in vivo. We confirmed that silencing of SSBP1 expression by RNAi in MDA-MB-231 and MDA-MB-468 breast cancer cells, can potentiate the proclivity to metastasize in Transwell assay and enhance lung colonization by tail vein injection in mice. In a reverse-complimentary approach, we determined that elevated SSBP1 expression in highly metastatic breast cancer cell lines can suppress the ability of invasion and metastasis in vitro and in vivo. Mechanistically, SSBP1 loss resulted in a significant decrease of mitochondrial DNA copy number in breast cancer cells, which initiated calcineurin-mediated mitochondrial retrograde signaling pathway. The activated mitochondrial retrograde signaling induced c-Rel/p50 nuclear localization, activated transforming growth factor-β (TGF-β) promoter activity and promoted TGF-β-induced epithelial-mesenchymal transition (EMT). Furthermore, Oncomine database and cBio database showed that SSBP1 is down-regulated in various human cancers. In addition, through examining tissue microarrays containing 250 breast tumor specimens, we figured out that patients with low SSBP1 expression were significantly associated with histological grade (grades III and IV; $P = 0.001$, Pearson $\chi^2$-test) and lymph node metastasis ($P = 0.009$). Importantly, low SSBP1 expression correlated with worse disease-free survival in breast cancer patients.

Conclusion: Cumulative results provide compelling evidence that SSBP1 is a critical tumor suppressor involved in human triple-negative breast cancer and provide a novel paradigm for regulation of TGF-β-induced EMT through SSBP1’s regulation of mitochondrial retrograde signaling pathway. Low SSBP1 expression has important prognostic power for patients with TNBC.
Title: The AKT-mTOR pathway as a potential organ-specific drug target signature of hepatic metastases from breast cancer

Body: Background: The identification of organ-specific targetable signatures may help design more effective treatment for patients with metastatic breast cancer (MBC). We took a multi-OMIC approach to assess whether the AKT-mTOR pathway is globally activated during metastatic progression or whether it represents an organ-specific target.
Methods: Snap frozen biopsies from 25 MBC patients enrolled in a prospective phase II trial were used. Sites of metastasis were classified as liver (n=8) and others (n=17), the latter including cutaneous, lung, lymph nodes, and intra-abdominal lesions. Signaling analysis of the 25 cases was performed using Reverse Phase Protein Microarray (RPPA) coupled with Laser Capture Microdissection. Activation of the AKT-mTOR pathway was quantified as phosphorylation of AKT (S473) and the mTOR target p70S6 (T389). Matched exome (WES) and RNASeq data were available for 17 of 25 patients, five with liver metastases. Sequencing data was processed using an in-house developed pipeline to identify somatic events including coding mutations, copy number alterations, gene fusions, and differential expression. Activation of the AKT-mTOR pathway and sequencing data were compared between hepatic and non-hepatic lesions using an integrated RPPA and genomic approach.
Results: Among liver metastases, AKT was activated in 4 of the 8 (50.0%) patients, while 6 of the 8 cases (75.0%) showed activation of p70S6. Sequencing data revealed mutation of PIK3CA in 4 of the 5 liver metastases (80.0%). Three of the PIK3CA mutated specimens with catalytic domain mutations (codons 1023 and 147) demonstrated co-activation of AKT and p70S6, while the fourth case, containing a helical domain mutation (E542K), had activation of p70S6 only. The PIK3CA wild-type liver metastasis demonstrated low activation of AKT and p70S6. For non-hepatic metastases AKT was activated in 2 of the 17 cases (11.8%) and p70S6 in 5 of the 17 patients (29.4%).
Discussion: Although these results need further validation, activation of the AKT-mTOR pathway appears to be a hepatic specific signature in MBC and could be used for the selection of targeted agents for hepatic lesions.
Title: Pre-clinical findings on obesity reversal and breast cancer progression: Targeting persistent inflammation

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Body: Background
Obesity is an established risk and progression factor for several intrinsic subtypes of breast cancer, including basal-like breast cancer (BLBC). Increased local and systemic levels of pro-inflammatory mediators, which typically accompany obesity, can independently influence tumor growth. Currently, it is unclear whether weight loss can reverse the enhancing effects of obesity on breast tumorigenesis. We hypothesize that chronic obesity results in epigenetic reprogramming, which may mediate residual inflammation, and lead to persistent mammary tumor growth despite moderate weight loss.

Methods
Female C57BL/6 mice (n=51) were administered a control (10% kcal from fat) or diet-induced obesity (DIO; 60% kcal from fat) regimen. After 17 weeks, DIO mice either continued on DIO diet or were switched to control diet to stimulate gradual weight loss, subsequently designated as Formerly Obese (FOb). Mice were orthotopically injected with Wnt-1 mammary tumor cells (a model of BLBC) at week 25, and monitored for an additional 9 weeks, then killed and their tumors excised, measured and stored for subsequent analysis. In an ongoing tumor study, we will randomize mice to the control or DIO regimen, switching half the DIO mice to the control diet after 15 weeks, resulting in normal weight, obese, and FOb mice. Within each of these groups, the mice will be further randomized to +/- supplementation with Sulindac, a non-steroidal anti-inflammatory drug (NSAID), starting at the time of the diet switch. After 10 weeks of +/- Sulindac supplementation, mice will receive orthotopic injections of a mesenchymal derivative of an MMTV-Wnt-1 transgenic tumor, the M-Wnt cell line, and will continue on diet and treatment until euthanization.

Results
In our initial study, body weight, adiposity, and serum levels of leptin and insulin were similar in FOb and control mice, but serum levels of IL-6 were similar in FOb and DIO mice, and significantly higher than controls. Moreover, tumor burden, the mammary gland expression of key inflammatory genes, and the prevalence of hypermethylated inflammation-related genes in DNA from mammary tissue were comparable in DIO and FOb mice and significantly higher than in control mice, and there was high concordance with DNA methylation profiles in breast DNA from obese versus normoweight women participating in the UNC Normal Breast Study.

Conclusions
Our preclinical findings suggest that modest weight loss may not be sufficient to reverse the effects of chronic obesity on epigenetic reprogramming and inflammatory signals that are associated with obesity-related mammary tumor progression. Moreover, we have identified several genes with concordant obesity-related hypermethylation in humans and mice; which were unchanged in FOb mice. Thus combining weight loss regimens with epigenetic or inflammatory modulators may be an effective strategy for breaking the obesity-breast cancer link. We are currently assessing whether combining moderate weight loss with anti-inflammatory interventions (Sulindac or omega-3 fatty acids), or reprogramming metabolism with a bariatric surgical intervention, is more effective than moderate weight loss alone at offsetting the persistent enhancing effects of chronic obesity on BLBC.
Title: TWIST1 silences FOXA1 transcription to promote breast cancer progression

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Body: TWIST1 promotes epithelial-mesenchymal transition (EMT), invasion and metastasis of breast cancer cells, but the underlying mechanism is still not well understood. We generated mammary gland tumor specific Twist1 knock out mouse model and found that TWIST1 does not affect PyMT-induced mammary tumor initiation and growth but promotes tumor lung metastasis. We identified FOXA1 as a novel direct target of TWIST1 in both mouse and human breast cancer. We further found that TWIST1 inhibits FOXA1 expression through direct binding to its proximal promoter region and recruiting Mi2/nucleosome remodeling and deacetylase (Mi2/NuRD) transcriptional repressor complex. Moreover, TWIST1 also diminished transcriptional activator AP1 binding to FOXA1 promoter. TWIST1 mediated FOXA1 down-regulation is essential for promoting breast cancer migration, invasion and metastasis. FOXA1 significantly inhibits TWIST1 dependent cell migration and invasion capability of MCF7 cells through inhibiting integrin α5, β1 and MMP9 expression. Importantly, TWIST1\textsuperscript{high} FOXA1\textsuperscript{low} correlates with the poorest prognosis in breast cancer patients.
Title: OPG+ bone marrow B cells induced by non-metastatic tumors inhibit the pre-metastatic bone niche induced by T cells

Bonomo A, Monteiro AC, Leal AC, Fontão AP, Spinetti E and Balduino A. Fiocruz, Brazil; UFRJ, Brazil; Universidade Veiga de Almeida, Brazil and Excellion Biomedical Services.

Body: Using the 4T1 model of experimental breast cancer we had recently shown that cancer induced bone disease starts before metastatic colonization and is mediated by RANKL expressed by tumor specific T cells. The role of anti-tumor B cell immune response in the context of cancer induced bone disease has never been investigated. There is evidence in the literature that B cells are good prognostic markers for metastatic breast cancer. B cells have an intimate relationship with bone cells as they differentiate from HSC present on endosteal surfaces; cross-talk with skeletal system through the RANK-RANKL-OPG signaling axis; and produce OPG, a decoy receptor of RANKL.

Here we used the BALB/c derived 4T1 (metastatic) and 67NR (non-metastatic) sibling cell lines of mammary mouse carcinomas. By day 7, 14 and 21 after tumor injection, B220+ BM B cells from 67NR+ animals produce high amounts of OPG in vitro in contrast to B220+ BM B cells of 4T1+ mice. In vitro, BM B cells from 67NR+ mice, but not from 4T1+, could inhibit the RANKL dependent, anti-4T1 T cell mediated-OC differentiation ascertained by TRAP enzymatic activity, morphology and osteolytic disk assay.

Transference of BM B cells from 67NR+ mice together with 4T1 tumor cells to BALB/c mice led to inhibition of osteoclastogenesis, increased numbers of bone lining cells and mesenchyme stem cell. Besides acting directly on the bone remodeling system, these B cells also modulated T cell activity evidenced by diminished RANKL and IL-17F production. All the anti-osteolytic and pro-osteogenic activity of B cells modulate and inhibits the pre-metastatic niche formation. Indeed transference of such cells to 4T1 animals inhibited LN and BM metastatic colonization.

We conclude that 67NR induced OPG+ B cells can inhibit pro-osteoclastic / pre-metastatic activity of tumor induced T cells, favoring a bone metastasis free-phenotype. These findings have implications, not only for the understanding of the direct contribution of B cells in the control of bone metastasis but also it might be a promising prognostic tool for predicting cancer-induced bone metastasis.
2015 San Antonio Breast Cancer Symposium

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Title: TIMP-4 expression correlates with disease progression among HER2-positive breast cancers

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Body: Breast cancer with HER2 gene amplification accounts for 20-25% of invasive breast cancer. HER2 activates the PI3K/Akt and MAPK pathways promoting survival and cell proliferation resulting in aggressive biological behavior and poorer prognosis for the patient. Although trastuzumab (Herceptin®), a recombinant humanized antibody, offers effective treatment of HER2-positive cancers, a proportion of patients will not respond to trastuzumab-based regimens and those who do respond can lose clinical benefit during the late stages of their treatment.

In this IRB-approved study we have assessed HER2 and LuminalB subtypes of invasive breast cancer from an archival collection together with specimens collected in an on-going prospective study for the expression of tissue inhibitor of metalloproteinase-4 (TIMP-4), an inducer of the PI3K/Akt pathway and its possible correlation to trastuzumab resistance. Circulating TIMP-4 in the stroma binds to CD63 on the tumor epithelial cells and initiates a signaling cascade through interaction with β1-integrin.

We have previously demonstrated that elevated levels of TIMP-4 confers poor survival prognosis for triple-negative breast cancers. Genomics data found at TCGA, cBioPortal for Cancer Genomics¹ have showed that TIMP-4 is amplified in 24% of breast cancer patients. Therefore, we propose that the reported ~20% of HER2-positive patients that will not respond to trastuzumab could in fact be TIMP-4 positive and have active PI3K/Akt signaling. In this on-going study, we have to date examined twenty-nine patients with HER2 gene amplification. Among these, eight had elevated levels of TIMP-4 as determined by immunohistochemistry, and seven had progression of their disease within one year of starting systemic therapy (all with metastasis to brain) while one had local recurrence 5 years after starting therapy. Two of these patients had stage IA disease, one had IIA, three had IIB and two were diagnosed with stage IIIC disease. These results suggest that TIMP-4 amplification could be, at least in part, responsible for the trastuzumab resistance due to TIMP4's ability to promote survival and cell proliferation by a continuous activation of the PI3K/Akt pathway.

In an attempt to test if a monoclonal antibody specific for human TIMP-4 could be used to prevent or alleviate the trastuzumab resistance, we are using human breast cancer cell-lines with amplified HER2, with and without CD63 expression as well as a CD63 knock-out line. From these experiments we hope to learn if treating for TIMP-4 could be an approach for future clinical intervention for the patients that have HER2 gene amplification and elevated levels of TIMP-4.

Title: Focal adhesion kinase is required for efficient tumor-induced osteoclastogenesis via control of macrophage colony stimulating factor expression

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Body: Breast cancer most commonly metastasizes to bone where metastases cause the potentiation of the vicious cycle. During this process, breast cancer bone metastases inhibit osteoblasts from forming new bone, while also activating osteoclasts to degrade bone. This in turn causes release of bone-matrix bound growth factors which propagate tumor growth. The overall net bone destruction can lead to significant adverse clinical consequences for patients including fractures or substantial pain. While agents such as bisphosphonates or the monoclonal antibody to RANKL denosumab, both of which inhibit the osteolytic activity of osteoclasts, are currently used in breast cancer bone metastatic patients to alleviate these adverse clinical outcomes, they have not been associated with increased patient survival. Given the lack of successful treatments for bone metastases providing survival advantages, we sought to evaluate the role of focal adhesion kinase (FAK), a novel therapeutic target, in breast cancer mediated osteolysis. FAK is a non-receptor tyrosine kinase that regulates many pathways that contribute to enhanced tumor progression and metastasis. FAK is also expressed in all the cell types involved in the vicious cycle. We thus hypothesized that FAK plays an important role in breast tumor-induced osteolysis and that its inhibition would lead to restoration of bone homeostasis, in addition to inhibition of tumor progression. Using in vitro siRNA-targeted depletion of FAK in breast cancer tumor cell lines, we found that production of numerous soluble growth factors, many of which are known contributors to osteoclastogenesis, was inhibited. We confirmed that FAK regulates the expression of the osteoclastogenic factor macrophage colony stimulating factor in breast cancer cell lines. Further, using conditioned media from FAK expressing versus depleted breast cancer tumor cells in osteoclastogenesis assays, we show that FAK-depletion results in impaired osteoclastogenesis. These data suggest that in addition to its proven direct anti-tumor effects, inhibition of FAK may also result in therapeutic blockade of bone degradation.
The ratio of progesterone receptor isoform A to B determines the effect of antiprogestins on preclinical models of breast cancer metastasis


Body: In previous studies using several experimental models expressing different progesterone receptor (PR) isoform ratios, we have shown that only those with high levels of isoform A (PRA) are inhibited by antiprogestins whereas those with high levels of isoform B (PRB) are resistant to antiprogestin therapy. Moreover, results obtained using tissue cultures of breast cancer patients confirmed the data observed in these experimental models (May and Rojas et al., ASCO meeting 2015).

The aim of this study was to evaluate the role of progestins and antiprogestins on the outgrowth of spontaneous metastatic foci of mammary carcinomas with different PR isoform ratios.

We used metastatic tumors from the murine medroxyprogesterone acetate (MPA)-induced breast cancer model: C7-2-HI (PRA>PRB) and C7-HI (PRA<PRB), and human MDA-MB-231 cells genetically modified to overexpress PRB. Tumors were orthotopically inoculated in the fourth mammary gland and Mifepristone (MFP; 6 mg), Telapristone (TLP; 6 mg), MPA (20 mg) or vehicle silastic pellets were sc implanted when the tumors reached a size of 25 mm2. Tumors were measured twice a week and animals were followed to detect the presence of axillary lymph node metastasis. Animals were euthanized before tumor size exceeded 2 cm at the largest diameter or showed a deterioration of the physical conditions.

Both antiprogestins had similar effects, MFP induced almost complete regression of the C7-2-HI tumor as already described, and TLP inhibited significantly its growth as compared with control tumors, while no changes in tumor size were observed in MPA-treated mice. Lung or lymph node studies confirmed the lack of metastatic foci in MFP-treated mice while no changes in tumor size were observed in MPA-treated mice. In C7-HI tumors, with higher levels of PRB than PRA, MFP clearly stimulated the number of lung foci as compared with control animals (p<0.05). The effects of TLP and MPA on tumor metastasis, although not inhibitory, need to be confirmed. Studies performed using MDA-MB-231-PRB xenografts growing in NSG mice showed that MFP stimulated the growth of lung foci, while MPA was inhibitory. No significant changes were observed in mice inoculated with empty vector transfected cells. To investigate the mechanisms underlying the metastatic process, we evaluated the expression of a metastatic suppressor gene (Nm23-H1) and a prometastatic enzyme (MMP-2). We found a lower expression by immunohistochemistry of the former and an increase in the latter by qPCR in MFP-treated MDA-MB-231-PRB xenografts compared with untreated tumors.

In conclusion, we have demonstrated that MFP impedes the metastatic process in tumors with higher levels of PRA than PRB while it might promote metastasis in those with the opposite ratio. We suggest that MFP, through PRB receptors, downregulate the expression of Nm23-H1 and increase the expression of MMP-2. The conclusive effects of TLP and MPA need further investigation. These data underscore the importance of isoform ratio determination before treatment of breast cancer patients with endocrine therapies that target PR.
Title: The impact of the plant lignin secoisolariciresinol diglycoside on preclinical models of estrogen receptor positive breast cancer

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Body: Background: Several preclinical studies indicate that secoisolariciresinol diglycoside (SDG), a polyphenolic plant lignin found most abundantly in flaxseeds, inhibits the progression of both estrogen receptor (ER) positive and negative mammary tumors. SDG is metabolized by gut bacteria into the biologically active metabolites enterolactone (ENL) and enterodiol (END), which are known to have anti-estrogenic activity. However, the mechanisms mediating SDG's anti-tumor effects remain poorly understood.

Methods: In a dose-determination pilot study linked to an ongoing clinical trial of SDG in women at high risk for breast cancer, 18 week old C57BL/6 mice were randomized to a control diet or SDG-supplemented diets (25 or 74 mg/kg of food) for 8 weeks prior to euthanization, and the levels of serum and tissue SDG metabolites (particularly ENL and END), metabolic hormones and inflammatory markers were measured. In an ongoing tumor study, 12-week old C57BL/6 and foxn1 nu/nu mice were randomized to the control or control plus SDG (100 mg/kg of food, a dose projected to match ENL and END metabolite levels achieved in the clinical trial) diet regimen. After 8 weeks on diet, they will receive orthotopic injections of E0771 mouse mammary tumor cells or BT-483 human breast cancer cells (both ER positive), continuing on the same diets until euthanization. Cell culture studies examining the impact of biologically relevant concentrations of ENL and END on E0771 and BT-483 cells are also in progress.

Results: In comparison to those maintained on the control diet, the higher dose SDG diet reduced estrogen and pro-inflammatory signaling in the pilot study mice, as evidenced by higher interleukin 10 and lower C-reactive protein mammary fat pad expression as well as lower circulating levels of the adipokines leptin and resistin, which have been linked to chronic inflammation. High dose SDG also decreased serum insulin and glucose levels, indicating improved metabolic function. Because serum ENL and END levels in the pilot study did not reach those achieved in the SDG clinical trial, a 100 mg/kg SDG dose was chosen for the tumor study. Cell culture studies indicate that ENL (150 nM) inhibits E0771 and BT-483 cell proliferation and ER alpha:beta expression ratio.

Conclusions: Preliminary data suggests that the anti-tumor effects of SDG's metabolites may be mediated through multiple mechanisms, including improvements in metabolic function and inflammatory signaling as well as modulation of breast cancer cell gene expression. The results of the ongoing tumor study will inform the design of additional cell culture studies aimed at further defining these mechanisms.
Title: Characterisation of C11orf67, an oncogenic driver in a new subtype of aggressive endocrine receptor positive breast cancer

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Body: The recent integration of both genomic and transcriptomic datasets have added a further dimension to the landscape of breast cancer (BrCa) subtyping, defining novel functional subgroups with distinctive oncogenic drivers that carry important implications for therapy. This integrative clustering has unveiled a novel subtype of hormone receptor positive (HR+) BrCa associated with high proliferation and very poor survival characterised by copy number amplification and overexpression of a cluster of candidate oncogenic drivers at the 11q13.5-14 locus (1). At the heart of this amplicon we have demonstrated the selective overexpression of C11orf67/AAMDC (Adipogenesis associated Mth938 domain containing) which encodes a hypothetical protein of 122 aa with unknown function. In a pilot tissue microarray of 75 BrCa cases C11orf67 amplification and expression were significantly correlated with hormone receptor positivity. These positive cases also demonstrated high risk features with 75% demonstrating lymph node involvement.

In functional elucidation studies knockdown of C11orf67 in the highly expressing T47D cell line lead to decreased cell proliferation, cell migration, anchorage independent cell growth and induction of senescence. T47D xenografts with stable shRNA-induced C11orf67 knockdowns introduced into BALB/c mice showed significantly lower tumour volumes relative to T47D with empty vector. A genome wide analysis of these T47D-C11orf67 shRNA cells compared to T47D-empty vector cells using the Illumina HumanHT-12 platform demonstrated 40 differentially expressed genes. Network analysis revealed a proliferation node, enriched in cell cycle proteins, and a metabolic node comprising several biosynthetic enzymes such as MTHFD1L involved in one-carbon folate metabolism. Supporting this link and pointing to potential utility in chemotherapy selection, induction of ectopic C11orf67 expression in MCF7 cells increased sensitivity to fluorouracil and methotrexate but not to paclitaxel.

Investigating potential novel binding partners and effectors, in yeast two hybrid screening C11orf67 was found to associate strongly with RABGAP1L, a protein involved in controlling GTPase signalling, protein trafficking, and autophagy. Exploring the molecular cues that control C11orf67 expression, our data suggest the locus is regulated by transcription factors associated with high proliferation and metabolic control, notably Myc and NFkB, as well as HRs. E2 lead to a significant down-regulation of C11orf67 in T47D cells, which was reversed by the antiestrogen drug tamoxifen, whereas PG significantly increased C11orf67 levels. In keeping with this MCF7 cells ectopically expressing C11orf67 were resistant to the anti-proliferative effects of tamoxifen compared to the parent cell line.

These observations endorse C11orf67 as a novel oncogenic driver with exciting therapeutic potential, which could serve to distinguish the HR+ tumours at high risk of relapse and guide both the selection of current chemotherapeutical and endocrine treatments as well as the design of future precision therapeutics, notably anti-folate/one carbon drugs and novel endocrine agents.

References
Publication Number: P2-06-02

Title: BIN3 is an 8p21 tumor suppressor regulating the epithelial attachment checkpoint

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Body: An important characteristic of multicellular organisms is the control that tissue architecture exerts on the growth and differentiation of individual cells. Epithelial cells sense their location through interactions with the extracellular matrix (ECM) as well as neighboring cells. These interactions generate input signals, including survival, that are critical to maintain tissue and cellular homeostasis. When attachment is compromised, epithelial cells undergo an intrinsically programmed cell death (apoptosis) that is termed anoikis (from the Greek "loss of home"). Importantly, failure to execute the anoikis program could result in adherent epithelial cells surviving in suspension or being able to proliferate at sites different from their original environment. Thus, anoikis is a line of defense that must be circumvented by cancerous epithelial cells for them to leave their home niche, thrive in inappropriate ECM environments, and establish long distance metastases. Thus, elucidating how epithelial cancer cells escape anoikis is critical to understanding cancer progression.

In order to uncover genes that modulate the anoikis response and are altered in human cancers, we performed a functional genomics study. We couple genome wide RNAi to identify gene functions that, when silenced, induce resistance to anoikis with a novel computational method, ISAR-DEL, specifically aimed at pinpointing candidate tumor suppressor genes based on recurrent loss of copy number. Our studies identified Bridging Integrator 3 (BIN3) as a novel tumor suppressor located on the chromosomal region 8p21.3, one of the most frequently lost regions in epithelial cancers.

Mechanistically, we link BIN3 tumor suppression function to its ability to sense changes in the curvature of the cell membrane and relocate to the cell membrane after cell detachment to induce a proapoptotic cascade. Once BIN3 has translocated to the cell membrane it modulates the relocation and function of CDC42. In these conditions, CDC42 transmits the signal that leads to the activation of the stress protein P38-α and programmed cell death mediated by accumulation of the apoptotic facilitator BimEL. Overall, our results explain how changes in cell geometry are integrated in the cellular signaling network and present, for the first time, BIN3 as a novel breast cancer tumors suppressor.
Leukemia inhibitory factor receptor as a tumor suppressor: A study on migration and invasion of breast cancer cells upon LIFR stimulation

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BACKGROUND: Tumorigenesis is the result of a step-wise process during which a mutation activates an oncogene or inactivates a tumor suppressor gene. Identification of these genes is critical in order to develop effective therapies for breast cancer patients. Our group previously discovered the Leukemia Inhibitory Factor Receptor (LIFR) as a novel tumor suppressor gene via an in vivo RNAi screen in HMLE cells. HMLE is a partially transformed non-tumorigenic cell line; these cells can become tumorigenic with a single mutation, such as the Ras mutation that creates the HMLER line. HMLEs were transduced using an shRNA library targeting the entire human genome, and stably transfected cells were xenografted into NOD/SCID mice. Genomic DNA from resultant primary tumors were analyzed for the shRNA sequences that, when integrated, made HMLEs tumorigenic. LIFR emerged from this screen as a novel candidate tumor suppressor gene in breast cancer. Here we report on the decreased migration and invasion of breast cancer cells activated by LIFR stimulation.

METHODS: HMLER cells were plated at 500,000 cells per well of a six-well plate. Twenty-four hours later, HMLERs were treated with 100, 25, 12.5, 5, 2.5, or 0 ng/ml recombinant hLIF. Protein lysates were analyzed for phospho-STAT3 induction upon LIF stimulation. Based on the results, we selected 25 ng/ml as the appropriate hLIF concentration to maximally stimulate LIFR in the migration assay described here. HMLERs were serum starved for 8 hours. DMEM with 10% fetal bovine serum was added to the bottom of the migration assay plate as a chemoattractant. The cells were suspended in DMEM with 0.1% bovine serum albumin and either treated with 25 ng/ml LIF or no LIF. Thereafter, 25,000 cells were added to either a Corning Biocoat Matrigel Invasion Chamber or a control insert lacking a migration matrix. The migration assay plate was incubated at 37°C and the cells were allowed to migrate for 20 hours. Migrated cells were enumerated under the light microscope and a migration percentage was calculated.

RESULTS: In the first portion of the study, we found that low concentrations of LIF (2.5 ng/ml) resulted in p-STAT3 induction in HMLERs, but that p-STAT3 was maximally induced with 25 ng/ml of LIF. In the invasion and migration assay, HMLER cells that had not been treated with LIF displayed an aggressively invasive and migratory phenotype with 61.1% migration in matrigel compared to control inserts without the migration matrix. When HMLERs were treated with 25 ng/ml LIF, the cells displayed decreased invasion and migration with only 50.0% of cells migrating. Based on these results, LIFR stimulation inhibits the invasion and migration of breast cancer cells.

CONCLUSIONS: As a tumor suppressor gene, LIFR is vital to the normal functioning of a non-cancerous cell, and its loss can produce a tumorigenic and metastatic phenotype. Treatment with LIF converts aggressively metastatic breast cancer cells to a less invasive phenotype. Through a deeper understanding of LIFR's tumor suppressor effects, we can harness the anti-tumorigenic and anti-metastatic properties of LIFR stimulation and develop targeted therapies to prevent growth and metastasis of breast cancer.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-06-04

**Title:** The power of next generation sequencing in the detection of breast and ovarian cancer susceptibility genes other than BRCA


**Body:** Background: Most cases of breast and ovarian cancer susceptibility remains unexplained. Testing multiple genes in one go with next generation sequencing is then an asset with the recent discovery of new genes involved in breast and ovarian cancer susceptibility.

Methods: We studied 457 patients originated from Burgundy (France) fulfilling the criteria for BRCA1/2 testing using a next generation sequencing 25-gene panel including 17 genes of predisposition for breast and/or ovarian cancer (ATM, BARD1, BRCA1/2, BRIP1, CHEK2, PALB2, RAD51C, TP53, PTEN, RAD50, MRE11, MLH1, MSH2, MSH6, PMS2, STK11).

Results: A pathogenic BRCA1/2 mutation was found in 8% (n=37) of patients. Besides, we found 39 deleterious or probably deleterious mutations in 13 different genes. The most frequently mutated genes were CHEK2 (n=10 ; 2.1 %), ATM (n=9 ; 2 %), and PALB2 (n=4 ; 0.9%). One patient had deleterious mutations in both TP53 and PALB2, and another one had deleterious mutations in both BRCA2 and CHEK2. The mutation could explain the phenotype in the majority of cases, but a pathogenic mutation was found in a different predisposing gene in 7 patients, and could be considered as incidental findings with the currently published spectrum of cancer locations.

Conclusion: Besides BRCA1/2 mutations, that remain the most frequent susceptibility genes for breast and ovarian cancer, gene panels remain a powerful tool for identifying the other less frequent susceptibility genes. The penetrance and spectrum of cancer associated to these other genes remain sometimes undefined, and further collaborative work is crucially needed to address this question. The possibility of double hits should led to careful genetic counseling.
LncRNAs may play a role in cancer and serve as new potential targets for improving cancer diagnosis, prognosis or treatment. Long intergenic non-protein coding RNA 478 (LINC00478), the miR99a-let-7c cluster host gene, has been shown to be downregulated in estrogen-independent ER-positive (ER+) breast cancer cells. However, its expression in breast tumors and its significance in recurrence has not been well characterized.

Methods:
The clinical relevance of LINC00478 in ER+ breast tumors was first determined by analysis of the expression levels in MiTranscriptome database (RNAseq-based), which contains ∼8000 most differentially expressed lncRNAs for each cancer and/or tissue type. Additionally, the expression of LINC00478 mRNA was determined in a) a cohort of matched pairs of primary and (nodal) metastatic tumors (n=18) (RNA-seq) and b) a cohort of 60 paraffin-embedded ER-positive, node-negative breast carcinomas with Oncotype DX recurrence scores (QRT-PCR).

Results:
LINC00478 was significantly downregulated in patients with ER-positive tumors compared to uninvolved normal breast tissue. In RNA-seq analysis, metastatic tumors had significant low expression as compared to matched primary tumors (FC: -2.2 fold-down; P=0.0027). Quantitative RT-PCR analysis showed that LINC00478 expression was significantly lower in ER+ node-negative patients with Oncotype DX high recurrence score when compared with low recurrence score patients (FC: -4.14-fold down; P=0.02).

Conclusion:
We document for the first time that LINC00478 functions as a tumor suppressor in ER-positive breast cancer. Its loss is associated with tumor progression and recurrence/metastasis. Novel strategies that focus on re-expression of LINC00478 might be useful for targeting breast cancer and preventing the metastases.
Body: Breast cancer is the most commonly diagnosed cancer and 2nd leading cause of cancer death in women in the United States. Despite high rates of diagnosis and numerous drug treatments, the response to treatment is difficult to predict due to different breast cancer subtypes with varying molecular signatures. Recurrence of breast cancer is now more prevalent and is often associated with drug resistance, which accounts for nearly 90% of all metastatic breast cancer deaths. Expression of the tumor suppressor protein Adenomatous Polyposis Coli (APC) could be one important molecular marker in breast cancer treatment. APC is lost by mutation or hypermethylation in up to 70% of sporadic breast cancers, and predicts resistance to cisplatin in other tumor types. Using the \textit{Apc}^{min/+} mouse crossed to the mouse mammary tumor virus Polyoma middle T antigen (MMTV-PyMT) transgenic model, we showed that APC loss enhanced PyMT-mediated breast tumorigenesis. \textit{In vitro} studies recently demonstrated that cells isolated from MMTV-PyMT;\textit{Apc}^{min/+} tumors are more resistant to doxorubicin- or cisplatin-induced apoptosis, have increased multidrug resistance protein 1 (MDR1), and have an enhanced tumor initiating cell (TIC) population. Reintroduction of full-length APC into the MMTV-PyMT;\textit{Apc}^{min/+} cells restores sensitivity to both cisplatin and doxorubicin. Therefore the current study focuses on determining the mechanism(s) responsible for APC-mediated chemotherapeutic resistance. We have found that cells from MMTV-PyMT;\textit{Apc}^{min/+} tumors have greater expression of phosphorylated signal transducer and activator of transcription 3 (STAT3) and production of interleukin 6 (IL-6) compared to cells from MMTV-PyMT;\textit{Apc}^{+/+} tumors. Given that IL-6 can activate STAT3, and both of these factors can contribute to increased MDR1 and TICs, our future studies will investigate whether STAT3 and IL-6 contribute to the development of APC-mediated chemotherapeutic resistance.
Title: PRKCQ regulates taxol sensitivity of triple negative breast cancer cells via IL-6/Stat3 signaling

Irie HY Y and Byerly J. Icahn School of Medicine at Mount Sinai, NY, NY.

Body: Background/Rationale. While some patients with triple negative breast cancer achieve long-term remission with chemotherapy, many have cancers that are chemotherapy resistant. The lack of targeted therapies for this subtype also makes some triple negative cancers difficult to treat and control. PRKCQ, a member of the novel protein kinase C family, is preferentially expressed in triple negative breast cancers compared to ER+/Luminal cancers. We previously reported that PRKCQ expression drives growth-factor independent growth, anoikis resistance and migration of breast epithelial cells. In addition, PRKCQ is required for in vivo growth of triple negative breast cancers tumor xenografts. We sought to determine if PRKCQ expression modulates sensitivity of triple negative breast cancer cells to standard of care chemotherapy and whether PRKCQ inhibition could be a strategy to induce death of chemotherapy-resistant triple negative breast cancer cells. Methods. We determined the effects of modulating PRKCQ expression, using PRKCQ cDNA or shRNA vectors, on Doxorubicin or Taxol treatment-induced effects on triple negative breast cancer cells, including those that are relatively chemotherapy resistant at baseline. We determined the mechanisms by which PRKCQ expression regulates sensitivity to Taxol. Results. Increased PRKCQ expression in MCF-10A breast epithelial cells suppresses the apoptosis-inducing effects of Doxorubicin or Taxol treatment. PRKCQ-induced Taxol resistance is dependent on PRKCQ kinase activity. PRKCQ-expressing MCF-10A cells secrete enhanced levels of IL-6, leading to the autocrine activation of Stat3; IL-6/Stat3 activation is necessary for PRKCQ-induced resistance to Taxol. Finally, downregulation of PRKCQ sensitized MDA-231-Luc cells to Taxol treatment and induced apoptosis of these cells which are relatively resistant to Taxol at baseline. Conclusions. PRKCQ regulates sensitivity to standard of care chemotherapies used in the treatment of triple negative breast cancer. IL-6/Stat3 signaling induced by PRKCQ kinase activity is responsible for resistance to the effects of Taxol treatment. Targeting PRKCQ therefore could be an attractive strategy to overcome chemotherapy resistance of a subset of triple negative breast cancers.
Title: Interplay of Smad2 and Smad3 during TGF-β induced TMEPAI/ PMEPA1 mediated triple negative breast cancer cell growth

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Body: Background: Despite the known effects of TGF-β mediated canonical and non-canonical Smad signaling in cancer cell growth and metastasis, the role played by individual R-Smads in mediating TGF-β dependent late growth and metastasis remained enigmatic. Previously we have reported that transmembrane prostate androgen induced (TMEPAI/PMEPA1), a TGF-β target gene has unique role in subverting TGF-β mediated growth suppression into growth promotion of triple negative breast cancer (TNBC) cells. But the relationship between Smads and TGF-β mediated TMEPAI induction and TNBC proliferation is not known. Therefore, we undertook the present study to know the differential role played by Smads in TGF-β mediated induction of TMEPAI and TNBC growth.

Materials and Methods: Cells were cultured as recommended by ATCC. Knockdown of TMEPAI and Smad2, Smad3 proteins was achieved by lentiviral or retroviral shRNA vectors. Transfections, luciferase assays, RT-qPCR and immunoblotting were performed according to standard methods. Cell proliferation was measured by quantitation of total cellular DNA.

Results: Although no significant differences were found in mRNA levels of Smad2 and Smad3 in normal human mammary epithelial cells (HMEC) and different TNBC cell lines, at the protein level aggressive TNBC cells expressed more Smad3 protein than Smad2 compared to normal cells. TMEPAI knockdown did not modify this profile in TNBC cells. However, HMEC that expressed more Smad2 protein than Smad3, produced little TMEPAI and growth arrested in response to TGF-β. In contrast, MDA-MB-231 (231) cells, which contained more Smad3 over Smad2 produced high levels of TMEPAI and grew robustly in response to TGF-β. To delineate the role of individual R-Smads in TGF-β mediated growth regulation and TMEPAI expression, Smad2 or Smad3 were selectively knocked down using shRNAs. Knockdown of either Smad2 or Smad3 rescued HMEC from TGF-β mediated growth arrest, suggesting that Smad signaling is growth suppressive in HMEC. In contrast, selective Smad2 or Smad3 knockdown had distinctive effects on 231 cell proliferation and TMEPAI expression. Interestingly Smad3 knockdown, which showed diminished TMEPAI expression in 231 cells, greatly inhibited their growth both in the absence or presence of TGF-β. In contrast, Smad2 knockdown in 231 cells caused augmented TMEPAI expression in response to TGF-β and increased cell proliferation at the rates similar to control cells either in the absence or presence of TGF-β. Notably, individual R-Smad deficiency caused a compensatory increase in the complementary R-Smad and its associated signaling in 231 cells. Therefore, the effect of Smad3 shRNA on TNBC proliferation was similar to that of TMEPAI shRNA reported by us earlier. Like TMEPAI knockdown, Smad3 knockdown also elevated PTEN protein levels with reduced Akt phosphorylation that reduced growth both in the absence or presence of TGF-β.

Conclusion: Our results suggest that growth of TNBC in the presence of TGF-β is unique to cancer cells and is pathological in a TGF-β-Smad3-TMEPAI axis dependent manner. Smad3 plays an important role in growth promoting TGF-β dependent non-canonical signaling not only with respect to the induction of TMEPAI but also by decreasing the PTEN.
Title: Co-expression plasmid carrying GRIM-19 and LKB1 genes acted synergistically on breast cancer in vitro and in vivo

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Body: Background:
The onset and progression of cancer is a multistage, multi-gene and multi-factor process. It is very difficult to cure the tumor by using a single therapeutic gene. Combination of many genes is more effective to inhibit the cancer growth than that observed with individual genes. Both GRIM-19 and LKB1 act as tumor suppressor genes, are ideal targets for cancer gene therapy. Increasing evidence demonstrated that GRIM-19 and LKB1, two kinds of tumor suppressor genes, combination other gene could inhibit several tumor growth.

Methods:
In the present study, a recombinant eukaryotic expression plasmid carrying GRIM-19 was constructed and then transfected into the MCF-7 to examine its effects on breast cancer cell growth, migration and invasion using several in vitro approaches, including qRT-PCR, western blot analysis, cell proliferation and colony formation, cell apoptosis, cell cycle distribution, cell migration and invasion assay, etc. In addition, tumor growth ability in nude mice was detected to define the Synergistic inhibition of tumor growth in vivo by co-expression plasmid pGRIM-19-LKB1.

Results:
In this study, we developed a dual expression plasmid that coexpressed GRIM-19 and LKB1, and then, we evaluated the synergistic effects of the two genes on anticancer activity in breast cancer in vitro and in vivo. We found that simultaneous expression of GRIM19 and LKB1 (pGRIM19-LKB1) in MCF-7 cancer cells significantly inhibited the proliferation, colony formation, migration and invasion compared with treatments of either pGRIM-19 or pLKB1 alone. We also found that treatment with a combination of GRIM19 and LKB1 (pGRIM19-LKB1) in MCF-7 cancer cells synergistically induced cell apoptosis and cell arrest at G0/G1 stage relative to pGRIM-19 or pLKB1 alone. In vivo study in MCF-7 xenograft tumor model demonstrated that intravenous injection of eukaryotic coexpression plasmid (pGRIM19-LKB1) caused an additive effect on tumor growth inhibition, compared to pGRIM-19 or pLKB1 alone.

Conclusion:
In summary, these findings suggest that combined therapy with eukaryotic coexpression plasmid carrying GRIM19 and LKB1 synergistically and more effectively suppressed tumor growth of breast growth in vitro and in vivo, and have therapeutic potential for treatment of human breast cancer.

Reference:
Title: Loss of COX5B inhibits proliferation and promotes senescence via mitochondrial dysfunction in breast cancer

Gao S-P, Sun H-F, Jiang H-L, Li L-D and Jin W. Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Background: COX5B, a peripheral subunit of the cytochrome c oxidase complex, is reported to maintain the stability of this complex and impact on cell viability. Recently, several studies demonstrated that cox5b may involve in cancer progression. In our study, we also found that COX5B was upregulated both in breast cancer tissues and cells. However, the role and mechanism of COX5B in breast cancer remain unclear. Therefore, the aim of this study is to validate COX5B functions and its underlying mechanisms in breast cancer cells.

Methods: SILAC (stable isotope labeling with amino acids in cell culture) assay was conducted in mammary epithelial cells and its paired breast tumor cells, and the results was validated by other two paired tissues. Forty tumors and twenty normal or benign samples were implied for COX5B expression by IHC. Online Kaplan Meier plotting tool was used to confirm the association between COX5B expression and clinical outcome. Three Cell lines (MDA-MB-231, MDA-MB-468 and MCF-7) were constructed by sh-RNA and the efficiency was confirmed by qPCR and western blot and then migration, proliferation and senescence characteristics were evaluated. Furthermore, microarray was implemented to detect potential mechanisms and the associated genes were assessed by qPCR. ELISA was carried out for IL8 secretion. Condition medium was collected from stable knock down cells and used as attractant in lower chamber of tranwell assay to examine microenvironment influence. Finally, the intracellular ATP glucose uptake and lactate secretion were evaluated, as well as mitochondrial membrane potential and ROS production.

Results: We found that the level of COX5B was elevated in breast cancer and cell lines. Patients with high levels of COX5B were likely to have a longer disease-free survival than patients with low levels. Furthermore, loss of COX5B inhibited proliferation and induced the senescence of breast cancer cells, which was accompanied by increases in the secretion of IL-8 and other cytokines. Additionally, conditioned medium from COX5B knockdown cells increased the migration of breast cancer cells compared with conditioned medium from control cells. The mechanisms underlying these processes were associated with mitochondrial dysfunction due to increased ROS production and decreased MMP and metabolism disorder.

Conclusion: Our results show that loss of COX5B inhibits proliferation and promotes senescence in breast cancer cells, which is associated with mitochondrial dysfunction. Indeed, loss of COX5B has different functions in target cells and the surrounding environments. These findings may provide a new perspective for the design of anti-cancer therapy combining with anti-IL8 treatment.
Wild type N-Ras, overexpressed in basal-like breast cancer, promotes tumor formation by inducing IL8 secretion via JAK2 activation


"Basal-like" breast cancer (BLBC) is a very aggressive subtype of breast cancer. BLBC has very poor prognosis — median time to distant recurrence is just 2.6 years vs. 5 years overall, and survival time from diagnosis of distant metastatic disease is 9 months vs. 22 months. BLBC tumors usually do not express ER, Her2, or progesterone receptor. As such, they cannot be treated by the current targeted therapies, which tar¬get these molecules. What drive the formation and progression of BLBCs is largely unclear.

Ras GTPases are best known for mediating growth factor signaling. Oncogenic mutations in the RAS genes, K-RAS in particular, are found in more than 30% of human tumors. Surprisingly, oncogenic RAS mutations are rare in breast cancer. However, we found that wild-type N-RAS is overexpressed in BLBCs, possibly partly via promoter demethylation, but not in other breast cancer subtypes. Repressing N-RAS inhibits transformation and tumor growth, while overexpressing it enhances these processes even in preinvasive BLBC cells. In contrast, in breast cancer cells of other subtypes, repressing N-RAS expression does not affect growth and transforming activities. We identified N-Ras-responsive genes, most of which encode chemokines and cytokines, e.g., IL8. High expression levels of these N-Ras-responsive genes as well as of N-RAS itself in tumors correlate with poor patient outcome. N-Ras, but not K-Ras, induces IL8 by binding and activating the cytoplasmic pool of JAK2; IL8 then acts on both the cancer cells and stromal fibroblasts.

In conclusion, N-Ras drives BLBC by promoting transformation in epithelial cells, which may in turn remodel the tumor microenvironment to create a proinvasive state. Although oncogenic mutations affecting RAS are common in many other human cancers, tumorigenesis in an important subset of breast cancers is driven instead by increasing activity of wild-type N-Ras. Thus, to fully assess the impact of Ras on tumorigenesis, the role of wild-type as well as mutant Ras proteins must be carefully examined.
**Title:** Association of TILs with clinical parameters, recurrence score, and prognosis in patients with early HER2-negative breast cancer (BC) – A translational analysis of the prospective WSG planB trial

Liedtke C, Gluz O, Heinisch F, Feuerhake F, Kreipe HH H, Clemens M, Nuding B, Kraemer S, Reimer T, Svedman C, Shak S, Nitz U, Kates RE E, Harbeck N and Christgen M. Westdeutsche Studiengruppe GmbH, Moenchengladbach, Germany; University Clinics Schleswig-Holstein/Campus Luebeck, Women's Clinic; Ev. Hospital Bethesda, Breast Center Niederrhein; Medical School Hannover, Institute of Pathology; Mutterhaus der Borromäerinnen Trier; Ev. Hospital Bergisch Gladbach; University Clinics Cologne, Breast Center; Clinics Suedstadt Rostock; Genomic Health, Inc.; Palleos Healthcare Services, Statistics and Breast Center, University of Munich and CCCLMU.

**Body:**

Introduction:
Tumor-infiltrating lymphocytes (TILs) have been associated with prognosis and with chemotherapy response among patients with BC, particularly in presence of high-risk features. The WSG planB trial randomized 2448 patients with HER2- N0/1 BC for comparison of anthracycline-free (6xTC) vs. standard anthracycline-taxane chemotherapy (4xEC-4xDoc). Recurrence Score® (RS) was incorporated for risk stratification in hormone receptor positive (HR+) BC. The present analysis focuses on the correlation of TILs with clinical/pathological parameters and their prognostic impact among planB patients.

Methods:
Stromal TILs were evaluated using a pathologist and two-observer approach. Three independent observers evaluated digital sections on H&E staining as previously suggested (Salgado et al., Ann Oncol. 2014); the median of the three values (TILmed) was used for statistical analysis. Spearman correlations of TILmed with clinical/pathological parameters (including central Ki67 expression, quantitative ER measurements, nodal involvement, and RS) and univariate impact on event-free survival (EFS) were analyzed.

Results:
Our analysis included 300 patients with HR- and 1124 patients with HR+ HER2- BC. Both in HR- and HR+ BC, a significant association between TILmed and (i) central grading (correlation coefficient r=0.147, p=0.012 and r=0.195, p<0.001, respectively) and (ii) central Ki67 expression (r=0.202, p=0.001 and r=0.152 and p<0.001) was observed. Among HR+ cases, a significant association between TILmed and quantitative ER measurements (r=−0.412, p=0.041) and RS (r=−0.190, p<0.001) was found. Furthermore, univariate Cox analysis revealed a significant association between TILmed (coded as fractional rank) and event-free survival (EFS). The hazard ratio of 75th to 25th percentile was 1.58 (95%CI: 1.06-2.36, p=0.025). This impact was not separately significant in HR subgroups due to lack of events.

Conclusion:
In this dataset, presence of stromal TILs was moderately associated with clinical features of high-risk breast cancer (including RS) and decreased EFS. TILs will be evaluated as a prognostic or predictive factor (in multivariate and subgroup analyses) when the outcome results are evaluated after prolonged follow up. Furthermore, an updated analysis including the complete planB dataset will be presented.
Tumor infiltrating lymphocytes density and coding mutations effects on the outcome of operable triple negative breast cancer patients


Background-Aim: Neoantigens are considered to trigger host immune responses against tumors, which may be reflected by tumor infiltrating lymphocytes (TILs) density within the tumor stroma. High TILs levels have been associated with favorable triple-negative breast cancer (TNBC) patient outcome. Herein we evaluated the presence of coding mutations and TILs density with regard to outcome in a cohort of TNBC patients treated with anthracycline-based adjuvant chemotherapy.

Patients and Methods: Paraffin TNBC tissues from 242 patients treated in the context of four prospective clinical trials were histologically reviewed and submitted to massively parallel semiconductor sequencing with a custom panel targeting 57 breast cancer (BC)-related genes. Mutations (mut) were evaluated in 210 informative samples as missense/nonsense amino acid changing variants, with minor allele frequency <1% in the case of single nucleotide polymorphisms. TILs density was morphologically evaluated as percent of the stromal area in 197 tumors; lymphocyte predominant (LP) BC tumors were called for TILs >50%. Disease-free survival (DFS) was used as the endpoint for the present analysis.

Results: 426 Mut were observed for 40 genes in 147 TNBC patients (70%). Among mutated genes, ranging from 1 in 97 tumors up to >10 in 8 tumors, the most frequently affected were TP53 (102 tumors, 69%) and PIK3CA (40 tumors, 27%). Intriguingly, mut rate (p=0.042) and number of mut genes (p=0.018) per tumor were inversely associated with TILs density. Nineteen tumors (10%) were LP-TNBC, carrying TP53 and PIK3CA mut as the only coding alterations in 10 and 3 cases, respectively. LP-TNBC patients did not experience any relapses during a follow-up period of 46-152 months (mean 66 months). For the 90% of non-LP-TNBC, the previously reported outcome benefit for 10% increments of TILs density was only demonstrated for tumors with 31-50% TILs. In non-LP-TNBC, upon adjustment for standard clinicopathological parameters, PIK3CA mut, TP53 mut and TILs density as a continuous variable, TP53 mut and nodal status independently conferred unfavorable DFS (HR=1.89, 95% CI 1.03-3.47, p=0.040 and HR=2.89, 95% CI 1.59-5.24, p=0.001, respectively). When continuous TILs density was added in the multivariate models in the entire cohort, 10% increments significantly predicted favorable DFS (HR=0.73, 95% CI 0.59-0.91, p=0.006), while high nodal status predicted unfavorable DFS (HR=2.75, 95% CI 1.51-4.99, p<0.0001).

Conclusions: In the present study, tumors with higher TILs density, including LP-TNBC, were not characterized by multiple mutations or mutated genes with the panel tested. In TNBC, increasing TILs density is a strong favorable and high nodal status a strong unfavorable prognosticator. Importantly, LP-TNBC may be regarded as a distinct subgroup with excellent prognosis concerning 10% of TNBC. In non-LP-TNBC, TP53 mut and nodal status were significant unfavorable prognosticators. These data may suggest that the level of morphologically assessed TILs density does not necessarily correspond to the tumoral mutational load and merit validation in larger cohorts.
Title: Nomograms to estimate long-term overall survival and breast cancer-specific survival of patients with luminal breast cancer

Sun W, Jiang Y-Z, Liu Y-R, Ma D and Shao Z-M. Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Purpose
Luminal breast cancer (estrogen receptor [ER] and/or progesterone receptor [PR] positive) represents approximately two-thirds of all breast cancer. This type of cancer constitutes a group of highly heterogeneous diseases with a sustained high risk of late recurrence. In this study, we aimed to develop comprehensive and practical nomograms for the first time to better estimate the long-term survival of luminal breast cancer. Thus, those patients with high risk of late recurrence and poor prognosis could be screened out and individualized treatments can be applied.

Methods
Patients with luminal breast cancer diagnosed between 1990 and 2006 were retrieved from the Surveillance, Epidemiology, and End Results (SEER) database, and randomly divided into the training (n=87,867) and validation (n=88,215) cohorts. The inclusion criteria we used to identify eligible patients were as follows: female; aged 18 to 79 years old at diagnosis; known time of diagnosis from January 1, 1990, to December 31, 2006; unilateral breast cancer; breast cancer as the first and only cancer diagnosis; diagnosis not obtained from a death certificate or autopsy; surgical treatment with either mastectomy or breast-conserving surgery; pathologic confirmation of invasive carcinoma; AJCC stages I-III; histological grade I-III and known ER and PR statuses. Patients with inflammatory breast cancer or Paget's disease and lack of data on the above inclusion criteria were also excluded. Univariate and multivariate survival analyses were applied to identify prognostic factors for overall survival (OS). The cumulative incidence function (CIF) and a competing-risks model were used to estimate the probability of breast cancer-specific survival (BCSS) and death from other causes. We integrated significant prognostic factors to build nomograms, and subjected the nomograms to bootstrap internal validation and external validation.

Results
We screened 176,082 luminal breast cancer cases. The mean age at diagnosis was 57.5 years, and mean survival time was 107.4 months. By the end of the last follow-up, 36,911 (21.0%) patients had died, including 17,855 (10.1%) died from breast cancer and 19,056 (10.8%) from other causes. The 5- and 10-year probabilities of overall death were 0.089 and 0.202, respectively. The 5- and 10-year probabilities of breast cancer specific-mortality (BCSM) were 0.053 and 0.112, respectively. Independent prognostic factors for both OS and BCSS were integrated to construct the nomograms, including age at diagnosis, race, tumor size, histology, grade, positive lymph nodes, ER/PR status and radiation. The calibration curves for the probabilities of 5- and 10-year OS and BCSS showed excellent agreement between the nomogram prediction and actual observation. The C-indexes of the nomograms were high in both internal validation (0.732 for OS and 0.800 for BCSS) and external validation (0.731 for OS and 0.794 for BCSS).

Conclusion
We established and validated nomograms that accurately predict OS and BCSS of luminal breast cancer based on a large, population-based cohort with long-term follow-up. The nomograms can identify patients with higher risk of late overall mortality and BCSM, helping physicians in facilitating individualized treatment.
A novel diagnostic androgen receptor gene signature links clinical outcomes and preclinical response to enzalutamide, paclitaxel or the combination in triple-negative breast cancer


Body: Background: The androgen receptor (AR) is expressed in ≈70% of all breast cancers (BCs) and may be necessary for proliferation and survival advantage in AR+ tumors. A novel gene signature associated with AR-signaling biology (PREDICT AR) was developed by sequencing triple-negative BC (TNBC) tumors collected in a phase 2 study evaluating enzalutamide (ENZA) monotherapy\(^1\); clinical outcomes were superior in patients (pts) with PREDICT AR+ vs PREDICT AR- tumors.\(^1,2\) ENZA blocks nuclear localization and suppresses its activity.\(^3\) Paclitaxel (PTX) stabilizes microtubules and may also block AR nuclear localization. Thus we hypothesize that response to PTX-based therapy may be additive in PREDICT AR+ vs PREDICT AR- disease. This study sought to identify independent clinical datasets with PREDICT AR+ gene signature to assess outcomes following PTX-based therapy. Preclinically, we investigated the antitumor activity of ENZA, PTX, or ENZA+PTX in AR-driven TNBC models.

Methods: We probed publicly available TNBC clinical databases from Gene Expression Omnibus datasets to assess PREDICT AR status and clinical outcomes. Similarly, we assessed 21 TNBC lines for PREDICT AR status. BT549, MDA-MB-436, and MDA-MB-453 were treated with ENZA, PTX, or ENZA+PTX to determine activity. Cell signaling and pathway activation were assessed by western blot. ENZA and PTX activity was assessed in PREDICT AR+ xenograft models. Tumor RNA sequencing and immunohistochemistry were used to identify gene signatures, potentially predictive biomarkers, and potential synergistic effects of ENZA+PTX.

Results: The prevalence of PREDICT AR+ tumors in one cohort of 182 pts with primary TNBC\(^4\) was 51%. Distant relapse-free survival following PTX-based adjuvant/neoadjuvant therapy was not statistically different between pts with PREDICT AR+ vs PREDICT AR- TNBC (p=0.605). Pathologic complete response rates were 12.5% for PREDICT AR+ vs 21.0% for PREDICT AR- TNBC. Additional sets of pts with primary TNBC are being evaluated. Preclinically, we observed a dose-dependent inhibition of cell viability with either ENZA or PTX in AR+ TNBC cell lines and additive effects from ENZA+PTX. In PREDICT AR+ xenograft models, ENZA or PTX treatment resulted in a dose-dependent antitumor response. Combination studies are underway. Tumor RNA sequencing are being evaluated for gene signature of synergistic antitumor response to ENZA+PTX.

Conclusion: Analyzing publicly available clinical datasets, we found that PREDICT AR+ status was not associated with differential outcomes following PTX-based therapy in primary TNBC, suggesting there is potential to provide additive benefits in PTX-based therapy with ENZA. In preclinical studies, we observed additive effects when combining ENZA+PTX in PREDICT AR+ TNBC lines compared with single agent treatments. Taken together, these data suggest ENZA combined with PTX might provide additive benefits in a clinical setting for pts with PREDICT AR+ TNBC.

References
Title: Class IIa histone deacetylase HDAC5 expression in human breast cancer and its role in tumor cell lines

Li M, Li A, Liu Z, Zhou S, Xu Y and Yang W. Fudan University Shanghai Cancer Centre, Shanghai, China and Fudan University Shanghai Medical College, Shanghai, China.

Body: Background: Previous studies have underlined the expression of class I histone deacetylases (HDACs) in breast cancer (BC). However, little is known about the specific contribution of class II HDACs in BC, due to their particular structure and relatively weak enzymatic activity. HDAC5 has recently been reported to play an important role in medulloblastoma and in BC cells growth, and may potentially be a novel drug target. Thus, we attempt to investigate HDAC5 expression in tumor samples of BC patients.

Methods: Quantitative real-time PCR was performed to examine HDAC5 mRNA that extracted from 149 fresh frozen tumor samples of BC. By western blot, HDAC5 protein expression was measured in another 8 paired breast tumor and non-tumor samples. Immunohistochemical staining for HDAC5 expression was performed on TMA with 350 breast tumor samples. All of the specimens in this study were retrieved from BC patients that underwent surgery and were diagnosed with primary invasive ductal carcinoma at Fudan University Shanghai Cancer Center between January 1st, 2001 and December 31th, 2009. Patient data, clinicopathologic features and prognostic factors were obtained from patients charts. In addition, siRNA-mediated knockdown of HDAC5 was conducted in BC cells.

Results: Using the median value as a cut-off point, a total of 75 (50.3%) patients had high mRNA expression of HDAC5. Patients with high HDAC5 mRNA expression was significantly associated with distant metastasis (P<0.028), lymph node status (P=0.017), and molecular subtypes (P=0.009). During a median follow up of 51.2 months (4.6 - 82.7 months), there were 23 (15.4%) metastatic relapses and 10 (6.7%) deaths. Multivariate cox regression analysis showed that HDAC5 mRNA expression was an independent factor that was associated with disease free survival (DFS) (HR=2.33; 95% CI, 1.00-5.30; p=0.04), as well as lymph node status (HR=1.37; 95% CI, 1.00-1.87; p=0.047). The Kaplan-Meier survival curve demonstrated that overexpression of HDAC5 correlated with decrease in DFS (HR=2.148; 95%CI, 1.037-4.449; p=0.0396), whereas its overexpression was not significantly correlated with OS (HR=0.9480; 95% CI, 0.287-3.129; p=0.9295). Western blot analyses showed that HDAC5 protein expression was substantially stronger in tumor tissues than in non-tumor tissues. Moreover, HDAC5 expression in luminal subtypes was relatively higher than that in HER2 and basal-like breast tumors. Immunostaining showed that HDAC5 was predominantly expressed in the cytoplasm of malignant epithelial cells. HDAC5 expression was divided into two groups according to cut-off value set by ROC analysis. HDAC5 overexpression was significantly associated with metastasis (p=0.001), which is consistent with mRNA results. The prognostic value of HDAC5 protein expression in terms of DFS and OS was similar with mRNA expression in 350 patients. siRNA-mediated knock down of HDAC5 inhibited cell proliferation, migration and invasion.

Conclusions: Class IIaHDAC5 was expressed extensively in human BC, and its expression was significantly associated with distant metastasis, lymph node status and patients' DFS, which indicat...
Title: Periostin is associated with breast tumor progression and epithelial periostin expression is correlated with poor survival in patients with invasive breast carcinoma

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Background: Invasion and metastasis are the direct causes of mortality in patients with breast cancer and require reciprocal interactions between cancer cells and the extracellular matrix (ECM). Periostin, a fasciclin-containing adhesive ECM glycoprotein, is frequently overexpressed in various types of human cancer, and its overexpression in cancer-associated stroma and/or cancer cells is usually associated with poor clinical outcomes. However, the expression of periostin in the successive steps of breast tumorigenesis and its association with outcome variables have not been well established in breast carcinoma. The purposes of the present study were to assess the role of periostin alteration in breast tumorigenesis and evaluate the putative prognostic value of periostin as a function of its compartmentalization.

Materials and Methods: Immunohistochemical staining with anti-periostin antibody was performed in a total of 300 patients (26 patients with normal breasts, 76 patients with ductal carcinoma in situ [DCIS], and 198 patients with invasive breast carcinoma [IBC]) using tissue microarray. Periostin immunoreactivity was assessed in both epithelial tissue and the surrounding stromal compartment. In addition, the mRNA and protein expression of periostin was analyzed in 10 paired normal/invasive cancer frozen specimens by quantitative real time-polymerase chain reaction and western blot analysis, respectively.

Results: Periostin mRNA and protein expression in cancer tissues was increased compared with that in adjacent normal tissues. Both epithelial and stromal periostin staining scores were significantly increased with disease progression in a stepwise manner to DCIS and IBC compared with those in normal breast tissues (P = 0.000 and 0.000, respectively). High epithelial and stromal periostin expression was observed in 109 of 189 (57.7%) and 158 of 189 (83.6%) cases of IBC, respectively. High epithelial periostin expression was more frequently observed in the distant metastatic relapse-positive group than in the distant metastatic relapse-negative group (41 [80.4%] of 51 cases versus 68 [49.3%] of 138 cases [P = 0.000]). Furthermore, high epithelial periostin expression was associated with reduced disease-free and overall survival on univariate and multivariate analyses.

Conclusion: Periostin might play an important role in the progression of breast tumor, and epithelial periostin expression may serve as a new parameter for prognostic prediction in patients with IBC. Further study of periostin expression and its potential as a target of therapy for IBC appears warranted.
Title: Prospective trial of endocrine therapy alone in patients with estrogen receptor positive, HER2-negative, node-negative breast cancer: Results of the TAILORx low risk registry

Sparano JA A, Gray RJ J, Makower DF F, Pritchard KI I, Albain KS S, Hayes DF F, Geyer Jr CE E, Dees EC C, Perez EA A, Olson Jr JA A, Zujweski J, Keane MM M, Gomez Moreno HL L, Reddi RP P, Goggins TF F, Mayer IA A, Brufsky AM M, Toppmeyer DL L, Kaklamani VG G, Atkins JN N, Berenberg JL L and Sledge Jr GW W. Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; Dana Farber Cancer Institute, Boston, MA; Sunnybrook Research Institute, Toronto, ON, Canada; Loyola University Medical Center, Maywood, IL; University of Michigan, Ann Arbor, MI; Virginia Commonwealth University School of Medicine and the Massey Cancer Center, Richmond, VA; University of North Carolina, Chapel Hill, NC; Mayo Clinic, Jacksonville, FL; Duke University Medical Center, Durham, NC; National Cancer Institute, Bethesda, MD; University College Hospital, Galway, Ireland; Oncosalud SAC, Lima, Peru; Via Christi Regional Medical Center, Wichita, KS; Fox Valley Hematology and Oncology, Appleton, WI; Vanderbilt University, Nashville, TN; University of Pittsburgh, Pittsburgh, PA; Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; Northwestern University, Chicago, IL; Southeast Clinical Oncology Research Consortium, Goldsboro, NC; University of Hawaii Cancer Center, Honolulu, HI and Stanford University, Stanford, CA.

Body: Background: The Trial Assigning Individualized Options for Treatment (TAILORx) is a prospective trial evaluating the role of endocrine therapy or chemoendocrine therapy in patients 18-75 years of age with estrogen receptor (ER)-positive, HER2-negative breast cancer, a primary tumor between 0.6-5.0 cm, and negative axillary nodes, a population for whom chemotherapy is typically recommended or least considered based on National Comprehensive Cancer Center Network (NCCN) guidelines.

Methods: The trial was designed to demonstrate non-inferiority of endocrine therapy compared with chemoendocrine therapy in the randomized group with an Oncotype DX Recurrence Score (RS) of 11-25. Patients with a low RS < 11 were assigned to endocrine therapy alone and with a high RS > 25 assigned to chemoendocrine therapy, and both groups were followed in a prospective registry. The definition of an intermediate RS differed in this trial (RS 11-25) from the original reports (RS 18-30) in order to reduce the risk of chemotherapy undertreatment in patients with a mid-range or low RS (Sparano & Paik. J Clin Oncol 2008; 26:721-728).

Results: The trial enrolled 10,273 patients between April 2006 and October 2010, of whom 6907 patients (67.2%) had a mid-range RS of 11-25, 1737 (16.9%) had a high RS > 25, and 1639 (15.9%) had a low RS of < 11. At the fourth planned interim analysis, the ECOG-ACRIN data monitoring committee recommended that the study continue as planned for the randomized group with a RS 11-25, and that the results be released to the investigators for the low risk group with a RS <11. The characteristics of the low risk registry population are as follows: age 50 or less (27%), 51-60 (35%), > 60 (39%); tumor size < 1 cm (8%), 1-2 cm (61%), > 2 cm (31%); histologic grade low (34%), intermediate (59%), high (7%); breast conservation (68%) or mastectomy (32%). Initial endocrine therapy included tamoxifen in 35%, aromatase inhibitors in 59%, ovarian function suppression in 3%, and unspecified therapy in 3%; 5 patients received adjuvant chemotherapy (1 of whom relapsed). Five-year rates (and 95% confidence intervals [CI]) for low RS group were 99.2% (98.5, 99.6%) for distant relapse free interval, 98.5% (97.7, 99.1%) for relapse-free interval, 93.7% (92.2, 94.9%) for invasive disease free survival, and 98.2% (97.3, 98.7%) for overall survival. Information regarding ER, PR, and HER2 RNA expression will be presented.

Conclusions: Despite meeting guidelines for recommending or at least considering adjuvant chemotherapy based on classical clinicopathologic features, the risk of recurrence was very low at 5 years in patients with ER-positive, HER2-negative, axillary node-negative breast cancer and a low RS of < 11 treated with endocrine therapy alone without chemotherapy.
Tumor-infiltrating lymphocytes (TILs) are a powerful prognostic marker in patients with triple negative breast cancer treated by induction chemotherapy with or without oral low dose cyclophosphamide-methotrexate maintenance chemotherapy (CMM).


Background: TILs have been reported to have a prognostic role in patients with triple negative breast cancer (TNBC) receiving AC-containing regimens, but their predictive role has not been demonstrated. IBCSG Trial 22-00 is a randomized, phase III clinical trial, assessing the efficacy of low dose CMM, following standard adjuvant chemotherapy, in hormone receptor negative breast cancer. Patients were randomly assigned to treatment with adjuvant induction chemotherapy plus oral CMM for 1 year, or induction with no further treatment.

Patients and Methods: Of 724 patients enrolled in Trial 22-00 with TNBC confirmed centrally per 2013 St Gallen criteria (<1% ER/PgR, Her2 negative by IHC or FISH), 672 (93%) had blocks/slides available and gave consent. TILs analysis was retrospectively performed on prospectively collected full face H&E slides, following recent TILs Working Group guidelines. The analytic cohort included 647 (96%) of the 672 samples with TILs results available. Endpoints were breast cancer free interval (BCFI) and overall survival (OS). Cox proportional hazards regression models tested for prognostic association of stromal TILs as a continuous (10% increase) or binary (<50% or ≥50 TILs, lymphocyte-predominant breast cancer, LPBC) variable in univariable (UVA) and multivariable analyses (MVA) adjusted for pathological and demographic factors (age, nodal status and tumor size), and for TILs-by-treatment interaction to assess predictive associations in subgroups.

Results: The median follow-up for the cohort was 6.9 (95% CI: 6.6-7.2) years. Median TILs score was 18%, and 119 (18%) patients had ≥50% TILs. Continuous TILs score was significantly associated with improved BCFI and OS in UVA, with an estimated HR for each 10% increase in TILs score accounting for 0.87 (95% CI: 0.79-0.95, p=.003) and 0.83 (95% CI: 0.74-0.92, p<.001), respectively. The MVA showed consistent results, indicating that TILs as a continuous variable was a powerful prognostic factor for BCFI (HR 0.88, 95% CI: 0.8-0.96, p=0.007) and OS (HR 0.84, 95% CI:0.75-0.93, p=.001) for every 10% TILs increase, independent of age, lymph node status and tumor size. When assessed as a binary variable (LPBC vs. non LPBC), the estimated HR for BCFI was 0.56 (95% CI: 0.33-0.94, p=.03) in UVA, and 0.6 (95% CI: 0.36-1.03,p=0.06) in MVA. Patients with higher TILs (LPBC) receiving CMM had breast cancer risk reduction (HR=0.65, 95% CI: 0.22-1.91) when compared with observation, less apparent in patients with <50% TILs (HR=0.94, 95% CI:0.65-1.37), although the TIL-by-treatment interaction was not statistically significant.

Conclusions: TILs are highly prognostic in patients with TNBC treated with different chemotherapy regimens. Interestingly, the risk reduction observed in CMM for patients with higher TILs suggests that TILs may predict clinical benefit from metronomic chemotherapy, a hypothesis deserving further investigation in the lymphocyte subpopulations involved in tumor immunity. The study further demonstrates the need for mandatory collection of tissue blocks from clinical trials.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-08-03

Title: Survival analysis of the prospective randomized Cher-Lob study: Correlation with tumor infiltrating lymphocytes

Dieci MV, Bisagni G, Cagossi K, Generali DG G, Sarti S, Piacentini F, Conte P and Guarneri V. University of Padova, Padova, Italy; Azienda Ospedaliera ASMN, IRCCS, Reggio Emilia, Italy; Division of Medical Oncology, Ramazzini Hospital, Carpi, Italy; U.O. Multidisciplinare di Patologia Mammaria, AO. Istituti Ospitalieri di Cremona, Cremona, Italy; Division of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori IRCCS, Meldola, Italy; University Hospital, Modena, Italy and Medical Oncology 2, Istituto Oncologico Veneto IRCCS, Padova, Italy.

Body: Background: We previously reported the correlation between tumor infiltrating lymphocytes (TIL) at baseline and pathological complete response (pCR) after neoadjuvant chemotherapy plus anti-HER2 agents for HER2-positive patients included in the prospective randomized CherLOB study (Dieci, SABCS 2014). Here we report the survival analysis results.

Methods: The phase II neoadjuvant CherLOB study (Guarneri, J Clin Oncol 2012) randomized 121 HER2-positive, stage II-IIIA breast cancer patients to anthracyclines/taxane-based chemotherapy plus trastuzumab, lapatinib, or both. A part from endocrine therapy for hormone receptor-positive patients, adjuvant treatment was left to physicians’ decision. This included trastuzumab for up to 1 year. Intratumoral (It) and stromal (Str) TIL were centrally evaluated on Hematoxylin and eosin-stained slides from pre-treatment biopsies and post-treatment surgical samples. For the present analysis, follow up was updated as of May 2015 (median follow up 68.3 months). Event-free survival (EFS) was calculated as the time interval from randomization to: recurrence, second primary cancer, death from any cause.

Results: Among the 118 patients evaluable for pathological response, pCR (ypT0/is ypN0) was confirmed as a strong prognostic factor for EFS (HR 0.15, 95% CI 0.04-0.64, p=0.01). Both pre-treatment ItTIL and StrTIL evaluations were available for 105 patients, median values were 5% and 17% (interquartile range 0%-15% and 9%-40%, respectively). Table 1 summarizes the results of the survival analysis for the association of TIL with EFS.

A non-significant trend for better EFS with increasing It-TIL (per 10%) was observed. Patients with ItTIL above the median value had a more prolonged EFS compared to patients with ItTIL below median (5-yrs EFS rate 89% vs 76%), with borderline statistical significance for the comparison between the two groups. No difference according to StrTIL above vs below median value was observed (5-yrs EFS rate 83% vs 82%, respectively). A comparison between lymphocyte predominant (LP) tumors, as defined by the generally accepted cut-off of ItTIL and/or StrTIL >=60%, vs non-LP cases was not performed due to low number of patients (n=17) and events (n=1) in the LP group. Among the 63 patients with residual disease, ItTIL and StrTIL evaluated on the surgical sample were not associated to EFS.

Conclusions: In this low-powered analysis, TIL did not provide significant prognostic information for HER2-positive breast cancer patients treated in the CherLOB study. However, a non-significant trend suggests a positive correlation between increased levels of It-TIL and better EFS. The evaluation of immune gene expression signatures and their correlation with survival is ongoing, results will be available for the meeting.
Title: Prognostic impact of tumor-infiltrating lymphocytes (TIL’s) and stromal-infiltrating lymphocytes (SIL’s) in triple negative breast cancer

Sherwell-Cabello S, Maffuz-Aziz A, Bautista-Piña V, Labastida-Almendaro S, Camacho-Ramírez DA A, Ríos-Luna NP P and Rodríguez-Cuevas S. Breast Disease Institute, FUCAM, Mexico City, Coyoacan, Mexico.

Body: Background. Immune response seems to improve outcome in women with Triple-Negative Breast Cancer (TNBC). Recent data suggests that the presence of Tumor-Infiltrating Lymphocytes (TIL’s) is an independent factor associated with favorable prognosis. In this study, we evaluate the prognostic impact of both the TIL’s and SIL’s in patients with TNBC.

Methods. Data on women diagnosed with triple negative breast cancer between 2005 and 2013, was collected by retrospectively reviewing at Breast Disease Institute, FUCAM. The rate of intratumoral and/or stromal lymphocytes was evaluated in all histopathologic biopsies according to Denkert et al. Lymphocyte infiltrate was assessed in hematoxylin and eosin-stained sections. TIL’s were reported as the percentage of tumor epithelial nests containing infiltrating lymphocytes, while SIL’s as the percentage of stroma area with lymphocytic infiltrate without contact with tumor cells. The five-year disease-free survival (DFS) and overall survival (OS) were compared between groups with the presence or absence of Tumor-Infiltrating Lymphocytes or Stromal-Infiltrating Lymphocytes. Demographic and clinical characteristics were assessed, and the variables with a statistically significant difference between groups were analyzed in a multivariate analysis.

Results. A total of 172 patients with Triple-Negative breast cancer treated this institution were included with a mean age of 49.8 years. A complete absence of tumor lymphocytes was found in 88 patients while the presence of intratumoral, stromal or both was found in 84 (48.8%). A mean follow-up of 46.12 months showed significantly higher rates of both disease-free survival and overall survival in women with SIL’s and TIL’s (p = 0.014 and 0.042 respectively) in locally advanced stages (LAS), regardless the rate of infiltrating lymphocytes found.

<table>
<thead>
<tr>
<th>Overall Survival</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>48.7 ± 7.8</td>
</tr>
<tr>
<td>SIL´s</td>
<td>74.2 ± 6.6</td>
</tr>
<tr>
<td>TIL´s</td>
<td>69.2 ± 7.3</td>
</tr>
</tbody>
</table>

Table 1. Statistical significance was defined as p < 0.05.

In these patients, Stromal Lymphocytes are strongly correlated with a better prognosis compared with Intratumoral Lymphocytes.

Multivariate analysis

<table>
<thead>
<tr>
<th>Variables with statistical significance</th>
<th>β Value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIL´s</td>
<td>4.846</td>
<td>0.028</td>
</tr>
<tr>
<td>TIL´s</td>
<td>2.850</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Table 2. Multivariate analysis. Statistical significance was defined as p = 0.10

Non-significant differences were found in early stages (p = 0.255).

Conclusion. Immunity seems to play a key role regarding the outcome in women with Triple-Negative Breast Cancer. We show
that the presence of SIL's or TIL's are strongly associated with higher rates of disease-free and overall survival in LAS, especially when stromal lymphocytes are found. Independent to the rate of lymphocytic infiltrate, its presence has a statistical significance. Because it is a feasible, reproducible and inexpensive test that can be used as a prognostic predictor, we recommend assessing both SIL’s and TIL’s histopathologic biopsies of patients with TNBC.
Introduction: A high neutrophil-to-lymphocyte ratio (NLR) has been reported to be a poor prognostic indicator in several malignancies including breast cancer. It is unknown whether the prognosis associated with high NLR can be explained by other prognostic factors such as proliferation or estrogen receptor signalling. Here we explore the association between NLR and the 21-gene recurrence score (RS).

Methods: The associations between RS, NLR, tumor size, histologic grade, and estrogen receptor (ER) and progesterone receptor (PgR) expression (assessed by immunohistochemistry) were explored in sequential women with early-stage, lymph node-negative (or with lymph node micrometastases), ER-positive and HER2-negative breast cancer treated at Princess Margaret Cancer Centre in Toronto, Canada and in whom results of the RS were available. NLR was measured prior to surgery. Patients with a documented history of pre-existing infectious/inflammatory condition were excluded. Associations were explored using simple linear regression and statistical significance was defined as p<0.05.

Results: A total of 130 women diagnosed between January 2006 and April 2015 were included in the analysis. Median age was 55 (range 32-79), 87% were lymph node negative and 13% had nodal micrometastases. The median NLR was 2.2 (range 0.9-9.1) and was collected at a median of 12 days prior to surgery (range 0-60). The median RS was 18 (range 0-41). There was no association between RS and NLR (R=-0.10, p=0.31), grade (R=0.13, p=0.15), age (R=-0.05, p=0.58) or tumor size (R=0.06, p=0.48). RS was negatively associated with the magnitude of expression of both ER (R=-0.22, p=0.01) and PgR (R=-0.44, p<0.001). There was no association between NLR and grade (R=0.20, p=0.15), age (R=-0.13, p=0.17), tumor size (R=0.14, p=0.93), ER (R=0.01, p=0.94) or PgR (R=0.13, p=0.23)

Conclusion: The poor outcomes associated with high NLR are unlikely explained by proliferation of estrogen receptor signalling.
Title: Usefulness of the pre-treatment neutrophil-to-lymphocyte ratio in predicting first-line progression free-survival in triple-negative breast cancer patients


Body: Background: The neutrophil-to-lymphocyte ratio (NLR) is an independent predictor of poor prognosis in unselected breast cancer patients with NLR >3.3. Moreover, pre-treatment NLR has been associated with disease-free and overall survival (OS) in patients with early triple-negative breast cancer (TNBC). We aimed to determine whether the NLR is predictive of progression-free survival (PFS) in metastatic TNBC.

Methods: We reviewed the records of 48 TNBC patients who received at least one administration of first-line (1stL) chemotherapy for advanced disease from October 2004 to April 2014. The NLR (absolute neutrophil count/absolute lymphocyte count) was calculated from the full blood count routinely performed immediately before the initiation of first-line treatment. The association between categorical variables was calculated by X2 test. PFS (from start of 1stL treatment to disease progression or death) and OS (from start of 1stL treatment to death) were estimated using Kaplan Meier method. Multivariable Cox regression was used to determine the independent prognostic significances of the NLR (co-variables stage at diagnosis, histology, and tumor grade).

Results: NLR was not associated with stage at diagnosis (p=0.214), histology (p=0.597), or tumor grade (p=0.775). After a median follow-up of 10.9 months (range 1.3-54.9), 88.6% of TNBC patients with NLR≤3.3 versus 0.0% of patients with NLR>3.3 had a 1stL PFS>3 months (p<0.001). Similarly, 62.9% of TNBC patients with NLR≤3.3 versus 30.8% of patients with NLR>3.3 had an OS>10 months (p=0.047). Metastatic TNBC patients with NLR≤3.3 had a longer median 1stL PFS (5.2 months) and median OS (13.5 months) compared with patients with NLR>3.3 (1stL PFS 2.1 months, p<0.001; OS 7.7 months, p=0.018). In multivariable analysis, NLR>3.3 is associated with a shorter PFS (hazard ratio [HR] 22.4; 95% confidence interval [CI] 6.7-75.1, p<0.001) and higher risk of death (HR 3.2, 95%CI 1.4-7.4, p=0.005).

Conclusion: Our study showed that pre-treatment NLR is associated with 1stL PFS and OS in patients with metastatic TNBC. However, further investigation in larger series of metastatic TNBC is warranted.
Title: Prognostic significance of immune checkpoint receptors in node-negative breast cancer

University Hospital, Mainz, Germany; Technical University, Dortmund, Germany and Leibniz Research Centre for Working Environment and Human Factors (IfADo) at Technical University, Dortmund, Germany.

Body: Background: Immune checkpoint blockade is increasingly discussed in breast cancer. We examined the prognostic significance of the immune checkpoint receptors cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) in node-negative breast cancer.

Methods: Microarray based gene-expression data for CTLA-4 (221331_x_at) and PD-1 (207634_at) were analysed in four previously published cohorts (Mainz, Rotterdam, Transbig, Yu) of node-negative breast cancer patients not treated with adjuvant therapy (n=824). A meta-analysis of previously published cohorts was performed using a random effects model. Prognostic significance of CTLA-4 as well as PD-1 for metastasis-free survival (MFS) was examined in the whole cohort and in different molecular subtypes: luminal (ER+/HER2-), basal-like (ER-/HER2-) and HER2+. Independent prognostic relevance was analysed using multivariate Cox regression.

Results: Higher RNA expression of CTLA-4 was related to better MFS in a meta-analysis of the whole cohort (HR 0.58, 95% CI 0.38-0.88, P=0.0102). Prognostic significance was most pronounced in the HER2+ positive molecular subtype (HR 0.23, 95% CI 0.08-0.65, P=0.0062) as compared to luminal (HR 0.68, 95% CI 0.39-1.18, P=0.1744) and basal-like (HR 0.53, 95% CI 0.25-1.15, P=0.1087) carcinomas of the breast. PD-1 RNA expression, however, was not associated with outcome in the whole cohort of patients (HR 0.88, 95% CI 0.32-2.43, P=0.1853). A trend for improved survival was noticed in basal-like breast cancer (HR 0.40, 95% CI 0.15-1.08, P=0.0701). Neither luminal (HR 0.81, 95% CI 0.28-2.36, P=0.2122) nor HER2+ (HR 0.85, 95% CI 0.27-2.68, P=0.7759) patients showed an association of PD-1 with MFS. CTLA-4 showed independent prognostic significance (HR 0.393, 95% CI 0.224-0.688, P=0.001) in multivariate analysis. In addition to CTLA-4, only histological grade of differentiation (HR 2.335, 95% CI 1.490-3.660, P<0.0001) and tumor size (HR 1.924, 95% CI 1.260-2.937, P=0.002), but neither PD-1 nor age nor HER2 status nor hormone receptor status retained an independent prognostic association with MFS.

Conclusions: The immune checkpoint receptor CTLA-4 has independent prognostic significance in node-negative breast cancer. Higher expression of CTLA-4 is associated with improved outcome. The prognostic impact of CTLA-4 differs between molecular subtypes and is most pronounced in HER2+ breast cancer.
Body: Background: The European Pooled Analysis of CTC (EPAC) in metastatic breast cancer, based on 1,944 individual data from patients with various tumor types and clinical settings (Bidard et al, Lancet Oncol 2014), has established CTC count (CellSearch) at baseline and during therapy as a level of evidence 1 independent prognostic biomarker and demonstrated its superiority over serum blood markers. As part of the study pre-planned objectives, we sought to establish nomograms allowing accurate individual survival predictions.

Methods: Using individual data from 17 centers, we built simplified multivariate prognostic models taking into account the independent prognostic clinico-pathological (CP) characteristics including CTC count, dichotomized using the 5CTC/7.5ml threshold, at baseline and at 3-5 weeks after the start of a new treatment regimen, and derived nomograms for progression-free survival (PFS) and overall survival (OS) prediction at baseline and after 3-5 weeks of treatment. We report here the internal validation of these nomograms. Discrimination of the models was assessed using the c-index estimated by a jackknife procedure and the calibration was visually assessed through 10-fold crossvalidated calibration plots at 1,2,3 years for OS and 1,2 years for PFS.

Results: Multivariate models at baseline for PFS and OS were fitted on 1501 and 568 individual patient data with CTC count at baseline and CTC count at baseline and after 3-5 weeks, respectively. Models include tumor subtype, the number of previous chemotherapy lines (0/1/>2), PS, age (<=50/>50-65/>65 years), metastasis-free intervals (0/>0-3/>3 years), metastatic sites (liver and CNS) and CTC count at baseline and eventually at 3-5 weeks of treatment. The C-index increased from 0.722 to 0.755 (increase in C-index:0.033, 95% CI [0.019;0.045]) when adding baseline CTC to the CP only model for OS (n=1501). For those patients with CTC values at 3-5 weeks (n=568), there was an additional increase in the C-index when adding CTC at 3-5 weeks to a model with already CP and baseline CTC from 0.731 to 0.743 (increase in C-index 0.013, 95% CI [-0.004;0.025]). The model with CP and baseline CTC counts showed a good calibration for OS at 1,2,3 years and the model with CP, baseline CTC and CTC count at 3-5 years a moderately good calibration. Similar results were obtained for PFS.

Conclusion: From the largest database with individual CTC data, we were able to build PFS and OS survival nomograms, with satisfactory discrimination and calibration. Our planned next step is to validate the nomogram in an additional cohort.
Title: Prognostic values of circulating tumor cell (CTC) enumeration and their clusters in advanced breast cancer

Ye Z, Mu Z, Wang C, Palazzo JP P, Biederman L, Li B, Jaslow R, Avery T, Austin L, Yang H and Cristofanilli M. Division of Population Science, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; Division of Solid Tumor Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA and Vanderbilt University, Nashville, TN.

Body: Background The enumeration of circulating tumor cells (CTCs) has been proven to have prognostic values in several solid tumors including breast cancer. It has been established that a cut-off of 5 CTCs in 7.5 ml of blood may significantly differentiate breast cancer patients with favorable and unfavorable survival. However, CTC enumeration has not been shown to further predict the prognosis in those patients with more than 5 CTCs in 7.5 ml of blood. There are several recent in vitro and in vivo studies suggesting that clusters of CTC can be identified in blood and those clusters may play an important role in tumor progression and metastasis. Few clinical studies have been reported to enumerate CTC clusters and evaluate their prognostic values. In the current study, we hypothesize that the enumeration of CTC clusters play an important role in the prognostication of advanced breast cancer patients by providing additional predictive performance independent of CTC enumeration.

Methods In an ongoing study of blood-based breast cancer biomarkers, we enrolled 114 patients with stages III and IV breast cancer. Among them, 68 patients had inflammatory breast cancer (IBC), an extremely aggressive form of breast cancer with a much lower survival rate than non-IBC breast cancer patients. The number of single CTCs and CTC clusters (two or more CTCs bound together) in 7.5 ml blood sample were counted using the CellSearch™ system (Janssen Diagnostic) at baseline study entry, and their associations with the progression-free survival (PFS) of patients were evaluated using Kaplan Meier curves and Cox proportional hazards modeling.

Results Baseline CTCs were detected in 67 (58.77%) patients. Thirty-five (30.70%) and 19 patients (16.67%) had elevated CTCs (≥5 CTCs/7.5 mL) and clusters, respectively. IBC patients had a slightly higher percentage of cluster (17.65%) compared to non-IBC patients (15.22%). Compared to patients with < 5 CTC and without cluster, those patients with elevated CTC without cluster, and those with elevated CTC with cluster had an increasingly higher risk of disease progression with an hazard ratio [HR] of 1.93 (95% confidence interval [CI] 1.01-3.67) and 2.91 (1.54-5.50), respectively (P for trend = 0.001). Moreover, the combined analysis of baseline CTC and cluster enumerations showed similar effect when the analysis was restricted to IBC patients (HR 3.03, 95% CI 1.34-6.86).

Conclusion Baseline enumerations of both individual CTCs and CTC clusters predict PFS in advanced stage breast cancer patients. CTC clusters provide further prognostic value in patients with elevated CTC and their molecular characterizations may provide novel insights into the metastasis process.
**Title:** Validation of prediction of local recurrence (LR) by Prosigna® (PAM50) in a Danish breast cancer cooperative group (DBCG) cohort of hormone receptor positive (HR+), postmenopausal early breast cancer (EBC) patients allocated to 5yr of endocrine therapy (ET)

Ole Eriksen J, Jensen M-B, Laenkholm A-V, Kibøll T, Bruun Rasmussen B, Knoop AS S, Ferree S, Haffner T, Buckingham W, Schaper C and Ejlertsen B. Region Zealand, Denmark; The Danish Breast Cancer Cooperative Group, DBCG Secretariat, Rigshospitalet, Copenhagen, Denmark; Herlev Hospital, Herlev, Denmark; Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark and NanoString Technologies, Inc., Seattle, WA.

**Body: Background:** HR+EBC patients are routinely treated with both adjuvant radiation therapy (RT) and ET. RT is considered an important tool for achieving local control of disease. A limited number of biomarkers have been demonstrated to predict LR. In a previously performed retrospective analysis of a randomized trial, Prosigna (PAM50) risk of recurrence (ROR) score identified low risk patients with a local recurrence rate of 1.6% at 9.5yr median follow-up. In this study, we seek to validate the ability of ROR to predict LR in a comprehensive nationwide cohort from Denmark. **Methods:** Using the population based DBCG database primary FFPE tumor blocks and follow-up data were collected from all postmenopausal Danish women diagnosed from 2000-2003 with HR+EBC (N=2,722). Prosigna (PAM50) on the NanoString nCounter® Dx Analysis System assigned each patient an ROR score and associated risk group based on pre-specified cutoffs. Patients are also assigned an intrinsic subtype (Luminal A, Luminal B, Her2-Enriched, Basal-Like) based on gene expression. Univariate and multivariate analyses were performed to assess the ability of Prosigna (PAM50) to predict LR. **Results:** 48 local recurrences were observed with median follow-up of 9.25 yr. Continuous ROR was significantly associated with LR in univariate and multivariate models including node status (0, 1, 2, or 3 positive nodes), tumor size (≤2 vs.>2cm), grade (I, II, III, or unknown), age (≤65 vs.>65yr), and local treatment (mastectomy (MX), lumpectomy+RT, or MX+RT) (p=0.036 and p=0.049, respectively). Clinicopathologic variables were not significant in the multivariate model alone or in combination (p=0.85 for full model excluding ROR). Utilization of a pre-specified LR cutoff, hazard ratio (HR) and [95%CI, p-value] for high risk vs. low risk patients in a univariate analysis was 1.96 [1.11-3.46, p=0.0205] and 2.04 [1.08-3.83, p=0.0275] in the multivariate analysis. 10-year cumulative incidence of LR for low risk patients was 1.7% [1.1%-2.6%]. Similarly, 10-year cumulative incidence of LR for Luminal A patients was 1.7% [1.1%-2.6%]. 10-year cumulative incidence for high risk patients was 2.3% [1.3%-3.2%]. **Conclusions:** In a large population-based study of n=2,722 patients, Prosigna (PAM50) predicted LR over standard variables. These data validate a pre-specified cutoff separating patients at high and low risk of LR. Additional studies of Prosigna (PAM50) in RT-untreated populations are ongoing.
Title: Abstract Withdrawn
Title: Integration of tumor size and grade with the breast cancer index (BCI) for prediction of distant recurrence in hormone receptor-positive breast cancer with 1-3 positive lymph nodes

Sestak I, Zhang Y, Schroeder B, Dowsett M, Sgroi D, Cuzick J and Schnabel CA A. Centre for Cancer Prevention, Queen Mary University, London, United Kingdom; BioTheranostics, Inc., San Diego, CA; Royal Marsden Hospital, London, United Kingdom and Massachusetts General Hospital, Boston.

Body: Background: BCI is a genomic signature (Molecular Grade Index (MGI) and HOXB13/IL17BR (H/I)) that significantly predicts risk of distant recurrence (DR) in hormonal receptor-positive, lymph node negative (LN-) breast cancer. As previously shown in the TransATAC and MA.14 studies, BCI was also prognostic for DR in lymph node-positive (LN+) patients. Here, a distinct BCI model that integrates tumor size and grade was evaluated for prediction of DR in women with 1-3 lymph node positive disease.

Methods: 219 primary tumor samples from hormonal receptor-positive patients with 1-3 positive lymph nodes treated with 5 years of tamoxifen or anastrozole were examined. Women with four or more positive lymph nodes were excluded. BCI was combined with tumour size and grade into a comprehensive risk score, BCIN+. Kaplan-Meier (KM) estimates of 10 year DR and hazard ratios (HR) and 95% confidence intervals (CI) were estimated. Change in likelihood ratio 2 (LR-2) values were used to measure prognostic information of each variable alone or combined in new score. New cutpoints for low versus high risk groups for the new model were determined to ensure the low risk patients had minimal 10-year residual disease.

Results: In 219 LN+ patients, BCI alone provided substantial additional prognostic information to tumor size (LR-2=11.83, P=0.0006) and grade (LR-2=8.33, P=0.004). Both clinical variables provided additional significant prognostic information to BCI alone (BCI alone: LR-2=9.59, P=0.0004; T: LR-2=7.09, P=0.008; G: LR-2=27.59, P<0.0001). Integration of tumor size and grade with BCI (BCIN+) provided additional highly significant prognostic information compared to BCI alone (Interquartile HR=3.15 [95% CI: 1.54-6.04]; LR-2=33.89, P<0.0001). A cut-point for a very low risk group in this LN+ population was determined, and included 55 (25%) women with no DR within 10 years. In contrast, 51 (31.1%) women developed a DR in the high risk group (N=164). 10-year DR risk for those in the high risk group was 35.4% (95% CI 28.0-44.1%).

Discussion: Integration of tumor size and grade significantly enhanced the prognostic ability of BCI to predict 10 year DR risk in hormonal receptor-positive patients with 1-3 positive nodes. A significant number of patients have been identified to have a very low 10-year risk for DR, who may choose to safely forego unnecessary adjuvant chemotherapy or extended adjuvant endocrine therapy. Validation of BCIN+ in other datasets is ongoing.
Title: Integrating multiplex and next generation sequencing (NGS) platforms in routine molecular profiling of metastatic breast cancer (MBC) patients (pts): Trends for enrollment in genotype-directed clinical trials (GDTs)

Body: Background/aims: Multiplex or NGS platforms increase the number of mutations (mut) detected in tumor samples respect to single-gene sequencing techniques. We aimed to assess the actionable molecular alteration (ActMA) detection rate and the enrollment in GDTs derived from the integration of these platforms in routine molecular profiling of MBC pts, in addition to FISH and IHC techniques already in use. Methods: Consecutive MBC pts screened for gene mut by Sequenom (Seq) or AmpliconSeq (ASeq) were identified. Data on FGFR1 amplification (amp), PTEN IHC, and enrollment in GDTs were collected. ActMA: any mut, PTENnull (IHC score=0), or FGFR1/HER2amp for which a matched targeted drug might be available. Targeted therapy: treatment with PI3K/mTOR, novel anti-HER2, FGFR, or AKT inhibitors (inh) irrespective of having ActMA. GDT: treatment matched to ActMA. Results: From Oct2010-Apr2015, 260 pts screened (Seq 207, ASeq 53). IHC subtype: HR+/HER2- (LUM) 65%, HER2+ 13.5%, TN 19.6%, unk 1.9%. 84 samples from a metastatic site (32.3%).

<table>
<thead>
<tr>
<th>ActMA / n (%):</th>
<th>LUM</th>
<th>HER2+</th>
<th>TN</th>
<th>P value (Fisher's exact test)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53*</td>
<td>11  (31.4)</td>
<td>1   (50)</td>
<td>9  (52.9)</td>
<td>0.34</td>
<td>21 (38.9)</td>
</tr>
<tr>
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<td>10  (28.6)</td>
<td>4  (7.8)</td>
<td>0.1</td>
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<tr>
<td>FGFR1amp</td>
<td>21  (17.4)</td>
<td>1   (5.3)</td>
<td>6  (16.7)</td>
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<td>28 (15.9)</td>
</tr>
<tr>
<td>PTENnull</td>
<td>12  (9.3)</td>
<td>2   (7.7)</td>
<td>9  (25)</td>
<td>0.03</td>
<td>23 (12)</td>
</tr>
<tr>
<td>AKT1</td>
<td>10  (5.9)</td>
<td>1   (2.9)</td>
<td>-</td>
<td>0.15</td>
<td>11 (4.3)</td>
</tr>
<tr>
<td>ERBB2</td>
<td>3   (1.8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>EGFR</td>
<td>1   (0.6)</td>
<td>-</td>
<td>2  (4.1)</td>
<td>-</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>ESR1*</td>
<td>1   (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (2)</td>
</tr>
<tr>
<td>KRAS</td>
<td>2   (1.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (0.8)</td>
</tr>
</tbody>
</table>

Denominators vary according to platform. *Amplicon only

Proportion of PIK3CAmut was similar irrespective of the site of analysis (primary 25.5%, metastasis 21.4%; P=0.63) and platform (Seq 22.2%, ASeq 24.5%, P=0.72). ASeq detected more mutations in actionable genes than Seq (36% vs. 29%, P=0.01). At least 1 ActMA (range 0-3) was found in 53.5% of pts, with non-significant differences in HER2- subtypes (LUM 48.5% vs. TN 39.2%, P=0.32).

<table>
<thead>
<tr>
<th>Subtype* / ActMA n (%):</th>
<th>≥1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>All</td>
<td>139 (53.5)</td>
<td>121 (46.5)</td>
<td>111 (42.7)</td>
<td>25 (9.6)</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>LUM</td>
<td>82 (48.5)</td>
<td>87 (51.5)</td>
<td>71 (42)</td>
<td>9 (5.3)</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>HER2+</td>
<td>35 (100)</td>
<td>-</td>
<td>22 (62.9)</td>
<td>12 (34.3)</td>
<td>1 (28)</td>
</tr>
<tr>
<td>TN</td>
<td>20 (39.2)</td>
<td>31 (60.8)</td>
<td>16 (31.4)</td>
<td>4 (7.8)</td>
<td>-</td>
</tr>
</tbody>
</table>

*5 pts with unk subtype not shown
Pts with ≥2 ActMA (excluding HER2amp): 11 LUM (interestingly, 3 pts with PIK3CAmut+FGFR1amp), 1 HER2+, and 4 TN. Overall, 56% of pts received ≥1 targeted therapy (range 0-4). From the 139 pts with ≥1 potential ActMA (including HER2amp if treated with a novel anti-HER2), 61.8% received a targeted therapy and 42.4% were enrolled in a GDT: PI3K/mTOR inhibitor (inh) 54 (64.3%), novel anti-HER2 16 (19.1%), FGFR inh 8 (9.5%), AKT inh 6 (7.1%). Of the 121 pts that did not have potentially ActMA, 50% received a targeted therapy. The OR for receiving targeted therapy if ActMA was present was 1.59 (95% CI 0.94-2.70, P=0.08). **Conclusion:** Integration of multiplex and NGS platforms in routine molecular profiling of MBC pts yields a detection rate of ActMA >50%, which translates into higher probability of receiving a targeted agent and enrollment in a GDT. This suggests that physicians are pushing towards matched targeted therapies for pts that participate in molecular screening programs and have ActMA. Results on the outcome of these pts will be presented.
Purpose
We have identified 34 genes of interest that can distinguish post-mastectomy cancer patients into high- and low-risk of locoregional recurrence (LRR) in 2006. In this study, we validate the performance of 18 of 34 genes in prediction of LRR after mastectomy and breast conserving surgery (BCS).

Materials and methods
A total of 124 breast cancer patients who underwent mastectomy from 2005 to 2012 and had DNA microarray study on the primary tumor tissues were chosen for this study. Eligible patients should have no post-mastectomy radiotherapy (PMRT) with a minimum of 2-year follow-up. Among the 34 genes in previous report, only 30 genes could be found in Affymetrix U95 to U133 Plus2.0 array. Eighteen of 30 genes (18-Gene classifier) were used to distinguish low-risk and high-risk patients. Patients with 18-Gene score 31 or more were defined as high-risk and score less than 31 defined as low-risk group. We then examined the performance of the 18-Gene classifier in breast conserving patients (n=87) who usually had adjuvant radiotherapy (94%) and were treated in the same study periods.

Results
The overall accuracy of the prediction of LRR is 87% (sensitivity 91% and specificity 87%). By the 18-Gene classifier, the 5-year LRR rate in high-risk group was 42%, and in low-risk group <1% (p < 0.0001).

LRR and 18-gene score in non-PMRT patients

<table>
<thead>
<tr>
<th>18-Gene score</th>
<th>No LRR #</th>
<th>LRR #</th>
<th>5-year LR control probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score &lt;31</td>
<td>97</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Score &gt;= 31</td>
<td>15</td>
<td>11</td>
<td>57.7%</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

LRR: Locoregional recurrence

Multivariate analysis of mastectomy patients revealed that 18-Gene score is the independent prognostic factor that predicts the likelihood of LRR regardless of nodal status and breast cancer subtype. The predictive ability of LRR by the 18-Gene classifier in BCS patients is independent from adjuvant radiotherapy, and is consistent with the performance in mastectomy patients.

Multivariate analysis in BCS patients

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Patient #</th>
<th>LRR #</th>
<th>LRR rate</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent/focal</td>
<td>73</td>
<td>4</td>
<td>5.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prominent</td>
<td>14</td>
<td>3</td>
<td>21.4%</td>
<td>4.3(1.0,19.3)</td>
<td>5.1*(1.1,23.1)</td>
</tr>
<tr>
<td>18-Gene score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>66</td>
<td>2</td>
<td>3.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>21</td>
<td>5</td>
<td>23.8%</td>
<td>9.4*(1.8,48.4)</td>
<td>10.4*(2.0,54.2)</td>
</tr>
<tr>
<td>Adjuvant R/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>2</td>
<td>40%</td>
<td>10.3*(2.0,53.9)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>82</td>
<td>5</td>
<td>6.1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
LVI: lymphovascular invasion; R/T: radiotherapy; *p<0.05

Discussion
This 18-Gene classifier is capable of identifying high-risk patients of LRR after mastectomy and breast conserving surgery.
Title: Impact of OncotypeDX on treatment decision for early stage, hormone-receptor-positive breast cancer

Anastasiadou L, Schneider M, Barinoff J, Khandan F, Falk S and Thill M. Agaplesion Markus Krankenhaus, Frankfurt am Main, Germany and OptiPath Gemeinschaftspraxis für Pathologie, Frankfurt am Main, Germany.

Body: BACKGROUND and OBJECTIVE
The OncotypeDX multigene assay analyzes the individual biology of an invasive breast cancer by examining the activity of 16 breast cancer related genes and 5 reference genes in the tumor tissue. It is used in patients with early hormone-receptor-positive breast cancer with up to 3 positive lymphnodes. The results of the analysis are fed into a formula that calculates a Recurrence Score® (RS) result. The RS result is a number between 0 and 100, and can provide information about how likely your breast cancer is to recur within 10 years of diagnosis. Since becoming available, the test is successfully validated in 18 studies for both N0 and N1.

The primary study objective was to evaluate the impact of the RS on physicians’ adjuvant therapy decision-making in a cohort of consecutive patients with early ER+, HER2-negative, N0/N1 (until two positive lymph-nodes) breast cancer in our multidisciplinary team (MDT). Secondary study objectives were to assess the impact of the RS on patients’ decisional conflict.

PATIENTS and METHODS
This prospective clinical study was carried out in the Breast Cancer Center at the AGAPLESION Markus Hospital. 140/890 (15.7%) patients were enrolled between 02/2013 and 04/2015 with early ER+, HER2-negative (IHC or FISH), N0/N1, T1-3, histological grade 1-3. We kept records from the treatment decision before and after having the result of OncotypeDX. Further inclusion criteria were age >18 years and no contraindication for receiving systemic chemoendocrine therapy. Also, the treating physician and the enrolled patient had to confirm that she was principally willing to consider her initial recommendation and decision.

Each patient's case was discussed twice within the respective institution's MDT. In the first meeting, adjuvant treatment was recommended based on available clinical and histopathological data according to the guidelines. In a second meeting, the case was rediscussed and treatment was recommended with the RS result as an additional piece of information.

RESULTS
100/140 (71.4%) patients were node negative (N0), whereas 40/140 (28.6%) were node positive (N+). Treatment recommendation changed in overall 34.3% of the cases (N0 33%, N+ 35%). In 48% of all patients (N0 43.5%, N+ 52%) with an initial recommendation for chemoendocrine therapy, the post-RS recommendation changed into endocrine therapy, in 25% (N0 30%, N+ 6.7%) of the cases with an initial recommendation for endocrine therapy only to combined chemoendocrine therapy, respectively. Only 48% of the patients with an initial endocrine therapy recommendation followed post-RS recommendation change to chemoendocrine therapy. On the other hand, 100% of the patients with an initial chemoendocrine therapy recommendation followed the post-RS recommendation change to endocrine therapy.

CONCLUSION
Our results show that OncotypeDX has an impact on the initial treatment decision on node positive as well as node negative patients. A substantial therapy change was evident in 34.3% of the cases. The data confirm, that OncotypeDX can help the doctor to formulate an individually treatment plan for patients with conflicting combination of clinical and pathological parameters.
Title: Prognostic and predictive abilities of intrinsic subtype in hormone receptor-positive metastatic breast cancer from the EGF30008 phase III clinical trial


Body: Background
Combination of letrozole and lapatinib improved progression-free survival (PFS) compared with letrozole and placebo in patients with hormone receptor-positive (HR+)/HER2+ metastatic breast cancer (MBC), but not HR+/HER2-negative (HER2-) disease (JCO 2009). However, HR+ disease is clinically and biologically heterogeneous with all intrinsic molecular subtypes (Luminal A, Luminal B, HER2-enriched [HER2E] and Basal-like) identified. Here, we tested retrospectively the prognostic and predictive ability of intrinsic subtype in tumor samples of the EGF30008 trial.

Methods
Expression profiling from FFPE tumor tissues was performed on the nCounter platform. Tumors were classified into each intrinsic subtype using the research-based PAM50 classifier (JCO 2009). Cox proportional hazard models for PFS and overall survival (OS) were used to generate point estimates of hazard ratios (HR) and corresponding 95% confidence intervals (CIs). Changes in likelihood ratio $\chi^2$ values were used to measure and compare the relative amount of information of each variable. Variables evaluated were: age, prior endocrine therapy, presence of visceral disease, number of metastatic sites, performance status, clinical HER2 status, and treatment. To determine whether the intrinsic subtypes were predictive of lapatinib benefit, we tested the interaction term of subtype by treatment arm in a Cox model that also included the main effects. Kaplan-Meier plots were used to depict the proportion of patients free from progression as a function of time.

Results
Tumor samples from 821 patients (63.8%) were profiled (85.7% primary and 14.3% metastatic tumor samples). Clinical-pathological features of this patient subset were well balanced compared with the original set. Within the entire cohort, all subtypes were identified: Luminal A (46.5%); Luminal B (29.7%); HER2E (7.4%); Basal-like (3.4%) and normal-like (12.9%). Within HER2+ disease, 28.6% of samples were HER2E. Intrinsic subtype was found the strongest prognostic factor independently associated with PFS and OS in all patients, and in patients with HER2-negative or HER2+ disease ($P<0.0001$). Median PFS and OS for each subtype within clinically HER2-negative disease were: Luminal A (16.85 and 45.0 months), Luminal B (10.97 and 37.0 months), HER2E (4.67 and 16.0 months) and Basal-like (4.14 and 23.0 months). Within clinically HER2-negative disease ($n=644$), 16 patients (2.5%) had HER2E disease. Patients with HER2-/HER2E disease benefited from lapatinib (6.5 vs 2.6 months; PFS HR =0.24, 95% CI: 0.07-0.86; $P=0.019$; HER2E vs not treatment interaction $P=0.016$). Finally, intrinsic subtype was not predictive of benefit from lapatinib within HER2+ disease.

Conclusions
HR-positive disease is biologically heterogeneous and intrinsic subtypes are strongly prognostic in a first-line MBC setting. HR+/HER2- disease with a HER2E profile may benefit from lapatinib. The clinical value of intrinsic subtyping in HR+ MBC warrants further investigation, but patients with Luminal A/HER2-negative MBC disease might be good candidates for letrozole monotherapy in the first-line setting regardless of visceral disease and number of metastases.
Title: Prognostic impact of breast cancer subtypes in elderly patients

Bergen ES S, Tichy C, Berghoff AS S, Rudas M, Dubsky P, Bago-Horvath Z, Mader RM M, Gnant M, Dieckmann K, Zielinski CC C, Steger GG G, Preusser M and Bartsch R. Comprehensive Cancer Center Vienna, Austria; Medical University of Vienna, Austria; Medical University of Vienna, Austria and Medical University of Vienna, Austria.

Body: Background
We aimed to analyze the impact of BC subtypes on the clinical course with special emphasis on the occurrence of brain metastases (BM) and outcome in an elderly breast cancer population.

Patients and Methods
571 patients ≥65 years receiving treatment for BC from 2007-2011 were identified from a BC database. BC subtypes and clinical characteristics including overall survival (OS) were obtained by chart review. Statistical analysis was performed using the Chi Square test, the log rank test and time depended covariate cox regression model as appropriate.

Results
Three-hundred-eighty/571 (63%) were grouped among the young-old (65-74 years), 182/571 (31.9%) among the old-old (75-84 years), and 29/571 (5.1%) among the oldest-old (≥85 years). 392/571 (68.8%) patients presented with luminal BC, 119/571 (20.8%) with HER2 positive and 59/571 (10.3%) with triple negative BC. After a median follow up of 38 months (range 0-204), 115/571 (20.1%) patients presented with metastatic recurrence. Highest recurrence rate was observed in HER2 positive BC patients (43/119 (36.1%)), followed by triple negative (15/59 (25.4%) and luminal BC (57/392 (14.5%); p<0.001; Chi Square test). BM occurred significantly more frequently in HER2 positive BC patients (9/119 (7.6%) compared to triple negative (2/59 (3.4%)) and luminal BC patients (6/392 (1.5%); p=0.003; Chi Square test). Occurrence of metastases (HR 7.7; 95% CI 5.2-11.4; p<0.001) as well as development of BM (HR 3.5; 95% CI 1.9-6.4; p<0.001) had a significant impact on OS prognosis as entered in a time depended covariate cox regression model.

Conclusions
In contrast to younger BC patients, HER2 positive BC subtype and not triple negative BC subtype was linked to the most aggressive clinical course including the development of metastatic disease and BM in our elderly cohort.
Body: Background: The genotype and phenotype of breast cancer may change during disease progression. But technical issues may affect biomarker assessment when comparing primary tumor tissues with biopsies of metastasis at distant sites. As the exact determination of molecular subtype in primary tumors for prevention of distant metastasis is of the utmost importance in clinical decision making, we compared RNA expression levels of ESR1, PGR, ERBB2 and MKI67 in pairs of PBC and MBC tissue using RT-qPCR and evaluated our results against conventional immunohistochemistry (IHC).

Methods: The tumor bank of a single institution was screened for paraffin-embedded pairs of PBC and MBC tissue samples and a total of 34 matched PBC and MBC pairs.

RNA was extracted using a bead-based extraction method (RNXtract® IVD kits, BioNTech Diagnostics GmbH). Multiplex RT-qPCR was performed using TaqMan® based primer probe sets for ESR1, PGR, ERBB2 and MKI67 (MammaTyper® IVD kits, BioNTech Diagnostics GmbH). Results were compared with IHC analysis of ER (clone 1D5), PR (clone PgR636) and HER2 (A0485) in both primary and metastatic tissue. Associated survival data will be presented at the meeting.

Results: Samples from 34 patients with MBC and PBC were available. Positivity of PBC for ER, PR & HER2 IHC was 71%, 76% and 7%, while positivity for RT-qPCR for ESR1, PGR and ERBB2 was 78%, 70% and 3%. The overall agreement between matched primary and metastatic lesions by IHC was 80%, 60% and 100% by IHC and 90%, 70% and 100% for RT-qPCR of ESR1, PGR and ERBB2. When comparing PBC with MBC the NPA was substantially lower for IHC (ER 56%, PR 50% and HER2 100%) than for RT-qPCR (ESR1 100%, PGR 100% and ERBB2 100%). Strikingly, there were some shifts from negative to positive for IHC based ER/PR determination, and from positive to negative for RT-qPCR, when comparing differences between PBC and matched MBC. By IHC several primary "non-luminal tumors" turned into metastatic "luminal" tumors exhibiting hormone receptor expression, while no such case was observed for RT-qPCR determination.

Conclusion: Overall concordance between PBC and MBC is high, particularly when tested by RT-qPCR. As shifts from non-luminal to luminal and aggressive to less aggressive subtypes does not seem to reflect the more aggressive nature of metastatic lesions, the RT-qPCR based methods seem to be more reliable. Metastatic tissue should therefore be reevaluated with regard to markers relevant to treatment such as ESR1 and ERBB2; preferably by standardized RT-qPCR methods. Validation of these findings in an independent cohort of similar size is ongoing and will be presented at the meeting.
Title: Prognostication of HER family gene expression collaborate with ESR1 expression in patients with triple negative breast cancer


Body: Background: Triple-negative breast cancer (TNBC) consists of heterogeneous sub-population. Although many investigators made an effort to categorize and classify TNBCs using genetic expressions, it is still needed to be defined for prognostication. Traditionally, HER family genes have been known to contribute mammalian glands formation and breast cancer generation as well as ESR gene. Moreover, target agents for HER family genes have been already developed. Accordingly, we investigated the expression profiles of HER family genes with ESR in patients with TNBC to categorize into sub-types and determine the prognostic value of HER family genes in search of clinical implications.

Methods: We investigated the results of the nCounter expression assay (NanoString®) for ERBB1, ERBB2, ERBB3, ERBB4 and ESR1 using mRNA extract from paraffin-embedded tumor tissues in 203 patients diagnosed as TNBC. We used the results of nCounter expression assay using 84 TNBC tissues for validation and 52 breast cancer tissues diagnosed as other subtypes to control the expression assay results of these five genes.

Results: Two-hundred and three patients were diagnosed as TNBC from 2000 to 2004 and received adjuvant chemotherapy after curative surgery. Eighty-four TNBC patients for validation set and 52 patients diagnosed as other subtypes for control set were selected from the patients diagnosed as breast cancer from 2005 to 2010 and received curative surgery. Through analyzing 5 genes using the nCounter expression profiles from 203 TNBC tissues, we found that increased expression of ERBB4 was associated with poor prognosis by survival analysis (5 year disease recurrence free survival (DRFS), low vs. high expression [cut-off: median]: 90.1% vs. 80.2%; p = .002). This trend was still remained in validation set composed of TNBC (5 year DRFS, low vs. high expression [cut-off : median]: 61.1% vs. 44.0%), whereas was not observed in other subtypes of breast cancer (44.4% vs. 80.8%). The Kaplan-Meier estimates of the rates of 5 year DRFS in the subgroups classified according to the level of 5 genes expression showed that the group of higher expression of all HER family genes and lower expression of ESR1 gene had dismal prognosis rather than other groups in patients with TNBC (5 year DRFS, this group vs. others: 50.0% vs. 88.2%; p < .001). In a multivariate Cox regression model, ERBB4 expression identified as a useful marker for predicting long-term prognosis in patients with TNBC although other HER family genes and ESR1 expressions did not predict prognosis of TNBC with statistical significance (Table 1).

Conclusions: The expression profile of HER family genes could be used as a prognostic marker in patients with TNBC. Further study is needed to identify the expression profiles of HER family gene as predictive marker of HER targeting treatment in patients with TNBC.

Impact of the mRNA expression levels of ERBB family and ESR1 on DRFS

<table>
<thead>
<tr>
<th>Stage</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.0</td>
<td>NA</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IIA</td>
<td>1.20</td>
<td>0.40-3.57</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>3.75</td>
<td>1.20-11.74</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>5.46</td>
<td>1.55-9.24</td>
<td></td>
</tr>
<tr>
<td>IIIC</td>
<td>59.92</td>
<td>14.30-251.12</td>
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<tr>
<td>EGFR</td>
<td>Low</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1.69</td>
<td>0.80-3.55</td>
</tr>
<tr>
<td>Protein</td>
<td>Low</td>
<td>High</td>
<td>p-value</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>ERBB2</td>
<td>1.0</td>
<td>0.43</td>
<td>0.057</td>
</tr>
<tr>
<td>ERBB3</td>
<td>1.0</td>
<td>2.20</td>
<td>0.061</td>
</tr>
<tr>
<td>ERBB4</td>
<td>1.0</td>
<td>2.68</td>
<td>0.016</td>
</tr>
<tr>
<td>ESR1</td>
<td>1.0</td>
<td>0.57</td>
<td>0.113</td>
</tr>
</tbody>
</table>
Title: Clinical outcomes among HR+/HER2- metastatic breast cancer patients with multiple metastatic sites

Xie J, Hao Y, Li N, Lin PL L, Ohashi E, Koo V and Wu EQ Q.  Analysis Group, NY, NY; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Analysis Group, Boston, MA.

Body: Background:
Hormone receptor-positive, human epidermal growth factor receptor-2-negative (HR+/HER2-) is the most common type of metastatic breast cancer (mBC). While overall the prognosis among these patients is poor with short progression-free survival (PFS) and overall survival (OS), those with multiple metastatic sites (multiple mets) may have even worse clinical outcomes due to multiple organ involvement. This real-world study examined clinical outcomes among HR+/HER2- mBC patients with multiple mets.

Methods:
In this retrospective chart review, a sample of postmenopausal women with HR+/HER2- mBC was collected from community-based oncology practices in the US. Patients were required to have failed a non-steroidal aromatase inhibitor and later initiated a new treatment (defined as the index therapy) for mBC between July 1, 2012 and April 15, 2013. Patients were classified into two mutually exclusive groups: multiple mets or single metastatic site (single met), based on the number of non-lymph-node metastatic sites at index therapy initiation. PFS, time on treatment (TOT), and OS were compared between the two study groups using Kaplan-Meier analyses with log-rank tests and multivariable Cox proportional hazards models adjusting for baseline characteristics, including age, race, insurance, mBC type, and months from initiation of last adjuvant endocrine therapy to mBC diagnosis, index therapy type, index therapy line, adjusted Charlson comorbidity index (CCI), Eastern Cooperative Oncology Group (ECOG) performance status, and prior chemotherapy for mBC. Patients without an event were censored at the last follow-up. In addition, separate Cox proportional hazard models were conducted including an interaction term between line of therapy and study group to assess the impact of multiple mets on clinical outcomes across different lines of therapy.

Results:
A total of 408 patients in the single met group and 291 patients in the multiple mets group were included. Patients with multiple mets had worse ECOG performance status and a higher rate of prior chemotherapy use for mBC compared with patients in the single met group. Relative to patients with single met, patients with multiple mets were associated with significantly shorter PFS (log-rank test p<0.001, hazard ratio (HR)=1.68, 95% confidence interval (CI): 1.32-2.14), TOT (log-rank test p<0.001, HR=1.37, 95% CI: 1.09-1.72) and OS (log-rank test p<0.001, HR=1.71, 95% CI: 1.12-2.63). Similar outcomes were observed in each line of therapy.

Table 1. Multivariable -adjusted comparisons of PFS, TOT, and OS between patients with multiple mets and single met by line of therapy

<table>
<thead>
<tr>
<th>Line of therapy</th>
<th>PFS</th>
<th>TOT</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Multiple mets vs.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single met</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line of therapy 1</td>
<td>1.51 (1.04,2.19)</td>
<td>0.030*</td>
<td>1.22 (0.86,1.73)</td>
</tr>
<tr>
<td>Line of therapy 2</td>
<td>1.79 (1.17,2.74)</td>
<td>0.008*</td>
<td>1.50 (1.02,2.21)</td>
</tr>
<tr>
<td>Line of therapy 3+</td>
<td>1.82 (1.18,2.83)</td>
<td>0.007*</td>
<td>1.46 (0.97,2.21)</td>
</tr>
</tbody>
</table>

*P < 0.05

Conclusion:
Among HR+/HER2- mBC patients, those with multiple mets had significantly worse clinical outcomes, highlighting substantial disease burden and unmet need for more efficacious treatment for these patients.
Stromal co-expression of urokinase-type plasminogen activator (uPA) and plasminogen activator Inhibitor (PAI-1) protein by IHC predicts poor disease outcome in endocrine-treated postmenopausal patients with receptor-positive early breast cancer

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Background Elevated levels of intratumoral uPA and PAI-1 in ELISA-based measurements are associated with a high recurrence risk and allow to identify patients who might particularly benefit from adjuvant chemotherapy. The clinical utility of ELISA-based protein analysis is, however, greatly limited by the requirement of fresh tumor tissue. We have therefore evaluated in a large clinical trial patient cohort with long-follow-up whether immunohistochemical detection of uPA and PAI-1 in FFPE archived tumor samples is also able to identify women with poor prognosis.

Patients and Methods 547 postmenopausal women with hormone receptor–positive, early breast cancer who had received at least 5 years of endocrine therapy in the prospectively designed ABCSG-06 trial, and in whom FFPE tumor tissue was available, were included in this analysis. uPA and PAI-1 protein expression was evaluated by immunohistochemistry, and correlated with distant-disease free (DDFS) and overall survival (OS).

Results Stromal co-expression of uPA and PAI-1 was detected in 166 of 276 (60%) evaluable tumor samples and was weakly associated with tumor size (p=0.012, Chi Square test) but not with age, nodal status, grading, or receptor status. Women with intratumoral uPA/PAI-1 expression were more likely to have a shorter DDFS in multivariate analysis (HR for relapse p=1.870; 95% CI 1.184-2.955; p=0.007 Cox regression analysis) and and OS (adjusted HR for death p=1.291; 95% CI 0.928-1.795; p=0.129) than women without. After a median follow-up of 10 years, women with uPA/PAI-1-positive tumors experienced a significantly shorter DDFS (p<0.0001 log rank test) and OS (p=0.020).

Conclusions Stromal co-expression of uPA and PAI-1 in breast cancer samples predicts poor DDFS and OS in postmenopausal women with hormone-receptor positive early-stage breast cancer who receive endocrine therapy.
**Title:** The ASCO-recommended biomarkers urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 define a subgroup of patients with very low risk of recurrence under routine conditions

Kantelhardt E-J, Thomssen C, Grosse R, Papendick N, Steer S, Buchmann J, Wickenhauser C and Vetter M. Martin-Luther-University, Halle (Saale), Germany; Institute of Pathology, Martha-Maria, Halle (Saale), Halle (Saale), Germany and Institute of Pathology, Martin-Luther-University, Halle (Saale), Germany.

**Body:**

**Background:**
ASCO Tumor Marker Guidelines 2007 recommended clinical routine use of the invasion markers uPA and PAI-1 for risk assessment in node-negative breast cancer patients (Harris et al. JCO 2007; 25:5287), and in some countries, e.g. Germany and France, risk assessment using these markers is broadly used. We wanted to evaluate the impact of uPA/PAI-1 on identifying patients with low risk of recurrence also in the daily routine in order to demonstrate that >25 years since their first description, these markers are still valuable.

**Material and Methods:**
We identified a cohort of 227 patients who were tested for uPA and PAI-1 in the clinical routine before 2012. Fresh frozen tissue of the primary tumor was obtained at biopsy or operation and processed for testing by a commercially available ELISA (FEMTELLE®, Sekisui Diagnostics GmbH) as previously described (Thomssen et al. JNCI 2009;101:1028). Tumor and patient characteristics were documented and all patients were regularly followed. Tumor concentrations below 3 ng/mg protein for uPA and below 14 ng/mg protein for PAI-1 were considered indicating low risk of recurrence (Harbeck et al. EJC 2013; 49:1825). Disease-free survival was defined as survival free from metastasis and loco-regional recurrence.

**Results:**
In our cohort, 86 patients had low tumor levels of uPA/PAI-1 (37.9 %). The median follow-up was 38.9 months (0.4 – 113.5 months). Adjuvant chemotherapy was delivered to 25 of 86 patients (29.1%) in the low risk group and to 85 of 141 patients (60.3%) in the high risk group; if steroid hormone receptor status was positive, generally adjuvant endocrine therapy for five years was advised. Using immunohistochemical subtyping, 73 of 86 patients with low uPA/PAI-1 values were luminal-like, 9 patients had a HER2-positive tumor and 3 patients had a triple negative breast cancer (TNBC). In patients with uPA or PAI-1 or both elevated, 105 of 141 patients had a luminal-like cancer, 12 patients were HER2 positive and 22 had TNBC; 1 case unknown. At 60 months of follow-up, patients with low uPA and/or PAI-1 tumor values had not experienced any recurrence, while in the high risk group 7 recurrences were observed although adequate adjuvant therapy was delivered (log-rank p=0.07). In node-negative pts with low uPA/PAI-1 values (n=72; pN0 70, cN0 2), no recurrences were observed, in 104 high risk patients 5 recurrences were observed (p=0.057).

**Conclusions:**
This observation confirms that also in daily routine, patients with a very low risk of recurrence can be identified by testing for uPA/PAI1. This group of pts comprises nearly 40% of pts and in these patients further evaluation with expensive predictive tests can be avoided and - above all - potentially toxic adjuvant chemotherapy can be spared.
Title: TP53 mutation is a biomarker for prognosis in triple-negative breast cancer patients treated with post-neoadjuvant cisplatin

Hancock BA A, Chen Y-H, Solzak JP P, Miller KD D and Radovich M. Indiana University School of Medicine, Indianapolis, IN.

Body: Introduction: Patients with Triple-Negative Breast Cancer (TNBC) who have residual disease (RD) after neoadjuvant chemotherapy are at an increased risk of relapse and have a poor prognosis. No adjuvant therapies are currently indicated for this group. BRE09-146 was a Phase II post-neoadjuvant clinical trial testing Cisplatin or Cisplatin + Rucaparib in TNBC patients with RD after neoadjuvant chemotherapy. As TP53 is mutated in 70-80% of TNBCs, and is well known to play a role in the DNA damage response, we sought to determine the prognostic capability of mutated TP53 in BRE09-146.

Methods: We performed full sequence and copy number analysis of 134 genes in 76 tumors from BRE09-146 using the Oncomine Research Panel along with Ion Proton Next Generation Sequencing. All patients included had RD. Somatic mutations were called by identifying mutations that were present in the tumor that were not present in the germ line DNA from a normal blood sample. Mutations were annotated using the IARC TP53 somatic mutation database. Gene copy numbers in tumors were identified using the Ion Reporter system from Thermo-Fisher Scientific and called as copy number loss, normal, or gain based upon a comparison to a reference range established from the normal blood samples. Survival analyses were generated using the Log-Rank and Kaplan-Meier methods.

Results: 84% (64/76) of our TNBC tumors harbored a somatic mutation in the TP53 gene. The majority were missense mutations (particularly in the DNA binding and tetramerization domains) followed by frameshift insertions/deletions, and copy number loss. Patients whose tumors harbored somatic TP53 mutations were observed to have a significantly inferior disease free survival (DFS) compared to non-mutated tumors (events = 29/64 vs. 1/12; median = 25.9 mos vs. NR (Not Reached); p=0.021, HR=7.28 (95% C.I.: 2.98-17.79). The same was observed for overall survival (OS) (events = 23/64 vs. 0/12, median = 33.78 vs. NR; p=0.017, HR = Not evaluable). There was no difference in DFS or OS when comparing the nature of the mutation (point mutation vs. indel vs. copy loss) at a p=0.88 and p=0.91, respectively. We then sought to determine if clonal status of TP53 mutations was also associated with survival. Cases were divided into non-mutated, subclonal (mutations present in a fraction of cells), or truncal (mutation present in most or all cells). Interestingly, tumors that harbored subclonal TP53 mutations had a superior OS compared to truncal mutations (events = 1/9 vs. 22/55; median = NR vs. 29.1, p=0.036, HR =0.16 (95% C.I.:0.06-0.42). OS for subclonal mutations was highly similar to non-mutated tumors (p=0.25).

Conclusions: While RD after neoadjuvant chemotherapy in TNBC is a well-known risk factor for poor prognosis, in our study, we observed a subset of RD patients defined by a lack of TP53 mutation or presence of a subclonal mutation that portended a superior survival outcome after post-neoadjuvant Cisplatin. If validated, these results reveal that the presence and clonal status of TP53 mutations is important to accurate prognostication and should be considered in decision-making algorithms for this patient population.
Title: High levels of serum C-terminal crosslinking telopeptide of type 1 collagen at baseline are associated with poor prognosis for breast cancer patients

Imamura M, Nishimikai A, Yanai A, Miyagawa Y, Higuchi T, Ozawa H, Murase K, Takatsuka Y and Miyoshi Y. Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.

Body: Background: It has been demonstrated that adjuvant treatment using bisphosphonate may reduce recurrence among breast cancer patients. However, these improved prognoses of patients are reportedly limited to breast cancers of estrogen receptor (ER)-positive and postmenopausal women. Although the mechanisms of the effects of bisphosphonate remain unknown, this finding seems to represent support for the hypothesis that suppression of bone resorption by bisphosphonate results in favorable prognoses at least for patients in this subset. In order to determine the prognostic significance of bone resorption in breast cancer patients, we investigated these markers c-terminal crosslinking telopeptide of type I collagen (1CTP) and N-telopeptide of type I collagen (NTX).

Patients and Methods: 469 breast cancer patients were recruited who were operated on Hyogo College of Medicine and histologically confirmed to have invasive carcinoma. Serum 1CTP and NTX were measured preoperatively with the two-antibody radioimmunoassay and enzyme-linked immunosorbent assay methods, respectively, and blood samples were obtained before treatment from patients who were treated with neoadjuvant chemotherapy or endocrine therapy. The area under receiver operating characteristic curves were applied and optimal cutoff values were set at 3.6 ng/ml for 1CTP, and 10.55 nmol BCE/L premenopausal and 14.05 nmol BCE/L postmenopausal for NTX. The relationships between these bone turnover markers and various clinicopathological characteristics were evaluated with the chi square or Fisher's exact test. The log-rank test was used to compare relapse-free survival (RFS) in Kaplan-Meier plots. Associations of RFS were assessed with a Cox proportional-hazards model based on the results of univariate and multivariate analyses. Differences were considered statistically significant if p<0.05.

Results: There were significantly more 1CTP-high patients among postmenopausal women and RFS of 1CTP-high patients was significantly worse than that of 1CTP-low patients (5-year RFS: 0.65 vs 0.86; p=0.0002). Similarly, NTX-high patients were significantly associated with postmenopausal status, but there was no significant association between NTX-high worse RFS (p=0.0976). Multivariate analysis of tumor size, lymph node metastasis and nuclear grade identified 1CTP (hazard ratio: 2.04, 95% confidence interval: 1.13-3.68; p=0.018) as a significant independent prognostic factor. Subset analyses of 1CTP showed that prognosis was consistently worse recognized for postmenopausal (p=0.0002), but not premenopausal (p=0.37) patients. Furthermore, prognosis for 1CTP-high patients was worse for the estrogen receptor (ER)-positive subset (p=0.0005) but not for the ER-negative subset (p=0.22).

Conclusion and discussion: High levels of serum bone resorption markers at baseline were identified as significant unfavorable prognostic factors for breast cancer patients. The prognostic significance of 1CTP seems to be prominent for postmenopausal patients with ER-positive breast cancers. These findings suggest the use of bone-modifying agents as an adjuvant therapy may be beneficial for breast cancer patients, especially for patients with high serum levels of 1CTP.
**Title:** Impact of kynurenine 3-monooxygenase expression on outcome in patients with triple-negative breast cancer undergone primary treatments

Wu C-Y, Chu P-Y, Wang W-L, Liu C-Y and Tseng L-M. Division of Hematology and Oncology, Taipei Veterans General Hospital, Taipei, Taiwan; School of Medicine, National Yang-Ming University, Taipei, Taiwan; Taipei Veterans General Hospital, Taipei, Taiwan; Show Chwan Memorial Hospital, Changhua City, Taiwan and School of Medicine, College of Medicine, Fu-Jen Catholic University, New Taipei City, Taiwan.

**Body:**

**Background**

Triple-negative breast cancer (TNBC), characterized by aggressive behavior and poor prognosis, represents an important clinical challenge because there is no well-established target therapy. Tryptophan-kynurenine metabolism in cancer is increasingly being recognized as a key factor that suppresses antitumor immune responses. Previous studies have focused on the expression and function of the rate-limiting enzyme indoleamine-2,3-dioxygenase in tumor cells, whereas the second step catabolic enzyme Kynurenine 3-monooxygenase (KMO) was rarely addressed in cancer. This study aimed to explore the clinical impact of KMO expression in patients with TNBC undergone primary treatments.

**Methods**

Primary tumors from 103 patients with TNBC who received surgical resection were enrolled for analysis. The expression of KMO and other clinicopathologic parameters, including tumor-infiltrating lymphocytes (TILs) and tumor expression of vascular endothelial growth factor (VEGF), was assessed semiquantitatively using immunohistochemistry-based scoring. Spearman rank correlation was used to examine the association between the expressions of KMO, TILs and VEGF. The clinical impact of KMO expression on survival was analyzed using Kaplan-Meier curve and Cox regression.

**Results**

The expression of KMO is significantly higher in breast tumor tissue, as compared with non-tumor tissue. In addition, a median of threefold higher KMO expression was observed in cancerous part comparing to non-cancerous part in resected specimens of TNBC, either in tumor/non-tumor paired or non-paired samples. The expression of KMO was negatively correlated with TILs ($r = -0.389, p < 0.001$) and positively correlated with VEGF expression ($r = 0.344, p < 0.001$). Patients with higher KMO expression had a more advanced disease and demonstrated a trend toward poorer disease-free survival (DFS) ($p = 0.065$)(Table 1). Interestingly, an inverse distribution of TILs that contacted with tumor cells showing high KMO expression was observed in higher power field of microscopic examination. Moreover, patients with both higher expressions of KMO and VEGF had a poorer DFS, as compared with those without both higher KMO and VEGF expressions (hazard ratio, 1.52; 95% confidence interval, 1.17-1.97), after adjusted for tumor stage.

**Conclusion**

Our data suggested KMO expression correlates with tumor infiltrating lymphocytes as well as VEGF expression, and may have a negative impact on outcome of patients with TNBC. The role of KMO in TNBC warrants further investigation.

(Grant supported by MOHW104-TD-B-111-02)

**Clinical pathological factors in correlation with KMO expression**

<table>
<thead>
<tr>
<th></th>
<th>High KMO H score (&gt;=60)</th>
<th>Low KMO H score (&lt;60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 92</td>
<td>N = 11</td>
<td></td>
</tr>
<tr>
<td>Age &gt;= 60</td>
<td>32 (34.8)</td>
<td>6 (54.5)</td>
<td>0.321</td>
</tr>
<tr>
<td>Primary tumor (T)</td>
<td></td>
<td></td>
<td>0.445</td>
</tr>
<tr>
<td>1</td>
<td>26 (28.3)</td>
<td>5 (45.5)</td>
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</tr>
<tr>
<td>2</td>
<td>62 (67.4)</td>
<td>5 (45.5)</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>4 (4.3)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number 1</td>
<td>Percentage 1</td>
<td>Number 2</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>----------</td>
</tr>
<tr>
<td>Nodal status (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>50 (54.3)</td>
<td>8 (72.7)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19 (20.7)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>23 (25.0)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16 (17.4)</td>
<td>3 (27.3)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>51 (55.4)</td>
<td>6 (54.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25 (27.2)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Disease recurrence</td>
<td>29 (31.5)</td>
<td>0 (0.0)</td>
<td>0.031</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>24 (26.4)</td>
<td>0 (0.0)</td>
<td>0.063</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>15 (16.5)</td>
<td>0 (0.0)</td>
<td>0.361</td>
</tr>
<tr>
<td>TIL (tumor-infiltrating lymphocyte) [median (range)]</td>
<td>15 (1-90)</td>
<td>40 (10-90)</td>
<td>0.001</td>
</tr>
<tr>
<td>VEGF [median, (range)]</td>
<td>120 (0-300)</td>
<td>45 (0-100)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Presented as number (percentage)
**Body:** Introduction: Tumor related inflammation plays an important role in breast cancer progression, tumor-associated macrophages (TAMs) being a crucial part of this microenvironment. Platelet-derived growth factor-C (PDGF-C) is abundant in the breast cancer microenvironment having an anti-apoptotic effect on macrophages. Previous reports suggest that tumor cell derived PDGF-C promotes TAM survival, enhancing tumor progression. In this study we analyzed the prognostic value of PDGF-C density associated with TAM infiltration in human breast cancer.

Materials and Methods: TAM and PDGF-C density was evaluated by immunohistochemistry of CD163+, CD68+ and PDGF-C myeloid cells in tumor stroma. Tissue microarrays from 140 invasive breast cancer cases were used. Survival analysis to evaluate the impact of TAM and PDGF-C density on disease free survival (DFS) was done using Kaplan-Meier and Cox regression analysis.

Results: Infiltration of CD163+ and CD68+ macrophages into tumor stroma had a tendency, but not significant, for reduced DFS (p=0.204, p=0.314 respectively). Whereas PDGF-C strong density was significantly associated with worse DFS (p=0.024). This inverse correlation with DFS was demonstrated stronger when PDGF-C density was combined with CD163+ and CD68+ macrophage infiltration (p=0.008, p=0.018, respectively). But, CD163+ or CD68+ infiltration with strong PDGF-C density did not demonstrate as an independent prognostic factor when adjusting for tumor size, lymph node metastasis, hormone receptor and histologic grade. This result is probably due to small number of patients having both TAM infiltration and strong PDGF-C density.

Conclusion: Our results show that PDGF-C infiltration adversely affects DFS and also enhances the inverse correlation between tumor-associated macrophages and DFS. To support these results, a study with larger numbers is on progress.
Title: Prediction of bone metastases of breast cancer using combined markers of bone metabolism and inflammation

Shimoda M, Nishimukai A, Shibata N, Kikuchi W, Hutawatari H, Ishihara H, Miyoshi Y and Noguchi S. Osaka University Graduate School of Medicine, Suita, Osaka, Japan; Hyogo College of Medicine, Nishinomiya, Hyogo, Japan and Nittobo Medical Co., Ltd, Koriyama, Fukushima, Japan.

Body: Introduction
Luminal breast cancer patients show a relatively favorable prognosis when treated with adjuvant hormonal therapy alone. However, some of these patients develop recurrence and they might derive benefit from adjuvant chemotherapy. Although several genomic profilings successfully developed to decide whether to administer adjuvant chemotherapy, clinically practical prediction methods of recurrence sites do not exist. Our previous study showed a possible prediction of bone metastases by using two serum markers; TRACP-5b as a marker of bone metabolism; likelihood of bone metastases, and CRP as a marker of inflammation; likelihood of distant recurrence. The incidence of bone metastases was significantly higher in high risk patients(+/+) than in the others(odds ratio: 10.9, P=0.040). In this study, we examined the potential of the two-marker prediction in the newly enrolled luminal patients.

Patients and methods
One hundred sixty luminal patients who underwent surgery were enrolled in this study. Their serum levels of TRACP-5b and CRP were measured in a blinded manner at the R & D laboratory of Nittobo Medical Co., Ltd. In the preliminary study, we identified that the median value of TRACP-5b in the premenopausal patients was lower than in the postmenopausal patients. We adjusted the value of TRACP-5b in the premenopausal patients and the cutoff value of TRACP-5b from 334 to 396mU/dL. The cutoff value of CRP was same as previous study(0.016 mg/dL). The odds ratio between +/+ and the others were calculated using MedCalc statistical software.

Results
One hundred sixty patients stratified into four classes according to the value of TRACP-5b and CRP: +/+ (n=43), +/- (n=38), -/+ (n=42) and -/- (n=37). Six of the 160 patients developed bone metastases as the initial site of relapse within five years from surgery. The incidence of bone metastases was 9.3%(4/43) in the +/+ patients and 1.7%(2/117) in the others. The incidence was significantly higher in the +/+ patients than in the others(odds ratio: 5.9, 95% CI 1.31 to 33.46, p= 0.045). When the other relapses than bone metastases were included in the analysis, no significant difference was observed between the two groups (odds ratio: 0.4, 95% CI 0.02 to 7.43, P=0.521). TRACP-5b concentration alone could not classify the patients into two groups according to significantly different incidences of bone metastases(odds ratio: 13.7, 95% CI 0.76 to 247.22, P=0.076).

Conclusion
The results in here show that the prediction of bone metastases by the combination of TRACP-5b and CRP concentrations is clinically relevant in the luminal patients. Reliable prediction of bone metastases would be realized by combination of our prediction method and one of genomic profilings. We plan to increase the number of patients to provide sufficient statistical power to confirm this diagnostic potential.
Title: Association between rim enhancement on magnetic resonance imaging and response of chemotherapy


Body: Background: Triple-negative breast cancers (TNBC) are defined as tumors that lack expression of estrogen receptor (ER), progesterone receptor (PR), and HER2. TNBC is characterized as a cancer with a high malignancy potential and a poor prognosis. Systemic therapy that is effective in TNBC is only chemotherapy. On magnetic resonance imaging (MRI), rim enhancement was frequently seen in TNBC. It is reported that rim enhancement on MRI may associated with long-term outcome of patients with triple-negative breast cancer and may potentially serve as a prognostic biomarker in these patients. It is not well known about the relationship of rim enhancement on the MRI and treatment effectiveness of TNBC.

Purpose: We investigated the relationship between rim enhancement on MRI and response of chemotherapy and outcome in patients with TNBC.

Methods: MRI findings of 144 consecutive female TNBC patients, who underwent surgery from 2007 to 2012 in our hospital, were retrospectively reviewed. All patients have taken the MRI in our hospital before treatment, and had undergone chemotherapy before or after surgery. Presence of rim enhancement on MRI was assessed. Rim enhancement was defined more pronounced at the periphery of the mass at early phase.

Association of the presence of rim enhancement on MRI and the pathological complete response (pCR) rate in patients who underwent neo adjuvant chemotherapy (NAC) was assessed using two-sided Pearson's Chi squared tests. Disease free survival (DFS) rates were calculated by the Kaplan-Meier method. Univariate analysis was performed using the log rank test. pCR was defined as the disappearance of invasive cancer.

Results: The median age was 51yo (26-82), and the median observation period was 49 months (5-92). Eighty-one patients (56.2%) underwent NAC and 63 patients (43.7%) underwent adjuvant chemotherapy. Twenty-six cases (18.0%) occurred recurrence or distance metastasis. The presence of rim enhancement were observed 68 cases (42.3%), and non-rim enhancement were 66 cases (57.6%). DFS were not significantly different according to the presence of rim enhancement on MRI(P=0.31).

In NAC patients, 28 patients (34.5%) were led to pCR and 53 (63.4%) were non-pCR. The presence of rim enhancement were observed 44 cases (54.3%), and non-rim enhancement were 37 cases (45.6%). In pCR rate, rim enhancement is higher than non-rim enhancement (40.9%, 27.0%, respectively). However, the presence of rim enhancement on MRI was not significantly associated with pCR in TNBC patients (p= 0.190).

Table1. Association between rim enhancement and pCR

<table>
<thead>
<tr>
<th></th>
<th>pCR (%)</th>
<th>non-PCR (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rim enhancement</td>
<td>18 (40.9)</td>
<td>26 (59.1)</td>
<td></td>
</tr>
<tr>
<td>non-rim enhancement</td>
<td>10 (27.0)</td>
<td>27 (72.9)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Conclusion: The presence of rim enhancement on MRI showed high pCR rate. While, it is not a significant predictor of pCR in TNBC patients.
Title: Defining a signature of residual risk following endocrine treatment in the tamoxifen and exemestane adjuvant multinational (TEAM) trial

Bayani J, Yao CQ Q, Quintayo MA, Haider S, Brookes CL L, Yan F, van de Velde CJH JH, Hasenburg A, Kieback DG G, Markopoulos C, Dirix L, Seynaeve C, Boutros PC C, Rea DW W and Bartlett JMS MS. Ontario Institute for Cancer Research (OICR), Toronto, ON, Canada; University of Birmingham, Cancer Research UK Clinical Trials Unit, Birmingham, United Kingdom; Leiden University Medical Center, Leiden, Netherlands; University Hospital, Freiburg, Germany; Helios Medical Center, Aue, Germany; Athens University Medical School, Athens, Greece; St. Augustinus Hospital, Antwerp, Belgium; Erasmus Medical Center-Daniel den Hoed, Rotterdam, Netherlands and University of Toronto, Toronto, Canada.

Body: Introduction: There are a number of commercially-available tests to stratify risk for women diagnosed with early breast cancer. While such “Generation I” tests are increasingly being used, a consensus is growing that these tests are moderately accurate in assessing risk. Moreover, Generation I tests fail to direct more personalized treatment. Therefore, there is a clear need for more informative "Generation II" tests that better assess risk, also on the long term, and provide theranostic targets. To this end, we have performed an mRNA abundance-based analysis trained in the 790 patients of the UK TEAM cohort to identify a signature of residual risk, to be validated in the remaining 3000 patients from the TEAM pathology study.

Methods: RNA extracted from the tumors of respective TEAM pathology study patients were profiled using a 165-gene NanoString code set. The gene list was compiled from targets that comprise many of the existing risk assessment tests, in addition to genes known to be of importance for breast cancer pathogenesis. Signal intensities were normalized using the R statistical environment; 336 different combinations of preprocessing methods were assessed and the most optimal method selected using unbiased criteria. A10-fold cross-validation approach, in combination with a network-based patient risk score calculation formula, was used to derive a 95-gene signature. Briefly, genes were first filtered based on a Cox regression p-value threshold of 0.25; the sum of the weighted mRNA abundance levels of the result genes was calculated for each patient as the risk score. Patient-wise risk scores were then used in a multivariate Cox proportional hazards model along with clinical covariates such as age, grade, HER2 status and nodal status, using DRFS truncated to 10 years as an end-point.

Results: Univariate survival analysis revealed a number of significantly prognostic candidates. The resulting 95-gene signature identified in the training set, stratified patients into high and low risk with an HRhigh of 2.74 (p<2.06 x10-4) when adjusted for age, grade, HER2 status and nodal status; resulting in an AUC of 0.73. Modular analyses of the genes comprising the 95-gene signature identified pathways associated with receptor tyrosine kinase signalling, regulation of cell cycle, and the spindle assembly checkpoint. Additionally, the composition of the gene-list made it possible to characterize the patients into their intrinsic subtypes and to determine their relative risk for recurrence relative to assessment tools available today. The validation of the 95-gene signature will be conducted in the remaining samples in the TEAM pathology study using the bioinformatics strategy described above.

Conclusions: The impact of test-guided therapy using multi-parametric tests is increasingly being felt in the clinic, and is reshaping modern health-care economics. A successful Generation II multi-parametric test will better discriminate those that are truly at high risk for recurrence following endocrine therapy and indicate potential therapeutic options for intervention for those who would not benefit from current modalities.
Title: The role of skeletal muscle volume on prognosis of breast cancer survivors using with cross-sectional image analysis


Body: Background: Obesity is one of the well-known cause and prognostic factor of breast cancer. Body mass index (BMI) is often used to estimate the magnitude of obesity. Currently, muscle weight itself, instead of higher BMI, is more closely related to poor outcomes in chronic metabolic disease. However, this question was not thoroughly questioned in breast cancer survivors. We aim to find out whether the prognosis of breast cancer survivors is affected by muscle mass and fat volume. We also present that the higher muscle mass and muscle fat ratio do have a good influence on the prognosis.

Methods and Materials: Between January, 2001, and December, 2009, all consecutive patients diagnosed the breast cancer in National cancer center, Republic of Korea were 3909. Of all populations, the patients who had available chest Computed Tomography (CT) images within two years after the time of diagnosis were extracted. CT images were analyzed for total skeletal muscle and adipose tissue in cross-sectional area of 10mm thickness at level 3 of the lumbar vertebrae.

Results: Of all consecutive 3909 patients, final eligible cases were 1493. The median age was 46.0 (range 25–77). Median follow up period was 96.8 months and the 5 year survival rate was 96.5%. Recurrence free survival rate was 92.1%, and local recurrence free survival was 98.6% in 5 years. Median skeletal muscle volume of all patients was 93.3cc (range 39.6–236.9). Median fat volume of the same sections was 419.7cc (range 19.5–1392.3) and the median muscle-fat ratio was 0.22 (0.08–3.18). There is no known standard value of muscle mass in Korean women, the median volume was used as an indicator for comparison of two groups in terms of overall survival and recurrence free survival. The group with muscle volume greater than median value appeared better outcomes in overall survival and recurrence free survival than those of the other group (p=0.007, p=0.019). There were several factors related to overall survival and recurrence free survival such as age, operation types, clinical stage, pathologic stage, and tumor size in univariate cox regression analysis. In multivariate analysis by adjusting those factors, the muscle volume showed a remarkable correlation with survival, especially in recurrence free survival (Hazard ratio 0.61, p=0.029). As the indicator using with median muscle-fat ratio, the analysis revealed that overall survival had no significant difference (p=0.177) between two groups. However, the group with higher muscle-fat ratio showed better recurrence free survival and local recurrence free survival (p-value <0.0001, 0.038 respectively).

Conclusions: Our study showed that the actual amount of skeletal muscle rather than fat volume has higher effect on the prognosis of breast cancer survivors. In our knowledge, this is the largest study to analyze the prognosis of breast cancer with skeletal muscle volume. In view of the different magnitude of obesity among multiple ethnics, the actual muscle mass could be the most important parameter to assess the amount of exercise and patient's health status.
Introduction: Evaluation of the benefit of primary chemotherapy (PC) is not easy to establish. Pathologic complete response (pCR) has been considered the main surrogate prognostic factor of patient's survival. However, patients achieving a pCR are not the only ones who benefit from PC. The purpose of our study is to find a measure of response that includes the maximum of patients that benefit from PC in terms of survival.

Patients and methods: 224 breast cancer patients were treated in Breast Cancer Unit from Institut Català d'Oncologia (ICO) L'Hospitalet with taxans and antracyclines-based PC +/- trastuzumab between 2009 and 2011. pCR was defined as no invasive carcinoma found in the tumor and in the axillary lymph nodes (ypT0/ypTis ypN0). Tumor and nodal downstaging (TNDS) was calculated according to the "neoadjuvant response index" (NRI) from Rodenhuis and also as a dichotomic variable: Positive includes those patients achieving downstaging of both T and N plus T downstaging N0 and negatives those patients without downstaging in any of both variables. Those parameters were related to patient's overall survival (OS). Statistical analysis was performed with SPSS version 15.

Results: Median age 45.5 years (24-83). Main tumor characteristics: T2 (62.6%); N1 (50%); ductal infiltrating carcinoma (95.5%) and grade III (57.1%). Biological sub-type according to the last St Gallen classification: luminal A: 28 patients (pts); luminal B/Her2-: 61 pts; luminal B/HER2+: 34 pts; HER2+: 33 pts and triple negative: 69 pts. Pathologic complete response was achieved in 49 pts (22.5%). TNDS was evaluated in 181 patients and of those 90 was positive. According to NRI 74 patients presented cut-off> 0.5 and 52 pts > 0.7. Parameters related to OS were: biological subtype (P: 0.007); achieving a pCR (p: 0.007); NRI cut-off 0.5 (P: 0.001) and TNDS (p:0.000). In the multivariate analysis only TNDS and biological subtype remained statistically significant. When comparing those patients with positive vs. negative TNDS, the HR for recurrence was of 10.05 (IC 2.33 -43.57). The median OS of the series has not been reached. OS at 5y was 82.7% (IC: 77.1%-88%) and specific breast cancer OS at 5 y was 85% (IC:79.5%-90%). The number of events (breast cancer deaths) for each biological subtype according to positive vs. negative TNDS was: luminal A: 0/5 vs. 0/18; luminal B Her2-: 0/10 vs. 8/43; luminal B HER2+:0/23 vs. 2/9; HER2+: 0/22 vs. 0/2 and TN: 2/30 vs. 8/18. Survival data per subtypes and TNDS is immature due to the scarce number of events. Estimated 5y OS for TNDS positive vs. negative in luminal A: 100% vs. 100%; luminal B Her2-: 100% vs. 82%; luminal B HER2+:100 vs.77.7%; HER2+: 100% vs. 100% and TN: 93% vs. 55%, respectively.

Conclusion: In our series, TNDS measured either with the NRI from Rodenhuis or as a dichotomic variable was the best parameter to evaluate response to PC in terms of OS. OS of luminal A and luminal B/Her2 negative is less influenced by PC than the rest of subgroups. In fact both subgroups have good prognosis despite their poor sensitivity to chemotherapy. Those tumors that benefit most from PC were luminal B/ Her2+; Her2+ and triple negative patients who achieved a positive TNDS.
**Title:** Patient reported outcomes of multiplex breast cancer susceptibility testing utilizing a tiered-binned counseling and informed consent model in BRCA1/2 negative patients


**Body:** Background: The risks, benefits and utilities of multiplex panels for breast cancer susceptibility are unknown and new counseling and informed consent models are needed. We sought to obtain patient reported outcomes of multiplex testing in BRCA1/2 negative patients utilizing a novel, previously piloted tiered-binned counseling model for multiplex testing.

**Methods:** BRCA1/2 negative participants completed pre(V1) and post-test counseling(V2) and surveys evaluating cognitive, affective and behavioral responses to a 25-gene multiplex testing panel. We used linear regressions with estimation by GEE where appropriate.

**Results:** 376 patients have been approached. To date, 124 participants(33%) have consented to the study, 21(6%) declined and 231(61%) are considering. Of 95 who have completed pre-test counseling(V1), 88(93%) elected to proceed with 25-gene panel testing and (81%) were classified as making an informed choice after tiered-binned counseling. 6/53(11%) participants received a positive result, including 1 mutation in MSH2 and 5 in moderate penetrance genes (2 ATM, 1 BARD1, 1 CHEK2, 1 PALB2). 22/53(42%) participants received a variant of unknown significance(VUS). General anxiety and perceived utility decreased significantly with pre-test counseling and after results (Table 1). Knowledge increased with pre-test counseling; cancer worry increased after receipt of multiplex results. Higher cancer worry was associated only with lower income (2.6 points/income category, p<0.01). Those with a VUS had greater decreases in perceived utility compared to negative (p=0.01) or positive (p=0.003) results. To date, there are no other significant differences in knowledge, distress or uncertainty by test result. Medical management recommendations for the proband changed in 3/6 with a positive result. Cascade testing in the family was discussed as an option in 3/6 with a positive result. Conclusions: Many BRCA1/2-negative patients proceed with 25-gene cancer susceptibility testing if offered and most make informed choices utilizing a tiered-binned genetic counseling model. The tiered-binned counseling model is associated with increased knowledge, decreases in general anxiety and uncertainty after pre-test counseling and disclosure of results, but an increase in cancer worry after result disclosure. The clinical utility, long-term outcomes and differences in patient reported outcomes by test result remain unknown.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Baseline, Mean(SD)</th>
<th>After V1, Mean(SD)</th>
<th>After V2, Mean(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=75;N=49&quot;</td>
<td>N=75;N=49&quot;</td>
<td>N=49&quot;</td>
</tr>
<tr>
<td>General Anxiety (0-21)</td>
<td>6.4(3.9)*;6.9(3.9)**</td>
<td>6.0(4.3)*;6.6(4.2)**</td>
<td>5.8(4.5)**</td>
</tr>
<tr>
<td>General Depression (0-21)</td>
<td>2.8(2.9);3.1(3.2)</td>
<td>3.0(3.5);3.1(3.6)</td>
<td>3.0(3.7)</td>
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<tr>
<td>State Anxiety (20-80)</td>
<td>35.5(11.2);36.6(11.9)</td>
<td>35.5(11.8);36.8(11.9)</td>
<td>36.1(12.2)</td>
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<tr>
<td>Cancer Worry (0-75)</td>
<td>18.2(13.5);20.1(13.8)**</td>
<td>16.7(12.5);17.2(11.9)**</td>
<td>21.0(13.9)**</td>
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<td>Knowledge (17-82)</td>
<td>65.7(5.0)<strong>;66.5(5.0)</strong></td>
<td>68.0(5.7)<strong>;68.4(5.6)</strong></td>
<td>67.3(4.9)**</td>
</tr>
<tr>
<td>Uncertainty (0-15)</td>
<td>5.8(3.9);6.2(4.3)</td>
<td>5.6(3.7);5.4(3.2)</td>
<td>5.6(3.5)</td>
</tr>
<tr>
<td>Perceived Utility (24-120)</td>
<td>75.2(14.0)<strong>;75.9(14.0)</strong></td>
<td>73.0(14.1)*;74.1(13.4)**</td>
<td>68.4(16.9)**</td>
</tr>
</tbody>
</table>

*p<0.05 **p<0.01. "completed V1. ""completed V1 & V2. To date, 53 have received results and 49 have completed post-disclosure surveys.
Gene panel sequencing is revolutionizing germline risk assessment for hereditary breast cancer. Despite scant evidence supporting the role of many of these genes in breast cancer predisposition, results are often reported to families as the definitive explanation for their family history. We assessed the frequency of mutations in 18 genes commonly included in hereditary breast cancer panels among 2,000 index cases from breast cancer families and 1,997 population controls. Cases were predominantly breast cancer-affected women referred to specialized familial cancer centers (BRCA1 and BRCA2 wild-type). Controls were cancer-free women from the LifePool study (www.lifepool.org). Sequencing data were filtered for known pathogenic or novel loss of function mutations.

The frequency of pathogenic mutations in BRCA1 and BRCA2 in the control group was 0.2% (4 mutations) and 0.4% (8 mutations), respectively, which is consistent with previous indirect estimates for Caucasian populations but to our knowledge this the largest direct assessment of their prevalence.

Excluding 18 mutations identified in BRCA1 and BRCA2 among the cases and controls, a total of 69 cases (3.5%) and 26 controls (1.3%) were found to carry an "actionable mutation". PALB2 was most frequently mutated (22 cases, 3 controls), while no mutations were identified in PTEN or STK11. Among the remaining genes, loss of function mutations were rare with similar frequency between cases and controls.

The frequency of mutations in most breast cancer panel genes among individuals selected for possible hereditary breast cancer is low and in many cases similar or even lower than that observed among cancer-free population controls. While multi-gene panels can significantly aid in cancer risk management, they equally have the potential to provide clinical misinformation and harm at the individual level if the data is not interpreted cautiously.
Title: Breast cancer prevention: Is it time for population-based mutation screening of high risk genes?


Body: The traditional model of familial breast cancer practice involves ascertaining high-risk individuals based on family history. However, most individuals who carry a BRCA1 or BRCA2 mutation will not have a family history of breast or other cancer in a close relative. Moving germline testing from the familial cancer center to the population will result in many essentially unanticipated findings of great significance in regards to the risk of breast cancer in healthy relatives. To date there have been no large studies that have directly measured the frequency of BRCA1 and BRCA2 mutations in a cancer-free general population. As a first step toward population based BRCA1 or BRCA2 screening, we sequenced the entire coding region of these genes in 1,997 cancer-free Australian women recruited from the lifepool study (www.lifepool.org) which is a cohort of women attending the Australian population based mammographic screening program. Sequencing data were filtered for known pathogenic or novel loss of function mutations. Twelve individuals were identified with pathogenic mutations in either BRCA1 (4 mutations) or BRCA2 (8 mutations), which is consistent with previous indirect estimates for Caucasian populations but to our knowledge this is the largest direct assessment of their prevalence. Interestingly, the self-reported cancer family history of the majority of the 12 mutation positive women was unremarkable. All 12 women subsequently accepted an invitation to attend a Familial Cancer Centre for advice on whether to proceed with formal clinical genetic testing.

A population carrier frequency of 0.6% for mutations in BRCA1 and BRCA2, coupled with the rapidly declining costs of gene panel sequencing, suggests that population-based screening for these genes will be a highly cost effective way of reducing the incidence of breast and ovarian cancer through preventative strategies.
Title: Implementation of next generation cancer gene panel testing in a large HMO


Body: Background: Next generation cancer gene panel testing is fairly new in clinical practice. Little is known about the diagnostic yield of multigene cancer panel testing in community based hospitals.

Objective: To describe characteristics of a diverse cohort who underwent high/moderate risk multigene panel testing for either a personal or a family history of cancer in a large health plan, and report the proportion of pathogenic/likely pathogenic variants (PV/LPV) and variants of unknown clinical significance (VUS) by race/ethnicity.

Methods: Subjects included all 586 female patients who were referred for genetic counseling and underwent multigene panel testing between July 2014 and January 2015. Based on a literature review, the custom-designed high/moderate risk gene panel included 20 cancer susceptibility genes (described below). All tests were performed by the same commercial laboratory (GeneDx).

Results: Of the 586 women, 78 (13.3%) tested positive PV/LPV; 316 (53.9%) tested negative; and 192 (32.8%) carried one or more VUS. Age at testing ranged from 22-81 years (median 50 years). More women with PV/LPV results tended to be obese than those who tested negative (39.7% vs. 31.2%), and had greater comorbidity (Charlson Index of ≥3, 35.9% vs. 33.2%). Of 586 women, 305 (52.0%) had a cancer diagnosis, mainly first primary breast cancer (n=290, 95.1%), while some also had a second primary breast cancer (n=67, 11.4%). Of the 305 women with cancer, 131 (42.9%) were diagnosed prior to the multigene testing implementation (1987-2013), while 174 (57.1%) were diagnosed after implementation.

The cohort was diverse in terms of race/ethnicity: Western/Northern European (31.2%), Latina/Caribbean (30.0%), Asian (14.8%), African-American (7.2%), Ashkenazi Jewish (6.3%), Native American (5.9%), and other (14.9%) (percent exceeds 100% due to mixed race/ethnicity). Of the 192 women who carried a VUS, 60.4% were Western/Northern European, and 46.4% were Latina/Caribbean. Pathogenic or likely pathogenic mutations were higher in Latina/Caribbean women (37.2%), followed by Western/Northern European (26.7%), and African (10.3%). We identified a total of 84 pathogenic mutations among the 78 women with PV/LPV in the following genes: BRCA1 (n=22), BRCA2 (n=17), MUTYH (n=16; all heterozygous), CHEK2 (n=9), ATM (n=4), PALB2 (n=4), PMS2 (n=3), MLH1 (n=2), VHL (n=2), and one mutation in each of the following genes: APC, CDH1, PTEN, TP53, and STK11. VUS were detected in 192 patients (32.7%) of the 586 tested. VUS in ATM (n=57), APC (n=32) and CHEK2 (n=25) comprised 59.4% of all VUS detected.

Discussion: The large percent of VUS was surprising, given that our panel included only high/moderate risk cancer genes. The over-representation of BRCA1/2 among all mutations (45.1%) likely reflected a greater proportion of patients referred for genetic counseling with a personal and/or family breast cancer history. Given that 35% of our positive results were dominant-acting pathogenic or suspected pathogenic mutations, our results suggest that multigene cancer panel testing is an appropriate method for detecting germline mutations in a high-risk cohort in a managed care setting.
Title: Multiple-gene panel sequencing prompts no change in care compared to BRCA1 and BRCA2 testing in male breast cancer patients and their families

Fostira F, Saloustros E, Konstantopoulou I, Tryfonopoulos D, Barbounis V, Mauroudis D, Georgoulias V, Fountzilas G and Yannoukakos D. NCSR Demokritos, Molecular Diagnostics Laboratory, Athens, Greece; University Hospital of Heraklion, Heraklion, Greece; Saint Savvas Anticancer Hospital, Athens, Greece; Metropolitan Hospital, Athens, Greece and Aristotle University of Thessaloniki School of Medicine, Thessaloniki, Greece.

Body: Male breast cancer is a rare cancer entity, representing just 1% of all cancers diagnosed in men. It shares clinical and biological characteristics with post-menopausal female breast cancer, although recent reports showed that they are not identical. Approximately 10% of unselected male breast cancer cases carry BRCA1 & BRCA2 mutations, with the most frequently mutated gene being BRCA2. Multiple-gene sequencing has already entered clinical practice and leads in detection of potential pathogenic variants in other cancer predisposition genes, in as many as 15% female breast cancer patients without BRCA1 & BRCA2 mutations. We evaluated the performance of a customized panel for cancer risk assessment in a representative sample of male breast cancer patients.

Ninety eight male individuals diagnosed with breast cancer (mean age 59y, range 38y-81y) and referred for genetic counseling between 2010 and 2015 were invited to donate a research blood sample. Patients were eligible irrespectively of family history and age at diagnosis. Genomic DNAs were used to prepare libraries for the capture of the entire coding regions of twenty six known -or suspected- breast cancer genes. These were: BRCA1, BRCA2, TP53, STK11, CDH1, PTEN, PALB2, ATM, CHEK2, NBN, BRIP1, BARD1, RAD51C, RAD51D, RAD50, BLM, SLX4, MRE11A, FAM175A, MLH1, MSH2, EPCAM, MHS6, PMS2, MUTYH, NF1.

Loss-of-function mutations have been found in three genes; BRCA2, BRCA1 and PMS2. Not surprisingly, the most frequently mutated gene was BRCA2 accounting for 5% of the cases, while BRCA1 and PMS2 mutations have been detected in single cases, accounting each for 1%. Interestingly, no mutations were identified in any of other genes that have been reported in female breast-ovarian cancer families (PALB2, TP53, ATM, CDH1, RAD51C, RAD51D, etc). Compared to BRCA1 & BRCA2 testing, gene panel prompted consideration of change in care (early colon cancer screening) in a single family (1%).

This is the first evaluation of the performance a multiple-gene panel in male patients with breast cancer. Our findings confirm that genetic counseling and BRCA1 & BRCA2 should be the primary concern for these patients and their families. Mutations in other genes been reported in female breast-ovarian cancer families were not identified in this cohort. If larger studies confirm our results, then multiple-gene sequencing panels may be of limited benefit for male breast cancer patients and their families.
Title: Multi-gene panel testing for hereditary cancer risk

Yadav S, Ladkany R, Fulbright J, Dreyfuss H, Reeves A, Campian S, Thomas V and Zakalik D. Beaumont Health System, Royal Oak, MI and Nancy and James Grosfeld Cancer Genetics Center, Beaumont Health System, Royal Oak, MI.

Body: Background: Multi-gene panels are widely available for assessing hereditary cancer risk in high risk individuals. Due to the use of these panels, many genetic mutations other than BRCA 1 or 2 can be detected which can potentially affect management. This study presents the results of multi-gene panel testing performed at Beaumont Health System.

Methods: All patients who underwent multi-gene panel testing at Beaumont Health System between November 1, 2012 and January 15, 2015 were included in this study. This cohort consisted of patients who met criteria for genetic testing due to personal or family history. All patients received comprehensive pre and post-test genetic counseling. The panels ranged from 5 to 43 genes associated with risk for breast and other cancers.

Results: 653 multi-gene panel tests were performed. The majority of these consisted of either a 5 gene high risk breast panel (25%), an 18 gene moderate to high risk breast panel (21%), or a 9 gene high risk breast and gynecologic panel (17%). 184 variants of undetermined significance (VUS) were identified with a pooled VUS rate of 28%. Among the commonly used panels, there was a positive correlation between VUS rate and the number of genes included in the panel (r = 0.86, p = 0.01, Range 6% to 70%). A pathogenic mutation was identified in one or more genes in 65 (10%) panels for a total of 67 mutations. Of these, 17 mutations were in BRCA1 or BRCA2 gene. Fifty non-BRCA deleterious mutations were identified with the following frequencies: CHEK2(12), MUTYH(7 monoallelic, 1 biallelic), TP53(4), PTEN(4), ATM(4), MSH6(3), PALB2(3), MSH2(2), CDH1(2), APC(2), NF1(2), BARD1(2), MLH1(1) and PMS2(1). Of these non-BRCA mutations, 41(82%) had a significant impact on management.

Conclusions: Our study demonstrates that multi-gene panel testing identifies several genes that can impact management and would likely not have been discovered by pedigree analysis alone. However, this added detection is associated with a higher VUS rate, especially using larger panels. Further research is needed to better define the role of multi-gene panel testing in high risk patients, with a focus on choosing appropriate genes, understanding the magnitude of cancer risk and delineating impact on management.
Title: The patient experience in a prospective trial of multiplex gene panel testing for cancer risk

Kurian AW, Idos G, McDonnell K, Ricker C, Sturgeon D, Culver J, Lowstuter K, Hartman A, Allen B, Rowe-Teeter C, Kingham KE, Koff RB, Lebensohn A, Chun NM, Petrovchich IM, Mills MA, Hong C, Ladabaum U, Ford JM and Gruber SB. Stanford University School of Medicine, Stanford, CA; University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA; Keck School of Medicine, University of Southern California, Los Angeles, CA and Myriad Genetics, Salt Lake City, UT.

Body: Background: Multiplex gene sequencing panels (MGP) are increasingly used for assessment of hereditary breast cancer risk. Compared to testing for BRCA1 and BRCA2 (BRCA1/2) only, testing more genes increases the likelihood of identifying a deleterious mutation (DM) and/or a variant of uncertain significance (VUS), which might cause distress, uncertainty or regret about testing. Little is known about the patient experience of MGP testing.

Methods: We conducted a prospective study of MGP testing, using a panel of 25 genes: APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, and TP53. Participants were enrolled at three medical centers and were eligible if they met standard genetic testing guidelines or if they had ≥2.5% probability of a DM in any gene on the panel, as calculated by predictive models (e.g. IBIS, Penn II, MMRPro). Participants were surveyed about their experiences with MGP testing including distress and uncertainty at baseline (before test results disclosure) and three months later. The 25-item Multidimensional Impact of Cancer Risk Assessment (MICRA) scale measured distress, uncertainty and positive testing experiences at three months after testing. We present a planned interim analysis after enrolling 500 of 2000 total participants.

Results: Of 500 participants, 332 (66%) were referred for suspicion of hereditary breast/ovarian cancer syndrome. Of these 332, 97% were female, 79% were white, 43% were Hispanic and 33% were Spanish-speaking only; for 25%, high school was their highest level of education. A total of 48% had breast cancer, 5% had ovarian cancer, and 7% had another cancer; 11% had a DM and 35% had VUS in one or more genes. At study entry most participants thought about cancer rarely or not at all (69%, 95% confidence interval (CI) 58%-77%), and few (7%, CI 3%-14%) had thoughts of cancer that affected their daily lives; results were unchanged three months later, after genetic results disclosure (Chi-squared test, p-value >0.1). MICRA scores at three months were low for distress (mean score 2 out of a possible 30) and uncertainty (mean score 7 out of 45), and high for positive testing experiences (mean score 9 out of 15). Most (82%, CI 72%-88%) participants wanted to know all of their MGP results even if the clinical relevance was not fully understood, and most (87%, CI 79%-93%) never regretted learning their MGP results.

Conclusions: Among diverse participants of a prospective, multi-center MGP testing trial, cancer- and genetic testing-related distress were low at entry and remained low three months later. These results provide no evidence for an increase in distress or uncertainty after MGP. Longer-term follow-up in a larger cohort is underway.
Title: Universal BRCA testing and family outreach for women with triple negative breast cancer

Emborgo T, Muse KI I, Bednar E, Oakley HD D, Litton J, Lu KH H and Arun BK K.  The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background Germline mutations in BRCA1 or BRCA2 significantly increase the lifetime risk for a woman to develop breast and ovarian cancers. Triple negative breast cancer (TNBC) is enriched for BRCA mutations, with approximately 20% of unselected TNBC patients having a BRCA1 or BRCA2 mutation. Current guidelines recommend individuals with TNBC age 60 or younger undergo BRCA genetic testing. Studies suggest that expanding testing beyond age 60 may capture additional BRCA positive women. Identification of a BRCA mutation has significant implications for early cancer detection and prevention, treatment options, and at-risk blood relatives. Many at-risk relatives are not aware of, or pursue genetic testing for mutations identified in their family. Our previous prediction models have shown that testing all at-risk first degree relatives of TNBC patients may reduce the risk of breast and ovarian cancers by 23%, and 41%. Few studies exist regarding the occurrence or success of intra-family cascade genetic testing. Study Design This study is conducted through the University of Texas MD Anderson Cancer Center’s Women’s Cancer Moonshot program. This is a five year prospective cohort study of unselected women with confirmed TNBC. It provides universal BRCA genetic testing for TNBC patients. TNBC patients are enrolled in a research registry which provides clinical BRCA genetic testing regardless of age of diagnosis. BRCA positive TNBC patients are consented for a separate prospective family outreach protocol (REACH registry). The REACH registry includes questionnaires and active outreach to at-risk family members using an innovative information-technology platform and a variety of web-based patient education tools. Results In year one of our study, a total of 439 patients with TNBC have been seen for genetic counseling and 377 (86%) have undergone BRCA genetic testing. Fifty-one (14%) patients were identified as having a BRCA mutation. Of those with a BRCA mutation, 48 (94%) have a BRCA1 mutation, 3 (6%) have a BRCA2 mutation and 1 (2%) would not have been identified by current testing guidelines. Further, 74 patients with identified BRCA mutations and 50 at-risk family members have enrolled in the REACH registry. Recruitment and data collection of patients and family members, and their communication, genetic testing, cancer risk reduction, and surgical choices are on-going. Using our innovative IT platform to collect information and to communicate with patients and families, we anticipate an increased study recruitment, patient and family participation, and ultimately improved awareness, education, and cancer-prevention and screening among our patients and their family members. Conclusion Through this study we have maximized awareness and identification of high-risk hereditary cancer patients through implementation of universal BRCA1 and BRCA2 genetic testing of all TNBC patients. We have shown initial feasibility to successfully recruit family members to our REACH registry. REACH registry is an innovative research platform providing education and awareness to patients and at-risk family members to aid communication and dissemination of BRCA test results and to assess the psychosocial and behavioral impacts of a mutation in a family.
**Title:** Risk-reducing surgery and cancer-related distress among female *BRCA1* and *BRCA2* mutation carriers

Liede A, Fairchild A, Friedman S, Amelio J, Hallett DC C, Mansfield CA A and Metcalfe KA A. Amgen Inc., CA; Facing Our Risk of Cancer Empowered (FORCE), Tampa, FL; University of Toronto, Toronto, ON, Canada and RTI Health Solutions, Research Triangle Park, NC.

**Body:**

**Background:** Distress levels among female *BRCA1* and *BRCA2* mutation carriers can be similar to levels reported among breast cancer patients. However, there is a lack of data on long-term psychosocial functioning, and it is not known if uptake of risk-reducing surgery influences long-term cancer related distress in women with a *BRCA* mutation who are unaffected with cancer. The objective of this study was to evaluate long-term cancer-related distress in women with a *BRCA* mutation, and to evaluate predictors of distress, including uptake of cancer risk reducing surgery.

**Methods:** Female *BRCA1* or *BRCA2* mutation carriers, ages 25-55, and without cancer were eligible to complete the survey online. A validated instrument, Impact of Events Scale (IES)-Revised (Horowitz 1979, Weis & Marmar 1995; 0-80 overall scale), was used to assess current levels of cancer risk-related psychological distress. Respondents were recruited through the Facing Our Risk of Cancer Empowered (FORCE) advocacy organization, which includes women at high risk of breast cancer. This interim analysis is part of a larger multi-center patient preference study of *BRCA* mutation carriers designed to assess women's willingness to adopt hypothetical treatments to prevent breast cancer. Linear regression was used to evaluate predictors of IES distress levels.

**Results:** Between January and April 2015, 259 women completed the survey. The mean age of the participants was 41 years, and the mean time since receipt of genetic test results was 3.5 years (range 0-16; median 2 years). One hundred thirty-six (52%) women elected for prophylactic bilateral mastectomy (PBM), 139 (54%) elected for bilateral salpingo oophorectomy (BSO) (93 [36%] women had both surgeries), and 77 (30%) had not undergone risk-reducing surgery. The mean total IES score was 15.1 (range 0-72; median 11). Overall, 54 (21%) women reported moderate or severe cancer-related distress, and those who had undergone risk-reducing surgery reported lower perceived risk of developing breast cancer. Results to date indicate that shorter time since notification of mutation status, not having PBM (with or without BSO) (table), and not completing post-secondary education were independent predictors of higher IES distress scores.

<table>
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<th>IES severity</th>
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<th>PBM only</th>
<th>BSO only</th>
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<tr>
<td>Moderate</td>
<td>18 (23)</td>
<td>5 (12)</td>
<td>6 (13)</td>
<td>11 (12)</td>
</tr>
<tr>
<td>Severe</td>
<td>6 (8)</td>
<td>2 (5)</td>
<td>3 (6)</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

**Conclusions:** This study measured cancer-related distress in a large population of women with *BRCA* mutations who participate in the FORCE online support community. Higher levels of distress were associated with not having PBM and more recent genetic test disclosure. These findings are specific to a more informed community of women with high levels of understanding of cancer risk than may be seen in the clinical setting.
Double heterozygosity for BRCA1 and BRCA2 pathogenic variants in a French metastatic breast cancer patient


Double heterozygosity is an extremely rare occurrence in hereditary breast and ovarian cancer syndrome (HBOC [MIM 604370; MIM 612555]) where two pathogenic variants, one in BRCA1 and one in BRCA2, are found in an individual. To date, only a few case reports and case series have been reported in the literature (1-3). Furthermore, little is known about the clinical characteristics, family history, and tumor histology in these patients. In this study, we utilized targeted gene testing with next-generation sequencing (NGS) technology in an early-onset metastatic breast cancer patient from France. We evaluated germline variants using Pathway Genomics' BRCATrueTM NGS test, which analyzes variants covering all exons and exon flanking regions in both the BRCA1 and BRCA2 genes. All variant calls were determined after alignment and mapping to the GRCh37/hg19 reference genome. Variant calls were confirmed by Sanger sequencing. In this patient, a c.1016dupA (p.V340GfsX6) frameshift variant was found in BRCA1 along with a c.6814delA (p.R2272EfsX8) frameshift variant in BRCA2. Both frameshift variants are predicted to truncate the BRCA proteins. The BRCA1 c.1016dupA variant is considered a Norwegian founder mutation but has also been observed in individuals who are of French-Canadian, French, Italian or Dutch ancestry (4-7). The BRCA2 c.6814delA (p.R2272Efs*8) pathogenic variant, also known as 7042delA, is predicted to truncate the BRCA2 protein and has been identified in individuals with a personal or family history of breast and/or ovarian cancer (8,9). To the best of our knowledge, the combination of these two pathogenic variants in an individual has not been previously reported. In a clinical diagnostic setting, the possibility of double heterozygosity of pathogenic variants in more than one susceptibility gene should be considered, especially in patients with early-onset metastatic cancers. Furthermore, genetic testing and genetic counseling should also be indicated for high-risk family members.

Title: Consistency of pathogenicity determinations for hereditary cancer gene mutations


Background: As the number of laboratories offering genetic tests grows, the potential for inconsistent variant classifications increases. New resources can help address this: (a) ClinVar, a rapidly growing public database of clinical variants to which many (but not all) laboratories contribute; (b) the public release of thousands of BRCA1/2 reports from Myriad Genetics through the Sharing Clinical Reports Project (SCRP), the Free the Data (FTD) initiative, and recent publications; and (c) ExAC, a greatly improved database of population allele frequencies. These complement longstanding efforts, e.g. the ENIGMA consortium. In addition, the American College of Medical Genetics (ACMG) recently updated guidelines for the interpretation of sequence variants. Using these resources, we sought to investigate the consistency of variant classifications to help inform ongoing practice.

Methods: Pathogenicity assessments for variants in hereditary cancer genes were collected from multiple sources. Among these were 15,364 BRCA1/2 submissions to ClinVar, including 5416 submissions from SCRP and 1062 from our prior work [1]. When 3 or more submissions for a variant were available, we determined a consensus interpretation requiring 2 of 3 submitters to agree (or 3 of 4, etc.) to identify outliers. For our own classifications, we established a point-based system based on the 2015 ACMG guidelines, and independently applied it to publicly available evidence of pathogenicity without regard to other labs’ classifications.

Results: Initially, discordance among ClinVar submissions appears high (20-30%). However, upon investigation much of this discordance is a result of (i) research submissions to ClinVar, (ii) differences in confidence (e.g. benign vs. likely benign), (iii) older data in ClinVar, (iv) single lab outliers, and (v) nuances in the detailed structure of the ClinVar database. A careful comparison using the consensus methodology of objectively filtered ClinVar data shows high concordance between our interpretations and consensus: 95% were identical and 99% were similar (e.g. benign and likely benign were considered similar). Where consensus was not achieved (8% of variants with 3 or more independent sources) or not possible (any variant with only 2 sources), pairwise comparisons showed that few of these remaining differences (5%) were clinically significant. Also, many variants with significant discordances appeared to be particularly rare in the human population, and thus would be present in few patients. The rate of discordances with SCRP/FTD data was similar with that of other ClinVar submitters.

Conclusions: Evaluations of inter-laboratory concordance need to be done carefully to avoid over-counting differences. Laboratories generally agree on the clinical significance of the vast majority of variants. Furthermore, the inter-laboratory consensus classification is often reached using a careful implementation of the ACMG guidelines and publicly available data. Thoroughly understanding the remaining differences is challenging when the evidence used by any laboratory is not available for peer review. Detailed data and methods from this study are available for review and alternate analyses.

Title: Prevalence of Hispanic BRCA1 and BRCA2 mutations among HBOC patients from Southern Brazil reveal differences among Latin American populations

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Body: Background. Breast cancer (BC) is the most common malignancy in women around the world, and 5% of BC cases and 15% of ovarian cancer (OC) cases can be attributed to BRCA1 and BRCA2 germline mutations, configuring Hereditary Breast and Ovarian Cancer Syndrome (HBOC). Considering that the genetic similarities between Latin American populations are largely unknown, we aimed to address if a panel of Hispanic BRCA mutations could be useful for a Brazilian population.

Methods. Unrelated patients fulfilling NCCN criteria for HBOC syndrome were recruited. ASCO, Myriad and Penn II clinical testing criteria were analyzed for all patients. Blood-derived DNA samples were screened for 114 BRCA mutations, in a panel estimated to account for up to 90% of all BRCA Hispanic mutations (HISPANEL), using the Sequenom MassArray platform. Results. Among a total of 233 unrelated patients included (225 women and 8 men), 85% were BC patients and 14 patients were cancer unaffected. The mean age at diagnosis was 43 years for BC affected individuals and 45 years for patients with OC. Fifty-three patients fulfilled ASCO criteria, and 182 had Penn II ≥ 10%, while only 64 had a Myriad score ≥10%. BRCA mutations were detected in 9 of 233 patients (prevalence of 3.86%). Among BC and/or OC affected individuals, this prevalence was 4.22%. Features of mutation carriers are depicted in table 1.

Table 1. BRCA mutations identified in Brazilian HBOC patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Gender</th>
<th>Tumor type and age at diagnosis</th>
<th>Family history of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 c.3331_3334delCAAG</td>
<td>Female</td>
<td>OvCa, 47; BrCa, 50</td>
<td>Daughter BrCa≤20; Brother PrCa 54; Cousin BrCa≥ 50</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Female</td>
<td>Bilateral BrCa, 35 and 45</td>
<td>Mother OvCa 58; Aunt OvCa 49; Cousin BrCa 49; Great-grandmother BrCa 90</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Female</td>
<td>BrCa, 36</td>
<td>Aunt BrCa 62; Cousin BrCa 45; Cousin BrCa 44; Cousin Bilateral BrCa 45; Cousin BrCa 44; Cousin OvCa 52</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Female</td>
<td>OvCa, 52</td>
<td>Mother OvCa 77; Sister BrCa 44; Aunt OvCa 66</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Female</td>
<td>Bilateral BrCa, 23 and 44</td>
<td>Mother BrCa 45; Aunt Bilateral BrCa 48; Cousin BrCa 30; Aunt Bilateral BrCa 58; Grandmother BrCa</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Female</td>
<td>Bilateral BrCa, 26</td>
<td>Sister BrCa 50; Niece BrCa 28; Niece BrCa 30</td>
</tr>
<tr>
<td>BRCA1 c.5266dupC</td>
<td>Male</td>
<td>BrCa, 64</td>
<td>Daughter BrCa 34; Sister BrCa≤ 50; Sister BrCa 32</td>
</tr>
<tr>
<td>BRCA2 c.2806_2809delAAAC</td>
<td>Female</td>
<td>BrCa, 49</td>
<td>Sister BrCa 32; Sister BrCa 44; Aunt Bilateral BrCa 40 and 55</td>
</tr>
<tr>
<td>BRCA2 c.2806_2809delAAAC</td>
<td>Female</td>
<td>Bilateral triple negative BrCa, 42 and 55</td>
<td>None</td>
</tr>
</tbody>
</table>

BrCa=breast cancer; OvCa=ovarian cancer.

Conclusions. The HISPANEL detects 59 BRCA1 and 55 BRCA2 mutations, including some mutations previously reported in Brazilian individuals. Most mutation carriers had the BRCA1 c.5266dupC (5382insC) mutation, a common founder mutation in several populations. Our results are largely different from other Latin American published data. Although low BRCA prevalence mutational rates were also seen in Peru and Mendellín (Colombia), in Mexico and Bogota (Colombia) a prevalence of 27% and 15%, respectively, was found in studies using HISPANEL and all studies included patients unselected for family history. Our
findings reinforce that different Latin-American populations have different \textit{BRCA} mutational profiles.
Title: BRCA mutations in South Texas and the Rio Grande Valley among Hispanics with a personal or family history of breast cancer

Mette LA A, Poullard NE E, Pulido Saldivar AM, Torres IC C, Seth SG G, Seigler D, Jatoi I and Tomlinson GE E. University of Texas Health Science Center at San Antonio, San Antonio, TX; Greehey Children’s Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX; Laredo Regional Campus, University of Texas Health Science Center at San Antonio, Laredo, TX; Regional Academic Health Center, University of Texas Health Science Center at San Antonio, Harlingen, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX and Cancer Therapy & Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: South Texas, including regions along the US-Mexico border, is characterized by a predominantly Hispanic population, most of who are of Mexican decent. In the past decade, the discovery of Mexican-American founder mutations in BRCA1/2 has established the importance of large rearrangement testing in this population. Recent studies estimate that 15-25% of Mexican women with early-onset breast cancer carry a BRCA mutation, with the BRCA1 EX9-12 deletion accounting for 10% of all mutations. Here we review the BRCA1/2 pathogenic and unclassified variants in our South Texas Hispanic population who underwent cancer genetic risk assessment and testing between June 2013 and May 2015. All mutation carriers had either a personal history of early-onset breast cancer (before age 50), or early-onset breast cancer in a first-degree relative. A total of 23 unique mutations were identified in 28 kindreds. Three affected individuals harbored mutations that had not previously been reported in the literature at the time of testing (BRCA1 134+3A>T; BRCA2 428C>G; BRCA2 8265A>T). Pathogenic mutations in BRCA1 were the most common finding, accounting for 64.3% of identified variants. The BRCA1 EX9-12 deletion was only found in only family. We also identified a co-segregating pathogenic mutation (1793delA) and variant of uncertain significance (1149G>A) in BRCA1 in two unrelated kindreds, suggesting a possible founder mutation. Although previous studies have identified recurrent BRCA mutations in Hispanic families with breast cancer in the Southwestern United States, this was not seen in our sample of South Texas Hispanics. While only one (3.5%) of our families had the BRCA1 EX9-12 deletion, other large rearrangements, such as BRCA1 EX1-2 deletion, BRCA1 EX21-22 duplication and whole gene deletions demonstrate the continued importance of comprehensive BRCA1/2 analysis in Hispanics. This work was supported by CPRIT Grant PP120089 and NIH 2P30CA054174.

BRCA gene mutations in South Texas Hispanics with a personal or family history of breast cancer

<table>
<thead>
<tr>
<th>Mutation (n%)</th>
<th>Frequency</th>
<th>Kindreds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA1 pathogenic (64.3%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>delEX1-2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>1793delA*</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>EX12del</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>815_824deupAGCCATGTGG</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>delEX9-12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>entire gene deletion</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3878delTA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>lVS16+3G-T</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2806_2809delAAAC</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>188insAG</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4936delG</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>dup21-22</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>BRCA2 pathogenic (9.5%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Variant</td>
<td>Count 1</td>
<td>Count 2</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>R2520X</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8377G&gt;A</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8591G&gt;A</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>BRCA1 variant of uncertain significance (16.7%)</strong></td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>1149G&gt;A*</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>7819C&gt;T</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5470A&gt;G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>134+3A&gt;T</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>BRCA2 variant of uncertain significance (9.5%)</strong></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>314+12A&gt;G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>428C&gt;G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3575T&gt;G</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>8265A&gt;T</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*co-segregating
Title: Surveillance program for women carrying BRCA1/BRCA2 genetic predisposition

Sidoni T, Coccioitone V, Cannita K, Di Giacomo D, Ciccozzi A, Bafille A, Pizzorno L, Resta V, Marsecano C, Ferrari F, Di Cesare E, Ficorella C and Ricevuto E. Medical Oncology, San Salvatore Hospital, University of L'Aquila, L'Aquila, Italy; University of L'Aquila, L'Aquila, Italy; Breast Imaging Unit, San Salvatore Hospital, L'Aquila, Italy; Breast Unit, San Salvatore Hospital, L'Aquila, Italy; Radiology, San Salvatore Hospital, University of L'Aquila, L'Aquila, Italy; Radiotherapy, San Salvatore Hospital, University of L'Aquila, L'Aquila, Italy and Oncology Territorial Care, Network ASL1 Abruzzo, San Salvatore Hospital, L'Aquila, Italy.

Body: Between January 2003 and January 2015, women at genetic risk of developing breast and/or ovarian cancer were selected for a surveillance program. The monitoring strategy consisted of the association of breast ultrasound (US), every six months, and Rx-mammography (XM) and breast MRI (MRI) to be performed annually. To date, 29 women have been included in the surveillance program: BRCA1 mutation carriers, 18; BRCA2 mutation carriers, 11.

Eight women (28%) had already developed breast and/or ovarian cancer, while 21 (72%) were unaffected carriers. At a median surveillance time of 33 months (range 0-144), 4 incidental breast cancers were diagnosed in 4 BRCA1/2 mutation carriers (4/29, 14%), 2 BRCA1+ and 2 BRCA2+; 3/21 (14%) unaffected carriers and 1/8 (13%) previously affected carriers. Two cancers (50%) were detected by all three diagnostic tools; 2 (50%) were identified only by US and MRI in patients aging 33 and 43, respectively.

Characteristics of patients with breast cancer diagnosed during surveillance

<table>
<thead>
<tr>
<th>age</th>
<th>mutated gene</th>
<th>previous cancer</th>
<th>US</th>
<th>XM</th>
<th>MRI</th>
<th>histology</th>
<th>surveillance time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>BRCA2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ductal</td>
<td>74+</td>
</tr>
<tr>
<td>43</td>
<td>BRCA2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ductal</td>
<td>40+</td>
</tr>
<tr>
<td>45</td>
<td>BRCA1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ductal</td>
<td>60+</td>
</tr>
<tr>
<td>40</td>
<td>BRCA1</td>
<td>yes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ductal</td>
<td>74+</td>
</tr>
</tbody>
</table>

One of these four patients, affected by breast cancer, died at 31 months of overall survival; three underwent surgery, chemotherapy and radiotherapy and are still disease free, in follow-up at 59, 28 and 26 months, respectively.

In conclusion, 29 BRCA1/2 carriers have been included in the surveillance program, the median age was 42.5 months (range 27-68) and the median surveillance time was 33 months (range 0-144). Our preliminary data confirm the 3% expected rate of diagnosed cancers and the effectiveness of performing the triple diagnostic surveillance with US, XM and MRI.
Title: Rapid BRCA analysis: Experience of a single institution


Body: The interest of rapid BRCA analysis, even before the initial treatment could modify the surgical indications (ie total mastectomy and immediate reconstruction even in case where tumorectomy would be suitable). The aim of this study was to evaluate the indications and consequences of this rapid determination of BRCA status in our institution.

Patients
Rapid BRCA analysis was conducted in 129 patients (pts) treated for breast cancer and addressed to the genetician at Oscar Lambret Center between 2009 and 2014: in 98 pts to discuss breast and axilla surgical modalities, in 4 cases to discuss oophorectomy, in 3 cases to have access to PARP inhibitors. In the other 24 pts, the reasons were debatable: patient leaving abroad, patient requiring reproductive assisted technology.....

Results
38 deleterious mutations (29.4%) were identified (31 BRCA1, 7 BRCA2) in 38 pts.
Cancer treatment was on going for 107 pts, programmed for 12 pts and finished for 10.
The median time between cancer diagnosis and genetic counseling was 81 days (5-5160), between blood sample collection and BRCA analysis 37 days (13-132), between availability of the laboratory results and the information to the patient: 39 days (1-234); 7 pts (5.4%) never came to obtain the laboratory results.
Treatment was modified after genetic counselling for 8 pts out of 119 (7%). Six of them had BRCA1 deleterious mutations. Three decided subsequent radical mastectomy and adjuvant radiation was cancelled, another had a radical mastectomy before radiation, another treated for bilateral breast cancer had a bilateral radical mastectomy before radiation and the last treated for unilateral breast cancer had a bilateral radical mastectomy before radiation.
Out of these 8 pts, two did not have a deleterious mutation. One had a bilateral radical mastectomy before laboratory result and one with BRCA variant of unknown significance had a homolateral radical mastectomy.

Conclusion discussion
Our experience shows that rapid BRCA analysis is feasible. In this selected population the mutation rate is very high. However, first, it appears that indications for rapid BRCA analysis were variable and not always relevant. Second, genetic result impacts on treatment only in 7% of pts. Indications could be justified by the use of PARP inhibitors. Up to now, the indications of rapid genetic analysis are rare.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-09-16

Title: Comparison of prediction models of breast cancer in high risk populations?


Body: Breast cancer is the most frequent female cancer with 48 800 new cases each year in France. A French national screening program in women without genetic risk factors has been initiated in 2004. Women with BRCA 1 or 2 mutations are included in surveillance programs. Women without such mutations can be assessed for breast cancer risk using risk prediction models. The aim of this study is to compare the performance of three breast cancer risk prediction models: GAIL2, BODICEA and IBIS in a population of patients (pts) who developed breast cancer.

Patients and Methods: The GAIL1 model was performed to 188 pts at high risk of breast cancer according to their family history and who developed breast cancer. Personal and familial data was regenerated at the day before the diagnosis of breast cancer and the three models were applied. From this population, 60 pts were selected: for whom all information necessary to use the 3 models was available and for whom the GAIL1 model provided sufficient variability in the relative risk of breast cancer.

The GAIL2 model takes into account individual risk factors: age, menarches, parity, age at first birth, history of breast biopsy, atypical hyperplasia or in situ lobular carcinoma. The BODICEA model considers age and familial risk factors: number of related affected by breast ovarian, prostate and pancreas cancer and age at diagnosis. Both personal and familial risk factors are used for the IBIS model.

We estimated lifetime breast cancer risk for each patient with the three models from the available software packages. We assessed Pearson's correlation coefficient between the three models.

Results

Median (range) age was 45 years (25-74). Risk prediction could not be evaluated by the GAIL2 model for 14 pts since they were less than 35 years of age.

Lifetime breast cancer risk was 16.1% (4.4-38.7) for GAIL2, 11.6% (2.2-39.5) for BODICEA and 16.5% (3.5-36.3) for IBIS. Pearson's correlation coefficient between BODICEA and GAIL2, IBIS and GAIL2 and between BODICEA and IBIS were 0.36, 0.38 and 0.69 respectively.

In most cases, IBIS risk predictions were higher than GAIL2's which were higher than BODICEA's as soon as lifetime risk was at least 20%. When the three models were applicable (46pts), IBIS estimated a higher risk in 31 cases (67%) versus 10 for GAIL2 (21.74%) and 5 for BODICEA (10.86%).

The median (range) time for use of the tools per patient was 30 seconds (16-80) for GAIL2, 588 (198-1804) for BODICEA and 86 (46-135) for IBIS.

Discussion

Results in this selected population of pts who developed breast cancer show that IBIS seems to be the better performer to predict breast cancer risk: Results are obtained faster and the risk predictions provide higher estimates. The GAIL2 model is quick and easy to use but with a limited number of items. Conversely, BODICEA requires a very large number of items, not always available, and does not consider incomplete data.

In conclusion, IBIS model seems to be the most suitable for practical use in the evaluation of breast cancer risk.
Title: Frequency of large rearrangements in BRCA1, BRCA2, ATM, CHEK2, and PALB2 in hereditary cancer testing

Bissonnette J, Doonanco K, Xu Z, Klein RT T and Hruska KS S. GeneDx, Gaithersburg, MD.

Body: Objectives
Large deletions and duplications are recognized as a cause of many inherited cancer syndromes; however, few studies have examined the frequency. In one study, large rearrangements accounted for 11-14% of pathogenic variants in BRCA1 and 1-3% of pathogenic variants in BRCA2 (Judkins 2012). Additionally, in a study of invasive ovarian cancer patients, large deletions and duplications accounted for 6% of BRCA1 and 3% of BRCA2 pathogenic variants (Zhang 2011). The frequency of large deletions and duplications in moderate penetrance genes, such as ATM, CHEK2, and PALB2, has not been carefully studied. We therefore sought to determine the frequency of pathogenic and likely pathogenic (P/LP) large deletions and duplications in BRCA1, BRCA2, ATM, CHEK2, and PALB2.

Methods
We analyzed a consecutive case series of patients who had BRCA1/2 and cancer panel testing. The data were analyzed to determine the percentage of deletions or duplications of greater than 250 bp identified on array comparative genome hybridization as a percentage of all pathogenic variants (PVs)/likely pathogenic variants (LPVs) in each of BRCA1, BRCA2, ATM, CHEK2, and PALB2.

Results
Large deletions/duplications accounted for 10.1% (55/545) of cases with a BRCA1 PV/LPV and 1.8% (10/556) of cases with a BRCA2 PV/LPV. By ethnicity, BRCA1/2 large deletions/duplications accounted for a higher fraction of PV/LPVs in Asians (11.3%) and Hispanics (8.1%). In Hispanic individuals, all of the large deletions/duplications were identified in BRCA1, accounting for 14.6% of all BRCA1 PV/LPV in this group. In the moderate penetrance genes, large deletions/duplications accounted for 4.6% (9/195), 5.1% (22/436), and 9.9% (13/132) of cases with a PV/LPV in ATM, CHEK2, and PALB2, respectively. There were two recurrent deletions in our cohort: the CHEK2 founder Slavic deletion of exons 9-10 observed in 16 of our cases, and a deletion of exon 11 in PALB2 observed in 6 cases.

Conclusions
Large deletions/duplications account for a substantial proportion of clinically significant variants in hereditary cancer genes. The proportion of large deletions/duplications in BRCA1 and BRCA2 reported here is similar to previous data. However, our cohort suggests that Asian individuals may have a higher proportion of mutations due to large deletions/duplications than previously reported. Among moderate risk cancer genes, PALB2 has the highest proportion of large deletions/duplications.
**Title:** Exploration of the diagnostic utility of next generation sequencing with TruSight cancer panel for BRCA negative hereditary breast and ovarian cancer patients

Dacheva D, Dodova R, Mitkova A, Kamenarova K, Tzveova R, Popov I, Vlahova A, Taushanova – Hadjieva M, Valev S, Dikov T, Timcheva K, Christova S, Mitev V and Kaneva R.  Molecular Medicine Center, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria;  General and Clinical Pathology Clinic, University Hospital "Alexandrovska"/Medical University of Sofia, Sofia, Bulgaria and  Clinic of Medical Oncology (Chemotherapy), Nadezhda Women's Health Hospital, Sofia, Bulgaria.

**Body:** Background. Breast cancer is the most commonly diagnosed malignancy and the most frequent cause of death in women due to cancer. About 5% to 10% of breast cancers are thought to be hereditary. Pathogenic mutations in BRCA1/2 genes across Hereditary Breast and Ovarian Cancer (HBOC) patients estimates are at around 15-20%. Other less common genes have also been associated with an increased risk of developing breast cancer, such as mutations in the TP53, PTEN, RAD51C, CDH1, ATM, CHEK2 or PALB2 tumor suppression genes. NGS based sequencing panels allow fast and simultaneous screening of large number of high- and low-penetrance susceptibility genes in these patients.

Methods. In the current study we included a group of 31 Bulgarian female breast cancer patients, selected following the strict BCLC and NCCN criteria for hereditary cancer. All of them were prescreened by direct sequencing and MLPA analysis, and tested negative for pathogenic mutations in BRCA1 and BRCA2 genes. Next generation target resequencing using a panel of 94 cancer related genes (Illumina TruSight cancer panel) was performed to explore the hereditary component beyond BRCA1/2 genes in these patients. All detected mutations and variants of unknown clinical significance (VUSs) were confirmed by Sanger sequencing method.

Results. Pathogenic and likely pathogenic mutations were found in 14 out of 31 BRCA1/2 negative patients: 1 new frameshift mutation in ATM gene; 6 new likely pathogenic missense mutations in PTCH1, RAD51C, MET, MUTYH, ATM and CHEK2; 7 previously reported pathogenic missense variants in WRN, ERCC4, PALB2, PRF1, RET, SDHB and AIP genes. In addition 27 VUSs (one new splice donor variant in ALK gene and 26 missense variants) were found.

Conclusions. The use of next generation target resequencing with TruSight Cancer panel lead to identification of clinically relevant pathogenic variants in 45% of the investigated patients. This could be the preferred diagnostic method in HBOC patients, carefully selected according the strict BCLC and NCCN criteria.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-09-19

Title: The performance of next generation panel testing in individuals assessed by a community-based genetics program

Daib S, Sedlacek S, Hamlington B, Brzeskiewicz L, Goetsch B, Tedesco K, Patel G, Sudhoff K, Mullineaux L and Langer L. Rocky Mountain Cancer Centers, Denver, CO; 207 Perry Parkway, Gaithersburg, MD; Texas Oncology; Compass Oncology and New York Oncology/Hematology.

Body: INTRODUCTION: Genetic testing identifies patients with an increased risk for hereditary cancer and assists in development of clinical management or cancer prevention strategies. Utilization of multigene panels for patients with a personal or family history of cancer has the ability to identify a significant number of pathogenic variants in high risk and moderate risk genes. Importantly, at-risk patients with previously negative single gene testing can be retested with multigene panels. We characterized the mutations found in patients who had inherited cancer multi-gene panel testing, including patients with negative BRCA testing, and explored the phenotypes associated with the most prevalently mutated gene.

METHODS: Patients were identified by a Community-Based Genetics Program and sent to a commercial laboratory for multi-gene panel testing. A retrospective query of multi-gene oncology tests was performed: patient history, family history and previous genetic test results were reported on the requisition. The following Genes were Utilized in the Inherited Cancer Panels: High risk genes (APC, BMPR1, BRCA1, BRCA2, CDH1, CDKN2A, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, TP53, VHL), moderate risk genes (ATM, CHEK2, PALB2), and genes with increase risk of unknown magnitude (AXIN2, BARD1, BRIP1, CDK4, FANCC, NBN, RAD51C, RAD51D, XRCC2). The combination of genes analyzed was depends on the panel ordered.

RESULTS: 529 individuals were tested with 38 individuals with pathogenic variant (PV) or variant expected to be pathogenic (VEP). Of the 529 individuals, 154 (29%) had previous testing and 12 (7.8%) had PV/VEP. Interestingly, 48% of PV/VEP were in the moderate risk genes ATM, PALB2, CHEK2, and CHEK2 being the most commonly mutated gene. The following table details the genes with Positive and VEP Results:

Table 1: Gene Distribution Patients with PV/VEP

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEK2</td>
<td>13</td>
<td>33.3</td>
</tr>
<tr>
<td>BRCA2</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>BRCA1</td>
<td>4</td>
<td>10.2</td>
</tr>
<tr>
<td>MUTYH</td>
<td>5</td>
<td>12.8</td>
</tr>
<tr>
<td>PALB2</td>
<td>4</td>
<td>10.2</td>
</tr>
<tr>
<td>ATM</td>
<td>2</td>
<td>5.13</td>
</tr>
<tr>
<td>RAD51C</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>MLH1</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>MSH2</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>MSH6</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>PTEN</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>BRIP1</td>
<td>1</td>
<td>2.56</td>
</tr>
</tbody>
</table>

The majority of patients with CHEK2 mutations had a personal history of breast cancer (69%), but the family histories exhibited varied phenotypic expression.
## Personal and Family History for Patients with CHEK2 PV/VEP or VUS (N=26)

<table>
<thead>
<tr>
<th>Site</th>
<th>Personal history</th>
<th>Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Uterine</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Colon</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pancreatic</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Gastric</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Esophageal</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Gynecologic</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION:** Inherited cancer panels provide patients with genetic information that would otherwise be missed by single gene testing and provides access to risk assessment and medical management. Using multigene panels, PV/VEP were discovered in 7.8% of patients with previous negative BRCA testing. CHEK2, a moderate risk gene, was identified in 33% of patients with a PV/VEP. The phenotypic expression for CHEK2 is primarily breast cancer with a wide spectrum of solid and hematological tumors.
Title: Evaluation on the mutation screening by next-generation sequencing in hereditary breast and ovarian cancer: Implementation of recurrent mutation panel

Body: Background: Hereditary disposition accounts for 10-15% in breast cancers and 20-25% in ovarian cancers, in which 5-10% of women have genomic alteration in breast cancer predisposition genes, BRCA1 and BRCA2, while the rest are likely due to less penetrant genes. In specific ethnicities such as Ashkenazi Jewish, three founder mutations have been identified which covers 95% of all the BRCA mutations identified in this race. These genes are screened prior to the gold standard Sanger Sequencing in order to reduce cost. Sanger Sequencing, however, still has the limitation on the necessity of laborious processing and results interpretation. Moreover, it limits the number of genes that can be analyzed in one setting. With the use of next-generation sequencing (NGS), identification of hereditary breast and ovarian cancer (HBOC) syndrome associated genes, other than BRCA, can be sequenced at the same time but yet a faster turnover time. This allows more timely targeted risk-reducing strategies and interventions to be implemented for mutation positive carriers and their family members.

Methods: In this study cohort, 948 high-risk breast/ovarian patients who met the HBOC selection criteria were recruited for mutation screening by our NGS pipeline. With the inclusion of 90 Sanger-validated known mutation cases, the performance of the NGS pipeline were proven to be comparable to Sanger sequencing. PTEN and TP53, other than BRCA1 and BRCA2, a 4 gene sequencing panel were included in the mutation screening for high-risk patients.

Results: The prevalence of BRCA1/BRCA2 germline mutations was 7.28% in our Chinese cohort and 47.8% of the mutation were recurrent mutations. Based on this finding, we further adopted a new workflow by screening the recurrent mutations including founder mutations from Chinese cohort prior to NGS for those who tested negative. In a testing cohort of 343 cases, the recurrent mutation pick-up rate was 3.5%, this implicated a more cost-effective method for mutation screening in the clinical setting. Moreover, the frequencies of PTEN and TP53 were 0.21% and 0.53% respectively in our population with breast and ovarian cases.

Conclusion: Taken together, our data demonstrated a strategic upfront screening for recurrent mutations in Chinese population which is highly applicable in most of the diagnostic laboratories. Multi-gene sequencing using the NGS technology will be the upcoming strategies for mutation screening for HBOC patients.
Background: In 2003, the American Society of Clinical Oncology (ASCO) issued a policy statement that addressed the oncologist’s role in integrating cancer genetic risk assessment and management into clinical practice. ASCO supports access to genetic counseling for patients offered genetic testing and after results disclosure. In 2014, Integrated Genetics (IG) began offering genetic counseling in partnership with OBGYNs and oncologists whose patients have a personal or family history of breast or ovarian cancer. The goal of the program was to provide an easily accessible and comprehensive genetic counseling service for Hereditary Breast and Ovarian Cancer (HBOC) to meet the needs of patients and to support clinicians who currently provide pre- and post-test counseling.

Methods: In this study we describe IG’s HBOC genetic counseling service and in a retrospective analysis, describe the characteristics of patients referred to the program in 2014. Clinicians were provided a toll free number for patients to call to schedule a genetic counseling session. The scheduling staff facilitated medical record requests and emailed a family history questionnaire. In person or telegenetic counseling (via WebEx) was offered. Telegenetic counseling allowed the patient and genetic counselor to see one another via webcam and view counseling visual aids and relevant medical records. Pedigree analysis and cancer genetic risk assessment were performed and genetic testing options discussed based on National Comprehensive Cancer Network (NCCN) testing criteria. Information discussed at the session, including patient decisions about testing, was documented for the referring physician and patient in the Genetic Counseling Consultation Report. The physician ordered testing and managed patient screening and surveillance.

Results: During 2014, 247 counseling sessions were provided to patients from 23 states. All patients had a personal or family history of breast or ovarian cancer and the average age of patients was 44 years (range: 16-85). Sixty four percent (64%) of sessions were done via WebEx and 36% in person. Of the 247 sessions, 198 (80.2%) were for pre-test genetic counseling; of those, 173 (87.4%) met NCCN HBOC genetic testing criteria and genetic testing for BRCA1/2 was offered. Of this group, 70.52% desired testing, 27.75% declined and 1.73% were undecided. The remaining sessions (n=49) included post-test counseling for pathogenic mutations or variants of unknown significance (n=39), and patients who had accepted testing but whose results were pending (n=10).

Conclusions: The IG HBOC genetic counseling program extended comprehensive genetic counseling to patients at increased risk for BRCA1/2 mutations who might not otherwise have access to this service. Physicians appropriately selected patients for referral (87% met NCCN testing criteria). The genetic counselors provided cancer genetic risk assessment, time-intensive patient education to facilitate informed decision making, and counseling about the implications of results, including the complexities of variants of unknown significance. This partnership model allowed physicians to retain patient care oversight, including management of screening and surveillance, while leveraging genetic counselor expertise.
Title: Long-term psychosocial consequences and counsellees' satisfaction after genetic counselling for hereditary breast- and ovarian cancer - A patient reported outcome study

Sztankay M, Meraner V, Martini C, Sperner-Unterweger B, Hubalek M, Weber I, Morscher R, Zschocke J, Egle D, Dünser M and Oberguggenberger A. Innsbruck Medical University, Innsbruck, Austria; Innsbruck Medical University, Innsbruck, Austria; Division of Human Genetics, Innsbruck Medical University, Innsbruck, Austria and Innsbruck Medical University, Innsbruck, Austria.

Body: Background: Genetic counselling and testing (GCT) for hereditary breast and ovarian cancer (BOC) has become a standard option in BOC care in Europe allowing for prognostic information on the individual risk for disease onset/ relapse as well as on treatment options comprising prophylactic surgery or surveillance programs. However, data on the psychosocial long-term consequences is limited, especially in high-risk counsellees opting against genetic testing. We aimed at investigating the long-term psychosocial consequences of GCT for hereditary BOC in all counsellees irrespective of their decision after counselling.

Patients and Methods: Counsellees for BOC with and without a previous disease who had undergone genetic counselling at Innsbruck Medical University between 2011 and 2014 were asked to participate in a cross-sectional Patient Reported Outcome (PRO) assessment (incl. Multidimensional Impact of Cancer Risk Assessment, Genetic counseling satisfaction scale, Satisfaction with Decision Scale, Breast Cancer Heredity Knowledge Scale, Hospital Anxiety and Depression Scale/ HADS, Short Form 12 Health Survey, Cancer Worry Scale/ CWS) targeting on psychological distress, cancer worry, patient knowledge and patient satisfaction with genetic counselling and decisions by means of an anonymous mail survey. Subsequent decisions for vs. against genetic testing and if eligible, for surveillance vs. prophylactic surgery were also assessed. A reference sample of BC survivors was recruited at the outpatient unit.

Results: An overall sample of 137 counselees was included in the analysis (67.9% decided to undergo genetic testing for a HCPS, 22.6% decided not to be tested, 9.5% were still uncertain about their decision). 22.6% of counsellees experienced clinically relevant levels of anxiety and 9.8% scored above the cut-off for clinically relevant depression according to the HADS. Mean CWS score was 11 (SD 3.6, 3-24). Counsellees did not differ from breast cancer survivors regarding anxiety and depression according to the HADS (depression: p<0.5). Mean patient satisfaction with decisions amounted to 25.4 (SD 5.78, min. 4 to max. 30); a mean satisfaction with counselling of 25 (5.4) was observed. Less overall satisfaction with genetic counselling (β=0.445, t=5.552, p=0.000) and lower certainty about decision for/ against genetic testing after counselling (β=-0.169, t=-2.105, p=0.037) were highly predictive for lower long-term patient satisfaction with decisions.

Conclusion: Our results indicate that genetic counselling for BOC has no overall deleterious psychosocial consequences in long-term. Levels of depression and anxiety were comparable to those of the general population, while distress levels did not differ from those of breast cancer survivors without a hereditary BOC predisposition. The overall satisfaction with counselling as well as the certainty with decisions on testing and related medical interventions are highly predictive for the long-term satisfaction with decisions. Hence, genetic counselling should focus on supporting counsellees in forming clear decisions and include identifying counsellees with increased cared needs in this regard by means of PRO assessment in follow-up.
Factors associated with BRCA genetic testing intention and uptake among Orthodox Jewish women


Background: Ashkenazi Jews have a 1 in 40 prevalence of carrying a BRCA1/2 mutation, mainly due to 3 founder mutations. Prior literature suggests that population-based genetic testing among Ashkenazi Jews is cost-effective and may detect over 50% more mutation carriers than family history-based screening. Orthodox Jewish women are an understudied population with unique social, cultural, and religious factors that may influence BRCA genetic testing. The aim of our study was to examine factors associated with BRCA genetic testing intention/uptake among the Orthodox Jewish community.

Methods: A one-time online survey was distributed to Orthodox Jewish women by 3 shuls from Washington Heights, NY (53% response rate) and through additional referrals. The questionnaire obtained information regarding demographics, breast cancer risk factors, genetic testing knowledge, decision self-efficacy, perceived breast cancer risk, breast cancer worry, and religious and cultural factors affecting medical decision-making. The Tyrer-Cuzick model was used to calculate lifetime breast cancer risk and accurate risk perception was defined as within +/-10% of actual lifetime risk. Descriptive statistics and multivariable logistic regression models were used to identify independent predictors of genetic testing intention/uptake.

Results: Among 342 evaluable participants, median age was 26 years (range, 19-77); 92% were Ashkenazi and 8% Ashkenazi/Sephardi; 98% had a college education, including 47% with post-graduate degrees. Despite being highly educated, only 54% of women had adequate genetic testing knowledge. Median lifetime breast cancer risk was 16% (range, 2.3-60.9) and only 44% had accurate breast cancer risk perceptions. Although 48% had a family history of breast cancer and 16% had a relative that tested positive for BRCA1/2 mutation, only 5% had undergone BRCA testing while 48% had the intention of undergoing genetic testing. Higher lifetime breast cancer risk, high decision self-efficacy regarding genetic testing, overestimation of breast cancer risk, and increased breast cancer worry were associated with genetic testing intention/uptake. The most important factors in the decision to have BRCA testing were to help prevent dying of cancer (55%), to help prevent getting cancer (54%), and effect on children (40%).

Multivariable analysis of factors associated with BRCA genetic testing intention/uptake

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decision Self-Efficacy (range, 0 [not confident] - 4 [very confident])</td>
<td>1.4</td>
<td>1.02-1.98</td>
<td>0.038</td>
</tr>
<tr>
<td>Actual Lifetime Breast Cancer Risk (range, 0 - 100%)</td>
<td>1.1</td>
<td>1.03-1.10</td>
<td>0.0005</td>
</tr>
<tr>
<td>Accuracy in Breast Cancer Risk Perception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accurate (referent)</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Underestimate</td>
<td>1.2</td>
<td>0.50-2.85</td>
<td>0.691</td>
</tr>
<tr>
<td>Overestimate</td>
<td>2.6</td>
<td>1.45-4.61</td>
<td>0.001</td>
</tr>
<tr>
<td>Breast Cancer Worry (range, 1 [none] - 7 [worry all of the time])</td>
<td>1.5</td>
<td>1.18-1.98</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusions: By understanding the religious and cultural issues regarding genetic testing in the Orthodox Jewish community, we can develop targeted interventions designed to enhance decision self-efficacy and improve accuracy of breast cancer risk perceptions to decrease unnecessary worry. This may in turn increase informed decision-making about BRCA genetic testing and...
implementation of cancer prevention strategies among Ashkenazi Jews.
Title: Information preferences and short-term psychological responses to multiplex genetic testing among individuals at risk for hereditary breast cancer


Body: Background: Multiplex genetic testing involves the simultaneous analysis of a panel of known cancer susceptibility genes. Although efficient and cost-effective, multiplex testing presents several challenges for patients and clinicians: these tests provide information about high and moderate penetrance genes of varying clinical utility, patients cannot choose which specific genes are tested, and multiple variants of uncertain significance can be identified at once. Multiplex testing is being increasingly integrated into clinical care, yet little is known about patients' preferences, uptake, or psychological responses to these tests.

Methods: To address this gap, we examined two data sources: Sample A) a cross-sectional clinical ascertainment of 189 patients evaluated for hereditary breast and other cancer syndromes (89% female), and Sample B) an ongoing prospective research study of multiplex testing among 194 breast cancer patients and survivors who previously received uninformative BRCA1/2 results (99% female, 84% white, ages 27-76, 60% had BRCA1/2 testing in the past year).

Results: In Sample A, 32% declined clinical multiplex testing in favor of more targeted testing. Female patients were more likely than males to decline ($p=0.004$). Self-reported reasons for declining included: concerns about uncertain clinical utility of moderate penetrance genes (51% of patients) and variants of uncertain significance (38%), feeling emotionally overwhelmed (23%), and not seeing the value of multiplex testing (20%). In Sample B, participants were allowed to select which information to receive from a multiplex test; 16% chose to learn less than all of the information available (e.g., not genes unrelated to breast/ovarian cancer, not genes without established clinical utility, not CDH1 or TP53). Information preferences were unrelated to demographic (age, race, time since cancer diagnosis and BRCA1/2 testing) and self-reported psychological factors (baseline genetic testing-related distress, uncertainty, and positive experiences; anxiety; depression). Participants who chose to learn all possible information reported greater concerns about their children's cancer risk than did those who chose to learn less information ($p=0.01$). Participants reported a small increase in both genetic testing-related distress and positive experiences from before testing to 1 week after receiving results ($p<0.001$). In multivariable analyses controlling for baseline psychological functioning, only non-white race was consistently associated with significantly increased post-result anxiety, depression, and genetic testing-related distress and uncertainty. Participants who had BRCA1/2 testing one or more years ago also reported fewer positive experiences 1 week after receiving results.

Conclusions: Together, these findings demonstrate that a sizable minority of patients have important concerns regarding multiplex tests that may limit their uptake of this novel testing, and suggest that some patients may prefer to customize the specific risk information provided. Results also highlight characteristics of those at risk for poorer emotional outcomes following testing; these individuals may benefit from additional support in this context.
Title: Constitutional mosaicism in hereditary cancer genes


Background: Mutations conferring cancer risk are typically inherited from one biological parent. Alternatively, a mutation may be constitutional mosaic, meaning that a fraction of cells in the body carry the mutation. This can result e.g. from a de novo mutation early in embryogenesis. Constitutional mosaicism may be an underreported cause of genetic disease [1,2] for 2 reasons: Family histories can be uninformative (mosaic mutations are typically not inherited, but can be passed on to children). Also, germline laboratory tests (if performed) are traditionally not optimized to detect low allele frequency events (in contrast to tumor tests for somatic mutations). Following our recent identification of a mosaic BRCA1 cancer patient [3], we sought to characterize additional such cases in our laboratory population.

Methods: We examined high-depth next-generation sequencing (NGS) data for pathogenic or suspected pathogenic mutations with unequal allele balance (i.e., not the expected 50-50 ratio) in blood-derived DNA. Because many factors can cause unequal allele balance in NGS, we reviewed the lab and clinical data in detail to remove cases likely due to (a) technical artifacts in NGS, (b) other somatic events, as may happen in response to chemotherapy, or (c) undiagnosed, residual or progressing blood cancers. An orthogonal assay was used for confirmation.

Results: To date, 14 cases with mosaic mutations (7-29% allele frequency) have been confirmed: 8 of these patients have breast/ovarian cancer and 6 have other cancers. These allele frequencies are unlikely to be caused by circulating tumor DNA or cells. These findings include our published BRCA1 case, 7 TP53 cases, 1 NF1 case, 2 CHEK2, 2 MLH1, and 1 PTCH1. 3 additional mutations were determined to be part of a haematopoietic neoplasm (1 previously undiagnosed). Cases of note include (i) a young breast cancer patient, with no cutaneous findings, positive for NF1 at 9%, and (ii) a patient presenting with the Muir-Torre variant of Lynch syndrome who had negative MLH1/MSH2 Sanger sequencing 14 years ago, identified to be positive for MLH1 at 25% by NGS.

Conclusion: Constitutional mosaic mutations may be an under-recognized cause of cancer and unique clinical considerations apply to such cases. First, a mosaic patient may not show the same gene-associated phenotypes as patients with inherited heterozygous mutations in the same gene. Second, patients with syndromic presentations who tested negative for the indicated gene(s) by traditional methodologies may warrant reexamination by NGS. Similarly, patients with heterozygous mutations (50-50) may have a mosaic parent, for whom the mutation could be missed by traditional testing. Testing of family members beyond first degree relatives of a mosaic patient is unlikely to modify risk assessments, unlike the situation for patients with inherited heterozygous mutations. Finally, apparent mosaicism may warrant an evaluation for a underlying haematologic malignancy. We note that the prevalence of mosaic findings in hereditary cancer genes is currently unclear but may higher than once thought.

Further clinical research on this topic is clearly warranted.

(1) Campbell et al., AJHG, 2014
(2) Acuna-Hildago et al., AJHG, 2015
(3) Friedman et al., SABCS 2014; BJC 2015.
Title: Women without significant claus model breast cancer risks may warrant breast MRI when a pathogenic/likely-pathogenic variant (PV/LPV) is detected in a hereditary cancer moderate risk gene

Alvarado M, Tiller GE E, Kershberg H, Solomon SR R, Mullineaux L and Haque R. Kaiser Permanente Southern California, Pasadena, CA and GeneDx, Gaithersburg, MD.

Body: Background: Hereditary cancer gene panel testing can assess breast cancer risk for women with significant family histories. The Claus risk model is another method to determine which women qualify for annual breast MRIs based on family history, and can be used for those with BRCA negative status or for those who do not qualify for BRCA testing. In July 2014, Kaiser Permanente Southern California, a large integrated health plan, began using hereditary cancer panels comprised of moderate and high-risk breast cancer genes (GeneDx). At the time of implementation, no clinical management guidelines existed for patients with PV/LPV in moderate risk genes. In March 2015, the National Comprehensive Cancer Network (NCCN) amended the Genetic/Familial High Risk Assessment Breast and Ovarian guidelines to include breast cancer surveillance for patients with PV/LPV in moderate risk genes including ATM, CHEK2 and PALB2.

Objective: To determine if the identification of PV/LPV in moderate risk genes versus Claus model calculation increases the number of women warranting breast MRI in a managed care setting.

Methods: We performed a retrospective query of our gene panel results from 6/2014 to 5/2015 to identify patients with ATM, CHEK2 and PALB2 PV/LPV. Personal and family histories were obtained from the test requisitions. Patients with personal histories of breast cancer were excluded from analysis. We calculated the lifetime breast cancer risk using the Claus model for all eligible female patients with a moderate risk gene PV/LPV. To calculate the risk, the Claus model included family history of breast cancer in first and second-degree relatives. A lifetime breast cancer risk of >20% indicates "high" risk.

Results: A total of 19 female patients without breast cancer had a PV/LPV detected in a moderate risk gene (ATM, CHEK2, and PALB2. Claus model calculation was feasible in 12 patients. Of these 12, 4 had a PV/LPV in ATM, 6 in CHEK2 and 2 in PALB2. Only one out of these 12 women was identified with >20% risk of breast cancer based on the Claus model, and was recommended a breast MRI.

A review of electronic medical records (EMR) notes to date (June 1, 2015) revealed that breast MRI was recommended for 10 of the 12 patients above, and completed in 6. MRI identified a suspicious breast lesion in one patient. Follow-up tests and lumpectomy revealed atypical ductal hyperplasia and she will be followed with annual MRI and mammogram. The remaining 2 of12 women had no mention of MRI in their EMR, and will be flagged for follow-up to determine MRI status.

Conclusions: Eleven out of 12 women with a PV/LVP in a moderate risk gene would not have been identified as having an increased breast cancer risk by the Claus model. In our small sample, utilization of a High/Moderate Risk Gene Panel identified more patients potentially warranting enhanced breast cancer surveillance with annual breast MRI than the Claus model. This finding suggests that the use of hereditary cancer gene panel testing may impact the medical management of women with a familial risk for breast cancer. Larger studies with outcome data are needed to determine optimal surveillance guidelines.
**Title:** The BRCA-1 polymorphism (major homozygous rs5820483) is associated with higher expression of phosphorylated -IGF-1 receptor

Singh B, Bochaca II I, Ruiz de Garibay G, Damiola F, Mazoyer S, Antoniou A, Chenevix-Trench G, Pujana MA A, Barcellos-Hoff MH H and Kleinberg D. New York University Langone Medical Center, NY, NY; Catalan Institute of Oncology (ICO), Bellvitge Institute for Biomedical Research (IDIBELL), L'Hospitalet del Llobregat, Barcelona, Catalonia, Spain; Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon–Centre Léon Bérard, Lyon, France and CIMBA and University of Cambridge, Cambridge, United Kingdom.

**Body:**

**Background:** Data from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) indicates that common variation in the BRCA1 locus modifies the penetrance of mutations. The rs5820483 variation is one such polymorphism in which patients homozygous for the major allele have a greater risk of breast cancer. The BRCA1 gene contains 24 exons that generate a full-length (FL) protein. Major alternatively spliced isoforms include skipping of exon 11 (Δ11) and a truncated form (IRIS), each of which appear to have distinct roles in cellular processes. rs5820483 is the only variant that maps to intron 10, flanking exon 11. Experimental models suggest that deregulation of the FL/D11 isoform ratio can have profound functional consequences. We have shown that cells from patients with the major rs5820483 allele express more FL relative to Δ11 compared to cells from patients with the minor allele. Breast cancer exhibit increased IGF-1 and IGF-1 activity in patients with BRCA1 mutations relative to breast cancer in women without BRCA1 mutations. IGF-1 produced in the mammary stroma mediates estrogen dependent proliferation. Here we test whether the rs5820483 allele affects IGF-1 activity in breast tissue of BRCA1 mutation carriers.

**Design:** We assessed the zyosity of the rs5820483 alleles in 28 BRCA1 mutation carriers. Phosphorylated IGF-1 receptor (p-IGF-1R rabbit polyclonal Ab39398, Abcam, MA) was measured by immunofluorescence and Ki-67 was measured by immunohistochemistry in sections from histologically normal breast tissue from these cases were compared to heterozygous controls. Image analysis was used to assess the intensity of the p-IGF-1R immunofluorescence at the epithelial cell membrane with appropriate controls. Ki67 immunohistochemistry was also performed on these specimens.

**Results:** The rs5820483 allele was heterozygous in 10 specimens, homozygous for the major allele in 11 and homozygous for the minor allele in 7. The intensity of p-IGF-1R was higher in major homozygous cases (140±54 SD) than in either the minor homozygous (99±24) or heterozygous cases (95±44). The frequency of Ki-67+ cells was higher in the major homozygous case (3.1±2.9 SD) than in either the minor homozygous (1.5±1.9 SD) or heterozygous cases (2.2±2.5 SD). However, neither p-IGF-1R immunoreactivity nor frequency of Ki-67+ cells was statistically different between groups.

**Conclusion:** Breast cancer risk in BRCA1 mutation carriers is modified by common genetic variation at the corresponding locus. We have identified a variation at rs5820483 that affects the isoform ratio. Our preliminary analysis suggests that IGF-1 activity increases in those women homozygous for the major allele, concomitant with increased Ki-67. Corroboration of this analysis in a larger series is ongoing. A statistically significant difference might have fundamental implications for cancer prevention in those carriers.
Introduction: We are conducting a phase II clinical trial of the GP2+GM-CSF vaccine for the prevention of breast cancer recurrence in disease-free, node-positive or high-risk node negative patients (pts). GP2 is an HLA-A2+-restricted immunogenic peptide from the HER2 protein (aa: 654-662) capable of stimulating CD8+ cytotoxic T-lymphocytes (CTLs) to recognize and destroy HER2-expressing tumor cells. Here, we present the final pre-specified analysis of our study, disease free survival (DFS) at one year from last enrolled pt.

Methods: The trial is a prospective, randomized, multi-center, placebo-controlled, single-blinded, phase I/II trial designed to evaluate the safety and clinical efficacy of GP2 in breast cancer patients. Pts with any level of HER2 (immunohistochemistry [IHC] 1-3+) expression were enrolled after standard of care therapy. Blinded HLA-A2+ pts were randomized 1:1 to receive GP2 + GM-CSF or GM-CSF alone. Pts in both treatment arms received 6 monthly intradermal inoculations during the primary vaccine series (PVS) followed by 4 booster inoculations administered every 6 months. Data was collected for demographic, safety, immunologic, and clinical recurrence. Per the statistical plan, DFS was analyzed for both intention-to-treat population (ITT; all randomized pts) and per-treatment population (PT; excluding recurrences during the PVS and second malignancies) using Kaplan-Meir methods. Continuous variables are compared using analysis of variance techniques and proportions compared with Fisher's exact test.

Results: This trial enrolled 180 pts (vaccine group n=89 and control group n=91). There were no differences in age, grade, hormone receptor status, tumor size or nodal status between groups (all p>0.2). With median follow up of 36.2 months, the five-year DFS estimates did not demonstrate a difference between vaccinated (VG) and control groups (CG) with ITT (82.6% v 80.4%, p=0.96, respectively) or PT (88.9% v 84.3%, p=0.52, respectively). On further subset analysis, in HER2 3+ by IHC (or positive via FISH) pts, ITT analysis showed a DFS of 93.8% v 87.2% (VG, n=50 v CG, n=51; p=0.67, respectively) while the PT analysis revealed a DFS of 100% v 87.2% (VG, n=48 v CG, n=50; p=0.052, respectively). In ER/PR+ pts, ITT analysis showed a DFS of 88.1% v 80.6% (VG, n=54 v CG, n=60; p=0.41, respectively) while PT analysis demonstrated a DFS of 91.5% v 85.2% (VG, n=52 v CG, n=57; p=0.42, respectively).

Conclusions: The final pre-specified analysis of this randomized phase II trial of the GP2+GM-CSF adjuvant breast cancer vaccine shows no statistical differences between treatment arms. However, it does identify a particular patient population where the vaccine may have efficacy; HER2 3+ patients appear to derive the greatest clinical benefit from GP2 vaccination after trastuzumab treatment with the PT analysis nearing statistical significance. Further studies are required to confirm these findings and investigate the potential link between hormone receptor status and the induction of clinically beneficial anti-tumor immunity with this vaccine.
Robust generation of T cell immunity to HER2 in HER2+ breast cancer patients with a degenerate subdominant HLA-DR epitope vaccine


Background: Recent studies have indicated that vaccination can protect against cancer development. One key aspect of developing vaccines is circumventing peripheral tolerance by identifying subdominant epitopes that are unique to the deregulated tumor microenvironment. While existing subdominant epitope vaccines are showing efficacy in preventing cancer, these vaccines are applicable only for subsets of patients with specific HLA subtypes. Therefore, we recently developed a degenerate HER2 subdominant epitope-based vaccine that should be useful in approximately 85% of all patients. The vaccine consists of a pool of four HLA-DR-restricted 15-amino acid epitopes (p59, p88, p422, and p885) that are naturally processed and are designed to elicit helper T cell immunity, the cornerstone of immune surveillance. Here we present Phase I trial results of this multi-peptide HER2 vaccine.

Methods: Eligible women had HER2+ breast cancer (Stages II-III) and had completed standard treatment (i.e. surgery, chemotherapy, and trastuzumab) at least 90 days prior to enrollment and were rendered disease free. Vaccine included the above epitope pool along with adjuvant GM-CSF. Patients were vaccinated six times over six months and were monitored for toxicity at each visit. Peripheral blood samples were collected for immune responses evaluating for T cell and antibody immunity. Endpoints were safety and immunogenicity leading to the development CD4 helper T cells that recognized naturally-processed HER2.

Results: Twenty-two subjects (age 33 to 69 years) were enrolled. At the present analysis, 21 have completed all 6 vaccination cycles; one patient withdrew after developing a grade 1 injection site reaction during the first vaccination cycle. Twenty patients have had LVEF measured after vaccination; only 2 patients had an LVEF drop of 10% or more but remained in the normal LVEF range. One severe toxicity was reported: a grade 3 INR increase considered unrelated to treatment. Mild to moderate (grade 1-2) toxicities included injection site reactions, fatigue, and white blood cell count decreases. All patients were alive at analysis and only one experienced a recurrence (median follow-up 507 days, range 22 – 844). Twenty patients have had immune response assessments. Vaccine induced T cell immunity was observed in 94% of patients to p59, in 94% of patients to p88, in 82% of patients to p422, and in 74% of patients to p885. Importantly, T cell immunity to naturally processed HER2 proteins occurred in 94% of patients. The mean number of T cells specific for each peptide, post vaccination, ranged from 349–528 T cells per million peripheral blood mononuclear cells (PBMCs). The mean number of T cells specific for whole HER2 protein was 783 T cells per million PBMCs compared to a mean of 898 T cells/million PBMCs specific for the foreign tetanus toxin. In contrast to T cell responses, modestly increased antibody immunity to HER2 occurred in 35% of patients, consistent with the T cell-inducing design of the vaccine.

Conclusion: Our results show that it is possible to develop vaccines with broad HLA coverage that circumvent natural tolerance and induce tumor antigen-specific immunity in the vast majority of patients.
Title: Phase I/II randomized study of combination immunotherapy with or without polysaccharide krestin (PSK) concurrently with a HER2 ICD peptide-based vaccine in patients with stage IV breast cancer receiving HER2-targeted monoclonal antibody therapy


Body: Natural killer (NK) cell defects, commonly seen in metastatic breast cancer (MBC) lead to decrease in dendritic cell (DC) maturation, proinflammatory cytokine production, and tumor infiltrating T-cells (TILs). This results in a protumorigenic Th2 immune microenvironment with low response rates to immunotherapy (i.e., immune checkpoint blockade) and standard chemotherapy. PSK, a potent TLR-2 agonist, activates NK cells to produce IFN-γ and IL-12 and promote DC maturation/differentiation toward a Th1 profile in the tumor microenvironment which results in antigen specific TIL that can eradicate tumor. The combination immunotherapy of PSK and HER2 directed therapy described here, aims at inducing Th1 immunity and tumor specific T-cells. This proposed regimen could eradicate microscopic residual disease and prevent recurrence in optimally treated HER2+ MBC patients. Moreover, the regimen could result in enhanced trafficking of TILs to the site of tumor and improve the efficacy of checkpoint inhibitors and other therapies. A phase I/II randomized 2 arm study of combination immunotherapy with oral PSK (or placebo) given with a HER2 peptide vaccine and HER2 mAb therapy (trastuzumab (TZ) +/- pertuzumab (PZ)) was initiated to assess the safety of the approach and evaluate the effect of PSK on NK cell activity, pro-inflammatory cytokine/chemokine profile; and HER2 vaccine-induced T cell immunity.

Methods: Up to 30 patients with HER2+ MBC who are without evidence of disease after definitive therapy and currently on maintenance TZ +/- PZ are enrolled and randomly assigned in equal numbers to 1 of 2 arms (15 patients/arm): Arm 1: HER2 ICD vaccine + placebo or Arm 2: HER2 ICD vaccine + PSK. All patients receive concomitant treatment with 4 months of daily oral PSK or placebo, 3 monthly intradermal HER2 ICD vaccinations and continued TZ +/- PZ. Toxicity is evaluated per CTEP CTCAE 4.0, during and post vaccination. Serial blood draws for immunologic evaluation of NK cell activity and antigen-specific T cell immunity via flow cytometry and IFN-γ ELISPOT, respectively; and pro-inflammatory cytokines/chemokines.

Results: 24 subjects have been enrolled and 60 vaccines have been given. 16 subjects have completed all 3 vaccines and PSK/placebo; and 6 subjects are currently in progress. 2 subjects received < 3 vaccines and were taken off study. Of 144 reported adverse events (AEs), 97% were Grade 1-2; 66 (46%) were possibly, probably, or definitely related to study treatment. Most common AEs are injection site reaction and flu-like symptoms. There have been a total of four Grade 3 AEs, 1 episode of self-limited nausea/vomiting attributed to study treatment; and cognitive disturbance, fatigue, and lymphopenia all in 1 subject and attributed to disease progression. There have been no Grade 4 AEs. Immunologic analyses are ongoing and will be presented along with completed clinical data on all patients.

Conclusion: Combination immunotherapy with PSK/placebo and concurrent HER2 directed therapy is safe and well-tolerated. Further ongoing immunologic studies will help define the immunogenicity of the approach.
Title: Serum IgG response against prostate-related antigen revealed by personalized peptide vaccination in patients with metastatic recurrent breast cancer

Toh U, Okabe M, Iwakuma N, Mishima M, Shichijo S, Yamada A, Noguchi M, Itoh K and Akagi Y. Kurume University School of Medicine, Kurume, Fukuoka, Japan and Kurume University Cancer Vaccine Center, Kurume, Fukuoka, Japan.

Body: Purpose: We have indicated that IgG and CTL boosting response could be a potential prognostic factor for overall survival (OS) and progression free survival (PFS) in metastatic recurrent breast cancer (mrBC) patients, who had received personalized selected peptide vaccine (PPV) therapy in our previously reported clinical phase II study. The aim of this study is to identify the prognostic role of serum value of IgG antibody against prostate related-antigen (PRA), including prostate-specific antigen (PSA), prostate specific membrane antigen (PSMA) and prostate acid phosphatase (PAP) in mrBC patients.

Methods: Peripheral blood samples of 77 patients with mrBC were analyzed for serum anti-PRA IgG levels before and after 6th and 12th PPV therapy prospectively. Most of the peptides using for PPV are derived from cancer associated antigens expressing in various types of advanced cancers, but the peptides derived from PRAs were not used in this study.

Results: After PPV therapy, total serum levels of anti-PRA IgG were significantly increased in 31 mrBC patients (Group 1) whereas in remaining 46 rmBC patients (Group 2). Either serum anti-PSA, anti-PAP and/or anti-PMSA IgGs showed a significant increase in patients of Group 1 after 6th (p=0.045) and 12th PPV treatment (p < 0.001), irrespective of their intrinsic subtypes. The median PFS and median OS of Group 1 patients were 8.1 and 14.3 months, while those of Group 2 patients were 5.1 and 10.8 months, respectively. Anti-PRA IgG levels were significantly associated with PFS (p=0.0073; HR: 0.37) and OS (p=0.025; HR: 0.43) between these two groups, whereas no significant relation was found with age, clinical response rate and recurrent metastatic status.

Conclusions: The Group 1 patients with elevated anti-PRA IgG may have better prognosis compared to Group 2 patients who showed no IgG elevation after PPV treatment for rmBC. These results indicated a clinical significance between pre-and post-treatment measurement of serum anti-PRA IgGs in mrBC patients receiving PPV therapy, and may be a useful prognostic marker for monitoring the outcome to PPV treatment of breast cancer.
**Title:** Overall survival in inflammatory breast cancer patients receiving Her-2 Neu directed tumor vaccine therapy: Matched comparison with SEER registry patients


**Body:**

**Background**
 Patients with inflammatory breast cancer (IBC) have a poor prognosis, primarily due to distant dissemination. Additionally, IBC patients have an increased rate of HER2 overexpression when compared to patients with non-inflammatory breast cancer. The forms the rationale for HER2 directed tumor vaccine therapy in these patients. The purpose of this study was to examine overall survival in IBC patients receiving HER2 directed tumor vaccine therapy when compared with matched control patients from the SEER Registry.

**Methods**
 Patients with diagnosis of Stage III or IV HER2 positive IBC having completed standard initial therapy and without evidence of disease received HER2 vaccinations after being enrolled on 5 prospective clinical trials. Overall survival data were pooled and analyzed. A control group of matched IBC patients were identified by querying the SEER database from 1997-2011. The control group was identified as any individual in the database with a code for IBC. A secondary analysis comparing survival in HER2 positive IBC vs HER2 negative IBC patients was performed by querying the SEER database from 2010 onwards, the time point when the HER2 status was coded in the database. Propensity score adjustment were made to the control group to account for any imbalances between groups in measured covariates such as stage, race, age, sex, and era of enrollment and the time interval from diagnosis to enrollment on vaccine trial (median ~2 years).

**Results**
 A total of 37 IBC patients received HER2 directed vaccine therapy and 676 patients were identified for the SEER control group; Stage at enrollment: stage IIIB: 30 patients in the vaccine group and 639 patients in the control group; stage IIIC: 1 patient in the vaccine group and 15 patients in the control group; stage IV 6 patients in the vaccine group and 22 in the control group. The median survival of the overall population was 112 months for the vaccine group and 47 months for the control group (p=0.04). After using propensity scores to adjust the control for imbalances in measured covariates, the median survival for the overall population was 112 months for the vaccine group and 37 months for the control group (p=0.03). There was no difference in survival between HER2 positive and HER2 negative IBC patients in the control group (p=0.6).

**Conclusion**
 These results demonstrate promising overall survival in HER2 positive IBC patients receiving HER2 directed vaccine therapy after initial therapy. Propensity matching was performed to adjust for imbalances in measured covariates and resulted in a modest decrease in survival of the control group after adjustment, suggesting that the vaccine trial group had relatively unfavorable pre-treatment characteristics. Despite these unfavorable characteristics, patients receiving vaccine had a median survival of 112 months. These results must be further confirmed in a prospective randomized trial.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-11-06

Title: Safety and clinical activity of atezolizumab (anti-PDL1) in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer

Adams S, Diamond J, Hamilton E, Pohlmann P, Tolaney S, Molinero L, Zou W, Liu B, Waterkamp D, Funke R and Powderly J. New York University Langone Medical Center, NY, NY; University of Colorado Anschutz Medical Campus, Aurora, CO; Sarah Cannon Research Institute, Nashville, TN; Georgetown Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; Dana-Farber Cancer Institute, Boston, MA; Genentech, Inc, South San Francisco, CA and Carolina BioOncology Institute, Huntersville, NC.

Body: Background: Metastatic triple-negative breast cancer (mTNBC) is associated with poor prognosis, and chemotherapy remains the mainstay of treatment. Cancer immunotherapy represents a promising treatment approach for mTNBC, which is characterized by a high mutation rate, increased levels of tumor-infiltrating lymphocytes and high programmed death ligand-1 (PD-L1) expression levels. Atezolizumab (atezo; MPDL3280A) is a humanized monoclonal antibody that can restore tumor-specific T-cell immunity by inhibiting the binding of PD-L1 to PD-1. Atezo has demonstrated durable responses as monotherapy in mTNBC (Emens et al, AACR 2015). In addition, high objective response rates (ORRs) and durable responses have been observed with atezo plus chemotherapy in patients with non-small cell lung cancer (Liu et al, ASCO 2015). This study is the first combination trial of a checkpoint inhibitor with chemotherapy in patients with mTNBC.

Methods: This arm of a multicenter, multi-arm Phase Ib study (NCT01633970) evaluated atezo in combination with weekly nab-paclitaxel in patients with mTNBC. Primary endpoints were safety and tolerability, with secondary endpoints of PK and clinical activity. Key eligibility criteria included measurable disease, ECOG PS 0/1 and ≤ 2 prior cytotoxic regimens. Patients received atezo 800 mg q2w (days 1 and 15) with nab-paclitaxel 125 mg/m² q1w (days 1, 8 and 15) for 3 weeks in 4-week cycles, continued until loss of clinical benefit. If nab-paclitaxel was discontinued due to toxicity, atezo could be continued as monotherapy. ORR was assessed by RECIST v1.1. PD-L1 expression was scored at 4 diagnostic levels based on PD-L1 staining on tumor cells and tumor-infiltrating immune cells with the SP142 immunohistochemistry assay.

Results: As of February 10, 2015, 11 patients were evaluable for safety. All patients were women with a median age of 58 y (range, 32-75 y). No unexpected or dose-limiting toxicities were observed. The median duration of safety follow-up was 79 days (range, 27-182 days). The efficacy-evaluable population consisted of 5 patients who had ≥ 1 scan and ≥ 3 months follow-up. Four PRs and 1 SD were observed. By the next data cutoff of June 15, 2015, 21 patients will have been enrolled (7 in the safety cohort and 14 in the expansion cohort). All patients in the expansion cohort were required to undergo serial biopsies for correlative analyses. Approximately 21 and 19 patients will be evaluable for safety and efficacy, respectively. Updated safety, efficacy and biomarker data will be presented.

Conclusions: Preliminary results indicate that the combination of atezo plus nab-paclitaxel is tolerable with promising activity in patients with mTNBC. Based on these results and the observed activity of single-agent atezo in these patients, the combination of atezo and nab-paclitaxel is being evaluated in a Phase III study (NCT02425891) of patients with previously untreated mTNBC. Sponsor: Genentech, Inc. ClinicalTrials.gov: NCT01633970.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-11-07

Title: Mutually exclusive expression pattern of the immune co-inhibitory molecules B7-H4 and PD-L1 in triple negative breast cancer


Body: B7-H4 (VTCN1, B7x, B7S1) is a transmembrane protein belonging to the B7 family of costimulatory proteins and has been shown to inhibit T cell proliferation, cytokine secretion, and cytotoxic lymphocyte (CTL) induction. B7-H4 expressed on tumor cells or macrophages has been associated with poor prognosis and impaired T cell function in renal cell and ovarian cancers. Here we show B7-H4 is abundantly expressed in human breast cancer with triple negative breast cancer (TNBC) having the highest overall B7-H4 mRNA expression. We developed a specific and sensitive immunohistochemistry (IHC) assay for evaluation of B7-H4 protein and quantified B7-H4 expression in 156 breast tumor samples. Approximately 70% of the breast tumor samples had detectable B7-H4 expression whereas none of the normal or benign breast tissues stained positive for B7-H4. Multiplex IHC and flow cytometry studies showed that the majority of B7-H4 expression was restricted to the tumor epithelial cells, the CD45+ immune cells were negative for B7-H4 expression. Interestingly none of the TNBC samples that were positive for B7-H4 showed detectable expression of PD-L1 suggesting that B7-H4 and PD-L1 checkpoint proteins may act in a mutually exclusive manner. To evaluate the role of B7-H4 on tumor immune evasion, we overexpressed murine or human B7-H4 on the mouse colon-26 (CT26) tumor cell line and injected these cells intravenously into Balb/c mice. By day 14 we observed significantly more tumors as well as larger percent tumor area in the lungs of mice given CT26 cells transduced with human or mouse B7-H4 as compared to vector control transduced cells. These data suggest B7-H4 expression in tumors can accelerate tumor growth in immune competent mice and that targeting B7-H4 may provide therapeutic benefit. Given the mutually exclusive expression patterns of B7-H4 and PD-L1 a B7-H4 targeting agent may provide particular benefit in those patients where current anti-PD-1/PD-L1 therapies are not effective.
Title: Abstract Withdrawn
Title: A phase 2 randomized trial of the IDO pathway inhibitor indoximod in combination with taxane based chemotherapy for metastatic breast cancer: Preliminary data

Tang S-C, Montero A, Munn D, Link C, Vahanian N, Kennedy E and Soliman H. Georgia Regents University, Athens, GA; Cleveland Clinic, Cleveland, OH; NewLink Genetics, Ames, IA and Moffitt Cancer Center, Tampa, FL.

Body: Background: Indoleamine 2,3 dioxygenase (IDO) is a tryptophan-catabolizing enzyme that plays a key role in the normal regulation of peripheral immune tolerance. Tumors also employ this mechanism to induce a state of immunosuppression, evading immune mediated destruction. Indoximod (D-1-methyltryptophan) is a broad IDO pathway inhibitor as it has been shown to potentially interfere with multiple targets within the IDO pathway. Preclinical studies in MMTV-neu mouse models have demonstrated that combining indoximod with cytotoxic chemotherapy had a greater in-vivo anti-tumor effect than either agent alone. A phase 1 trial combining docetaxel and indoximod demonstrated safety and responses in metastatic breast cancer (mBC) patients. Based on these data a phase 2 trial evaluating indoximod in combination with taxane chemotherapy as first line therapy in patients with metastatic breast cancer was initiated.

Methods: The study is a 1:1 randomized, placebo controlled, four arm phase 2 trial. The study treatment is docetaxel 75mg/m2 IV D8 plus indoximod 1200mg PO BID D1-14 every 21 days or matching placebo in study arms 1A and 2A or paclitaxel 80 mg/m2 IV weekly 3 out of 4 weeks with indoximod or matching placebo given BID Days 1-14 in arms 1B and 2B. The primary endpoint is progression free survival. Secondary endpoints include overall survival, response rate, safety, and immune response correlative assays. Patients with measurable, histologically confirmed mBC, no prior chemotherapies (hormonal therapies allowed) in the metastatic setting, ER+ or ER –, HER2 -, ECOG PS 0-1, no active CNS disease, no active autoimmune disease are eligible. Target enrollment is 154 patients.

Results: At submission, 65 patients have evaluable safety data on trial. The pooled, blinded data was evaluated for any safety signals. Patient demographics include median age 58 years, 95% female, 17% African American, 25% triple-negative disease, and 82% with multiple sites of metastatic disease. 28% of patients had at least one Grade 3 or 4 adverse event. Those occurring in multiple patients include dyspnea 5%, pleural effusion 3%, non-cardiac chest pain 3%, and febrile neutropenia 3%. Most patients had at least one adverse event (86%). The most common were anemia 14%, tearing 11%, constipation 19%, diarrhea34%, nausea 44%, vomiting 22%, fatigue 48%, peripheral edema 17%, increased liver function tests 10%, lymphopenia 17%, hyperglycemia 15%, myalgia 14%, dizziness 14%, dysguesia 16%, headache 20%, peripheral neuropathy 12%, dyspnea 19%, alopecia 34%, and rash 14%, all compatible with the side effect profile of single agent taxanes. No immune specific serious advents were reported. No treatment related deaths have been reported.

Conclusion: The aggregate safety data for these 65 patient are similar to what is typically observed with docetaxel and paclitaxel. No unexpected serious immune linked adverse events were reported. The trial is currently open at multiple clinical sites in the US and Europe and actively enrolling patients. Updated data will be presented. NCT01191216.
Title: Epigenetic immune modulation by entinostat in breast cancer: Correlative analysis of ENCORE 301 trial


Body: Background: Entinostat, a class I HDAC inhibitor (HDACi), has shown promising activity in ENCORE 301, a randomized, placebo-controlled, phase II trial of entinostat + exemestane (EE) vs. exemestane + placebo (EP) in advanced hormone receptor-positive breast cancer progressed on nonsteroidal aromatase inhibitors. ENCORE 301 met the primary progression free survival endpoint and showed a median 8.3-month improvement in the overall survival (OS) exploratory endpoint for the EE arm. Emerging preclinical work suggests that entinostat has immunomodulatory effects and can eradicate modestly immunogenic mouse tumors in combination with immune checkpoint blockade agents via reduction of circulating myeloid-derived suppressor cells (MDSC). Based on these data, we conducted an analysis of immune subsets in blood samples from ENCORE 301 breast cancer patients.

Method: Blood was collected from a subset of 49 patients (27 EE and 22 EP) representative of the 130 patients enrolled in ENCORE 301 on cycle 1 day 1 (C1D1; pre-treatment), C1D2, C1D8, and C1D15 for biomarker analysis. Of these, 34 patient samples (20 EE and 14 EP) were analyzed for circulating immune subsets. The percent change in subsets at C1D15 vs. baseline was assessed based on the following surface markers: Lin-MDSC (lin; CD3, CD19, CD56)-HLA-DR-CD11b+CD33+), granulocytic MDSC (CD14-CD11b+CD33+), monocyctic MDSC (Lin-HLA-DR-CD11b+CD33+CD14+), immature MDSC (Lin-HLA-DR-CD11b+CD33+CD14-), CD8+ T-cells (CD4-CD8+), Foxp3-CD4+ T-cells (CD8-CD4+Foxp3-), and Tregs (CD4+CD8-CD25hiFoxp3+). Monocytes were analyzed for three populations: CD14+, CD14+HLA-DRhi, and CD14+HLA-DRlow/negative. In addition, PD-1, CTLA-4, and TIM-3 were measured on T-cell subsets, and CD40 was measured on MDSCs.

Results: In line with preclinical data, we observed a significant reduction in granulocytic MDSC (-14.67% vs. +20.56%, p 0.029) and monocyctic MDSC (-62.3% vs. +1.97%, p 0.002) in EE. Entinostat did not alter immature MDSC levels (-20.9% vs. -15.0%, p 0.93) suggesting a downstream effect of entinostat on MDSC subsets. Interestingly, CD40, a costimulatory receptor required for MDSC-mediated immune suppression was significantly down-regulated in all MDSC subsets except granulocytic MDSC where a downward trend was observed. Entinostat did not significantly impact the ratio of CD8+ T-cells per CD4+ T-cells or per Tregs or alter expression of CTLA4, PD-1, or TIM3 on T-cell subsets. Reduced expression of HLA-DR on monocytes has been associated with poor prognosis in cancer. Consistent with entinostat-mediated immunomodulatory effects, a significant increase in the number of HLA-DR+ monocytes (34.1% vs. -11.38%, p 0.0004) and level of HLA-DR expression on monocytes (16.3% vs. -4.7%; p 0.015) was observed.

Conclusion: Data with entinostat combined with exemestane in ENCORE 301 provide the first evidence of HDACi-mediated reduction of immunosuppressive MDSCs and increased immunocompetent CD14+HLA-DRhi monocytes in patients. These findings may explain the improved OS seen with EE in ENCORE 301 and provide strong rationale for planned combination studies of entinostat with immune checkpoint blockade agents.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-11-11

Title: Phase II trial of exemestane with immunomodulatory oral cyclophosphamide in metastatic hormone receptor (HR)-positive breast cancer: Prolonged progression-free survival (PFS) in patients with distinct T regulatory cell (Treg) profile


Body: Background: Resistance to endocrine therapies in HR-positive breast cancer is a significant challenge. The steroidal aromatase inhibitor (AI) exemestane (EXE) has demonstrated short-term efficacy in metastatic HR-positive HER2-negative breast cancer (mHR⁺BC) that has progressed during treatment with a non-steroidal AI. Combination strategies have not shown a survival benefit. Immunotherapy represents a promising approach as it may increase durability of responses. Low dose cyclophosphamide (CTX) has demonstrated efficacy in combination with neoadjuvant letrozole in HR⁺BC, conceivably by enhancing anti-tumor immune responses. Here we investigated whether EXE combined with immunomodulatory CTX could provide durable responses in heavily pretreated patients and assessed immunological profiles (NCT01963481).

Methods: Phase II trial of EXE (25mg PO daily) with CTX (50 mg PO daily) enrolled postmenopausal women (n=23) with mHR⁺BC who had progressed on prior endocrine therapy (including nonsteroidal AI, tamoxifen, and/or fulvestrant); prior chemotherapy was allowed. The primary endpoint was PFS (per RECIST 1.1) at 3 months; secondary endpoints were response rate, tolerability, and immune correlates. Detailed functional immune profiling of peripheral T cell subsets were performed by flow cytometry at baseline, 1, 3, 6, 9 & 12 months, with healthy donors available as controls.

Results: All 23 patients have been enrolled, and 21 are evaluable for response. Median age was 54 (range 31-77), median prior lines of endocrine therapy was 2 (1-3) and chemotherapy was 1 (0-5). The majority (15/23) had visceral organ involvement. Combination treatment was well tolerated with one grade 3 urinary tract infection but no grade 4 or 5 toxicity. An objective response was observed in 19% of patients (4/21, 1 CR and 3 PR) and an additional 33% (7/21) had SD, resulting in a 3-month-PFS of 48.5% (95% CI, 30.5-77.1). Responses were durable in all patients, lasting ≥/> 9 months and included patients with liver metastases. Comparison of peripheral immune cell subsets of patients (n=16) at baseline to age/sex-matched healthy controls demonstrated an increased proportion of CD4⁺ memory T cells with central memory phenotype (CD45RO⁺CD27⁺, p<0.0001). When patients were stratified based on PFS at 3 months, the proportion of naïve Tregs (CD4⁺CD45RO⁻FOXP3⁺Helios⁺) at baseline was significantly lower (p=0.003) in the non-progressor group compared to patients with progression. Remarkably, when these patient groups were compared for changes in T cell subsets during treatment, the proportion of both naïve and memory Treg subsets increased from baseline to 3 months (p<0.01), but only in the non-progressor patient group. While preliminary, these findings are possibly indicative of novel predictive biomarkers.

Conclusion: EXE and CTX had a favorable safety profile with evidence of clinical activity in patients with heavily pretreated mHR⁺BC, including durable responses in liver and bone. Correlative studies are ongoing to identify potential biomarkers of response or resistance to therapy.
**Title:** Novel protocol combining metronomic nant-paclitaxel with HER2-targeted natural killer cells (innate immunotherapy) for HER2-positive metastatic breast cancer

Rabizadeh S, Simon B, Klingemann H, Sims D, Weiss R and Soon-Shiong P. NantCell, Inc, Culver City, CA; NantKwest, Inc, Culver City, CA and Windber Medical Center, Windber, PA.

**Body:**

**Background.** Natural killer (NK) cells are an important effector cell type for adoptive cancer immunotherapy. Phase 1 clinical trials in patients with advanced cancers demonstrated the safety of unmodified, activated NK-92 cells (aNK), with no evidence of cytokine storm from 18 infusions delivered over 6 months; clinical responses were observed in a subset of patients. Like T cells, NK cells can be engineered to express chimeric antigen receptors (CARs) to enhance their antitumor activity. A stable clonal HER2-specific NK-92 cell line (HER2.taNK) mediated selective and sequential killing of HER2-expressing MDA-MB-453 cells in vitro (Schönfeld. *MolTher.* 2015;23:330-338). In addition, HER2.taNK cells were enriched in MDA-MB-453/EGFP xenografts and reduced the number of pulmonary metastasis in a renal cell carcinoma model, suggesting that HER2.taNK cells are a promising clinical candidate for use in adoptive cancer immunotherapy. Metronomic (low-dose, continuous) chemotherapy can be more effective than high-dose therapy in patients with advanced breast cancer (Montagna. *Canc. Treat. Rev.* 2014;40:922-950). Here we evaluate HER2.taNK cells in combination with metronomic nant-paclitaxel (lyophilized polymeric micellar formulation of paclitaxel) in a mouse model of HER2-positive breast cancer to determine the feasibility of a human clinical trial of HER2.taNK in combination with metronomic nant-paclitaxel.

**Methods.** HER2.taNK cells were generated as described previously (Schönfeld. *MolTher.* 2015;23:330-338). MDA-MB-453 cells were implanted into the mammary fat pads of female nude mice. When tumors reached 100 mm$^3$, mice were divided into 6 groups of 5 mice and dosed (IV) with saline (10 mL/kg, qd x 15), nant-paclitaxel (2.5-4 mg/kg q2d x 15), γ-irradiated (5 Gy) HER2.taNK cells (1 x 10$^7$ cells, days 1, 3, 5, and 8), or nant-paclitaxel + γ-irradiated (5 Gy) HER2.taNK cells—γ-irradiation is a potential safety measure for clinical application and prevents HER2.taNK cell replication while preserving antitumor activity. Tumor size and animal weights were measured every other day post-implantation.

**Results:** Results obtained 20 days post-treatment are shown in the table. Nant-paclitaxel alone and HER2.taNK alone significantly inhibited tumor growth. The combination of nant-paclitaxel + HER2.taNK led to significant tumor regressions (p<0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>T/C (%)</th>
<th>P-Value</th>
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<tr>
<td>Saline</td>
<td></td>
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<tr>
<td>nant-paclitaxel</td>
<td>5 mg/kg</td>
<td>-26.7</td>
<td>P &lt; 0.05 (vs saline)</td>
</tr>
<tr>
<td>HER2.taNK</td>
<td>1 x 10$^7$ cells</td>
<td>-22.2</td>
<td>P &lt; 0.05 (vs saline)</td>
</tr>
<tr>
<td>nant-paclitaxel + HER2.taNK</td>
<td>5 mg/kg + 1 x 10$^7$ cells</td>
<td>-60.0</td>
<td>P &lt; 0.05 (vs nant-paclitaxel)</td>
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<td></td>
<td></td>
<td></td>
<td>P &lt; 0.05 (vs HER2.taNK)</td>
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</table>

**Conclusions:** Single agent nant-paclitaxel and HER2.taNK were similarly effective at inhibiting tumor growth in this mouse model of HER2+ breast cancer. The combination of nant-paclitaxel + HER2.taNK appeared to be synergistic resulting in tumor regressions and significantly better efficacy vs each agent alone. This study illustrates the potential for combining metronomic low-dose chemotherapy with NK-based immunotherapy in a clinical trial of patients with metastatic breast cancer.
Title: 3 years follow-up results of arm node preserving surgery

Park J, Yeu KJ, Choi JE, Kang SH, Bae YK and Lee SJ. Yeungnam University College of Medicine, Daegu, Republic of Korea and Yeungnam University College of Medicine, Daegu, Republic of Korea.

Body: Background: Arm nodes can be identified by using axillary reverse mapping technique during axillary lymph node dissection. Many articles reported about feasibility of arm node preserving surgery for preventing lymphedema in breast cancer patients. But there are only a few studies about long-term follow-up results. The purpose of this study is to evaluate the incidence of lymphedema and the rate of regional recurrence and distant metastasis after arm node preserving surgery for 3 years follow-up period.

Methods: From January 2009 to October 2014, 167 breast cancer patients who underwent axillary reverse mapping were included. Before axillary lymph node dissection, 2.5mL of blue dye was injected in the medial intermuscular groove of the ipsilateral upper arm, subcutaneously. After elevation of the arm for at least 15 minutes, axillary lymph node dissection was performed. Blue-dyed lymph nodes and lymphatics were identified and preserved unless they showed radioactivity or suspiciousness for metastasis. Bilateral upper arm circumferences were measured preoperatively and postoperatively in all patients. Over 2cm circumference difference between ipsilateral and contralateral upper arm was defined as lymphedema. Follow-up studies such as ultrasound, mammography and/or PET were checked every 6 months for 5 years and then annually.

Results: 125 patients had their arm node preserved and 42 patients had their arm node removed. The mean number of harvested nodes was 17.85±6.74 in arm node preserved group and 20.17±6.08 in arm node removed group (p=0.050). The mean number of identified arm nodes was 1.35±0.84. The mean follow-up period was 40.25±20.57 months. 15 patients complained subjective symptoms of lymphedema, 3 patients in arm node preserved group and 12 patients in arm node removed group (2.4% vs 28.6, p<0.001). Among them, only 6 patients in arm node removed group were diagnosed with lymphedema by objective criteria whereas in arm node preserved group, there was no actual lymphedema (14.3% vs 0%, p<0.001). The mean measured circumference difference between ipsilateral and contralateral upper arm was 0.19±0.66 in arm node preserved group and 0.67±0.92 in arm node removed group (p=0.003). There were 11 cases of distant metastasis with a median time to metastasis of 30 months, 9 cases in arm node preserved group, 2 cases in arm node removed group (7.2% vs 4.8%, p=0.732). Among them, two patients whose arm nodes were preserved had ipsilateral axillary recurrence simultaneously (p=1.000). 5-year disease-free survival was 92.7% in arm node preserved group and 85% in arm node removed group (p=0.963)

Conclusion: Arm node preserving surgery during axillary lymph node dissection in breast cancer patients can reduce the incidence of lymphedema and it can be performed safely without increase of the rate of regional recurrence and distant metastasis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-12-02

Title: Axillary reverse mapping: A feasibility study

Khare S, Singh G, Das A and Bal A. PGIMER, Chandigarh, Chandigarh, India.

Body: Background

The mainstay of the therapy in breast cancer has been mastectomy or wide local excision along with axillary lymph node dissection (ALND). There are a lot of adverse effects associated with ALND, lymphedema being the most devastating. Sentinel lymph node biopsy (SLNB) can identify women who may or may not need axillary dissection and translate into a smaller number of patients undergoing axillary dissection. However, patients having positive lymph node on SLNB will still have to undergo ALND. To reduce the occurrence of lymphedema and its associated complications the technique of Axillary Reverse Mapping (ARM) has been developed. It is based on the hypothesis that the lymphatic pathway of the arm is not involved by the metastasis of the breast primary and after accurately identifying and preserving the arm lymphatics, there would be risk of neither lymphedema, nor leaving behind metastatic cells in the lymph nodes. This prospective study was carried out to evaluate the feasibility of ARM in patients undergoing ALND.

Methods

This prospective study was carried out in the Department of General Surgery, PGIMER, from July 2010 to Dec 2011. There were seventy five Biopsy/FNAC confirmed breast cancer female patients between 18 to 75 years. All patients were undergoing ALND as part of their treatment. Patients with any prior surgical treatment for primary invasive breast cancer, bilateral breast cancer and metastatic breast disease were excluded. Patients with heart diseases, active or uncontrolled infection, dementia, altered mental status or any psychiatric condition, pregnant or lactating women and with known allergies to blue dye were also excluded. All patients were treated as per the standard treatment for stage. All ALNDs were performed by a single surgeon. ARM was performed in all the patients by giving 5 ml of Isosulfan Blue Dye (Sterlized 1% solution) in the upper inner arm of the involved site approximately 60 minutes before exposure.

Results

The results were analyzed clinically during surgery and post-operatively by histo-pathological examination of lymphnodes. Intra-operatively, the identification of lymphnodes and lymphathics were noted in 17(22.7%) and 33 patients(44%) respectively. In none of the patient any attempt was made to preserve the blue nodes and all the lymph nodes were resected and sent for histopathological examination. In only 2 of 17 patients (11.76%) the lymph node was positive for malignant tumour cells. The tumour burden in both these patients was very high (more than 10 nodes involved). No association was found in the identification rates of ARM nodes and lymphatics to age, BMI, size of tumour, site of tumour, pathological status of axilla and timing of dye.

Conclusion

The identification rate of ARM lymphatics and nodes is low when only blue dye is used. The method appears it to be oncologically safe in patients with low tumour burden. However there is need for further studies will before this procedure can be universally applied without compromising the oncologic safety.

Once the technique is proven to be oncologically safe even in a selected group of patients, a large randomized trial will be needed to give a satisfactory answer whether the problem of lymphedema be eliminated or minimized in the patients in whom arm lymphatics are successfully preserved.
Title: Endofascial axillary lymphadenectomy – Towards a drainless protocol

Meredith IC C, Popadich A, Mouat CH H, Barrett K and King B. North Shore Hospital, Auckland, New Zealand; Wellington Hospital, Wellington, New Zealand and The Breast Centre, Wellington, New Zealand.

Body: Background:
The pathogenesis of seroma formation following axillary dissection continues to be poorly understood, although it seems that the greater the surgical disruption of the axilla, the higher the incidence of seroma and lymphoedema. We have previously described the laminated, three-dimensional structure of the clavipectoral fascia (CPF) that is evident during axillary ultrasonography and dissection. We propose that reconstituting the CPF reduces dead space, partially restores pressure gradients and facilitates collateralization to improve lymphatic flow, thereby reducing the incidence of seromas. Herewith, is a description of our technique for reconstitution of the CPF and our experience thus far.

Method:
Technique:
Following mastectomy or breast conservation surgery, the lateral border of pectoralis major is defined. Here, the medial, anterior laminae of the CPF are identified but not incised. Once the anterior extent of the CPF is displayed, a longitudinal incision is made through the midpoint of the CPF to access the axillary contents. If there is a substantial axillary tail, then the CPF is incised along the perimeter of the tail to include intra-mammary lymph nodes. A loose areolar tissue plane is encountered; the edges of the CPF are grasped and elevated and this areolar tissue plane developed by blunt and sharp dissection. Medially, this loose areolar tissue plane leads directly to a posterior gutter, and the long thoracic nerve on serratus anterior is identified and preserved. Superiorly, a deeper lamina of the CPF along the inferior border of the axillary vein has to be incised to find the thoracodorsal nerve. Identification of the intercostobrachial nerves is standard, as is the lateral dissection. Identification of the long thoracic nerve and thoracodorsal bundle results in definition of a vertical sheet, ‘the interneural tissue’. This can be grasped between the thumb and index finger and is excised en bloc. This tissue contains fat, lymph nodes and lymphatic vessels and is lined by thin fascial layers that we consider related to the CPF3.
At this stage, the anterior laminae of the CPF and axilla are carefully palpated for any residual nodes. After haemostasis, the CPF is reconstituted with a running, absorbable ‘lymphostatic' suture. No drain is placed in the axilla.

Results:
Between 2012 – 2015, 64 patients have undergone axillary dissection with reconstitution of the CPF in our unit. The average age was 54 years (range 29-87 years). An average of 12 nodes were procured (range 2-26 nodes). Only 5 women (8%) required seroma aspiration.

Conclusion:
We have dispensed with axillary drains in those who have had reconstitution of the CPF and only a minority of our patients required axillary seroma aspiration. We believe this technique should be given consideration to decrease the use of drains following axillary dissection.
Title: Is there any benefit to perform extensive nodal dissection in primary or recurrent aggressive form of breast cancer?

Berliere M, Duhoux F, Nardai P, Schmitz S, Taburiaux L, Galant C, Leconte I, Piette P and Lengele B. Cliniques Universitaires St Luc, King Albert II Cancer Institute, Brussels, Belgium and Grand Hopital de Charleroi, Charleroi, Belgium.

Body: Background: Breast oncologic surgery and especially nodal surgery has become ever more minimally invasive. However, some aggressive breast cancers exhibit at their primary or recurrent presentation extensive nodal invasion at the axillary, retropectoralis and sometimes supraclavicular and cervical levels. Surgical treatment of these tumors is not standardized.

Material and methods: Between January 2012 and April 2015, 7 primary breast cancer patients (group I) and 7 recurrent breast cancer patients (group II) were included in a prospective, non randomized study approved by our local ethics committee. All the patients had cytologically proven retropectoralis and infraclavicular lymph node invasion and 7 of them had cytologically proven cervical lymph node invasion (5 in the group of primary tumors and 2 in the group of recurrences). Four of the 7 primary tumors were triple negative and 3 were HER2 positive tumors, while 4 out of the 7 recurrent tumors were triple negative and 3 were HER2 positive. All the patients underwent PET/CT and breast MRI at baseline. Visceral metastases were absent in all cases. In the group of primary tumors, all the patients were treated with neoadjuvant chemotherapy (plus trastuzumab for the 3 HER2 positive tumors); in the group of recurrent tumors, neoadjuvant chemotherapy associated with trastuzumab was administered in 3 patients, while the 4 other patients underwent complementary mastectomy plus extensive nodal surgery followed by chemotherapy. Radiotherapy was administered in all primary breast cancer patients and cervical radiotherapy was administered in 3 of the 7 recurrent diseases. The following parameters were assessed: disease-free survival, overall survival and adverse events of surgical treatment.

Results: All the patients are still alive after a relatively short mean duration of follow-up [24 months in group I (6 to 40 months) and 29 months in group II (3 to 39 months)]. Six of the 7 patients in group I have no signs of recurrence, one has metastatic evolution (bilateral cervical and mediastinal node evolution) and is currently receiving chemotherapy in combination with a PARP inhibitor. In group II, 6 of the 7 patients have no signs of recurrence and one has metastatic evolution (inguinal nodes and bone metastases), treated with chemotherapy and HER2-targeted therapy. The major adverse event is arm lymphedema, affecting 4 out of 14 patients (28%). No persistent pain nor motor troubles are noted.

Discussion: Patients with nodal metastases outside the axilla seem to benefit from extensive surgery integrated in a multidisciplinary therapeutic approach. Some studies have demonstrated survival benefits for patients undergoing surgical resection of these nodes.

Conclusion: In aggressive breast tumors (HER2 positive or triple negative tumors) presenting with extensive nodal invasion, surgical excision of these nodal metastases must be integrated in the multidisciplinary treatment and patients need to be followed prospectively for a long time to confirm survival benefits.
Trans-peri-areolar breast conservative surgery followed by endoscopic axillary lymph node dissection: A novel surgical option


Objective
Endoscopic surgery currently is a standard method in most surgical fields. Although endoscopic breast surgery has been introduced since 1993, it is not widely applied due to limited benefits. Changing the conventional local treatment may require new attempts that enhance cosmetic outcomes and diminish complication rates. We report the cosmetic outcomes and postoperative complications of a breast conservative surgery (EBCS) procedure, which consists in using a 3cm peri-areolar incision to perform lumpectomy, and followed by endoscopic axillary lymph node dissection without liposuction.

Patients and methods
From Nov 2013 till May 2015, 53 patients diagnosed with early stage breast cancer were categorized into the conventional breast conservative surgical group (CBCS) and EBCS group.

Indications for EBCS were
1. tumor/breast ratio below 20%
2. no distant metastasis
3. Tumor mass not located in the lower-inferior quadrant.

Inform consents were obtained from all patients. 25 patients were enrolled in the EBCS and 28 patients in the CBCS group.

Number of lymph nodes harvested, tumor size, intra-operative bleeding, drainage volume, pain score (10scale survey) were collected.

The breast symmetrical score (BSS, direct distance from nipple to fovea jugularis, midclavicular, midsternal, inframammary fold and anterior axillary line of both breast was measured 3days after surgery and distance difference ratio of operated to contralateral site was calculated. BSS is the sum of each absolute value of distance difference ratio) and patient satisfaction (4scale survey) were used to evaluate cosmetic outcomes.

Results
There was no statistical difference in intra-operative bleeding (EBCS:36.00±17.08ml, CBCS:29.29±19.99ml, P=0.20) and drainage volume (EBCS:124.60±50.22, CBCS:120.82±74.58, P=0.832). An increased number of harvested lymph nodes was observed in the EBCS group (EBCS:17.88±6.21, CBCS:14.25±5.82, P=0.03). No statistical difference reported for lymph metastasis among two groups (EBCS:1.68±3.59, CBCS:0.96±1.45, P=0.34). The average tumor size showed no difference (EBCS: 2.41±0.87, CBCS:1.96±0.95, P=0.07). The pain score (EBCS:3.88±1.53, CBCS:4.39±0.74, P=0.04) meanwhile BSS was significantly superior in the EBCS group (EBCS:0.097±0.084, CBCS:0.20±0.13, P=0.002). Patient satisfaction survey reported a positive feedback (EBCS:4.00±0.00, CBCS:3.68±0.48, P=0.001).

Conclusions.
We evaluated cosmetic outcome using a thorough BSS as an index plus adding the lack of scar in the axilla. EBCS patients
displayed a significantly superior outcome in comparison with CBCS, which was further appraised by a considerable satisfied patient's feedback.

Evaluation of long-term survival, complication rates and cosmetic results are mandatory in order to further estimate the applicability of this surgical management in treatment of breast cancer. EBCS may be a feasible surgical alternative with minimal invasiveness, suitable to patients demanding excellent cosmetic outcome.
Publication Number: P2-12-06

Title: Prevention of seroma formation after mastectomy by local methylprednisolone injection - A randomized controlled clinical trial

Qvamme G, Axelsson CK, Lanng C, Wegeberg B, Mortensen MB, Okholm M, Arpi MR and Szecsi PB. Copenhagen University Hospital Herlev, Herlev, Denmark; Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; Copenhagen University Hospital Herlev, Herlev, Denmark and Copenhagen University Hospital Gentofte, Hellerup, Denmark.

Body: Objective: To investigate the effect of local steroid administration on seroma formation until 14 days of dryness.

Background: Seroma formation, the most prevalent postoperative complication after mastectomy, is an inflammatory process that is potentially preventable via local steroid administration.

Methods: This double-blind, randomized, placebo-controlled intervention study included 212 women who were scheduled for mastectomy for primary breast cancer. The patients were classified according to the surgical axillary procedure: mastectomy with sentinel lymph node biopsy (M+SLNB) or mastectomy with level I-II axillary lymph node dissection (M+ALND). The participants received either 80 mg of methylprednisolone or saline intracavitary via the drain orifice upon drain removal.

Results: After M+SLNB, 46% (32 of 69) of the patients developed seromas in the steroid group vs. 78% (52 of 67) in the saline group (p<0.0001). The mean cumulative seroma volume in the intention-to-treat material for the first 10 and 30 days was significantly lower in the steroid group than in the saline group (24 vs.127 mL and 177 vs. 328 mL, respectively) (p<0.0001). After M+ALND, 94% of the patients developed seromas in both the steroid (35 of 37) and saline (34 of 36) groups, and steroid administration displayed no significant effect on seroma formation. Additionally, no difference in the infection rate was observed.

Conclusion: Methylprednisolone administered intracavitary on the first postoperative day after M+SLNB exerted a highly significant preventive effect against seroma formation during the first 30 days. Future studies may clarify whether higher or repeated steroid doses enhance these effects.
Title: Treatment of the breast in occult breast cancer: Results of a prospective Dutch national cohort study with 5 years follow up

de Maat M, Bretveld R, Steevens J, Vissers Y and Hulsewé K. Maastricht University Medical Center, Maastricht, Netherlands; Dutch National Cancer Registry, Utrecht, Netherlands; Maastricht University, Maastricht, Netherlands and Atrium-Orbis Medical Center, Heerlen - Geleen, Netherlands.

Body: Introduction
Occult breast cancer (cT0N+M0) is a rare diagnosis and there is insufficient evidence on which to base treatment of the ipsilateral breast.

Methods
From 2006 till 2008 the Dutch National Cancer Registry identified patients nationwide with possible occult breast cancer. These patients were diagnosed with axillary nodal metastases with no evidence of the primary tumor on physical examination, mammography and ultrasound. Data on histopathology, imaging diagnostics and treatment were collected. After a minimum of 5 years follow up, data on recurrence and survival were gathered and analyzed.

Results
A group of 71 patients with occult breast cancer was identified. Of these, 7 patients received no local therapy. The remaining 64 patients all underwent surgery with axillary lymph node dissection. Ninety-two percent had MRI of the breast. Three groups were formed: Group A (n=25) had surgery of the breast, group B (n=28) received breast irradiation and group C (n=11) consisted of patients who had no treatment of the breast. Recurrence in the ipsilateral breast was seen in 4 patients (14%) in group B and in 3 patients (30%) in group C. There was 1 local recurrence in group A after breast conserving treatment and no significant differences between groups. After a median of 85 months of follow-up, overall survival (72%, 82% and 82% for group A, B and C, respectively) and distant recurrence rates (12%, 11% and 10% for group A, B and C, respectively) were not significantly different between groups assessed by log-rank test.

Conclusion
In occult cT0N+M0 breast cancer, conservative treatment of the breast is an acceptable modality which results in similar distant recurrence and overall survival rates. Surgery and breast irradiation appear to reduce the risk of ipsilateral breast recurrence. This is the first prospective study that provides evidence in the current clinical setting to guide treatment for patients with occult breast cancer.
Oncologic outcomes after nipple-sparing mastectomy: A single-institution experience


**Body: Introduction:** Nipple-sparing mastectomy (NSM) is the latest advancement in the treatment of breast cancer. Long-term oncologic outcomes in nipple-sparing mastectomy (NSM) continue to be defined. Rates of locoregional recurrence for skin-sparing mastectomy (SSM) and NSM in the literature range from 0 to 14.3%. We investigated the outcomes of NSM at our institution.

**Methods:** Patients undergoing NSM at our institution from 2006 to 2014 were identified. Patient demographics, tumor characteristics, and outcomes were collected. Locoregional recurrence was compared to previously published NSM and SSM results compiled from 14 and 11 studies in the literature. Institutional review board approval was obtained prior to the initiation of this study.

**Results:** From 2006 to 2014, 319 patients (555 breasts) underwent NSM. 149 patients (248 breasts) had long-term follow-up available. Average patient age and BMI were 47.4 and 24.28. Eighty-five percent of patients underwent mastectomy primarily for a therapeutic indication. Average tumor size was 1.41 centimeters with the most common histologic type being invasive ductal carcinoma (66.7%) followed by DCIS (23.8%). Nodal disease was present in 14.8% of patients. Average patient follow-up was 30.72 months. There was one (0.7%) incidence of ipsilateral chest-wall recurrence in a 44 year-old (p<0.0001, compared to aggregate NSM and SSM data). There were 0.36 complications per patient. There were 3 incidences of nipple-areola complex (NAC) necrosis: 2 partial thickness necrosis and 1 full thickness necrosis.

**Patient Demographics and Tumor Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Race</th>
<th>BMI</th>
<th>Tobacco History</th>
<th>Radiation History</th>
<th>BRCA 1/2 Status</th>
<th>Family History</th>
<th>Unilateral vs. Bilateral NSM</th>
<th>Indication for Mastectomy</th>
<th>Neoadjuvant Therapy</th>
<th>Follow-Up (months)</th>
<th>Tumor Size (cm)</th>
<th>Histologic Type (Percent of Therapeutic NSM)</th>
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<tbody>
<tr>
<td></td>
<td>47.7</td>
<td>Caucasian: 127 (85.2%)</td>
<td>24.28</td>
<td>7 (4.7%)</td>
<td>8 (5.4%)</td>
<td>10 (6.7%)</td>
<td>38 (25.5%)</td>
<td>Unilateral: 76 (51.0%)</td>
<td>Therapeutic: 126 (84.6%)</td>
<td>6 (4.0%)</td>
<td>30.72 (57.6-8.28)</td>
<td>1.41</td>
<td>IDC: 82 (66.7%)</td>
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<td>Non-Caucasian: 22 (14.8%)</td>
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<td>Bilateral: 73 (49.0%)</td>
<td>Prophylactic: 23 (15.4%)</td>
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<td>DCIS: 30 (23.8%)</td>
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<td>Ind: 7 (5.6%)</td>
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<td>ILC: 7 (5.6%)</td>
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<td>Invasive Other: 6 (4.8%)</td>
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<td>Mixed Type: 1 (0.8%)</td>
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<td>Stage 0: 52 (34.9%)</td>
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<td>Stage I: 54 (36.2%)</td>
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<td>Stage IIA: 14 (9.4%)</td>
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<td>Stage IIB: 8 (5.4%)</td>
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<td>Stage IIIA: 3 (2.0%)</td>
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<td>Stage IIIC: 1 (0.7%)</td>
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<td>PR (+): 79 (53.0%)</td>
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<td>Her 2/neu (+): 6 (4.0%)</td>
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<td>Ki-67 (High): 35 (23.5%)</td>
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<tr>
<th>Positive Nodal Status</th>
<th>22 (14.8%)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>NSM Complications per Patient</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen Section: 6 (7 breasts) (4.0%)</td>
<td></td>
</tr>
<tr>
<td>Permanent Section: 2 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Mastectomy Flap Necrosis: 12 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>Nipple-Areola Complex Necrosis: Partial-Thickness: 2 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Full-Thickness: 1 (0.67%)</td>
<td></td>
</tr>
<tr>
<td>Nipple-Areola Complex Excision (Patient Preference): 1 (0.67%)</td>
<td></td>
</tr>
<tr>
<td>Implant Extrusion: 4 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Cellulitis: Oral Antibiotics: 12 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>Hematoma: Intravenous Antibiotics: 2 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Seroma: 3 (2.0%)</td>
<td></td>
</tr>
<tr>
<td>Wound Dehiscence: 1 (0.67%)</td>
<td></td>
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<tr>
<td>Capsular Contracture: 2 (1.3%)</td>
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<tr>
<td>Thoracodorsal Nerve Spasm: 1 (0.67%)</td>
<td></td>
</tr>
<tr>
<td>Microvascular Free Flap Failure: 1 (0.67%)</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** We examined our institutional outcomes with NSM and found a locoregional recurrence rate of 0.7% with no nipple-areolar complex recurrence. This rate is significantly lower than aggregate published rates for both NSM and SSM.
Title: Long-term follow-up of nipple-sparing mastectomy for early-stage breast cancer without radiotherapy: A single-center study at a Japanese institution

Sakurai T, Suzuma T, Yoshimura G, Jinta E, Umemura T and Sakurai T.  Wakayama Medical University Kihoku Hospital, Ito, Wakayama, Japan; Kushimoto Arida Hospital, Kushimoto, Wakayama, Japan; Kishiwada City Hospital, Kishiwada, Osaka, Japan; Kiwa Clinic, Hashimoto, Wakayama, Japan and Sakurai Breast Clinic, Wakayama, Japan.

Body: Introduction: Achievement of a good cosmetic outcome is one of the most important goals of surgical treatment of breast cancer. However, most patients must undergo a mastectomy if the outcome of breast-conserving surgery is discordant. All potential techniques that maintain oncological safety should be considered to maximize the cosmetic outcome for patients who require a mastectomy. We began performing nipple-sparing mastectomy (NSM) in 1978. Recent reports have suggested that NSM is oncologically as safe as mastectomy and affords a better cosmetic outcome. Conversely, the surgical complications and recurrence associated with NSM remain controversial.

Objective: In the present study, we review the safety of the NSM surgical technique, discuss nipple–areola recurrence and skin flap recurrence after NSM, and compare recurrence and prognosis between NSM and mastectomy based on our long-term follow-up data.

Patients and Methods: We retrospectively analyzed 723 patients with early-stage breast cancer who underwent NSM from 1985 to 2007. The patients' median age, tumor size, and tumor–areola distance were 50 y, 2.1 cm, and 1.5 cm, respectively. We used a thick skin flap method to avoid surgical complications including nipple and skin flap necrosis. We analyzed nipple–areola recurrence and skin flap recurrence after NSM. We also analyzed 100 patients who underwent mastectomy for early-stage breast cancer during the same period as those who underwent NSM. We compared the local recurrence rate (LRR), disease-free survival (DFS) rate, and overall survival (OS) rate among all 723 patients who underwent NSM and 100 patients who underwent mastectomy. No patients in either group received radiotherapy.

Results: Among all patients who underwent NSM, stage 0, 1, 2A, and 2B disease was present in 21, 320, 253, and 129 patients, respectively. Notably, no nipple necrosis occurred during the average 114-month follow-up period. Local recurrence developed in 49 patients (6.7%), including recurrence at the nipple–areola complex in 24 (3.3%) and recurrence at the skin flap in 25 (3.4%). The average disease-free interval in patients with nipple–areola recurrence was 50 months, and that in patients with skin flap recurrence was 68 months. The clinical features of nipple–areola recurrence were a low rate of ER positivity (27%), high rate of Her2/neu positivity (60%), Paget type recurrence rate of 52%, and small tumor–areola distance (0.5 cm). The clinical features of skin flap recurrence were a relatively high rate of ER positivity (55%), solitary type recurrence rate of 88%, and diffuse type recurrence rate of 12%. The prognosis of diffuse type skin flap recurrence was significantly worse than that of solitary type recurrence (p = 0.01). There were no significant differences between the NSM and mastectomy groups in the LLR (6.7% vs. 4.0%, respectively), 10-y DFS rate (88% vs. 90%, respectively), or 10-y OS rate (92% vs. 91%, respectively).

Conclusion: Our long-term follow-up data show that NSM should be considered as an alternative option for mastectomy when the outcome of breast-conserving surgery is discordant in patients with early-stage breast cancer.
Title: The oncological safety of nipple sparing mastectomy: A pooled analysis of 12358 procedures


Body: The nipple sparing mastectomy is an increasingly popular procedure for the treatment of breast cancer and as a prophylactic procedure for those at high risk of developing the disease. However, it remains a controversial option due to questions regarding its oncological safety and concerns over locoregional recurrence. In order to assess its safety, a literature review and pooled analysis was performed using the current literature regarding nipple sparing mastectomy. This resulted in seventy-three studies which met inclusion criteria, yielding a total of 12358 procedures. The overall pooled locoregional recurrence rate was found to be 2.38%, the overall complication rate was 12.3% and the incidence of nipple necrosis, either partial or total, was 4.6%. Through analysis of this data, we concluded that nipple sparing mastectomy appears to be an oncologically safe option in appropriately selected patients, where tumours are less than 5cm in diameter, more than 2cm away from the nipple margin an are Her-2 negative, although separate histopathological examination of subareolar tissue remains essential to rule out malignancy at this site and provide safe oncological practice.
Title: Does postmastectomy radiotherapy improve survival in patients with 1-3 positive axillary lymph nodes? A systematic review and meta-analysis of the current literature


Body: In breast cancer with more than four positive axillary lymph nodes, it is common practice to deliver radiotherapy to the affected site following a mastectomy. However, less is known about the benefits this might offer in women with 1-3 positive lymph nodes. In order to assess whether postmastectomy radiotherapy has any benefit in these women, a meta-analysis was performed to assess whether postmastectomy radiotherapy improved overall survival or reduced locoregional recurrence in this group of women. It was found that postmastectomy radiotherapy significantly reduced the risk of locoregional recurrence, with a relative risk ratio of 0.3 (95% confidence interval 0.23-0.38), and resulted in a small benefit in overall survival, with a relative risk ratio of 1.03 (95% confidence interval 1.00-1.07). Therefore, in women with 1-3 positive lymph nodes, postmastectomy radiotherapy reduces the risk of locoregional recurrence and is associated with a small benefit in overall survival, so should be recommended within this group after careful multidisciplinary discussion.
Title: MarginProbe device use and re-excision rates for breast conservation surgeries


Body: Background: Current methods of intraoperative assessment of lumpectomy margins are limited. Previous studies have found a lower rate of re-excisions with the adjunctive use of the MarginProbe device (Dune Medical Devices Ltd, Israel). The purpose of this study was to compare the tumor characteristics and re-excision rates before and after the use of MarginProbe for patients who had breast conservation surgery (BCS) at our institution.

Methods: The Breast Cancer Database of our medical center was queried for patients who underwent BCS from 1/2010-3/2015 by three breast surgeons. 2 surgeons used the MarginProbe to direct excision of additional margins at the time of primary lumpectomy surgery and 1 surgeon performed routine 6-surface cavity shavings. We compared our historical data (1/2010-12/2014) to MarginProbe data (1/2015-4/2015). The following variables were included: age, mammographic breast density, tumor characteristics, and re-excision rates. Statistical analyses were performed using Pearson's Chi-Square and Fisher's Exact Tests.

Results: We had a total of 1201 women who had BCS among the 3 breast surgeons. The median age was 61 years. The median invasive size was 1.2 cm. Majority of cancers were early stage (stage 0, I), invasive ductal carcinoma (61%), ER-positive (86%), PR-positive (74%), and Her2Neu-negative (88%). These tumor characteristics were not statistically different in the pre- and post-MarginProbe groups. The majority of patients had dense breasts (51%) and density did not differ among the pre- and post-MarginProbe groups (p=0.86). For the surgeons who used the MarginProbe for margin assessment at the time of surgery, the re-excision rate fell from 17% to 0% and 35% to 20% during the 4-month period. In contrast, the surgeon who routinely performed 6-surface shavings had a re-excision rate that fell from 13% to 12% in the same time period. 88% of MarginProbe readings were false positive. There was one false negative reading.

Table 1. Tumor Characteristics

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Total N=1201</th>
<th>%</th>
<th>No MarginProbe (N=1144, 95%)</th>
<th>%</th>
<th>MarginProbe (N=57, 5%)</th>
<th>%</th>
<th>P-value</th>
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<tbody>
<tr>
<td>TUMOR STAGE</td>
<td></td>
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<tr>
<td>0</td>
<td>292</td>
<td>24</td>
<td>278</td>
<td>24</td>
<td>14</td>
<td>24</td>
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<tr>
<td>I</td>
<td>644</td>
<td>54</td>
<td>614</td>
<td>54</td>
<td>30</td>
<td>53</td>
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<tr>
<td>II A, II B</td>
<td>223</td>
<td>19</td>
<td>211</td>
<td>19</td>
<td>12</td>
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<td>III A, III B, IIIC</td>
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<td>1</td>
<td>2</td>
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<td>IV</td>
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<td>HISTOLOGY</td>
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<td>Ductal carcinoma in situ</td>
<td>305</td>
<td>25</td>
<td>289</td>
<td>25</td>
<td>16</td>
<td>28</td>
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<tr>
<td>Invasive ductal carcinoma</td>
<td>730</td>
<td>61</td>
<td>695</td>
<td>61</td>
<td>35</td>
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<tr>
<td>Invasive lobular carcinoma</td>
<td>112</td>
<td>9</td>
<td>107</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td></td>
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<tr>
<td>Invasive other</td>
<td>54</td>
<td>5</td>
<td>53</td>
<td>5</td>
<td>1</td>
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<tr>
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<td>Negative</td>
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<td>157</td>
<td>14</td>
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<td>PROGESTERONE RECEPTOR STATUS</td>
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<td>Negative</td>
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<td>HER2-NEU STATUS</td>
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<td>788</td>
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<td>753</td>
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<td>35</td>
<td>81</td>
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<tr>
<td>Negative</td>
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<td>2</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>5</td>
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</tbody>
</table>

**Conclusions:** Routine use of the MarginProbe device was associated with lower re-excision rates compared to historical data and concurrent 6-cavity shaving approach. Better intraoperative margin assessment and lower re-excision rates will decrease the burden of breast cancer on patients and the health care system and support the practice of breast conserving surgery.
**Title:** Modified pectoral nerves block for postoperative analgesia after modified radical mastectomy: A comparative study

Kanitkar R, Mane A, Agashe A, Kulkarni M and Deshmukh S. Ruby Hall Clinic, Pune, Maharashtra, India and Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

**Body:**

**Background:** The modified pectoral nerves block (Pecs II) as a method of analgesia for breast cancer surgery has shown excellent results in recent publications. This technique blocks the long thoracic nerve, thoracic intercostal nerves from T2-T6 & thoracodorsal nerve.

**Aims:** To evaluate the effectiveness of the Pecs II block for pain relief in the postoperative period of patients undergoing modified radical mastectomy (MRM).

**Methods:** A prospective comparative study was conducted at our institution between November 2014 and March 2015. Patients scheduled to undergo MRM were randomly assigned to the test group (endotracheal anaesthesia along with Pecs II) and the control group (endotracheal anaesthesia only). In the postoperative period the patients were evaluated using a visual analogue scale to determine pain scores at 6, 12 & 24 hours.

**Results:** Fifty patients (25 in each group), between the ages of 24 to 76 years (54.76 ± 10) were included in the study. There was no significant difference in ages between test & control groups. In the postoperative period, the test group had significantly lower median pain scores at 6, 12 & 24 hours as compared to the control group.

**Table 1: Comparative analysis for Pecs II block**

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Control</th>
<th>p value</th>
<th>effect size (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>54.44 ± 10.78</td>
<td>55.08 ± 9.37</td>
<td>0.824</td>
<td>-</td>
</tr>
<tr>
<td>Median postoperative pain scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 hours</td>
<td>2 (1)</td>
<td>6 (2)</td>
<td>&lt;0.001*</td>
<td>- 0.83</td>
</tr>
<tr>
<td>12 hours</td>
<td>3 (1)</td>
<td>5 (2)</td>
<td>&lt;0.001*</td>
<td>- 0.62</td>
</tr>
<tr>
<td>24 hours</td>
<td>3 (1)</td>
<td>3 (1)</td>
<td>0.034*</td>
<td>- 0.30</td>
</tr>
</tbody>
</table>

* statistically significant

**Conclusion:** The modified pectoral nerves block (Pecs II) is a novel & effective technique for postoperative analgesia in patients undergoing modified radical mastectomy.
Therapeutic mammoplasty reduces high incomplete excision rate in lobular cancer

Romics L, Kabir SA A, Mansell J, Mallon EA A, Stallard S and Doughty JC C. Victoria Infirmary Glasgow, Glasgow, United Kingdom; Royal Infirmary Glasgow, Glasgow, United Kingdom; New Southern University Hospital Glasgow, Glasgow, United Kingdom and Western Infirmary Glasgow, Glasgow, United Kingdom.

Body: Introduction: Incomplete excision rate for lobular cancer is much higher compared to other types of breast cancer, since lobular cancer is frequently occult on imaging. This, and the inability to downstage lobular cancer with neoadjuvant therapy lead to the highest mastectomy rate of all subtypes. Here, we investigated the association between histopathological characteristics and incomplete excision as well as mastectomy rates.

Further, we investigated whether the application of level 2 therapeutic mammoplasty (TM) would extend the indication for conservation with lobular cancers.

Methods: Data of 1389 consecutive patients underwent surgery for (non)invasive breast cancer between January 2008 and June 2012 was analysed. Pathological and preoperative radiological results were analysed in the context of final surgery and tumour excision margins. Statistical significance was calculated using Chi-square, Mann-Whitney and Z-tests with a significance<0.05.

Results: Overall incomplete excision rate was 13.74% (131/953), and mastectomy rate was 35.35% (491/1389). Higher incomplete excision and mastectomy rates were strongly associated with lobular subtype (IE: 26.03% (19/73)); M: 51.22% (63/123); p<0.01 vs. other subtypes), node positivity (IE: 25% (36/144) vs. 10.43% (68/652); p=0 and M: 60.69% (193/318) vs. 25.65% (216/842); p=0) and tumour size (IE: T3 80% (4/5) vs. T2 22.51% (43/191) vs. T1 9.23% (55/596); all p<0.01; and M: T3 95.35% (41/43) vs. 59.46% (242/407) vs. 16.16% (112/693); all p=0). Incomplete excision rates were independent of hormonal and HER-2 expressions (ER+: 12.55% (89/709) vs. ER-: 16.67% (15/90); p=0.27 and HER2 neg.: 12.67% (91/718) vs. HER2 pos.: 16.67% (13/78); p=0.32) and it was just higher in grade 2 and 3 cancers (14.6% (60/411); p=0.037 and 16.22% (36/222); p=0.021 vs. G1:6.86% (7/102). However, hormonal and HER-2 expressions as well as tumour grade were in strong association with mastectomy rate (ER pos.: 33.28% (335/1007) vs. ER neg.: 48.75% (78/160); p<0.01; HER2 neg.: 33.43% (341/1020) vs. HER2 pos.: 49.65% (71/143); p<0.01; G3: 50.49% (205/406) vs. G2: 30.77%(172/559) vs. G1: 14.28% (17/119); all p=0).

135 patients underwent surgery for lobular cancer (simple wide excision: 66; TM:19; mastectomy: 50). TM was offered for significantly larger tumours than lumpectomy (28.29mm (10-62) vs. 19.96mm(5-57);p<0.01; vs. mastectomy: 37.56 mm(5-110);p=0.096). Incomplete margins were found with significantly smaller tumours when lumpectomy was applied compared to TM(25.94 mm(6-56) vs. 38.6 mm(30-45);p=0.031). Conservation was achieved with significantly bigger tumours when TM was used (25.46mm (10-62) vs. 17.66mm (5-57); p=0.032). Multifocality, however, significantly increased the chance for incomplete excision even after TM (4/7; p=0.019).

Conclusion: Higher incomplete excision rate is strongly associated with lobular subtype, node positivity and tumour size, but independent of hormonal and HER-2 expression, while tumour grade is not a strong predictor. All histopathological characteristics are strong predictors of final mastectomy rate. Using TM, breast conservation can be achieved for significantly larger lobular cancers, and incomplete excision rate decreased in smaller cancers, which are routinely treated with wide excision.
Title: The role of neck dissection in breast cancer patients with synchronous and metachronous ipsilateral supraclavicular lymph node metastasis

Xiao C, Qi X, Chen A, Zhang W, Zhang P and Cao X. Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Tianjin, China; Key Laboratory of Cancer Prevention and Therapy, Tianjin, China and Key Laboratory of Breast Cancer Prevention and Therapy, Ministry of Education, Tianjin, China.

Body: Purpose: To evaluate the effect of neck dissection in breast cancer patients who present with ipsilateral supraclavicular lymphnode metastasis (ISLM) without distant metastasis and to reveal the outcomes of neck dissection in different molecular subtypes.

Methods: A total of 90 patients with synchronous ISLM and 36 patients with metachronous ISLM without distant metastasis between 2000 and 2009 were retrospectively analyzed. Combined-modality treatments were performed, and patients were respectively divided into two parts according to whether they received neck dissection or not.

Results: In the synchronous ISLM group, there was no significant difference between the neck dissection and non-dissection group with respect to age, menstrual status, tumour size, and histological type, PR and HER2 status. Patients with negative ER status and a higher number of positive axillary nodes were more common in the neck dissection group. The five-year locoregional relapse free survival (LRFS) was 63.6% in the neck dissection group VS. 48.9% in the non-dissection group, respectively (P=0.359). The 5-year distant metastasis free survival (DMFS) was 37.3% in the neck dissection group VS. 38.5% in the non-dissection group, respectively (P=0.882). Further analyses were performed by the site of metastases. Results showed that the incidence of bone metastasis was lower in neck dissection patients (14.7% vs. 28.6%, P=0.132). Due to the limited amount of patients, subgroup analysis was just performed in the subtypes with negative ER status, negative PR status and negative HER2 status, respectively. The five-year LRFS of patients receiving neck dissection in the subtypes with negative ER status, negative PR status and negative HER2 status was 63.7%, 59.8% and 61.2%, which was much better than their matched non-dissection group, respectively. However, the difference was not statistically significant. The 5-year overall survival (OS) was similar between the neck dissection and non-dissection group in the subtypes of negative ER status and negative PR status, respectively. However, the 5-year OS of HER2 negative subtype significantly decreased in the neck dissection group (37.2% vs. 65.4%, P=0.032). Besides, it's worth mentioning that in the HER2 positive subtype, the mean time to relapse, metastasis and death was shorter in the neck dissection subgroup compared with the non-dissection subgroup. In the metachronous ISLM group, a trend of better regional control, with similar PFS and OS, was achieved in the neck dissection group.

Discussion: Neck dissection is an effective approach to improve the regional control for the patients with ISLM, especially for the subtypes with negative ER status, negative PR status and probably positive HER2 status in the synchronous ISLM. But, it might not be comfortable for the patients with negative HER2 status, because of the unfavorable effect on its overall survival. In addition, neck dissection, with better regional control, might be helpful for the control of bone metastasis, which is beneficial for long term survival.
2015 San Antonio Breast Cancer Symposium

Title: Current trend and indications of endoscopy-assisted breast surgery for breast cancer: Experience from Taiwan endoscopic breast surgery cooperative group

Lai H-W, Kuo Y-L, Hung C-S, Chen S-T and Chen D-R. Changhua Christian Hospital, Changhua, Taiwan; National Cheng Kung University Hospital, Tainan, Taiwan and Taipei Medical University Hospital, Taipei, Taiwan.

Body: Background: Endoscopy-assisted breast surgery (EABS) performed through minimal axillary and/or periareolar incisions is a possible alternative to conventional surgery for certain patients with breast cancer. In this study, we report the early results of an EABS program in Taiwan.

Methods: The medical records of patients who underwent EABS for breast cancer during the period May 2009 to December 2014 were collected from the Taiwan Endoscopic Breast Surgery Cooperative Group database. The Taiwan Endoscopic Breast Surgery Cooperative Group (T-EBSCG) was established to monitor the effectiveness of and clinical outcome associated with EABS in Taiwan. The T-EBSCG comprises members from three major endoscopic breast surgery centers, namely Changhua Christian Hospital, National Cheng-Kung University Hospital, and Taipei Medical University Hospital in Taiwan. Data on clinicopathologic characteristics, type of surgery, method of breast reconstruction, complications and recurrence were analyzed to determine the effectiveness and oncologic safety of EABS in Taiwan.

Results: A total of 315 EABS procedures were performed in 292 patients with breast cancer, including 23 (7.8%) patients with bilateral disease. The mean tumor size of these 315 EABS was 2.2 ± 1.8 cm, and 44 (13.9%) of them were multifocal/multicentric breast cancer. Lymph node metastasis was found during 23.3% of the procedures. The stage distribution of them were stage II cancer (n=103, 34.4%), followed by stage I cancer (n=92, 30.7%), ductal carcinoma in situ (stage 0) (n=86, 28.7%), and stage III breast cancer (n=19, 6.3%). The number of breast cancer patients who underwent EABS increased initially from 2009 to 2012 and then stabilized during the period 2012-2014. The most commonly performed EABS was endoscopy-assisted total mastectomy (EATM) (85.4%) followed by endoscopy-assisted partial mastectomy (EAPM) (14.6%). Of the 269 patients who underwent EATM, 198 (73.6%) received immediate breast reconstruction. The majority (72.2%) of them received implant-based (cohesive Gel implant or tissue expander) reconstruction and the remaining 27.8% received autologous pedicled TRAM flap for breast reconstruction. Endoscopic assisted nipple sparing mastectomy with Gel implant reconstruction was the most frequently performed EABS now. During the six-year study period, there was an increasing trend toward more frequently use of EABS in the management of breast cancer when total mastectomy was indicated (EATM) than breast conserving surgery (EPM). Overall, the rate of complications associated with EABS was 15.2% and all were minor and wound-related. There were no major or life threatening complications. The positive surgical margin rate was 1.9%. During a median follow-up of 26.8 months (range, 3.3-68.6 months), there were 3 (1%) cases of local recurrence (1 ipsilateral breast recurrence, 1 axillary local recurrence, and 1 core needle biopsy tract recurrences), 1 (0.3%) case of distant metastasis, and 1 death.

Conclusion: The EABS program in Taiwan showed that EABS is a safe procedure and results in acceptable cosmetic outcome, these findings could help to promote this under-used surgical technique in the field of breast cancer.
Title: Mini latissimus dorsi flap increases breast conserving surgery rate in early stage breast cancer patients

Ozmen V, Sarsenov D, Ozmen T, Ilgun S, Alco G, Ordu C, Agacayak F, Elbuken F, Erdogan Z and Pilanci KN Nur. Istanbul Florence Nightingale Hospital, Istanbul, Turkey; Gayrettepe Florence Nightingale Hospital, Istanbul, Turkey; Marmara University Medical Faculty, Istanbul, Turkey and Haseki Training and Research Hospital, Istanbul, Turkey.

Background
Oncoplastic techniques enabled performing breast conserving surgery (BCS) to larger tumors. Mini latissimus dorsi flap (MLDF) is a novel approach promising successful cosmetic outcome after wide excisions for breast cancer surgery. The aims of this study are to evaluate the effect of MLDF on BCS rate in early stage breast cancer patients, and to compare patient and tumor characteristics between BCS patients with and without MLDF.

Patients and Methods
Data of early stage breast cancer patients (cT1-3N0-1M0) operated between years 2005 and 2015 were prospectively collected. We started to perform MLDF after 2010, and 157 consecutive patients who underwent BCS + MLDF reconstruction composed the study group. To prevent any selection bias, we comprised the control group from patients, who underwent BCS before our clinic started performing MLDF. 152 tumor stage-matched patient underwent BCS before 2010 composed the control group. The MLDF reconstruction was performed after lumpectomy and sentinel lymph node biopsy and/or axillary dissection via axillary incision. The volume of prepared latissimus dorsi muscle flap was depended on volume of the tumor cavity. Ink on the tumor in the specimen was accepted as negative positive surgical margin. All patients took whole breast and boost radiation. Systemic treatment decision was given after assessment of patients on the tumor conference.

Results
Between 2005 and 2015 data on 994 patients who underwent BCS were prospectively gathered with 371 patients registered in between 2005-2009 and 631 patients in between 2010-2015. The BCS rate increased from 66.5% to 78% after our clinic started performing MLDF in 2010 with MLDF rate being nearly 12%. The mean age and the mean body mass index were higher in the control group (54.2 vs. 46.7, p<0.001 and 27.85 kg/m² vs. 25.01 kg/m², p<0.001, respectively). Mean tumor size was larger in the study group (27.8 mm vs. 21.0 mm, p<0.001). Multifocality rate was higher in the study group (26.75% vs. 9.79%, p=0.001). Early complications (seroma, prolonged wound drainage, wound infection, hematoma, wound dehiscence) rates were similar in both groups (Table 1).

Conclusion
The MLDF significantly increased breast conserving surgery rate in patients with early stage breast cancer. It should be considered in patients with young age (<40 years), having large and multifocal tumors.

<table>
<thead>
<tr>
<th>Study group (n=157)</th>
<th>Control group (n=152)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.66±10.35 (28-83)</td>
<td>54.17±11.18 (30-85)</td>
</tr>
<tr>
<td>Age &lt;40 years old (%)</td>
<td>26.1</td>
<td>9.1</td>
</tr>
<tr>
<td>BMI* (kg/m2)</td>
<td>25.01±3.55 (19.6-43.0)</td>
<td>27.85±4.74 (20.0-41.2)</td>
</tr>
<tr>
<td>Right upper quadrant (%)</td>
<td>73.2</td>
<td>56.6</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>27.84±16.68 (1=100)</td>
<td>21.04±11.19 (4-70)</td>
</tr>
<tr>
<td>Surgical margin width (mm)</td>
<td>6.91±4.58 (1-25)</td>
<td>8.17±4.11 (1-20)</td>
</tr>
<tr>
<td>Number of metastatic lymph nodes</td>
<td>3.17±5.32 (0-30)</td>
<td>1.39±2.97 (0-16)</td>
</tr>
<tr>
<td>pN0 rate (%)</td>
<td>44.59</td>
<td>68.53</td>
</tr>
<tr>
<td>Multifocality rate (%)</td>
<td>26.75</td>
<td>9.79</td>
</tr>
<tr>
<td>Morbidity rate (%)</td>
<td>8.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Data are presented as mean±SD [range] unless noted otherwise
*BMI: body mass index.
**Title:** Outpatient mastectomy with reconstruction in a freestanding surgery center using multimodality opioid-sparing perioperative analgesia including liposomal bupivacaine

Rock DT T, Jandik AL L, Wittenborn WS S, Fairfax K and Sandadi S. Regional Breast Care, Fort Myers, FL; Medical Anesthesia and Pain Management Consultants, Fort Myers, FL; Wittenborn Plastic Surgery, Fort Myers, FL and Lee Memorial Health System, Fort Myers, FL.

**Body:**

**HYPOTHESIS:**
The introduction of effective non-narcotic analgesics and the long-acting local anesthetic bupivacaine liposome (Exparel) has resulted in improved postoperative pain control and decreased reliance on opioid analgesics that often have the undesirable adverse effects of sedation, nausea, respiratory depression, and dysphoria or confusion. This has resulted in shorter postoperative care unit stays, earlier return to normal activity, and improved patient satisfaction without sacrificing appropriate pain control. Based on a positive experience with these agents in the inpatient setting, we hypothesized that mastectomy with immediate implant-based reconstruction could be performed safely in a freestanding outpatient surgery center using a multimodality opioid-sparing analgesic regimen.

**METHOD:**
Over a 6 month period we performed unilateral or bilateral mastectomy with concurrent implant-based reconstruction on 20 patients. One patient had a unilateral mastectomy and 19 patients had bilateral mastectomy performed. Sixteen of the patients had nipple sparing mastectomy procedures. Reconstruction was performed with tissue expanders in 6 patients and as a single stage procedure with silicone gel implants in 14 patients. Acellular dermal matrix was used to support the implant in all but 1 patient. All patients were given gabapentin 600mg on the evening prior to and morning of surgery. Acetaminophen 1gm IV was given prior to induction of anesthesia in the preoperative area and again 6 hours later in the post anesthesia care unit. Ketorolac 30mg IV was given during the last half of the surgical procedure. All patients had general anesthesia with standard inhalational agents and IV fentanyl as needed. The retropectoral and serratus fascia were infiltrated with liposomal bupivacaine after surgical removal of the breast tissue, before reconstruction was started. The drain sites were also infiltrated with liposomal bupivacaine. Patients were discharged with prescriptions for gabapentin 300mg twice daily for 7 days followed by 300mg nightly for 7 days, carisoprodol 350mg every 6 hours as needed for muscle spasms, ibuprofen 800 mg every 8 hours for 5 days, hydrocodone/acetaminophen every 4 hours as needed for pain, and oral antibiotics of the surgeon's choice.

**RESULTS:**
All 20 patients completed their surgery and were discharged home after a brief stay in the postoperative care unit. No patient required readmission for pain control or any other complication in the perioperative period. No patient reported inadequate pain control. All patients were highly satisfied with their perioperative care as reported during postoperative follow-up phone calls the day after surgery and during their post-operative follow up visit.

**CONCLUSION:**
In our experience, outpatient mastectomy with reconstruction in a freestanding surgery center is safe and has a high degree of patient satisfaction when using a multimodality opioid-sparing analgesia regimen including liposomal bupivacaine. By avoiding opioid-related adverse effects, patients have a more rapid recovery, earlier return to activities of daily living, and therefore improved quality of life. Proper patient selection requires a multidisciplinary team approach for success.
Title: Comparison between ultrasound-guided-vacuum-assisted excision and surgical excision of benign phyllodes tumor of the breast: A single center retrospective study

Ouyang Q, Chen K, Zhu L, Song E and Su F.  Sun Yat-Sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.

Body: Background: Wide local excision (WLE) is the recommended treatment for patients with phyllodes tumor of the breast, regardless of the tumor subtype (benign, borderline and malignant). However, the association between margin status and local recurrence is still controversial. In our institution, surgical excision (SE) alone (margin width<1cm) is the standard treatment for patients with benign phyllodes tumor. In addition, ultrasound-guided-vacuum-assisted excision (UGVAE) is also employed as an alternative, minimally invasive way to remove benign phyllodes tumor. We hypothesized that UGVAE alone is adequate and as safe as SE alone for benign phyllodes tumor.

Method:
We searched our database for patients with benign phyllodes tumor diagnosed in Sun Yat-sen Memorial Hospital between 2000 and 2014, and identified 136 patients with valid follow-up information. Formalin fixed slices of each patient were reviewed for pathology diagnosis. In UGVAE group, we used 8G Mammutome to remove all lesions detected by ultrasound and surgical re-excision after pathology diagnosis was not performed. In SE group, patients received SE with no intention to achieve a surgical margin >=1cm. Macroscopically negative margins is guaranteed by gross examination. Comparison of patients' features were performed using student t-test, Mann–Whitney u-test or Chi-square test, when appropriate. Association between surgery (UGVAE & SE) and local recurrence was analyzed using univariate (Kaplan-Meier analysis) and multivariate approaches (Cox-regression analysis). Age, tumor size and the presence of accompanied fibroadenoma were included in the multivariate analysis.

Results:
Patients had significantly smaller tumor in the UGVAE group and those in the SE group (Median size: 2.1 vs. 3.0cm, P<0.01). There were more patients in the UGVAE group that had accompanied fibroadenoma, when compared with those in the SE group (67.2% vs. 29.1%, P<0.01). The median age was 38 and 37 in the UGVAE and SE group, respectively (P=0.66). With a median follow-up of 43 months, the 3-year and 5-year recurrence-free survival was 89.6% and 86.5% in UGVAE group, 97.3% and 90.0% in SE group, respectively (Log-rank test: P=0.26). In multivariate analysis, Surgery type (UGVAE or SE) (HR=0.37, P=0.19), age(HR=0.97, P=0.45), tumor size (HR=0.88, P=0.58) and the presence of accompanied fibroadenoma (HR=0.38, P=0.16) were not associated with recurrence-free survival.

Conclusion: UGVAE alone did not significantly increase the local recurrence rate of benign phyllodes tumor, when compared with SE, in this study. A prospective study with more patients and longer follow-up is needed in future.
Title: Rapid evaporative ionisation mass spectrometry towards real time intraoperative oncological margin status determination in breast conserving surgery


Body: Introduction: Positive tumour margins following attempted breast conserving surgery (BCS) is an important risk factor for local recurrence. Nationally in the United Kingdom on average approximately 25% of patients undergoing BCS require additional surgery for positive margins. Traditional techniques such as specimen xray, frozen section & imprint cytology to optimise margin clearance have significant limitations. Various research methods under investigation include optical spectroscopy, high resolution imaging and radiofrequency spectroscopy. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a new method that uses mass spectrometric analysis of the tissue specific ionic content of the surgical diathermy smoke plume for the rapid identification of dissected breast tissues as an intelligent knife (iKnife). We investigate the ability of the “iKnife” to analyze heterogeneous breast tissue intraoperatively using mass spectrometric techniques.

Method: The study involved three stages that comprised: method development, tissue specific ex-vivo database construction and intraoperative analysis. Smoke aerosol produced as a result of electrosurgical diathermy from a variety of frozen, fresh and in-vivo breast samples were aspirated into a mass spectrometer via a modified surgical handpiece. Tissue diagnosis was confirmed by subsequent histopathological validation. The data underwent computational analysis using multivariate statistics –predominantly Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA), along with leave one patient out cross-validation. A total of 128 patients (n=40 method development, n=66 ex-vivo database, n=22 intraoperative analysis) undergoing breast surgery were enrolled in this study. Ethical approval was obtained from the Research Ethics Committee.

Results: 40 patients contributed breast samples (normal and cancerous) for method optimisation to enable analysis of high intensity spectra from heterogeneous breast tissue. Following optimisation an ex-vivo database was constructed from 89 excised fresh breast tissue samples from 66 patients using 330 spectra (246 Normal, 60 Tumour – IDC, ILC, IMC and 24 Benign - fibroadenoma). Multivariate statistical analysis of data revealed classification of tumour compared to normal tissue with sensitivities of 93.0% and specificity of 91.9%. The iKnife was used intraoperatively during the entire operation of 25 surgeries. Spectral data was obtained within 1-2 seconds. Specific margin analysis correctly identified negative margins in 10 cases.

Conclusions: The iKnife has been successfully developed for analysis of intraoperative heterogeneous breast tissue. Preliminary data suggests that this technique is suitable with high accuracy for the separation of normal, benign (fibroadenoma) and cancerous (invasive ductal and invasive lobular carcinoma) breast tissues. In comparison to the normal breast, cancerous tissues exhibit statistically different spectral profiles. Further work is aimed at the development of a real time algorithm able to match intraoperative data with the pre-existing database for the rapid interpretation and real time feedback of intraoperative data towards detecting positive margins intraoperatively.
Title: The “penny farthing” incision for mastectomy: A novel technique to reduce “dog ear” deformity and improve access to the axilla

Poole G and Sheikh L. Middlemore Hospital, Auckland, New Zealand.

Body: Introduction:
Medial and lateral “dog ear” deformities are a common problem with mastectomy incisions. The challenges include different skin tensions superiorly and inferiorly and variable BMI. Also, the standard mastectomy incision is also not in the optimal place for sentinel node biopsy access.

No published technique is universally applicable and some advanced techniques require excess dissection and operative time.

Aims:
1) To perform a literature search for all published techniques
2) To pilot test a new, simple incision

Methods:
A literature search using PubMed was performed that revealed eight previous techniques (1).

A new, simple technique was developed to incorporate most of the historical principles. Circles of different sizes are marked out medially (small) and laterally (large). The lateral circle incorporates the optimal position for access to the axilla for sentinel node biopsy or axillary clearance. These circles are joined by asymmetrical superior and inferior lines to encourage rotation during closure. Tumour size and position are taken into consideration. Wound closure begins from the centre which allows the wound to “choose” its own corners and tuck them inwards therefore minimising “dog ear” formation.

Results:
Seventy-five consecutive patients had the procedure by a single surgeon over an 18 month period.
At the conclusion of each procedure there was no residual "dog ear" deformity at either wound end.
Of the 36 patients seen for 12 month follow up only one (BMI 42) has required revision of the wound.

Conclusion:
The "Penny Farthing" incision is a novel, simple and promising technique to deal with "dog ear" deformity in mastectomy.

Reference:
Introduction
Atypical ductal hyperplasia (ADH) is known to be associated with underlying pre-malignant or malignant breast conditions. Here we report our 10-year data on ADH found in core biopsy, lumpectomy and mastectomy specimens.

Methods
From 1st January 2005 to 31st December 2014, a total of 104 core needle biopsy specimens and 218 lumpectomy / mastectomy specimens were found to contain ADH. Clinical, radiological and pathology data were retrieved and analysed from a prospectively-maintained database.

Results
Out of the 104 patients with core biopsy showing ADH, 101 patients received excision and 3 refused operation. 34 patients turned out to have ductal carcinoma in-situ (DCIS) on excision, while 6 had invasive ductal carcinoma (IDC), 1 had lobular carcinoma in-situ (LCIS) and 1 had angiosarcoma resulting in an upstaging rate of 40.4% (42/104). The remaining patients had benign lesions including papillary lesions, fibrocystic disease, or ADH alone. Multivariate analysis found that the only correlating factor for the presence of DCIS or IDC was suspicious mammographic features (BIRADS 4 or above) (P = 0.008 and 0.02 respectively) but not other parameters such as extent of micro-calcifications (P = 0.12) or age (P = 0.11).

Histopathological diagnosis after excision of lesions containing ADH on core biopsy

<table>
<thead>
<tr>
<th>Excisional Biopsy Result</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>34 (32.7%)</td>
</tr>
<tr>
<td>ADH</td>
<td>32 (30.8%)</td>
</tr>
<tr>
<td>Benign</td>
<td>27 (26%)</td>
</tr>
<tr>
<td>IDC</td>
<td>6 (5.8%)</td>
</tr>
<tr>
<td>LCIS</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Refuse operation</td>
<td>3 (2.9%)</td>
</tr>
</tbody>
</table>

218 lumpectomy or mastectomy specimens were found to harbor ADH. 62 (28.4%) had co-existing pre-malignant or malignant breast diseases. The only determining factor for the presence of malignant or pre-malignant condition was suspicious breast imaging features (BIRADS 4 or above) after multivariate analysis (P = 0.0003).

Co-existing conditions in the lumpectomy / mastectomy specimens with ADH

<table>
<thead>
<tr>
<th>Co-existing Patholog(ies)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Ductal Hyperplasia alone</td>
<td>97 (44.4%)</td>
</tr>
<tr>
<td>Fibroadenoma / Fibrocystic disease</td>
<td>31 (14.2%)</td>
</tr>
<tr>
<td>Intraductal papilloma</td>
<td>22 (10%)</td>
</tr>
</tbody>
</table>
Phyllodes tumor 6 (2.8%)
Ductal carcinoma in-situ 36 (16.5%)
Invasive ductal carcinoma 23 (10.6%)
Lobular carcinoma in-situ 1 (0.5%)
Invasive lobular carcinoma 1 (0.5%)
Malignant phyllodes tumor 1 (0.5%)

Conclusion
ADH is closely related to the presence of invasive cancer or DCIS especially when suspicious breast imaging features are present. The upstaging rate is still high in patients where initial biopsy was ADH.
Social networks and the decision to undergo contralateral prophylactic mastectomy

Baptiste D, Venetis MK K, MacGeorge EL L, Lagoon J, Mouton A, Pastor R, Friley LB Brooke, Clare SE E and Bowling MW W. Indiana University School of Medicine, Indianapolis, IN; Purdue University Brian Lamb School of Communication, West Lafayette, IN; Pennsylvania State University, University Park, PA and Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: Background: Multiple studies indicate that the choice of contralateral prophylactic mastectomy (CPM) is increasing, predominantly in women younger than 50. This is despite the lack of demonstrated survival advantage for removing the non-cancerous breast, and contradicts expert medical recommendations, such as those of The National Comprehensive Cancer Network, that discourage CPM unless the patient is at elevated risk for breast cancer (e.g., BRCA1/2 mutation). An underexplored area with regard to selection of CPM is the influence of patients’ social and support networks. Breast cancer patients typically involve one or more intimates in their treatment decisions. Further, breast cancer is rarely stigmatized, making it acceptable to disclose the condition, even to non-intimates. Thus, others, in person or using social media, are likely to know when patients are making treatment choices, and may provide information, opinions, or advice that affects these choices. The scope and strength of social network influence on CPM decision-making remains unexplored to date.

Methods: Potential participants for this study were identified from Indiana University and Wishard Health Services billing records using the procedure code for bilateral mastectomy during the years 2007-2012. The lists were then curated to identify patients who had undergone CPM. These patients were mailed an introductory letter and study information sheet, after which they were contacted by telephone and the questionnaire administered to those who agreed. Questions focused on the people with whom participants discussed the CPM decision.

Results: 117 women of 326 invited agreed to participate. 88% of participants were Caucasian and 11% African-American. The mean age at diagnosis was 50. Most respondents (97%) discussed their surgical options with at least one person other than their surgeons (median = 3, mean = 6.7). The individuals most frequently consulted were spouses/partners, and friends or relatives who had experienced breast cancer. Children, relatives and friends who had not experienced breast cancer, and health care providers or counseling professionals were also engaged in discussion. Most respondents (92%) reported that at least one of the people they talked with (median = 2, mean = 2.6) had some degree of influence on the CPM decision. The average influence of these individuals was 3.2 on a 5-point scale (5 = played a strong role in the decision). Children had the strongest average influence (3.7/5), followed by spouses, and friends or relatives who had experienced breast cancer. Consistent with the sample (all respondents had elected CPM), most people who influenced the respondents were reported to be positive or neutral toward CPM. In the few instances in which the advisor was negative with regard to CPM, respondents reported that this person did not influence their decision.

Conclusion: The current study corroborates prior research indicating that breast cancer patients discuss their treatment options with others, and underscores the potential for social and support networks to influence CPM decisions. To reduce the incidence of CPM, it may be necessary for health care professionals to educate and inform large segments of the lay public about the actual benefits and risks of CPM.
Title: An innovative risk-reducing approach to post-mastectomy radiation delivery following autologous breast reconstruction


Body: Introduction:
There is no consensus among radiation oncologists regarding delivery of post-mastectomy radiation therapy (PMRT) after immediate autologous breast reconstruction, and plastic surgeons rarely participate in this decision-making process. However, radiation-induced changes markedly influence flap outcomes and affect the flap permanently. We present an innovative approach for PMRT delivery, through the use of custom bolus. This technique provides individualized, targeted PMRT to the reconstructed breast to minimize flap-related complications.

Methods:
All patients who underwent mastectomy with immediate autologous reconstruction between 2005 and 2014 at our institution were identified. Radiation was delivered to the reconstructed autologous breast in 29 patients. Post-irradiation complications and reconstruction outcomes were compared for patients treated with custom bolus, standard PMRT, and historical controls.

Results:
Over the past 10 years, 157 patients (226 breasts) underwent immediate autologous tissue breast reconstruction following mastectomy. Of the 29 patients who received PMRT, 10 were treated with custom bolus. The custom bolus uses perforated Aquaplast and a nearly tissue-equivalent wax to form a cast which conforms to the irregular contours of the chest wall, allowing for easy application through the duration of treatment. Pre-irradiation computed tomography was used to optimize dose distribution, evaluate the internal mammary vessels, and target the deeper tissues adjacent to the chest wall (minimizing dose inhomogeneity to the skin). Custom bolus resulted in fewer radiation-induced skin changes and less skin tethering/fibrosis than standard bolus (0% vs 10% and 20% vs 35%, respectively), and less volume loss and contour deformities compared with historical controls (10% vs 22.8% and 10% vs 30.7%, respectively).

Conclusion:
The use of custom bolus tailors radiation delivery to the internal mammary vessels, anastomoses, and skin; uniformly doses the surgical incision; and provides the necessary radiation dose to prevent recurrence, thus not compromising oncologic safety. It is easily fabricated, cost-effective and placement is straightforward and reproducible. Because radiation has negative effects on autologous breast reconstruction and often results in vascular intimal fibrosis and fat necrosis, plastic surgeons should participate in radiation planning. Our custom bolus PMRT technique reduces the incidence of these radiation effects.
Body: Background: Rates of implant failure, wound healing delay, and infection are higher in patients having RT after tissue expander (TE) and permanent implant reconstruction. Some have suggested greater complications with increased body mass index (BMI) and with diabetes.

Patients and Methods: 127 patients had bilateral TE reconstruction and radiation from 2003 to 2013 at two centers. In 95% (121/127) of cases RT was performed while the TE was in place with the permanent implant inserted after RT. 3D-CRT technique included 50 Gy with daily or every other day bolus and forward planned segments. The supraclavicular and/or internal mammary lymph node bearing regions were treated in 82.7% (105/127) cases. The non-irradiated breast provided an internal control. Chi-squared testing of pretreatment factors included radiation, chemotherapy, and medical history of hypertension, diabetes, cardiovascular, and pulmonary disease. BMI, tobacco and alcohol use, use of antiestrogen, statin, antidepressant, antihypertensive, anxiolytics, and antidiabetic medications were also studied. Comparison of differences in means for continuous variables used analysis of variance, then multiple pairwise comparisons with Bonferroni correction of p-value.

Results: Mean age was 53 ± 10.1 years with just 14.6% African-American. Twelve (9.4%) were BRCA positive (9 BRCA1, 4 BRCA2, 1 Both). Nearly all complications were in the radiated breast. Complications were: Grade 0 (no complication; 43.9%), Grade 1 (tightness and/or drifting of implant or Baker grade II capsular contracture; 30.9%), Grade 2 (infection, hypertrophic scarring, or incisional necrosis; 9.8%), Grade 3 (Baker grade III capsular contracture, wound dehiscence, or impending exposure of implant; 5.7%), Grade 4 (implant failure, exchange of implant, or Baker grade IV capsular contracture; 9.8%). 15.3 percent (19 cases) experienced grade 3 or 4 complication and 9.8% (12 cases) had grade 4 complication. Considering non-irradiated breasts, there were two (1.6%) Grade 3-4 complications. For BMI, there was no significant difference by category as defined by the CDC (p=0.91). Patients history of HRT tended to be more likely to have Grade 3 or 4 complications (31.8% vs 12.4% respectively; p=0.08). Patients with depression were more likely to experience a Grade 3 or 4 complication (29.4% vs 13.2%; p=0.01). Multiple logistic regression was used to predict the probability of a Grade 3 or 4 complication with HR use and depression as independent variables. Patients with depression were 4.2 times more likely to have a Grade 3 or 4 complication (OR=4.2, p=0.03) and patients with a history of HRT use were 3.4 times more likely to experience a Grade 3 or 4 complication (OR = 3.4, p=0.04). Only clinical diagnosis of depression was considered as 12/31 (38.7%) with antidepressants used only venlafaxine. Neither BRCA status (p=0.72) nor chemotherapy factors (p=0.42) were associated with complication rates.

Conclusions: Higher rates of TE reconstruction complications may be expected in patients receiving radiotherapy. Patients reporting medical history of depression or HRT use showed statistically significant increase in complication rates. This effect might be attributed to a drug effect or to patient factors such as body image.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-13-03

Title: Complications following total skin-sparing mastectomy and expander-implant reconstruction: Effects of radiation therapy on the stages of reconstruction


Body: BACKGROUND
With increasing numbers of patients requiring post-mastectomy radiation therapy (PMRT), many patients undergoing total-skin sparing mastectomy (TSSM) and immediate two-staged expander-implant (TE-I) reconstruction will receive radiation therapy (XRT) during the course of their reconstruction. Additionally, many patients undergoing TSSM for recurrent cancer have a history of prior lumpectomy and XRT. While the increased risk of reconstructive complications in the setting of XRT has been well-documented, few studies have looked at the impact of XRT on the stages of TE-I reconstruction.

METHODS
All patients undergoing TSSM and immediate two-staged TE-I reconstruction between 2006 and 2013 were identified from a prospectively maintained database. The incidences of TE-I loss and severe infection requiring admission for IV antibiotics were assessed in the subsets of patients with a prior history of XRT and those who received PMRT. Complications were divided into those following the first stage of reconstruction (TSSM and TE placement) and those following the second stage (TE-I exchange).

RESULTS
A total of 218 TSSM and TE-I reconstruction cases were included in the analysis, 85 (39%) with prior XRT and 133 (61%) with PMRT, all of whom received PMRT prior to TE-I exchange. Mean follow-up time was 2.5 years. Nearly all cases of prior XRT occurred in patients who developed a local recurrence and then underwent TSSM; mean time from prior XRT to TSSM was 7 years (range: 2 months to 22 years). Patients with prior XRT were much more likely to develop complications following the first stage of reconstruction than after the second stage (TE-I loss: 15% vs. 4%, p = 0.02; infection: 20% vs. 8%, p = 0.02). Patients who received PMRT had low rates of complications following the first stage of reconstruction, when they had not yet received any radiation exposure (TE-I loss: 3%; infection: 8%). However, rates increased significantly following TE-I exchange, with an 18% TE-I loss and 30% rate of infection, which was nearly 4-fold higher than patients with a prior history of XRT.

CONCLUSIONS
Patients with prior XRT are at significantly increased risk of reconstructive complications following the first stage of TE-I reconstruction after TSSM, even with a remote history of XRT. However, if these patients are able to successfully maintain their reconstruction through tissue expansion, their risk of complications at the second stage is comparable to patients without radiation exposure and significantly lower than patients receiving PMRT. Careful patient selection and appropriate pre-operative counseling for TSSM and TE-I reconstruction is critical to optimize outcomes and set appropriate expectations.
Title: Clinical outcome and patient satisfaction with the use of bovine-derived acellular dermal matrix (SurgiMendTM) in implant-based immediate reconstruction following skin sparing mastectomy: A prospective observational study


Body: The advent of acellular dermal matrix devices (ADMs) has facilitated immediate breast reconstruction (IBR) with mammary implants following skin sparing mastectomy (SSM) for breast cancer treatment or risk reduction. This is a prospective observational single institution study of 118 consecutive patients undergoing a total of 164 SSM and IBR procedures using an implant and bovine-derived ADM (SurgiMend) for breast cancer or risk reduction purposes during 2012-2014. The primary endpoint was the explantation rate and secondary endpoints included patient quality of life, patient satisfaction, objective assessment of aesthetic outcome, surgical complications, recurrence and mortality.

The mean age of the patients was 50.1 years (median age of 48, range of 27-78). Median follow up time was 21 months (mean of 21.4 months, range of 2-40 months). 46 patients had a bilateral SSM and IBR, 5 of whom had bilateral breast cancer and 3 for risk reduction due to a significant genetic mutation. The remaining 37 patients had unilateral breast cancer and a contralateral risk reducing mastectomy. 27 (37.5%) of the 72 patients who had unilateral SSM underwent contralateral adjustment procedures to optimise symmetry, including 9 augmentation mammoplasty, 12 mastopexy and 4 reduction mammoplasty procedures and 2 combined augmentation-mastopexies. 61 patients (51.7%) received chemotherapy, 5 of whom had primary systemic therapy prior to surgery. 32 (27.1%) patients received radiotherapy (10 patients had prior radiotherapy and 22 patients had post mastectomy radiation: PMR). Those with ER positive disease received hormonal therapy. Those with Her2 positivity received Herceptin +/- Pertuzumab.

Over the study period, 2 implants had to be removed resulting in an explantation rate of 1.2%. Overall, wound complications were observed in 6 (3.7%) cases. There were 2 cases of local recurrence (1.7%), one distant recurrence (0.8%) and one patient died of metastatic breast cancer (0.8%). Overall survival was 99.2% and locoregional disease free survival (LRFS) was 98.3%. One patient (0.8%) developed a mild inflammatory reaction secondary to the underlying mesh. Wound complications were observed in 3 other patients (2 haematomas and wound dehiscence/persistent seroma requiring implant replacement).

Patient satisfaction with the procedure was very high. The mean Breast Q Score was 85 and mean overall patient satisfaction was 9 out of a possible 10. The mean objective assessment score was 8.9 out of a possible 10 and the mean subjective capsular contracture severity score was 2.9 out of 10.

In patients undergoing reoperations, the incorporation rate of the mesh was found to be very high almost approaching 95%. SurgiMendTM is an effective adjunct to IBR using implants following SSM for breast cancer or risk reduction, with a very low rate of implant loss and a high level of patient satisfaction. Furthermore, this ADM seems to incorporate readily and is associated with a very low incidence of inflammatory reactions. Neither prior radiotherapy nor PMR radiation represents a contraindication to its use.
Title: A head to head comparison between SurgiMend® - Fetal bovine acellular dermal matrix and Epiflex® - Decellularized human skin tissue in breast reconstruction in 127 cases

Eichler C, Vogt N, Brunnert K, Sauerwald A, Puppe J and Warm M. Breast Center, Municipal Hospital Holweide, Cologne, NRW, Germany; Municipal Hospital Holweide, Cologne, NRW, Germany; Clinic for Senology, Osnarbrueck, Germany; Hospital Düren GmbH, Dueren, NRW, Germany and University of Cologne, Cologne, NRW, Germany.

Body: Introduction: The use of acellular dermal matrices (ADM) has become a widely used option in breast reconstruction. A great deal of literature is available, totaling over 2400 ADM reconstructions. Nonetheless, head to head comparisons between SurgiMend® and Epiflex® are not yet reported. In fact, this is the first clinical data report on the use of Epiflex®. This work will therefore compare postoperative complication rates and costs for these ADMs.

Methods: This analysis is a retrospective review of a single surgeon's 6-year experience with both SurgiMend® – an acellular bovine dermal collagen matrix for soft-tissue reconstruction and Epiflex® – a decellularized human skin tissue from 2008 to 2013.

Results: One hundred patients had a total of 127 implant based reconstructions using SurgiMend® (64 cases; 50.4 %) or Epiflex® (63 cases; 49.6 %). Gross complication rates were 11.1 % for SurgiMend® and 40.6 % for Epiflex® including hematoma, postoperative skin irritation, infection, necrosis and revision surgery. The most common complication was postoperative red breast syndrome. Severe complications requiring revision surgery were significantly increased in patients treated with Epiflex® (12.5 %) compared to SurgiMend® (4.8 %). Conclusions: This retrospective analysis favors the use of SurgiMend® over Epiflex® due to significantly lower gross complication rates. Severe complication rates are comparable to those reported in literature for both products. Although results promote the use of SurgiMend®, the single surgeon, retrospective nature of this work limits its clinical impact.
**Title:** Acellular dermal allograft fenestrations decrease outpatient expander fills and increase direct to implant incidence in implant-based immediate breast reconstruction


**Body:** Introduction: The innovation of fenestrated allograft (acellular dermal matrix, ADM) has improved patient outcomes in two-stage tissue expander/implant breast reconstruction. This technical alteration utilizes optimal fenestration overlap and has enhanced the efficiency of the reconstructive experience. We present a follow-up study of one- and two-stage breast reconstruction with a more refined, standardized method of surgeon-designed fenestration of ADM. Methods: We conducted a retrospective review of 52 patients (91 breasts) having undergone one- and two-stage breast reconstruction using fenestrated ADM at our institution from 2013 to 2014. Results: Mean intra-operative fill volume (IOFV) measured 402cc (SD=118cc), and IOFV as a percent of tissue expander size averaged 79.1% (SD=16.7%). Ten breasts were expanded to 100% and completed reconstruction in one stage with implant placement. IOFV as a percentage of total fill volume at completion of expansion averaged 73.6% (SD=16.6%). Two-stage reconstruction patients underwent 1.8 post-operative expansions on average (range 0-4) and averaged 81.2cc (SD=29.3cc) per in-office expansion. Days to full expansion averaged 45.1 days, while days to exchange averaged 137.8 days (Table 1). Mean days to exchange between our first 24 breasts to complete reconstruction vs. our last 23 breasts to complete reconstruction differed significantly, with 205 ± 43.8 days vs. 137.7 ± 138.1 days, respectively (p=0.03). The major complication rate requiring re-operation within 30 days post-operatively was 11.0%. Four breasts experienced partial mastectomy flap necrosis requiring re-operation with implant salvage (4.4%). Six breasts (6.6%) underwent explantation due to: infection (three), flap necrosis (two), and patient preference (one) (Table 2). Conclusion: Our fenestrated technique is demonstrated to increase intra-operative fill volume, decrease number of post-operative expansions and time to full expansion, and improve expansion rate with subjectively less pain. We believe our patients benefited from improved cosmetic outcomes with better shape, maintenance of breast footprint, and enhanced comfort due to the decreased number of intra-office fills and increased intra-operative expansion.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE size</td>
<td>300</td>
<td>700</td>
<td>512.6</td>
<td>108.3</td>
</tr>
<tr>
<td>Intra-op fill (cc)</td>
<td>150</td>
<td>650</td>
<td>402.1</td>
<td>118</td>
</tr>
<tr>
<td>Intra-op/TE size (%)</td>
<td>44.1</td>
<td>108.3</td>
<td>79.1</td>
<td>16.7</td>
</tr>
<tr>
<td>Total fill (cc)</td>
<td>310</td>
<td>825</td>
<td>37.6</td>
<td>120.3</td>
</tr>
<tr>
<td>Intra-op/Total fill (%)</td>
<td>34.1</td>
<td>100</td>
<td>73.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Total fill/TE size (%)</td>
<td>76.9</td>
<td>141.3</td>
<td>108</td>
<td>13.2</td>
</tr>
<tr>
<td># of post-op expansions</td>
<td>0</td>
<td>4</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Office fill per expansion (cc)</td>
<td>31.3</td>
<td>225</td>
<td>81.2</td>
<td>29.3</td>
</tr>
<tr>
<td>Days to full expansion</td>
<td>0</td>
<td>204</td>
<td>45.1</td>
<td>41.8</td>
</tr>
<tr>
<td>Days to exchange</td>
<td>0</td>
<td>554</td>
<td>142</td>
<td>117.9</td>
</tr>
<tr>
<td>Implant size (cc)</td>
<td>335</td>
<td>800</td>
<td>547.3</td>
<td>126.9</td>
</tr>
</tbody>
</table>

Table 1. Fill volume characteristics for 91 breasts after immediate reconstruction with fenestrated ADM. Data on tissue expander size was excluded.
Table 2. Complication rates in 91 breasts after immediate reconstruction with fenestrated ADM.

<table>
<thead>
<tr>
<th>Complications</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minor 30-day complication</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Major 30-day complication</td>
<td>10</td>
<td>11.0</td>
</tr>
<tr>
<td>30-Day take back for necrosis with salvage</td>
<td>4</td>
<td>4.4</td>
</tr>
<tr>
<td>30-Day explant</td>
<td>6</td>
<td>6.6</td>
</tr>
<tr>
<td>Explant due to infection</td>
<td>3</td>
<td>3.2</td>
</tr>
<tr>
<td>Explant due to necrosis</td>
<td>2</td>
<td>2.2</td>
</tr>
<tr>
<td>Explant due to patient preference</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Total 30-day complication</strong></td>
<td>12</td>
<td>13.2</td>
</tr>
</tbody>
</table>
Title: Clinical application of adipose derived stem cells in reconstructive breast surgery: A decade and a half later… are we there yet?

Westbroek D, Dontu G and Roberts JW W. KHP, London, United Kingdom.

Body: Background:
The unbroken narrative of the past three decades of modern ablative and reconstructive breast surgery has been (i) the minimising of surgical insult (integument preserving resection and staging) and; (ii) decreasing donor site morbidity (perforator flap surgery) respectively with equivocal oncological outcomes. Reconstructive surgical oncology aims to advance loco-regional disease control whilst approximating pre-morbid status ("like for like tissue replacement") with the least possible morbidity. Adipose derived mesenchymal stem cells (ASC’s) first reported in 2001, hold great promise to drive this singular trend forward. ASC’s are pluripotent cells, abundantly present in the vascular stromal fraction of the body’s fat tissue. They hold the key to unlocking the body’s regenerative potential by replacing & repairing the sequelae of surgery and adjuvant radiation therapy. ASC harvest is a relatively straightforward procedure with minimal donor site morbidity (Coleman's lipofilling). Evidence of a further ASC- driven turn of the evolutional wheel is currently lacking. We aim to review the evidence to date of the clinical utility of ASC’s in breast surgery and evaluate options available to enhance their further clinical application.

Method:
We searched the Cochrane Central Register of controlled trials, Medline® (2000 – June 2015), Embase® (2000 – June 2015) and Evidence Search Results (last search June 2015). Search terms used: "adult and/or pluripotent stem cells"; and "adipose tissue". Results were limited to "English language, Humans and Clinical and/or Randomised Controlled Trial" yielding 60 items. We also hand searched conference proceedings, reference lists and review bibliography. Two review authors independently screened articles for primary outcome - clinical efficacy in breast surgery (assessed as "volume retention") at follow-up (minimum of six months). Secondary outcomes included: donor site morbidity and neoplastic transformation (recurrent disease at final follow-up).

Results:
There are to our knowledge, no reported RCT’s or matched controlled studies in humans of ASC assisted lipofilling. There is one single-arm study with no controls (RESTORE-2, Cytori Therapeutics. 2011) and several case series reports with variable clinical endpoints. No adverse oncological events were reported. No donor site complications beyond Clavien-Dindo Grade I were recorded.

Conclusions:
ASC assisted lipofilling theoretically has game-changing potential to improve outcomes following ablative breast surgery. Clinical efficacy and oncological safety are unresolved. A controlled study in humans with long-term outcomes remains the way forward.
2015 San Antonio Breast Cancer Symposium

**Publication Number:** P2-13-08

**Title:** Breast lipofilling: A systematic review of current practice and oncological safety


**Body:**

**Background:** Lipofilling is a reconstructive and aesthetic technique that has recently grown in popularity and is increasingly being used in breast surgery. Concerns had been raised regarding its safety when used for remodelling and reconstruction of the breast.

**Methods:** We reviewed the current literature by systematically searching PubMed and Google Scholar databases regarding the current evidence regarding the oncological safety of the procedure in patients seeking aesthetic breast enhancement and in patients requiring oncoplastic reconstruction.

**Results:** Among the 864 patients included in the currently available studies on breast cancer patients who underwent lipofilling, only 14 (1.6%) recurrences were identified. However, evidence has emerged suggestive that the use of lipofilling in the background of ductal carcinoma in situ (DCIS) may be associated with an increased risk of neoplasia.

**Conclusions:** Over the subsequent two decades, little evidence has been found to support these early theoretical concerns, and growing numbers of proponents of the procedure are confident in its safety. Further study is required to better delineate the effect of lipofilling on DCIS.
Title: Is nipple-sparing mastectomy with implant reconstruction for breast cancer safe and worthwhile?

Regis C, Mesdag V, Tresch E, Chauvet MP P, Boulanger L, Collinet P and Giard S. Centre Oscar Lambret, Lille, France; Biostatistics Unit, Centre Oscar Lambret, Lille, France and Hospital Jeanne de Flandre, University Hospital, Lille, France.

Body: Background: Nipple-sparing mastectomy (NSM) is a standard for bilateral prophylactic surgeries. However for cancer treatment, the preservation of the nipple areolar complex (NAC) is still discussed because of suspected increase of local recurrence and surgical specific complications as nipple or mastectomy flap necrosis. The aim of the study was to investigate both the relapse risk associated with NSM for breast cancer and women's satisfaction with preservation of the NAC.

Methods: We included retrospectively all patients who had skin-sparing mastectomy (SSM) or NSM from 2007 to 2012 for breast cancer or ductal carcinoma in situ (DCIS). We compared NSM and SSM group for oncological (overall survival (OS) and disease-free survival (DFS)) and surgical outcomes. Patients' satisfaction and quality of life has been evaluated by a specifically designed questionnaire, addressed by mail and inspired by the Breast-Q questionnaire with specific assessment of global esthetic result, harmony with the native breast and need for psychological support.

Results: During the study, we operated 5600 patients for a breast cancer, among them, 152 had NSM (n=63 / 41.5%) or SSM (n=89 / 58.5%) with immediate implant breast reconstruction. Eighty-nine (58.6%) patients had DCIS, and the other had invasive disease (86.9% of T1). The mastectomy has been indicated for primary cancer (81%) or recurrence (19%). The two groups did not differ significantly according to histological type (p=0.10), grade (p=0.84), hormonal receptor (p=0.7), HER2 (p=1.00), Ki67 (p=0.75) or node metastases (p=0.64). Median follow-up was 42 (IQR: 18-58 ) months. No cancer-related death occurred during the study. Local recurrence rate was 1.7% (n=1) in NSM group and 0% in SSM group (p=0.35). The recurrence did not appear on the preserved nipple. Severe complication requiring surgery (Grade 3 of Clavien-Dindo classification) occurred in 9.9% of the cases. In the NSM group, one patient had complete NAC necrosis and three patients suffered partial necrosis. Severe skin-flap necrosis leading to implant removal was more frequent in the SSM group (SSM: 6.7% (n=6) ; NSM: 0% (n=0); p=0.042). One hundred and four (80%) patients answered the questionnaire. Satisfaction about the aspect of the NAC was higher in the NSM group compared to SSM with delayed reconstruction of the nipple (75% vs 59%, p=0.14). Patients with NSM needed less psychological support before (p=0.028) and immediately after surgery (p=0.14) than patients in the SSM group, which may suggest a better acceptation of the surgery in this group.

Conclusion: NSM for breast cancer surgery was not associated with significant increase of local recurrence rate or surgical complications. Patient's satisfaction was high. Therefore, nipple-sparing mastectomy with immediate implant reconstruction can successfully and safely be performed for pre-invasive and small invasive breast cancer. Besides esthetic aspects, preserving the nipple may ease the acceptation of these radical surgeries.
**Title:** Expanding the scope of implant based reconstruction; Good results can be achieved in challenging and high-risk patients


**Body:** Aims: Implant based reconstruction can be challenging, especially in higher risk patients, resulting in implant loss, poor cosmesis and delayed wound healing. We assessed outcomes of higher risk patients with a multi-centre study, including 5 hospitals in the North West of England. Our trainee-led, regional data collection removes any single surgeon or institution bias. Methods: A multi-centre, retrospective review of implant-based reconstructions between 01/01/12 & 31/12/12 was performed. Rates of implant loss from complications, unplanned explantation for cosmesis and delayed wound healing were assessed for those deemed at high risk (obese BMI>30, Smokers, previous chest wall radiotherapy (RT), elderly >65years, underweight BMI<20 & neo-adjuvant chemotherapy(NAC)).

Results: 216 reconstructions were assessed, with follow-up for minimum of 24 months. 117/216 patients had at least one of the high-risk features of which; 59 were obese, 50 smokers, 28 previous RT, 18 elderly, 10 underweight & 7 had NAC. Reconstructive strategy in the High Risk group was implant/expander only 42 (36%), implant & flap 35(30%), implant- ADM 27(23%), implant & dermal-Sling 11(9%), & other 2(2%), vs. the Low Risk group (n=99); implant/expander only 29(29%), implant & flap 22(22%), implant-ADM 35(35%), implant & dermal-sling 9(9%), & other 4(4%).

Of the 59 obese patients 10(17%) implants were explanted (6 for complications and 4 for unplanned cosmetic revision) and 14(24%) had delayed wound healing, compared to 18(11%) explant and 22(14%) delayed wound healing in non-obese; suggesting obesity increases risk in implant reconstruction (p=0.36 & p=0.1 respectively).

Smoking is associated with increased risk, with 13(26%) implants lost (9 from infection & 4 for unplanned cosmetic revision) compared to 15(9%) in non-smokers (p=0.01) and 12(24%) having delayed wound healing compared to 24(14%) (p=0.1).

Of the 28 patients who had previous RT the predominant reconstructive strategy was implant with LD-flap (17/28, 61%). Of all RT patients, 5(18%) implants were lost (3 for complications, 2 unplanned revision) compared to 12% in non-RT patients. Delayed wound healing was common; 10(36%) compared to 26(13%) (p=0.01).

Implant based reconstruction in the elderly, underweight and NAC groups have similar implant loss and delayed wound heal rates as the non-RT comparison groups, see table 1.

Only 6/216(3%) patients were diabetic, suggesting surgeons may negatively select this patient group for implant reconstruction.

<table>
<thead>
<tr>
<th>Risk Factor [RF] (n)</th>
<th>Implant loss in patients with RF(%)</th>
<th>Implant loss in patients without RF</th>
<th>p</th>
<th>Delayed wound healing in patients with RF(%)</th>
<th>Delayed wound healing in patients without RF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese (59)</td>
<td>17</td>
<td>11</td>
<td>0.36</td>
<td>24</td>
<td>14</td>
<td>0.10</td>
</tr>
<tr>
<td>Smoking (50)</td>
<td>26</td>
<td>9</td>
<td>&lt;0.01</td>
<td>24</td>
<td>14</td>
<td>0.10</td>
</tr>
<tr>
<td>Previous RT (36)</td>
<td>18</td>
<td>12</td>
<td>0.37</td>
<td>36</td>
<td>13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Older age (18)</td>
<td>0</td>
<td>14</td>
<td>0.48</td>
<td>10</td>
<td>18</td>
<td>1.00</td>
</tr>
<tr>
<td>Low BMI (10)</td>
<td>6</td>
<td>14</td>
<td>0.37</td>
<td>6</td>
<td>17</td>
<td>0.30</td>
</tr>
<tr>
<td>NAC (7)</td>
<td>14</td>
<td>13</td>
<td>1.00</td>
<td>14</td>
<td>17</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Conclusions; Implant based reconstruction is feasible in many of the higher risk, challenging patients that are encountered in a UK NHS practice. However even with the limited numbers of high-risk patients in this study smoking and RT, are associated with increased risk.
2015 San Antonio Breast Cancer Symposium

Publication Number: P2-13-11

Title: Assessing the impact of post-surgery areola repigmentation and 3-dimensional nipple tattoo procedures on body image and quality of life among medically underserved breast cancer survivors

Burke NJ J, Orenstein F, Napoles T, Chaumette S and Luce J. University of California, San Francisco, San Francisco, CA and San Francisco General Hospital, San Francisco, CA.

Body: Background: The benefits of 3D nipple tattooing include restoration of a natural looking breast as well as the avoidance of additional surgeries to create a breast that more closely resembles its pre-surgical appearance. 3D nipple tattooing forms part of completion of the physical reconstruction process, offers women opportunity for a greater sense of closure around the loss of their breast, and plays an important role in helping a woman feel "whole again" physically, sexually and emotionally. The project's aim was to evaluate the experience of 3D-nipple/areola tattooing for medically underserved women who have undergone breast reconstruction and remain without a nipple or areola.

Methods: In-depth interviews were conducted with 20 women who had undergone 3D-nipple/areola tattooing. Interviews were conducted in English and Spanish, recorded, translated and transcribed into English for analysis. Two research team members coded all interview transcripts and the research team met monthly to discuss emergent themes.

Results: Interview narratives addressed the often unexpected impact 3D nipple-tattooing had on body image, self-esteem, emotional well-being, intimacy, and inter-personal relationships. Women described their decision-making processes as weighing concern about the needle, the pain, and the uncertainty of the outcome with the opportunity for returning to a more 'normal' appearance. Women also discussed the impact of the experience on their survivorship and sexuality in positive ways.

Conclusions: 3-D nipple and/or areola tattooing is an acceptable and meaningful reconstruction process for medically underserved public hospital patients. The decision to undergo 3-D nipple and/or areola tattooing can be complex and is impacted by a woman's surgical history and outcomes. The emotional response and positive impacts of the tattooing on body image and self-esteem illustrate the value of the procedure in the context of women's lives.
Body: Background
The scheduling of mastectomy with immediate reconstruction (M-IR) procedures requires coordination between breast and plastic surgical teams that can contribute to delays in breast cancer treatment and subsequently impact patient outcomes and satisfaction. The breast center leadership at our comprehensive cancer center established a time-to-treatment target of 28 days from initial consultation with a breast surgical oncologist to M-IR. We sought to determine if a centralized breast surgical coordinator (BC) could reduce preoperative delays.

Methods
We initiated a 60-day pilot program to evaluate the impact of a BC on the workflow, efficiency, and timeliness for patients seen at our breast center. All reconstructive surgery candidates were referred to the BC, who had access to the clinic and operating room schedules of the breast and plastic surgeons. The BC worked with patients and both surgical services to identify the earliest consult and surgery dates and facilitated case booking. Interval days between initial surgical consult and M-IR were calculated. The median time to M-IR and the proportion of M-IR cases that met the time-to-treatment goal was determined. These results were compared to a reference cohort of breast cancer patients undergoing M-IR during the same time period (January-March) in 2013 and 2014, who had their consults and surgeries scheduled independently by breast surgery administrative staff. Patients who received neoadjuvant therapy or did not have a definitive cancer diagnosis at initial consultation were excluded from the time-to-treatment calculation.

Results
A total of 99 patients were referred to the BC (62% cancer, 21% neoadjuvant, and 17% prophylactic) during the pilot period. Focusing exclusively on patients with a definitive breast cancer diagnosis at initial consultation, an 18.5% increase in the percentage of cases that met the target (p=0.04), and a 7 day decrease in the median number of days to M-IR (p=0.02) was observed with the implementation of the BC (Table 1).

Table 1: Days to M-IR Pre and Post Implementation of BC

<table>
<thead>
<tr>
<th>Patients (N)</th>
<th>Median Days to M-IR (IQR)</th>
<th>% M-IR within 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (59)</td>
<td>40.0 (17.0)</td>
<td>23.7%</td>
</tr>
<tr>
<td>BC (45)</td>
<td>33.0 (20.0)</td>
<td>42.2%</td>
</tr>
<tr>
<td>p-value</td>
<td>0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusion
The coordination of care between breast surgical and reconstructive services presents timeliness challenges which may be partially alleviated through the implementation of a BC role. Establishing a centralized position to coordinate co-surgeon cases has improved time-to-treatment for M-IR at our cancer center. Further research is warranted to validate these preliminary findings, and determine the impact the BC has on operational efficiency and workflows.
Title: CDK7: A marker of poor prognosis and tractable therapeutic target in triple-negative breast cancer


Body: Triple-negative breast cancer (TNBC) is defined by absent expression of estrogen receptor (ER), progesterone receptor (PR) and non-overexpression of human epidermal growth factor receptor 2 (HER2), representing a heterogeneous subgroup of breast cancer with substantial genotypic and phenotypic diversity. TNBC patients commonly exhibit poor prognosis and high relapse rates at early stages after conventional treatments. Currently, there is a lack of biomarkers and targeted therapies for the management of TNBC. During tumour development and progression, alterations in cellular behaviour are frequently linked with kinase expression and activity. Here, we aimed to identify novel kinase targets that may play a pivotal role in the progression of TNBC and, thus, offer new therapeutic vantage points.

We initially focused on identifying kinases correlated with differential outcome. Using publicly available transcriptomic data from a collated set of TNBC patients (n = 483), we identified 9 kinases that were significantly associated with survival at the mRNA level. From this in silico screen, CDK7 (cyclin-dependent kinase 7) was found to be correlated with poor recurrence-free survival. CDK7's trait as a marker of poor prognosis was further validated within another TNBC cohort (n=109) via assessment of a tissue microarray generated as part of the RATHER Consortium (www.ratherproject.com). At the protein level, high CDK7 expression was associated with poor breast cancer-specific, recurrence-free and distant recurrence-free survival.

To evaluate CDK7 as a therapeutic target in TNBC, two TNBC cell lines (BT-549 and MDA-MB-231) were selected to evaluate phenotypic alterations post shRNA-mediated CDK7 knockdown. CDK7 silencing led to decreased cell proliferation, colony formation and migration in vitro. CDK7 down-regulation also increased TNBC cell sensitivity to doxorubicin. BS-181 and THZ1, two highly specific CDK7 inhibitors, attenuated TNBC tumour growth by inducing G2/M phase cell cycle arrest and apoptosis, as well as down-regulation of RNAPII phosphorylation, an indication of global RNA transcription inhibition. Moreover, the covalent CDK7 inhibitor THZ1 demonstrated 1000-fold higher potency than BS-181. Inhibition of global RNA transcription preferentially affects proteins with short half-lives. Accordingly, we detected a reduction in the expression of the anti-apoptotic protein MCL-1 in both cell lines.

Next, we assessed anti-apoptotic dependence in MDA-MB-231 cells following treatment with THZ1 via BH3 profiling technology, and observed an increased response to the BAD and HRK peptides, inferring an elevated survival dependence on BCL-2/BCL-XL. We subsequently evaluated the combination of the BCL-2/BCL-XL inhibitor ABT-263 with THZ1 and discovered a synergistic inhibition of cell growth and apoptosis. Resulting combination index (CI) values demonstrated that synergistic cell death occurred following combined treatment with THZ1 and ABT-263/ABT-199 at various doses in both TNBC cell lines tested. Our data implicate high CDK7 expression as a promising biomarker of poor prognosis in TNBC. Moreover, these findings suggest that targeting CDK7, combined with the BCL-2/BCL-XL inhibitor ABT-263, may be a useful therapeutic strategy for TNBC.
Second-generation selective glucocorticoid receptor modulators in triple-negative breast cancer


Body: Triple-negative breast cancer (TNBC) lacks expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2. A subset of primary TNBCs (at least 30%) expresses moderate to strong glucocorticoid receptor (GR) in greater than 10% of invasive tumor cells; in addition, we have reported that increased GR signaling promotes TNBC cell survival. Furthermore, our laboratory recently observed that patients with early stage/high-GR-expressing TNBCs have a relatively poor prognosis. Therefore, GR is being explored as a target for improving outcome in TNBC. Indeed, mifepristone, a well-characterized non-selective steroidal GR/PR antagonist, has shown promise in reversing GR-mediated tumor cell-survival signaling in TNBC. In vivo, increased TNBC xenograft chemosensitivity was observed when mifepristone was added to chemotherapy treatment. Most recently, a Phase I clinical trial demonstrated the safety and potential efficacy of mifepristone added to nab-paclitaxel chemotherapy in Stage IV TNBC patients.

Three highly selective non-steroidal GR antagonists (GRAs) have been investigated, CORT108297, CORT125134 (two aryl pyrazole-fused azadecalins) and CORT118335 (a pyrimidine dione). These new GRAs have 1) far less cross-reactivity than mifepristone with other nuclear receptor family members and 2) lower interaction profiles for drug metabolizing CYP enzymes. We hypothesized that selective GRAs would not only inhibit GR-induced pro-survival gene expression, but also increase TNBC chemosensitivity, and may eventually be a valuable adjunct therapy for treating chemotherapy-resistant TNBC.

To understand how GR modulators interact with the GR ligand-binding domain (LBD), we are employing two approaches: 1) computational modeling of the GRA-GR LBD based on published crystal structures of the GR LBD with mifepristone (in collaboration with UIUC), and 2) crystal structure analysis of the GR LBD with GR modulators. Preliminary computational results indicate that CORT108297 docks into the LBD and antagonizes GR activity by indirectly disordering Helix 12. Experiments are ongoing using various buffer conditions to produce crystals of the GR LBD with CORT108297 and CORT118335 for x-ray crystallography.

Secondly, we are functionally characterizing CORT108297, CORT125134, and CORT118335 for their ability to inhibit glucocorticoid induction of key GR-mediated target genes. Over the course of eight hours, we observed that the compounds exhibited temporal antagonism of the expression of GR target genes encoding anti-apoptotic proteins such as SGK-1, MCL-1, and MKP-1/DUSP1. Lastly, we are examining the selective GRAs’ ability to enhance tumor cell cytotoxicity from paclitaxel in cell culture and in TNBC xenograft models. Preliminary results indicate that CORT125134 and CORT108297 significantly increase GR+ TNBC sensitivity to paclitaxel, and are well-tolerated in our in vivo models.
Title: Inhibition of the autocrine IL6-JAK2-STAT3-calprotectin axis as targeted therapy for HR-/HER2+ breast cancers


Body: Although HER2+ tumors are commonly considered as a single entity there is increasing evidence indicating that important intrinsic differences associated to hormone receptor status (HR) exist. Indeed, while HR+/HER2+ patients benefit from anti-hormonal and HER2 targeted therapies, the outcome of HR-/HER2+ patients strongly depends on their response to chemotherapy as well as anti-HER2 therapy. To identify genes that represent novel mechanistic dependencies in HR-/HER2+ breast cancer cells, we designed an integrative approach that combines functional genomic (RNAi screens) and computational algorithms to interrogate regulatory networks reverse engineered.

Our analysis identified STAT3 as a de novo master regulator (MR) gene associated to HER2 mediated transformation in HR-breast cancer cells. Importantly, we demonstrate that aberrant STAT3 activity is necessary to maintain HR-/HER2+ tumor state, thus representing a non-oncogene dependency. Mechanistically we found that HR-/HER2+ breast tumors secrete high levels of IL-6. This acts in an autocrine way to induce the activation of STAT3 via the canonical JAK2/STAT3 pathway. Aberrant STAT3 activity induces upregulation and secretion of the S100A8/9 complex (Calprotectin) triggering a second autocrine stimulus that enhances proliferation and survival via ERK1/2 and AKT. As a result, disruption of the "IL6-JAK2-STAT3-S100A8/9 cascade" compromises HR-/HER2+ cell viability suggesting that the components of this pathway represent putative therapeutic targets in HR-/HER2 tumors.

Importantly, small molecule inhibitors and blocking antibodies for components of this double autocrine loop are already FDA approved or in clinical trials. Here, we demonstrate in vivo that blocking the IL-6 receptor (IL-6R) with the humanized monoclonal antibody Tocilizumab, STAT3 inactivation with the dual JAK1/2 inhibitor Ruxolitinib or Calprotectin inhibition with the small molecule inhibitor Tasquinimod), alone or in combination with anti HER2 therapies, compromises the viability of HR-/HER2+breast cancer cells. Availability of FDA-approved inhibitors to target this novel mechanism represents an exciting opportunity for rapid translation of these findings to the clinics.
Title: PAK-1 amplified breast cancer cell lines are preferentially sensitive to PAK inhibition with G-5555


Body: The small GTP-binding proteins Rac1 and Cdc42 stimulate activity of the serine/threonine kinase p21-activated kinase-1 (PAK-1) to drive growth factor signaling networks and Ras-driven tumorigenesis. Genomic amplification and over-expression of PAK1 are prevalent in luminal breast cancer and correlate with poor clinical outcome. Here we use a novel and selective small molecule inhibitor, G-5555, of the group I PAKs (PAK1, 2, and 3) to evaluate the importance of PAK1 in promoting growth of PAK1 amplified breast cancer cells. Cell lines with amplification of PAK1 were found to be more sensitive to PAK1 inhibition than non-amplified cell lines. Additionally, reverse phase protein array (RPPA) was used to assess the effects of PAK1 inhibition on a wide range of signaling pathways in both amplified and non-amplified cell lines. Reduced levels of phosphorylation of MEK S298 was observed in all cell lines exposed to G-5555 irrespective of amplification status, consistent with PAK1 inhibition in these cell lines. However, modulation of this downstream PAK1 substrate did not correlate with inhibition of cell proliferation or induction of cell death. Cell lines that showed inhibition of proliferation in response to G-5555 also showed enhanced levels of cell death along with apoptosis. Moreover, G-5555 reduced tumor growth in the PAK1 amplified MDA-MB-175 xenograft tumor model. Finally, we compared the in vitro activity of G-5555 with palbociclib, a recently approved inhibitor of the cyclin-dependent kinases CDK4 and CDK6, in PAK1 amplified luminal breast cancer cell lines. Our data supports PAK1 as an attractive target in PAK1 amplified cells and tumors and suggests that inhibiting PAK1 rather than CDK4/6 in this context may be a more attractive therapeutic strategy.
Title: Inhibition of fatty-acid oxidation as a therapy for triple-negative breast cancer


Body: Expression of the oncogenic transcription factor MYC is disproportionately elevated in triple-negative breast cancer (TNBC) compared to estrogen, progesterone and/or human epidermal growth factor 2 receptor-positive (RP) breast tumors. We and others have shown that MYC alters metabolism during tumorigenesis. However, the role of MYC in TNBC metabolism remains largely unexplored. We hypothesized that pharmacologic inhibition of MYC-driven metabolic pathways may serve as a therapeutic strategy for this clinically challenging subtype of breast cancer. Using an unbiased metabolomics approach, we identified fatty acid oxidation intermediates as dramatically up-regulated in MYC-driven models of TNBC. A lipid metabolism gene signature was identified in patients with TNBC in the TCGA and multiple other clinical datasets, implicating fatty acid oxidation as a deregulated pathway critical for TNBC metabolism. We find that MYC-overexpressing TNBC, including transgenic models and patient-derived xenografts (PDX), display increased bioenergetic reliance upon fatty-acid oxidation (FAO). Pharmacologic inhibition of FAO catastrophically decreases energy metabolism of MYC over-expressing breast cancer, blocks growth of a MYC-driven transgenic TNBC model and MYC-overexpressing patient-derived xenografts. In vivo inhibition of FAO induced proliferation arrest and increased cell death in PDX models of TNBC. Our results demonstrate that inhibition of FAO is a novel therapeutic strategy against TNBCs that over-express MYC.
Title: Safety and efficacy of anti-Trop-2 antibody drug conjugate, sacituzumab govitecan (IMMU-132), in heavily pretreated patients with TNBC

Bardia A, Diamond JR R, Mayer IA A, Starodub AN N, Moroose RL L, Isakoff SJ J, Ocean AJ J, Guarino MJ J, Berlin JD D, Messersmith WA A, Thomas SS S, O'Shaughnessy JA A, Kalinsky K, Maurer M, Chang JC C, Forero A, Traina T, Gucalp A, Wilhelm F, Wegener WA A, Maliakal P, Sharkey RM M, Goldenberg DM M and Vahdat LT T. Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA; University of Colorado Cancer Center, Aurora, CO; Vanderbilt-Ingram Cancer Center, Nashville, TN; Indiana University Health Center for Cancer Care, Goshen, IN; University of Florida Health Cancer Center, Orlando, FL; Weill Cornell Medical College, NY, NY; Helen F. Graham Cancer Center & Research Institute, Newark, DE; Baylor Sammons Cancer Center, Texas Oncology, Dallas, TX; Columbia University Medical Center, NY, NY; Houston Methodist Cancer Center, Houston, TX; University of Alabama Medical Center at Birmingham, Birmingham, AL; Memorial Sloan Kettering Cancer Center, NY, NY and Immunomedics, Inc., Morris Plains, NJ.

Body: Background: Triple-negative breast cancer (TNBC) comprises about 15% of all breast cancer types, and has a particularly aggressive course. Following first-line therapy, the median PFS is <3 months, and OS is <10 months. Therefore, new treatment strategies are needed. Since Trop-2 is expressed in >90% of TNBC, as measured by IHC, we conducted a trial to evaluate the safety and efficacy of a humanized anti-Trop-2 monoclonal antibody conjugated to a high concentration of SN-38, a camptothecin that is a topoisomerase I inhibitor and the active metabolite of the prodrug irinotecan, with 2-3 logs higher potency than the prodrug.

Methods: After establishing the optimal repeated dose in a Phase I trial (ClinicalTrials.gov, NCT01631552) involving many different solid cancer types, an expanded Phase II was undertaken in a number of cancers, including TNBC. Patients received 8 or 10 mg/kg IMMU-132 i.v. on days 1 and 8 of 21-day repeated cycles. Assessments of safety and response by RECIST1.1 were made weekly and bimonthly, respectively. Tumor biopsies (archival, at baseline prior to treatment, and at disease progression) were obtained when safe and feasible.

Results: As of May 10, 2015, 58 patients with TNBC, with a median of 4 prior therapies (range, 1-11), were treated with IMMU-132. Grade 3-4 toxicities included neutropenia (26%), febrile neutropenia (2%), diarrhea (2%), anemia (4%), and fatigue (4%). No patient developed antibodies to SN-38 or the antibody, and no patient discontinued therapy due to toxicity. Tumor responses were defined as ORR (CR+PR) in 31% of 49 evaluated patients, including 2 with CR, and a clinical benefit ratio (CR+PR+SD>6 mo) of 49% (63% with SD>4 mo; 23 patients continuing treatment after 1st assessment). The current median progression-free survival is 7.3 months with 44% maturity in 50 patients treated at the 8 or 10 mg/kg dose level. Overall survival data are still not mature 20 months after enrollment of first patient. Clinical efficacy correlated to biomarker studies, including Trop-2 expression (target of antibody), topoisomerase-1 expression (target of SN-38), and homologous recombinant deficiency (HRD) assay (marker of DNA repair), is being studied. Immunohistochemistry results in archival specimens currently show 97% positivity of Trop-2 among 34 specimens evaluated, with 79% having high intensity (2+/3+) staining.

Conclusions: The Trop-2-targeting IMMU-132, delivering cytotoxic doses of the topoisomerase I inhibitor, SN-38, shows manageable toxicity, and encouraging anti-tumor activity in relapsed/refractory patients with TNBC. This ADC appears to have a high therapeutic index in heavily pretreated patients.
Title: TEM8 specific CAR T cells serve as a novel targeted therapy for triple negative breast cancer and its supporting endothelium

Byrd T, Fousek K, Pignata A, Szot C, Bielamowicz K, Wakefield A, Koch J, Landi D, Seaman S, Wels W, Fletcher B, Hegde M, St Croix B and Ahmed N. Baylor College of Medicine, Houston, TX; National Cancer Institute, Frederick, MD; Georg Speyer Haus, Frankfurt am Main, Germany and University of Florida, Gainesville, FL.

Body: Background: Triple Negative Breast Cancer (TNBC) refers to an aggressive subtype of breast cancer negative for HER2, estrogen and progesterone receptors. Lacking these receptors, individuals with TNBC do not benefit from many of the targeted therapies for breast cancer. Tumor endothelial marker 8 (TEM8), originally identified as a tumor endothelium associated antigen, has more recently been implicated in TNBC pathogenesis and as a marker of breast cancer stem like cells. Here we report that T cells expressing a TEM8-specific chimeric antigen receptor (CAR) serve as a novel approach to target both TNBC cells and its supporting endothelium. CARs combine the specificity of a monoclonal antibody with the signaling properties of a T cell. Methods: We designed two novel TEM8-specific CAR molecules. A CAR molecule containing an exodomain derived from the anti-TEM8 L2 antibody, followed by CD28 and CD3-zeta signaling domains (second generation CAR) and CD28, 41BB and CD3-zeta signaling domains (third generation CAR), respectively. Retroviral transduction was used to express the TEM8 CAR transgene constructs on HEK 293T cells, then on primary T cells. Results: Immunofluorescence staining revealed that in a panel of primary TNBC breast cancer samples, TEM8 was overexpressed in comparison to normal adjacent breast tissue (6/6). Costaining with the pan-endothelial cell marker CD31 revealed that this overexpression was not confined to the endothelial compartment, but also present on tumor parenchymal cells. The immortalized TNBC cell lines (MDA-MB-231, MDA-MB-436, MDA-MB-468 and Hs578T) expressed endogenous levels of TEM8 protein as revealed by western blot. Greater than 90% transduction of primary human T cells was achieved using both of our CAR constructs, as detected by flow cytometry. TEM8 specific T cells displayed significantly higher killing of TEM8 positive TNBC and tumor endothelial cells (2H11 and bEND.3) in standard four hour chromium release assays when compared to both non-transduced or irrelevant (CD19) CAR T cells and secreted immunomostimulatory cytokines upon encounter of TEM8 positive cells in coculture assays. In a vascularized xenograft model, MDA MB468 cells were injected subcutaneously with matrigel into athymic nude mice and followed via bioluminescence imaging over the course of two months. Established tumors were treated with either, second or third generation TEM8 specific T cells, HER2 specific T cells, non-transduced T cells or left untreated. Relative to non-transduced T cells, TEM8 specific second and third generation CAR T cells significantly delayed tumor growth by 36 days and 50 days, respectively. Conclusion: TEM8 specific CAR T cells could serve as a novel targeted therapy for TNBC and supporting endothelium.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD3-08

Title: Modulation of immunity with 2-fluorofucose (2FF) for breast cancer treatment and prevention

Disis ML L, Rastetter L, Gad E, Koehnlein M, Senter PD D, Gardai S and Okeley NM M. Tumor Vaccine Group, University of Washington, Seattle, WA and Seattle Genetics, Inc., Bothell, WA.

Body: The majority of patients with breast cancer have robust Type II immune responses directed against their tumors with little to no Type I immunity. The dominance of a Type II microenvironment is established early in breast tumorigenesis with a Type II immune signature prevalent even in pre-invasive lesions such as ductal carcinoma in situ. As a result, breast cancer is associated with abundant autoantibodies directed against tumor associated antigens and few infiltrating T-cells in the majority of patients. A recently reported inhibitor of protein and cellular fucosylation, 2-fluorofucose (2FF) has been shown to enhance immune cell function, in part through the generation of fucose-deficient antibodies which result in enhanced antibody dependent cell mediated cytotoxicity, as well as apparent modulation of T-cell dependent activity [Ca Res, Oct 1, 2014 74;2890].

For treatment studies, TgMMTV-neu (MMTVneu; luminal) and C31-Tag (C3T; triple negative) mice were treated orally with vehicle alone and 20mM 2FF when spontaneous tumor volume reached 75-100mm3 until sacrifice (n=20/grp). To assess the ability to prevent breast cancer development, mice were treated orally with vehicle alone, 20 or 50mM 2FF for up to 200 days starting at 6-8 weeks of age (n=20/grp). Tumor kinetics, disease free survival, and overall survival were calculated. Immune responses were evaluated by both DTH and IFN-gamma ELISPOT. To determine immune mechanism of action, NK, B, CD4, or CD8 cells were depleted concurrently with tumor implant.

When tumor bearing mice were treated with 2FF, tumor growth was significantly inhibited in both groups over the course of therapy (MMTVneu p<0.0001 and C3T p<0.0001 compared to controls). In addition, survival was significantly prolonged in both models (MMTVneu p<0.0001 and C3T p=0.0014). In the prevention setting, 2FF significantly delayed the average age of tumor onset in both models at both doses with the higher doses showing greater efficacy; MMTVneu 20mM, p=0.001, 50mM, p=0.0002. At the time of study termination (300d), 45% of 2FF-treated MMTVneu mice had no evidence of mammary tumors. 2FF also significantly improved the average age of tumor onset in C3T mice when comparing untreated to 20mM (p=0.007), and 50mM (p=0.0007) treated mice. At study termination, 33% of all 2FF-treated C3T animals were disease free. 2FF anti-tumor activity was associated with the induction of tumor antigen specific immunity. After treatment both the MMTVneu (p<0.0001) and C3T (p<0.0001) developed a DTH response to syngeneic tumor lysates. Of note, the DTH response was inversely associated with the rate of tumor growth (p<0.0001). 2FF anti-tumor activity was associated with the induction of tumor antigen specific immunity. Depletion of immune cell subsets using targeting antibodies demonstrated that the anti-tumor response was mediated in large part by CD4 T-cells which are involved in stimulating both humoral and CD8 T-cell responses.

2FF, a novel inhibitor of fucosylation has potent anti-tumor effects in 2 transgenic models of breast cancer; luminal and triple negative mammary tumors. The agent is active in these mouse models in both the treatment and prevention setting and thus may represent a rational therapeutic approach to evaluate in breast cancer patients.
Title: EndoPredict (EPclin) score for estimating residual distant recurrence (DR) risk in ER+/HER2- breast cancer (br ca) patients treated with 5 years adjuvant endocrine therapy alone: Validation and comparison with the oncoyte DX recurrence score (RS)

Dowsett M, Sestak I, Buus R, Kronenwett R, Denkert C, Krappmann K, Scheer M, Petry C, Dubsky P and Cuzick J. Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom; Queen Mary University of London, London, United Kingdom; Sividon Diagnostics, Cologne, Germany; Charité Universitätsmedizin Berlin, Berlin, Germany and Medical University Vienna, Vienna, Austria.

Body: Background: RS is widely used for estimating 10-yr risk of DR for br ca patients receiving 5yrs adjuvant endocrine treatment alone. EndoPredict is a prognostic test which unlike RS combines its eight-gene expression signature (EP score) with tumor size and nodal status to provide the EPclin score with a single low/high risk cut-off at 10% risk of DR at 10yrs similar to the low/intermediate risk cut-off with RS.

Aims: To determine the prognostic value of EP/EPclin over 10yrs, 0-5yrs and 5-10yrs in ER+/HER2- br ca patients and to compare this with the RS (i) alone (primary objective) and (ii) in addition to the clinical treatment score (CTS [developed in TransATAC]: nodes (N); tumour size; grade; age; tam or AI).

Methods: mRNA from 928 ER+/HER2- primary br ca from patients treated with anastrozole or tamoxifen in the ATAC trial on which RS was already available were assessed for the EP/EPclin. Primary end point was DR. Kaplan–Meier and Cox regression analyses were used to determine DR risk for 0-10, 0-5 and 5-10yrs follow-up. Likelihood ratio tests (LR-chi sq) were used to assess the prognostic information provided by EP, EPclin, and RS alone and in combination with CTS.

Results: Median follow-up was 9.95 yrs. The table shows the LR-chi sq for the prognostic information provided by each marker alone (univariate) and the information on top of CTS for all patients, and N- and N+ subgroups in 0-10, 0-5 and 5-10yrs. EP and EPclin provided substantially more prognostic information than RS across all three time periods and subgroups, except for N-patients in 0-5yrs. EP and EPclin also provided more information in addition to CTS than RS in 0-10yrs due to better performance of EP and EPclin in 5-10yrs where RS added no significant information to CTS. Using predefined cut-offs, EPclin and RS identified 546 (58.8%) and 573 (61.7%) patients as low risk respectively (HR (95%CI) low vs. non-low risk: 5.9 (3.9-9.1) and 2.7 (1.9-3.8), respectively). The EP score significantly added prognostic information to the RS over 0-10yrs and 5-10yrs in the overall, N- and N+ populations. Cases classified discordantly by EPclin and RS followed the EPclin classification more closely than RS classification.

Table: Prognostic information from EP, EPclin, RS

<table>
<thead>
<tr>
<th>LR-chi-sq</th>
<th>All patients</th>
<th>Node-negative</th>
<th>Node-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10yrs</td>
<td>0-5; 5-10yrs</td>
<td>0-10yrs</td>
</tr>
<tr>
<td>Univariate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP</td>
<td>49.3</td>
<td>25.7; 23.6</td>
<td>30.8</td>
</tr>
<tr>
<td>EPclin</td>
<td>139.3</td>
<td>80.0; 59.3</td>
<td>40.0</td>
</tr>
<tr>
<td>RS</td>
<td>29.1</td>
<td>26.1; 5.6</td>
<td>21.3</td>
</tr>
<tr>
<td>CTS</td>
<td>149.8</td>
<td>85.0; 64.7</td>
<td>35.6</td>
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<tr>
<td>added to CTS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EP</td>
<td>16.4</td>
<td>6.9; 9.8</td>
<td>15.5</td>
</tr>
<tr>
<td>EPclin</td>
<td>20.3</td>
<td>10.5; 9.9</td>
<td>17.0</td>
</tr>
<tr>
<td>RS</td>
<td>12.8</td>
<td>11.8; 2.3</td>
<td>18.7</td>
</tr>
</tbody>
</table>

LR-chi sq >3.8, p<0.05

Conclusions: EPclin was highly prognostic for DR in all patients, N-, N+, early and late metastasis. EPclin provided more...
prognostic information than RS in part but not solely because of the integration of the EP score with N and tumour size parameters. The data stress the importance of including clinicopathological factors in deriving an overall estimate of risk.
**Title:** Molecular predictors of outcome on adjuvant CAF plus tamoxifen (T) vs T in postmenopausal patients (pts) with ER+, node+ breast cancer – Transcriptome expression analysis of the phase III trial SWOG-8814

Albain KS S, Crager MR R, Barlow WE E, Baehner FL L, Bergamaschi A, Rae JM M, Ravdin PM M, Tripathy D, Gralow JR R, Livingston RB B, Osborne CK, Ingle JN N, Pritchard KL I, Davidson NE E, Carey LA A, Cherubavaz DB B, Sing AP P, Shak S, Hortobagyi GN N and Hayes DF F. Loyola Univ Chicago Stritch School of Medicine, Maywood, IL; Genomic Health, Inc., Redwood City, CA; Cancer Research and Biostatistics, Seattle, WA; Genomic Health, Inc. and Univ of California, San Francisco, Redwood City and San Francisco, CA; University of Michigan, Ann Arbor, MI; University of Texas Health Science Center Cancer Therapy and Research Center, San Antonio, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; University of Washington, Seattle Cancer Care Alliance, Seattle, WA; University of Arizona Cancer Center, Tuscon, AR; Baylor College of Medicine, Houston, TX; Mayo Clinic, Rochester, MN; Sunnybrook Odette Cancer Centre and the University of Toronto, Toronto, ON, Canada; University of Pittsburgh Medical Center, Pittsburgh, PA and University of North Carolina at Chapel Hill, Chapel Hill, NC.

**Body:** BACKGROUND: In SWOG-8814A, pts with ER+ node+ breast cancer and low 21 gene recurrence scores (RS) had good prognosis and no CAF benefit, but high RS predicted longer survival from CAF followed by T (CAF-T) vs T (Albain, Lancet Oncol 2010). The aims of SWOG-8814B were to identify novel genes and networks for 1) prognosis of early and late relapse and 2) prediction of CAF benefit, using whole transcriptome expression analysis with next generation RNA sequencing (NGS).

METHODS: Stored RNA previously extracted for SWOG-8814A (T, CAF-T arms; T, 5 yrs) was analyzed for RNA/library yield (see companion abstract Cherubavaz et al. for methods). Genes were sequenced and expression of mRNA species was related to disease-free survival (DFS) using Cox proportional hazards. Discovery analyses controlled false discovery rate (FDR) at 10%. Genes were identified for prognosis on T and prediction on CAF-T vs T. Networks of genes/pathways were explored. Early (0-5 yrs) and late (5-13+ yrs) time periods were studied. Gene Ontology, Cytoscape, pathway and hierarchical clustering were used for functional gene and metagene analyses.

RESULTS: Of 367 samples, 354 (96%; 142 T, 212 CAF-T; 141 DFS events) had sufficient RNA/library yield, with 20,101 genes sequenced. For prognosis on T, there were 2327 and 568 genes discovered in early and all-yrs follow-up, with only 9 genes prognostic after 5 yrs. Prognosis analyses for residual risk after CAF-T were uninformative. Functional mapping found that genes prognostic for worse DFS were enriched for proliferation (G2M, M-phase), cellular metabolism, DNA repair, stress response and EMT; whereas, those with better DFS involved transcription regulation/repression via zinc finger proteins. Hierarchical clustering (T arm) found significant DFS prognostic metastatic signatures for ER-related genes, immune response, ECM/stroma, chromatin remodeling-transcription factor activity and TGFβ pathway. All varied for early vs late DFS events. For example, low ER/high stroma expression signatures correlated with high proliferation gene expression and were strongly associated with early events (standardized [st] HR 2.94, p<0.001). Late recurrence was associated with high proliferation, both individually (stHR 1.51, p=.035) and in combination with higher ER expression (stHR 1.51, p=0.09). Fifteen genes predicted CAF benefit (9 better DFS, 6 worse), or 129 genes if FDR relaxed to 20%. Cluster analysis for CAF prediction is ongoing.

CONCLUSIONS: Unique genes, clusters and pathways were identified by NGS of archival material in ER+ N+ breast cancer, including previously unreported signatures. While ER, stroma and proliferation-related signatures were associated with early prognosis, proliferation best predicted worse DFS after 5 yrs. NGS of the primary tumor is most informative for early events in pts with only 5 years of T, with few genes selecting only for late relapse. If validated, these signatures may identify pts with excellent DFS despite positive nodes for endocrine therapy alone as well as others for whom chemotherapy and/or biologics are also required.

SUPPORT: NCI CA 180888, 180819, 180821, 180820, 180863; in part, Genomic Health, Inc.
Title: Nuclear FGFR1 interaction with estrogen receptor (ER) α is associated with resistance to endocrine therapy in ER+/FGFR1-amplified breast cancer

Body: Background: Estrogen receptor (ER)-positive breast cancers (BC) initially respond to antiestrogens but eventually become hormone-independent and recur. FGFR1 is amplified in ~10% of ER+ BC and is associated with early recurrence on antiestrogen therapy. Notably, one third of FGFR1-amplified tumors have simultaneous amplification of CCND1, FGF3, FGF4 and FGF19 on chromosome 11q12-14. Herein, we investigated the mechanisms by which FGFR1 amplification confers resistance to antiestrogen therapy in ER+ BC cells.

Results: We performed whole exome sequencing in tumor biopsies from 130 patients with an operable ER+/HER2- BC who had received letrozole for 10-21 days prior to surgery. Tumors were categorized by the natural log (ln) of post-letrozole Ki67 as sensitive (ln ≤ 1 or ≤ 2.7% Ki67+ cells; n=68) or resistant (ln ≥ 2 or ≥ 7.4%; n=18). We found amplifications in FGFR1 and/or 11q12-14 in 6/11 (55%) resistant tumors compared with 5/34 (15%) in sensitive tumors (p=0.006); all cases were confirmed by FGFR1-fluorescence in situ hybridization (FISH). Resistant tumors with FGFR1 and/or 11q12-14-amplification showed a marked increase in nuclear FGFR1 with letrozole. ER+/FGFR1-amplified CAMA1 and MDA134 cell lines also exhibited co-localization of ER and FGFR1 in the nucleus. Cell proliferation was partially reduced by estrogen deprivation, and FGFR1 siRNA further reduced cell growth in hormone-depleted medium. We generated CAMA1 and MDA134 cells resistant to long-term estrogen deprivation (LTED). These cells exhibited overexpression of FGF3/4/19 and ERα with a concomitant increase in ligand-independent ER transcriptional activity and growth. An ER-FGFR1 interaction was observed in the nucleus and cytosol of CAMA1 parental cells with enhanced interaction in CAMA1 LTED cells. Genetic (with siRNA) and pharmacologic (with lucitinib) inhibition of FGFR1 reduced a) nuclear localization of FGFR1; b) ER transcriptional activity; and c) cell proliferation. Nuclear localization and ER-FGFR1 interaction were disrupted by a kinase-deficient FGFR1. Conversely, addition of FGF3 ligand stimulated ER-FGFR1 interaction and ER transcriptional activity, suggesting FGFR activation can regulate ER function. Inhibition of FGF receptor-specific substrate (FRS2), a principal mediator of FGFR1 signal transduction to the MAPK and PI3K pathways, with siRNA or pharmacologic inhibition of PI3K with buparlisib or MEK with GSK1120212 did not reduce ER transcriptional activity suggesting that, in ER+/FGFR1-amplified cancer cells, ER function is not modulated by FGFR signal transducers. Finally, using chromatin immunoprecipitation (ChIP) we showed that FGFR1 binds directly to estrogen response elements (ERE). This association was reduced with lucitinib. We are currently investigating genes modulated by ER/FGFR1 in ER+ BC and the in vivo anti-tumor efficacy of dual inhibition of FGFR1 and ER in ER+/FGFR1-amplified patient-derived breast cancer xenografts.

Conclusions: These data support a critical role of ER and FGFR1 interaction in endocrine resistance in ER+/FGFR1-amplified breast cancer. Targeting of FGFR1 in combination with antiestrogens may abrogate resistance to endocrine therapy in these tumors and is worthy of clinical investigation.
2015 San Antonio Breast Cancer Symposium

Publication Number: S3-04

Title: ESR1 coregulator binding inhibitor (ECBI) as a novel therapeutic to target hormone therapy resistant metastatic breast cancer


Body: BACKGROUND: Estrogen contribute to the progression of breast cancer via estrogen receptor 1 (ESR1) and current therapies involve either antiestrogens or aromatase inhibitors. However, most patients develop resistance to these drugs. Critically, therapy-resistant tumors retain ESR1-signaling. Mechanisms of therapy resistance involve the activation of ESR1 in the absence of ligand or mutations in ESR1 that allow interaction between the ESR1 and coregulators leading to sustained ESR1 signaling and proliferation. For patients with therapy-resistant breast cancers, there is a critical unmet need for novel agents to disrupt ESR1 signaling by blocking ESR1 interactions with its coregulators.

METHODS: Using rational design, we synthesized and evaluated a small organic molecule (ESR1 coregulator binding inhibitor, ECBI) that mimics the ESR1 coregulator nuclear receptor box motif. Using in vitro cell proliferation and apoptosis assays, we tested the effect of ECBI on several breast cancer and therapy-resistant model cells. Mechanistic studies were conducted using established biochemical assays, reporter gene assays, RT-qPCR and RNA-Seq analysis. Differentially expressed genes were analyzed using Ingenuity Pathway Analysis (IPA). ESR1 positive (MCF7 and ZR75) xenografts were used for preclinical evaluation and toxicity. The efficacy of ECBI was tested using ex vivo cultures of freshly extirpated primary human breast tissues.

RESULTS: In estrogen induced proliferation assays using several ESR1 positive model cells, ECBI significantly inhibited growth and promoted apoptosis. Importantly, ECBI showed little or no activity on ESR1 negative cells. Further, ECBI also reduced the proliferation of several ESR1 positive hormonal therapy resistant cells. Mechanistic studies showed that ECBI interacts with ESR1, efficiently blocks ESR1 interactions with coregulators and reduces the ESR1 driven ERE reporter gene activity. Further, ECBI directly interacted with mutant-ESR1 with high affinity and significantly inhibited mutant-ESR1 driven oncogenic activity. RNA sequencing analysis revealed that ECBI blocks multiple ESR1 driven pathways, likely representing the ability of a single ECBI compound to block multiple ESR1-coregulator interactions. Treatment of ESR1-positive xenograft tumors with ECBI (10 mg/kg/day/oral) significantly reduced the tumor volume compared to control. Further, ECBI also significantly reduced the tumor growth of coregulator-overexpressed breast cancer cells in xenograft model. Using human primary breast tissue ex vivo cultures, we have provided evidence that ECBI has potential to dramatically reduce proliferation of human breast tumors.

CONCLUSIONS: The ECBI is a novel agent that targets ESR1 with a unique mechanism of action. ECBI has distinct pharmacologic advantages of oral bioavailability, in vivo stability, and is associated with minimal systemic side effects. Remarkably, ECBI block both native and mutant forms of ESR1 and have activity against therapy resistant breast cancer cell proliferation both in vitro and in vivo and against primary human tumor tissues ex vivo. Thus development of ECBI represents a quantum leap in therapies to target ESR1.
Title: Higher 10-year overall survival after breast conserving therapy compared to mastectomy in early stage breast cancer: A population-based study with 37,207 patients

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WITHELD PENDING PRESS CONFERENCE
2015 San Antonio Breast Cancer Symposium

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Title: Treatment outcomes in patients with invasive breast cancer treated with neoadjuvant systemic therapy and breast MR imaging: Results of a secondary analysis of TBCRC 017

De Los Santos J, Hyslop T, Alvarado M, Forero A, Golshan M, Hieken T, Horton J, Hudis C, McGuire K, Meric-Bernstam F, Nanda R, Zagar T and Hwang S. University of Alabama at Birmingham, Birmingham, AL; Duke Cancer Institute, Durham, NC; University of California San Francisco, San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA; Mayo Clinic, Rochester, MN; Duke University School of Medicine, Durham, NC; Memorial Sloan Kettering Cancer Center, NY, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Chicago Medicine, Chicago, IL and University of North Carolina at Chapel Hill, Chapel Hill, NC.

Body: Background: Neoadjuvant chemotherapy (NCT) is used frequently to downstage locally advanced tumors and facilitate breast conservation. However, we have previously reported that achievement of radiographic complete response (rCR) or pathologic complete response (pCR) does not impact choice of surgery for many patients. This secondary analysis reports treatment outcomes across 9 NCI comprehensive cancer centers in women receiving both NCT and breast MR imaging to assess whether treatment outcomes among women receiving NCT differs according to choice of locoregional treatment.

Methods: 1077 women from 9 institutions were retrospectively identified as having undergone NCT with MR imaging obtained both before and after systemic treatment. Systemic treatment regimen was not prespecified, but receipt of at least 80% of all planned cycles was required prior to final MR imaging. We performed a univariate analysis as well as a multivariable Cox proportional hazard regression to identify covariates associated with overall survival (OS), disease-free survival (DFS) and time to recurrence (TTR). rCR was defined as no residual enhancement on post-treatment breast MRI.

Results: 1077 patients diagnosed and treated with NCT for stage I-III invasive breast cancer from January 1, 2002 to June 16, 2014 were analyzed for all endpoints. Median follow-up was 4.2 years, (range 0.1 to 13 years). Median age of the cohort was 50 years, (range 19-87 years). 473 (43.9%) had ER(+) and/or PR(+)/HER2(-) disease, 348 (32.3%) had HER2(+) disease, and 256 (23.8%) had ER(-)/PR(-)/HER2(-) (triple negative) disease. Mastectomy or breast conserving therapy (BCT) was recorded as the definitive surgery in 675 (62.7%) and 402 (37.3%) of patients, respectively. Radiation receipt was confirmed in 84.1% of BCT and 68.3% of mastectomy patients. Overall there were 134 recurrences, 168 disease events and 89 deaths. Among patients with pCR, there were 7/161 (7.2%) recurrences in those undergoing mastectomy and 6/143 (5.1%) in those undergoing lumpectomy (p=0.81). Among patients who achieved an rCR, there were recurrences in 5% of those undergoing mastectomy and 2.9% in those undergoing lumpectomy (p=0.53). In multivariable analysis of the entire cohort, only clinical stage, ER status and pCR remained independently associated with DFS. Notably, subset analysis showed that lumpectomy was independently associated with improved TTR (HR 0.40; 95% CI 0.17-0.97) in the triple negative group only, but this did not translate into improved DFS with lumpectomy in this group. Radiographic CR as determined by breast MRI accurately predicted presence or absence of pCR in 74% of cases, but was not independently associated with DFS, OS or TTP.

Conclusions: Among a contemporary cohort of women receiving neoadjuvant systemic therapy and breast MR imaging at 9 NCI designated cancer centers, type of surgery did not impact DFS, OS or TTP. The only exception was found in the triple negative group in which the lumpectomy group had a more favorable TTP compared to the mastectomy group. These findings provide additional evidence that in women who are appropriate candidates for lumpectomy after NCT, BCT does not compromise long-term cancer outcomes.
Title: Complication and economic burden of local therapy options for early breast cancer

Smith BD D, Jiang J, Shih Y-CT, Giordano SH H, Huo J, Jagsi R, Caudle AS S, Hunt KK K, Shaitelman SF F, Buchholz TA A and Shirvani SM M. The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Michigan, Ann Arbor, MI and Banner MD Anderson Cancer Center, Gilbert, AZ.

WITHHELD PENDING PRESS CONFERENCE
Identification of early versus late drivers of breast tumors and metastasis


Body: Background: The molecular characterization of primary breast cancers has led to signatures identifying risk of future metastasis and survival; however the underlying biology driving metastasis development is largely unknown.

Methods: Utilizing a Rapid Autopsy Program, we have collected 61 metastatic breast cancer tumors from 7 individuals (4 triple negative, 2 HER2+, 1 ER+/HER2-) including primary tumors and 3-6 metastases/patient. We performed mRNA and DNA exome sequencing. We next used DawnRank, a novel network-based method that integrates DNA and RNA data to identify computationally determined "driver" genes (i.e. a DNA variant that significantly alters its gene expression-network) in each individual sample. Phylogenetic tree and clonal analysis were also performed, with the computationally determined drivers mapped onto these trees.

Results: The breast cancer primaries were molecularly subtyped as 5 Basal-like, 1 HER2-Enriched, and 1 Luminal A; in all cases, the metastases clustered immediately adjacent to their primary tumor by hierarchical clustering analysis. Widespread DNA copy number alterations identified in the primary tumors were typically maintained throughout metastasis. On average, 1.9 ± 1.3% of DNA copy number altered genes, and 2.4 ± 0.95% of the somatic mutations per tumor were identified as "drivers" by DawnRank. There were an average of 199 ± 72 total drivers per tumor due to copy number alterations (amplifications or deletions) and 12 ± 23 drivers per tumor from somatic mutations.

Phylogenetic tree analysis demonstrated that the majority of DNA copy number events occurred early in tumor development. Founding clones were defined as genetic events present in the primary and all matched metastases. Chr5q13 loss and TP53 mutation were the only consistent alterations in the founding clones of all 7 patients. Drivers on chr5q13 identified in this cohort include CCNB1, CDK7, and TAF9. Among the basal-like patients, all 5 patients' TP53 mutations were identified as a driver by DawnRank.

39% and 20% of drivers from copy number gains and losses, respectively, were identified in the primary tumor, while another 34% and 30% were not seen in the primary but were present in more than 1 metastasis within each patient. Metastasis-enriched copy number drivers not seen in any primary included FLT1, MAP2KR, and ARNT.

38% of the drivers resulting from somatic mutations were established in the primary and maintained in metastases. Of the remaining drivers from somatic mutation, only 18% were shared among metastases but not seen in the primary while 47% were not seen in any other tumor within a given patient (i.e. private to a single sample). TP53, PSEN1, CDC27, HDAC1, and BRCA1 were somatic mutation drivers established early in metastatic development, while CCNH was a consistent late driver.

Conclusions: We present a novel computationally determined genetic "driver" analysis of matched breast cancer primaries and multi-organ metastases. In this cohort, our results suggest that most genetic drivers in a single tumor are based on copy number aberrations, are established early, and are maintained in metastases. In contrast to copy number, drivers from somatic mutations are acquired later, and most of the metastases continued to acquire new genetic driving features.
Title: A comprehensive analysis of fusion transcripts in breast cancer reveals associations between number of fusion transcripts, copy number events, gene expression profiles, and potentially clinical outcome

Thompson EA, Asmann YW W, Su X, Ellis MJ J, Shao J, Hu Y, White KP P, Cherniack AD D, Hoadley KA A, Serie DJ J, Perez EA A and Perou CM M. Mayo Clinic Comprehensive Cancer Center, Jacksonville, FL; MD Anderson Comprehensive Cancer Center, Houston, TX; Baylor College of Medicine, Houston, TX; Washington University, St. Louis, MO; Sage Bionetworks, Seattle, WA; University of Chicago, Chicago, IL; Broad Institute, Cambridge, MA and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

Body: Fusion transcript analysis by five groups, each using "in house" analytical workflow to analyze RNA-seq data from 813 breast tumors from The Cancer Genome Atlas project detected 2514 high confidence RNA fusions (2 or more junction spanning reads detected by 2 or more groups). A subset of tumors with fusions were evaluated for chromosomal rearrangement using whole genome sequence data (WGS), and about half of the high confidence fusions could be validated, with the remainder likely subclonal and therefore below the limits of detection by WGS at the relatively low depth of coverage (30X) available. The majority of tumors contained one or more fusion transcript (range 0-24 fusions/tumor), but recurrence of specific chimaeric transcripts was low. Only ESR1->CCDC170, C20orf3->ACSS1, and USP22->MYH10 fusions were detected in 10 or more tumors. Additional ESR1 fusions were detected with AKAP12, BNC2, C6orf211, GNAS, POLH, USP25, and UTRN, within 19 tumors, primarily of the Luminal B subtype. RARA was the second most common 5' fusion partner with 17 tumors, predominately of the HER2-enriched subtype, expressing out of frame RARA fusions to 19 different 3' fusion partners. Other common 5' fusion partners included FBXL20, USP22, USP32, BCAS3, MED1, BPTF, ERBB2, MED24, and PPF1A1 (expressed in 10 or more tumors each). The most common 3' fusion partners were ERBB2, CCDC170, PITPNC1, ACSS1, MYH10, IKZF3, and RAB6A (10 or more tumors each). HER2-enriched tumors expressed significantly more fusions than other subtypes (4.9 fusions/tumor vs 3.3 for Basal-like, 1.3 for LumA, and 3.2 for LumB). Almost all HER2-enriched tumors expressed fusions (97%), whereas 85% of Basal-like tumors and 79% of Luminal B tumors expressed one or more fusions. Among Luminal A tumors, only 48% expressed one or more fusion transcript. Those Luminal A tumors with multiple fusion transcripts contained significantly more copy number aberrations and were enriched for expression of mitotic cell cycle genes (p=3.07E-18), characteristic of the recently described high risk CNH subclass of Luminal A tumors. We conclude that, consistent with other observations, breast tumors generally exhibit a high level of genomic instability, associated with multiple copy number alterations and multiple fusion transcripts. However, recurrence of specific fusion transcripts is low. About half of Luminal A tumors express fusion transcripts; and these tumors exhibit multiple copy number events, consistent with high level genomic instability, as well as enriched expression of mitotic cell cycle genes. These tumors appear to be related to the relatively high risk Luminal A CNH subclass, and express high levels of aurora kinases and polo-like kinases, which might be considered as therapeutic targets. Given the increasing emphasis on transcript profiling of clinical samples and the clinical significance of high risk Luminal A tumors, it may be timely to consider inclusion of fusion transcripts in such analyses as surrogate markers of genomic instability and potential therapeutic and/or prognostic indicators.
Title: A functional assay for homologous recombination (HR) DNA repair and whole exome sequencing reveal that HR-defective sporadic breast cancers are enriched for genetic alterations in DNA repair genes


Body: Background: Germline mutations in the BRCA1 and BRCA2 genes lead to hereditary breast cancers that are defective in homologous recombination (HR) repair and sensitive to DNA damaging agents. HR deficiency (HRD) also occurs in sporadic breast cancers, but its incidence and etiology are unclear. Genomic signatures of HRD recently were employed as biomarkers in clinical trials with modest success. We posited that sporadic breast cancers displaying functional HR deficiency would harbor genetic alterations affecting HR DNA repair genes. To test this hypothesis, we applied a functional assay to define lack of competent HR DNA repair and sequenced the exomes of consecutive sporadic breast cancers.

Methods: We developed an assay to assess the ability of cancer cells to localize RAD51 into sub nuclear foci in response to ex-vivo irradiation (IR) in fresh sporadic breast cancer tissue specimens from 60 patients. RAD51 focus formation was compared between mock and IR conditions to determine relative fold induction. Twenty-nine tumors with sufficient DNA underwent whole-exome sequencing. Structural genomic signatures of HRD (i.e. large state transitions (LST), telomeric imbalance (NtAI), loss-of-heterozygosity (LOH)) and a previously reported mutational signature related to BRCA1/2 hereditary breast cancers were assessed. HR deficient tumors were defined as those with both RAD51 foci defects and a genomic signature of HR deficiency (LST>15 or presence of a BRCA mutational signature). Somatic, germ-line, and copy number changes in HR genes were investigated. BRCA1 methylation status was determined.

Results: Seventeen of 60 (28%) tumors displayed defective RAD51 recruitment following ex-vivo IR (RAD51-DEF). RAD51-DEF was seen in all breast cancer subtypes , including 7 of 33 (21%) ER+/HER2-, 4 of 14 (29%) HER2+, and 6 of 13 (48%) triple-negative cases. Of the 29 sequenced tumors, 13 (45%) were RAD51-DEF and 16 (55%) were competent for inducing RAD51 foci. LST was elevated in 10 tumors (LST >15) and associated with RAD51-DEF (p=0.02), whereas NtAI (p=0.10) and LOH (p=0.052) did not show a significant association with RAD51-DEF. The BRCA1/2 mutational signature was evident in 4 tumors, all were RAD51-DEF (p=0.03) and 2 were BRCA2 mutated. Nine of 29 (31%) sequenced tumors were determined to have HRD by the RAD51 assay and presence of a genomic scar. Eight of these 9 (88%) cases with HRD had a genetic alteration of both alleles of a bona fide HR gene due to a pathogenic mutation (somatic or germline) coupled with loss of heterozygosity or a homozygous deletion compared to 1 (5%) tumor without HRD (p<0.001). Both alleles of a gene were affected for BRCA2 (n=4), FAAP100 (n=2), CHEK2 (n=1), [italic]TP53BP1 (n=2) and BRCA1 (n=3). BRCA1 gene promoter methylation was found not to be significantly associated with HRD.

Conclusion: Combined functional and genomic analyses of breast tumors demonstrated that genetic loss of an HR gene may underpin HRD in sporadic breast cancers. Our findings warrant further comprehensive genetic assessment (somatic, germline, and copy number) of HR genes as potential biomarker for HR-directed therapies.
Title: Lobular carcinoma in situ displays intra-lesion genetic heterogeneity and its progression to invasive disease involves clonal selection and variations in mutational processes

Reis-Filho JS S, Schizas M, Piscuoglio S, Sakr RA A, Ng CKY K Y, Lim RS S, Carniello JVS S, Towers R, Martelotto L, Giri DD D, de Andrade VP P, Viale A, Solit DB B, Weigelt B and King TA A. Memorial Sloan Kettering Cancer Center, NY, NY; Memorial Sloan Kettering Cancer Center, NY, NY and Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, NY, NY.

Body: Introduction: Lobular carcinoma in situ (LCIS) is considered both a risk factor and non-obligate precursor of invasive breast cancer. We sought to determine the genomic landscape of LCIS and the mutational processes involved in the clonal evolution and progression from LCIS to ductal carcinoma in situ (DCIS) and invasive lobular carcinoma (ILC).

Methods: Patients with a history of LCIS undergoing therapeutic or prophylactic mastectomy were prospectively enrolled in an IRB approved protocol. Frozen tissue blocks were collected, screened for lesions of interest (LCIS, DCIS, ILC, invasive ductal carcinomas (IDC)) and subjected to microdissection and DNA/RNA extraction. Matched germline DNA was available for all cases. Whole exome sequencing was performed on a HiSeq2000 and data were aligned to the reference human genome and processed using GATK. Single nucleotide variants (SNVs) and small insertions/deletions were identified using MuTect and Varscan, respectively. Purity and ploidy estimates were calculated using ABSOLUTE. Clonal frequencies were estimated using Pyclone and the clonal structure of each sample was reconstructed using SubcloneSeeker. Shannon index and Simpson index metrics were used to calculate heterogeneity levels. Mutational signatures were defined according to their mutational trinucleotide context, and the expression levels of APOBEC gene family members were assessed by quantitative reverse transcription (qRT)-PCR.

Results: 30 LCIS, 10 ILCs, 7 DCIS and 5 IDCs from 15 patients qualified for data analysis. CDH1 was the most frequently mutated gene and found to be targeted by mutations in 26 LCIS samples (23 somatic, 3 germline). The repertoire of somatic mutations in LCIS was similar to that of luminal A breast cancers, with the exception of the significantly higher frequency of CDH1 mutations and the lower prevalence of TP53 mutations. ILCs were clonally related to at least one LCIS in 10 patients, and in 3/7 patients, DCIS was clonally related to at least one LCIS. Clonal composition analysis revealed that the presence of a minor clone(s) in LCIS, and the levels of intra-tumor genetic heterogeneity were significantly higher in LCIS clonally related with DCIS/ILC than in LCIS unrelated to DCIS/ILC. In two cases, a minor LCIS subclone constituted the major clone in the associated DCIS/ILC. A comparative analysis of the mutational signatures in the truncal and branch mutations of these cases revealed that whilst the truncal mutations displayed an aging signature, branch mutations were enriched for the APOBEC signature. qRT-PCR analysis demonstrated that cases displaying the APOBEC signature also harbored significantly higher levels of APOBEC3B expression than samples with the aging signature.

Conclusions: LCIS displays intra-lesion genetic heterogeneity, and the progression from LCIS to DCIS or ILC may involve the selection of clones resulting from distinct mutational processes during clonal evolution. Our findings also suggest that cytidine deamination driven by the overexpression of APOBEC3B may drive the progression of LCIS to DCIS/ILC in a subset of cases.
**Title:** Interrogating the landscape of long noncoding RNAs in breast cancer to identify predictors of tamoxifen resistance


**Body:**

*Background:* We previously performed an informatics-based analysis on RNA sequencing libraries from 7,256 tumor and normal tissue specimens to delineate the landscape of long noncoding RNAs (lncRNAs) in the human transcriptome. This analysis identified 58,648 lncRNAs, including over 45,000 novel transcripts (Iyer MK et al, *Nature Genetics*, 2015). We now interrogate this lncRNA compendium to identify top candidate estrogen receptor (ER)-associated lncRNAs in breast cancer and characterize their association with disease progression.

*Methods:* To prioritize differentially expressed lncRNAs in cancer vs normal tissue, and in ER+ vs ER- disease, we performed Sample Set Enrichment Analysis (SSEA) on >1000 RNA Seq libraries, from breast cancer and normal tissue samples from The Cancer Genome Atlas project. The effect of the top prioritized lncRNA on cancer phenotypes was studied via in vitro proliferation, colony formation, invasion and tamoxifen resistance assays in MCF7 and T47D cells, and via in vivo mouse xenograft studies and chick chorioallantoic membrane (CAM) assays. To study the mechanism by which this lncRNA promotes tumor progression, we identified its top protein interactors and subdomains responsible for function, and then studied the effects of disrupting function of this lncRNA on cancer phenotypes. Finally, in a "guilt-by-association" study, we developed a signature of 150 protein coding genes most strongly associated with our lncRNA of interest, and investigated the association of this signature with clinical outcomes using Oncomine analyses.

*Results:* SSEA analysis on over 1000 TCGA samples nominated Breast Cancer Associated Transcript (BRCAT 431) as the top overexpressed ER-regulated lncRNA in breast cancer. *In vitro* experiments demonstrate that siRNA-mediated knockdown of BRCAT431 resulted in significantly decreased proliferation, colony formation, and invasion (by >50% in most assays). Tamoxifen resistance was associated with significantly increased BRCAT431 levels in both MCF7 and T47D cells, and knockdown of BRCAT431 reversed tamoxifen resistance. *In vivo* xenograft and CAM studies demonstrate that knockdown of BRCAT431 also significantly decreased xenograft growth and tumor invasion by >50%. RNA pulldown followed by mass spectrometry identified the RNA binding protein hnRNPL as a key protein interacting with BRCAT431. Deletion studies identified a 27 base region of BRCAT431 necessary for its interaction with hnRNPL, and loss of this region abrogated BRCAT431- induced invasion. Finally, guilt-by-association studies demonstrate a strong association between BRCAT431 overexpression and tumor grade, recurrence, and metastases.

*Conclusion:* In this study, we develop the largest reported compendia of breast cancer lncRNAs. We prioritize BRCAT431 as the top lncRNA upregulated in ER-positive breast cancers, and demonstrate that it confers aggressive oncogenic phenotypes *in vitro* and *in vivo*. We identify a novel mechanism by which this lncRNA functions. Our results suggest that by promoting tamoxifen resistance, BRCAT431 increases the clinical risk of recurrence and metastases in breast cancer. Overall, this study supports the rationale for investigating lncRNAs as novel biomarkers and therapeutic targets in breast cancer.
**Title:** HER2 status as predictive marker for AI vs Tam benefit: A TRANS-AIOG meta-analysis of 12129 patients from ATAC, BIG 1-98 and TEAM with centrally determined HER2

Bartlett JMS MS, Ahmed I, Regan MM M, Sestak I, Mallon EA A, Dell’Orto P, Thürlimann BJK J.K., Seynaeve C, Putter H, Brookes CL L, Forbes JF F, Colleoni MA A, Bayani J, van de Velde CJH J.H., Viale G, Cuzick J, Dowsett M, Rea DW W and On Behalf of the Translational Aromatase Inhibitor Overview Group (Trans-AIOG). Ontario Institute for Cancer Research, Toronto, ON, Canada; University of Birmingham, Birmingham, United Kingdom; Dana-Farber Cancer Institute, Boston, MA; Queen Mary, University of London, London, United Kingdom; Western Infirmary, Glasgow, United Kingdom; Breast Center, Kantonsspital St. Gallen, St. Gallen, Switzerland; Erasmus Medical Center Cancer Institute, Rotterdam, Netherlands; Leiden University Medical Center, Leiden, Netherlands; The University of Newcastle, Newcastle, New South Wales, Australia; University of Milan, Milan, Italy; Royal Marsden Hospital, London, United Kingdom and European Institute of Oncology, Milan, Italy.

**Body:** There is now significant evidence emerging from the pivotal trials of AIs versus Tamoxifen (AIOG) demonstrating the value of meta-analysis of key clinical questions. The "Trans-AIOG" group has been tasked with the exploration of key molecular/biomarker questions that are pertinent to meta-analyses of biomarkers (past/present/future) in AIOG trials. HER2 has been long proposed as a marker of endocrine "resistance". Data from three trials, before the era of HER2-directed therapy, suggest a potential role for HER2 to select patients for treatment with upfront AIs. However the individual trials lack power to test treatment-by-HER2 interaction due to sample size and low HER2+ve rates. A meta-analysis of the predictive value of HER2 status, specifically within the first 3 years of endocrine therapy, has the potential to inform patient selection for upfront or sequential strategies with AIs. The pre-existing standardization of methodology for HER2 (IHC/FISH) facilitates analysis of existing data from BIG-1-98, TEAM and ATAC for this key marker.

**Analysis plan:** Following a prospectively-designed analysis plan, patient-level data from 3 randomized phase III trials (ATAC, BIG 1-98, TEAM) comparing AIs to tamoxifen during the first 2-3 years of adjuvant treatment were collected at the CRCTU (Birmingham UK), accounting for both the established time-dependency of relapse in HER2+ve, anti-endocrine treated patients and to address the clinical question of "upfront" vs "sequential" strategies for AIs. For each trial, covariate-adjusted Cox models estimated HER2-by-treatment (Al vs Tam) interaction on distant recurrence-free interval-censored at 2-2.75 years follow-up. A meta-analysis of the HER2-by-treatment interaction terms and of treatment effects according to HER2 status was performed. Results: 12129 patients with centrally-confirmed ER and HER2 status, 1092 (9%) HER2+ve, with 473 (4%; 111 among HER2+ve) distant recurrences were analyzed. The meta-analysis estimated a pooled HER2-by-treatment interaction of 1.61 (95% CI 1.01,2.57), reflecting treatment effect hazard ratio(Al/Tam) of HR=1.13 (0.75,1.71) among HER2+ve and HR=0.70 (0.56,0.87) among HER2-ve. There was heterogeneity among interaction terms (I-squared=59%, p=.09) that resulted from treatment effect heterogeneity among HER2+ve subgroup (I2=71%, p=.03), not the HER2-ve subgroup (I2=0%). The results for disease-free survival were similar.

**Conclusion:** An individual patient data meta-analysis across 3 trials (ATAC, BIG 1-98, TEAM) conducted prior to standard use of HER2-directed adjuvant therapy demonstrated a marginally-significant interaction between HER2 status and treatment with AIs vs Tamoxifen in the 2-2.75 years prior to potential "switching" between Tamoxifen and AIs. Patients with HER2-ve cancers experienced improved outcomes when treated with AIs vs Tamoxifen whilst patients with HER+ve cancers fared no better, or slightly worse, during AI treatment. However, the small number of HER2+ve cancers and events even in this meta-analysis may explain a large degree of heterogeneity in the treatment effects within the HER2+ve subgroups across the 3 trials. Other causes, perhaps related to subtle differences between AIs, cannot be excluded.
Title: Tamoxifen resistance driven by the DNA cytosine deaminase APOBEC3B in recurrent estrogen receptor positive breast cancer


WITHheld pending press conference
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-01

Title: Endocrine therapy alone as primary treatment for elderly patients with clinical stage I to III estrogen receptor positive breast cancer with low recurrence score

Aft R, Trinkaus K and Ma C. Washington University, St. Louis, MO.

**Body: Background:** Approximately 20% of breast cancers are diagnosed in women 75 years of age or older. Elderly patients with breast cancer are at a higher risk for over- or under-treatment. In general, older patients are more likely to die from comorbidities than from their breast cancer. The 21-gene Recurrence Score (RS) predicts 10-year distant recurrence rate in the adjuvant setting in women with early stage ER+ breast cancer. In elderly patients, a large percentage of the cancers diagnosed are ER+. It is likely that a subpopulation of elderly women with good prognosis ER+ tumors can be identified who may benefit from endocrine therapy alone, without surgery, to treat their cancer without compromising clinical outcome. We hypothesize that endocrine therapy alone will provide adequate local and systemic control of breast cancer in the subpopulation of women 75 or older with ER+ breast cancer and low RS.

**Trial Design:** This is a single arm phase II trial in pts with newly diagnosed early stage ER+ breast cancer, age 75 or greater. RS will be obtained on core biopsies collected at the time of diagnosis. Patients whose scores are < 18 will be enrolled into the trial and receive endocrine therapy only. Patients whose scores are ≥ 18 will receive standard of care (SOC) treatment. Tumor assessment will occur every 6 months. Patient can continue on endocrine therapy for up to 10 years. If there is progression of disease, then an alternative endocrine agent may be prescribed or the patient can opt for SOC treatment.

**Objectives:** Primary: To determine the rate of loco-regional progression in women with early-stage ER+ breast cancer, 75 years or older with low RS who are treated with endocrine therapy alone. Secondary: To determine the breast cancer-specific survival and overall survival of women with early-stage ER+ breast cancer who are 75 years or older treated with endocrine therapy alone. To correlate response to treatment with RS.

**Eligibility:** Newly diagnosed invasive breast cancer defined as cT1 or T2, N0-1, M0, age 75 years of age or older and ECOG performance status ≤ 2. Disease must be ER+ (Allred score ≥ 5) and HER2- and measurable defined as lesions that can be accurately measured in at least one dimension by ultrasound or mammogram.

**Statistical Methods:** A Kaplan-Meier model will be used to estimate the 5-year local or systemic progression rate with a Brookmeyer-Crowley confidence interval. If the true 5-year progression rate is 10%, a sample size of 50 will provide power = .90 at a one-sided .05 significance level to demonstrate that the rate is less than 25.5%. A point estimate of the cumulative incidence of local progression at 5 years will be calculated using a Kaplan-Meier model or, if covariate adjustment is desired, a Cox proportional hazards model. The upper bound of a 95% confidence interval will be used to determine whether the rate may plausibly fall above 25.5%.

**Accrual:** Target 50 patients with an RS score < 18 will be enrolled. Anticipated opening is July 2015.
Title: Phase III study of ribociclib (LEE011) in combination with fulvestrant for the treatment of postmenopausal patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2–) advanced breast cancer (aBC) who have received no or only one line of prior endocrine treatment: MONALEESA-3

Fasching PA A, Jerusalem G, Pivot X, Martin M, De Laurentiis M, Blackwell K, Esteva FJ J, Paquet-Luzy T, Tang Z, Lorenc KR R and Slamon DJ J.  Universitätsklinikum Erlangen, Erlangen, Germany;  Centre Hospitalier Universitaire du Sart Tilman Liège and Liège University, Liège, Belgium;  CHRU Besançon – IRFC, Besançon, France;  Hospital General Universitario Gregorio Marañón, Madrid, Spain;  National Cancer Institute Fondazione Pascale, Naples, Italy;  Duke University Medical Center, Durham, NC;  Laura and Isaac Perlmutter Cancer Center, NYU Langone Medical Center, NY, NY;  Novartis Pharma AG, Basel, Switzerland;  Novartis Pharmaceuticals Corporation, East Hanover, NJ and  UCLA Medical Center, Santa Monica, CA.

Body: Background: Endocrine therapy (ET) is the predominant first-line treatment for HR+, HER2– aBC; however, resistance eventually occurs in a large number of cases. Dysregulation of the cyclin D–cyclin dependent kinase (CDK)4/6–inhibitor of CDK4 (INK4)–retinoblastoma (Rb) pathway has been associated with endocrine resistance. Inhibition of CDK4/6 may overcome such resistance and enhance the efficacy of existing endocrine regimens. Combination of ribociclib (a type of CDK4/6 inhibitor) with fulvestrant (a selective estrogen receptor downregulator) has demonstrated potent tumor regressions in preclinical HR+ breast cancer (BC) models.

Design & Objectives: MONALEESA-3 (NCT02422615) is a randomized, double-blind, placebo-controlled study of oral ribociclib (600 mg QD on Days 1–21 of each 28-day cycle) in combination with fulvestrant (500 mg intramuscularly on Days 1 and 15 of Cycle 1 and Day 1 of each cycle thereafter) in postmenopausal pts with HR+, HER2– aBC. Pts may have newly diagnosed aBC that is treatment-naive, relapsed BC that has progressed at any time during or after (neo)adjuvant ET with no treatment for metastatic disease, relapsed BC that has progressed >12 months after adjuvant ET and then subsequently progressed after one line of ET for metastatic disease (with either an antiestrogen or an aromatase inhibitor), or newly diagnosed aBC that has progressed after one line of ET (with either an antiestrogen or an aromatase inhibitor). Pts must have measurable disease or at least one predominantly lytic bone lesion, and an Eastern Cooperative Oncology Group performance status (ECOG PS) of ≤1. Prior treatment with chemotherapy (except for [neo]adjuvant chemotherapy), fulvestrant, or any CDK4/6 inhibitor is prohibited. Randomization (2:1) to receive ribociclib plus fulvestrant (Arm A) or placebo plus fulvestrant (Arm B) is stratified by visceral disease status and previous ET. Tumor assessments will be performed every 8 weeks for the first 18 months and every 12 weeks thereafter until disease progression, death, withdrawal of consent, loss to follow-up, or patient/guardian decision. The primary endpoint is progression-free survival (PFS; local, RECIST 1.1); secondary endpoints include overall survival, PFS (central, RECIST 1.1), overall response rate, clinical benefit rate, time to response, duration of response, safety and tolerability, ECOG PS, pt-reported outcomes, and pharmacokinetics. Molecular alterations of genes associated with HR+ BC and the cyclin D–CDK4/6–INK4–Rb pathway will also be explored in tumor and blood samples.

Statistical Methods: PFS between Arms A and B will be compared using a stratified log-rank test; 364 PFS events will be required to detect a hazard ratio of 0.67 with 90% power using the log-rank test and a 3-look (interim look futility only, interim look efficacy only, then final analysis) group sequential design at one-sided cumulative 2.5% level of significance.

Target Accrual: Approximately 660 pts at 300 sites worldwide.
Title: A phase 1/2 study of once-daily oral VT-464 in patients with advanced androgen receptor (AR) positive triple negative (TNBC) or estrogen receptor (ER) positive breast cancer (BC)


Body: VT-464, an oral dual lyase-selective CYP17 inhibitor and AR antagonist (wild-type and mutated forms [e.g., F876L and T877A]), is in multiple Phase (Ph) 2 studies as treatment for men with castration-resistant prostate cancer (CRPC). VT-464 inhibits the growth of multiple BC cell lines in vitro including MCF7 (ER+/AR low), tamoxifen-resistant MCF7, and MDA-MB-453 (ER-/AR+) in a dose-dependent manner and with greater potency/efficacy than enzalutamide (submitted, Ellison et al., 2015). A subset of TNBC and most ER+ BC express AR, making them potential targets for VT-464 since it directly inhibits both androgen/estrogen synthesis and AR transcriptional activity.

Objectives: The primary objective of Ph 1, now enrolling, is to establish the once-daily dose of VT-464 in women. Secondary objectives include safety, PK and efficacy endpoints, including determination of clinical benefit rate (CBR) which is the primary objective of Ph 2. Exploratory objectives include the determination of the extent of AR expression and signaling in breast tissue and to evaluate the relationship of expression with VT-464 effects on circulating tumor biomarkers, circulating hormones and clinical outcomes.

Study Design: This study is an open-label, single arm, Ph 1/2 study of VT-464 in women with AR+ TNBC or ER+/HER2 normal unresectable locally advanced or metastatic BC. Ph 1 will follow a modified 3+3 Fibonacci design with cohort expansion to 6 patients following a single DLT in the first 28-days of treatment. Approximately 2-3 dose-levels will be explored in Ph 1. Ph 1 start dose will be the MTD for men with CRPC. AR+ TNBC and ER+/HER2 BC cohorts will be expanded in Ph 2 using the MTD from Ph 1. Ph 2 will follow a Simon’s two-stage design with pre-determined futility parameters. Eligible patients will have ER≥1% BC or AR≥1% (as determined by central IHC testing using the Dako antibody) TNBC. ER+ patients must be postmenopausal and must have received at least 1 prior line of endocrine therapy. Additional eligibility criteria include: ≥ 18 years of age, ECOG PS ≤ 1, unresectable locally advanced or metastatic BC, available representative tumor specimen to enable correlative science.

Treatment Plan: Eligible patients will receive VT-464 once-nightly with dinner in a continuous dosing schedule. Adverse events and concomitant medications will be collected from the time of signing of informed consent until 30 days after end of study visit (EOS). Safety labs will be monitored monthly through EOS. Dense PK will be collected after the first dose of study drug in Ph 1 and single morning samples collected approximately every two cycles thereafter in Ph 1 and Ph 2 until EOS. Blood samples for steroid, circulating tumor DNA and circulating tumor cells will be collected through Cycle 2 and then at EOS. Tumor biopsy will be collected at baseline and at disease progression. Radiographic response will be assessed every 8 weeks and EOS.

Patient Accrual: Accrual is ongoing with 12-18 patients expected to be enrolled in Ph 1.
E2112: Randomized phase III trial of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer. A trial of the ECOG-ACRIN cancer research group

Connolly R, Zhao F, Miller K, Tevaarwerk A, Wagner L, Lee M, Murray J, Gray R, Piekarz R, Zujewski JA and Sparano J. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; Dana-Farber Cancer Institute, Boston, MA; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; University of Wisconsin Carbone Cancer Center, Madison, WI; Wake Forest University Health Services, Winston-Salem, NC; National Cancer Institute, Bethesda, MD; Cancer Therapy Evaluation Program (CTEP) National Cancer Institute, Bethesda, MD and Albert Einstein College of Medicine, Montefiore Medical Center, NY, NY.

Background: A potential mechanism of resistance to endocrine therapy in breast cancer involves changes in gene expression secondary to epigenetic modifications, which might be modulated with the use of histone deacetylase (HDAC) inhibitors such as entinostat. ENCORE 301, a phase II study evaluating the addition of entinostat to the steroidal aromatase inhibitor (AI) exemestane in patients with hormone receptor (HR)-positive advanced breast cancer who had experienced disease progression after a non-steroidal AI (NSAI), showed a significant improvement in progression-free survival (PFS), and overall survival (OS). Entinostat has been designated a Breakthrough Therapy by the FDA.

Methods: E2112 is a multicenter randomized double-blind placebo-controlled phase III study (NCT02115282) enrolling patients with advanced HR-positive, HER2-negative breast cancer with prior disease progression on a NSAI (n=600). Patients receive exemestane 25mg po daily and entinostat/placebo 5mg po every week. Eligibility: Postmenopausal women and men, ECOG 0-1, locally advanced/metastatic invasive adenocarcinoma of the breast: ER/PR-positive, HER2-negative, measurable or non-measurable (20% cap) disease. Disease progression after NSAI use in the metastatic setting OR relapse while on or within ≤ 12 months of end of adjuvant NSAI therapy.

Statistics: Both PFS (central review) and OS are primary endpoints, and the study is designed to show an improvement in either PFS or OS. Secondary endpoints include: Safety and tolerability, objective response rate, changes in lysine acetylation status in peripheral blood mononuclear cells, patient-reported symptom burden and treatment toxicities, adherence. One-sided type 1 error 0.025 split between two hypotheses tests: 0.001 for PFS test and 0.024 for OS. PFS is tested in the first 360 pts, 88.5% power to detect 42% reduction in the hazard of PFS failure (median PFS 4.1 to 7.1 months); OS is tested in all 600 pts, 80% power to detect 25% reduction in the hazard of death (median OS 22 to 29.3 months).

E2112 was activated in March 2014 and accrual is anticipated to complete in 40 months.
**Title:** A phase 2 randomized, double-blind, placebo-controlled trial of hormone therapy ± radium-223 dichloride in human epidermal growth factor receptor 2–negative, hormone receptor–positive breast cancer patients with bone metastases

Coleman RE E, Huang L, Petrenciuc O, Zaccarini P and Rugo HS S. University of Sheffield, Weston Park Hospital, Sheffield, United Kingdom; Bayer HealthCare, Whippany, NJ; Bayer S.p.A., Milan, Italy and UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

**Body:**
**Background:** Treatment options for bone-metastatic breast cancer (MBC) are limited. Multimodality therapy may improve symptom control and survival. In a phase 2a study of advanced breast cancer patients with bone-dominant and no visceral disease, radium-223 dichloride (radium-223), a first-in-class α-emitter selectively targeting bone metastases, reduced baseline bone biomarker levels with favorable safety (Coleman et al. *Breast Cancer Res Treat* 2014). This study (NCT02258464) evaluates efficacy and safety of radium-223 versus placebo in human epidermal growth factor receptor 2–negative (HER2–), hormone receptor–positive (HR+) bone-MBC patients receiving single-agent hormone therapy.

**Trial design:** Patients receive (1:1) radium-223 50 kBq/kg IV or placebo q 4 wk (6 cycles) + concurrent single-agent hormone therapy + best supportive care. Stratification is by geographic region, prior lines of hormone therapy for MBC, and number of prior skeletal events.

**Main eligibility criteria:** Eligible patients are pre- or postmenopausal with estrogen receptor–positive, HER2–, bone-dominant MBC with ≥ 2 bone metastases and ≥ 1 or 2 prior symptomatic skeletal events (external beam radiotherapy for bone pain, pathologic bone fracture, spinal cord compression, orthopedic surgery). Patients had ≥ 1 line of hormone therapy for MBC; are taking bisphosphonates or denosumab for ≥ 1 month before study; are eligible for endocrine treatment; and have evaluable disease (RECIST v1.1), asymptomatic or mildly symptomatic bone disease (Brief Pain Inventory), ECOG performance status 0-1, and adequate hematologic, renal, and liver function. Patients may not have had visceral or brain metastases or leptomeningeal disease, need for chemotherapy for metastases, and untreated spinal cord compression.

**Specific aims:** Patients are assessed for efficacy and safety, and followed to symptomatic skeletal events, radiologic progression, death, or withdrawal. Primary endpoint is symptomatic skeletal event–free survival.

**Statistical methods:** Assuming a 1-sided α of 0.1, power of 90%, ~ 119 symptomatic skeletal events are required for the analysis. Time-to-event variables will be analyzed using a log-rank test, accounting for stratification. Kaplan-Meier estimates and survival curves will be given for each treatment group. Safety analyses will be descriptive.

**Present and target accruals:** As of May 2015, 5 patients have been screened and 3 enrolled. Target enrollment is 227 patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-06

Title: Phase 2 open label, multinational, randomized, parallel design study investigating the efficacy and safety of GTx-024 on metastatic (MET) or locally advanced (LA) ER+/AR+ breast cancer (BC) in postmenopausal (PM) women

Overmoyer B, Rugo H, Schwartzberg L, Palmieri C, Taylor R, Hancock M, Small S and Johnston MA. Dana Farber Cancer Institute, Inflammatory Breast Cancer Program, Boston, MA; University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; The West Clinic, Memphis, TN; University of Liverpool, Liverpool, Merseyside, United Kingdom and GTx Inc., Memphis, TN.

Body: Background: Historically, androgens have been utilized for the treatment of BC as the androgen receptor (AR) is the most highly expressed steroid receptor (~95% in estrogen receptor positive (ER+), ~50% in ER negative). However, steroidal androgens often exhibit virilizing side effects, thus limiting clinical use. A non-steroidal, tissue-selective, AR modulator (SARM), GTx-024, offers a targeted approach of AR activation in ER+/AR+ BC without virilization or estrogenic effects. A previous Phase 2 study of 9 mg GTx-024 in MET ER+ BC demonstrated proof-of-concept, with 6/17 ER+/AR+ patients (pts) exhibiting clinical benefit (CB) following 6 months (m) of treatment. Increasing the dose to 18 mg has the potential for greater efficacy without compromising safety.

Trial Design: Open label, multinational, randomized, parallel design Phase 2 study to assess the efficacy and safety of GTx-024 in PM ER+/AR+ BC. Pts will be randomized to receive GTx-024, 9 mg or 18 mg orally (PO) daily. Therapy continues until disease progression. Pts achieving a CB can be treated for 12 m following the initiation of study treatment; those demonstrating continued CB are offered continuation in a safety extension study under a separate protocol.

Eligibility Criteria:
Inclusion: Informed consent, female, ≥18 years (yr), PM, MET or LA ER+ (≥1% staining) BC, HER2 negative, ≥1 prior hormonal treatment for BC (≥6 m response for MET; ≥3 yr response for adjuvant), provide archived tumor tissue for AR determination, measurable or bone-only disease, evidence of PD within 30 days (d), ECOG 0 or 1.
Exclusion: >1 prior chemotherapy regimen for MET, uncontrolled CNS metastases, radiotherapy ≤14d prior to enrollment, major surgery ≤28d prior to enrollment, currently receiving hormone replacement, hepatitis B/C positive, HIV positive, another active cancer.

Specific Aims: Primary endpoint: proportion of AR+ pts in each arm achieving a CB response (CBR) at 24 weeks (wks). CBR defined as pts with a complete response (CR), partial response (PR), or stable disease (SD); per modified RECIST 1.1. Secondary endpoints: objective response rate, progression free survival, time to progression, and duration of response. Statistical Methods: Simon's two-stage (optimal) design will be used to assess primary efficacy, requiring up to 88 evaluable pts; i.e., pts with centrally confirmed AR+ who receive at least one dose of study drug. The trial will test for an unacceptably low CBR of ≤10% versus a CBR ≥30%. There is no intent to statistically compare the two dose arms, but to determine whether either or both doses result in an acceptable CBR.

Target Accrual: Up to 118 pts will be enrolled. The first stage will be assessed in each arm among the first 18 evaluable pts. If at least 3/18 exhibit CB at 24 wks, then the arm will proceed to the second stage of recruitment up to a total of 44 pts. Otherwise, the arm will be discontinued for lack of efficacy.

Trial Information: www.gtxinc.com
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-07

Title: A phase 2 open label, multi-center, multinational study investigating the efficacy and safety of GTx-024 on advanced, androgen receptor-positive triple negative breast cancer (AR+ TNBC)

Rugo H, Overmoyer B, Schwartzberg L, Palmieri C, Taylor R, Hancock M, Small S and Johnston MA. University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Dana Farber Cancer Institute, Inflammatory Breast Cancer Program, Boston, MA; The West Clinic, Memphis, TN; University of Liverpool, Liverpool, Merceyside, United Kingdom and GTx, Memphis, TN.

Body: Background: The androgen receptor (AR) is the most highly expressed steroid receptor in breast cancer (BC) with expression seen in up to 95% of estrogen receptor positive (ER+) and up to 50% of ER negative disease. Historically, steroidal androgens exhibited virilizing side effects, thus limiting clinical use. In TNBC, the expression of AR and androgen synthesizing enzymes is associated with lower proliferation, lower tumor grade, better overall survival, and more favorable clinical outcomes as compared to those patients with TNBC not expressing AR. Data from two trials targeting AR in TNBC indicates low level but encouraging clinical activity. A non-steroidal, tissue-selective, AR modulator (SARM), such as GTx-024, offers a targeted approach of AR activation in AR+ TNBC without virilization or estrogenic effects.

Trial Design: Open label, multicenter, multinational, Phase 2 study for the treatment of advanced or metastatic TNBC. Subjects will receive GTx-024, 18 mg orally (PO) daily, continued until evidence of disease progression or toxicity. Subjects whose tumors demonstrate Clinical Benefit (CB) will be treated on study drug until progression.

Eligibility Criteria:
Inclusion: Female, ≥18 years old, confirmed AR+ (≥10% staining) TNBC (confirmed by medical history), up to 1-2 prior chemotherapy regimens, available archived tumor tissue, measurable or bone-only disease, ECOG 0 or 1, with prior toxicities from chemotherapy resolved.

Exclusion: Life expectancy <4 months, uncontrolled CNS metastases, radiotherapy ≤14 days prior to enrollment, active hepatitis, HIV positive, prior treatment with anti-androgens, testosterone or testosterone-like agents, or estrogens or megesterol acetate, or prior treatment for a different cancer (other than BC or non-melanoma carcinoma of the skin) within the past 2 year

Specific Aims: The primary aim is to measure the proportion of AR+ subjects with CB at 16 weeks; defined as subjects with a complete response (CR), partial response (PR), or stable disease (SD); per modified RECIST 1.1. Secondary endpoints include: objective response rate, progression free survival, and time to progression.

Statistical Methods: Simon's two-stage (optimal) design will be used to assess primary efficacy, requiring up to 41 evaluable subjects; i.e., subjects with centrally confirmed AR+ who receive at least one dose of study drug. The first stage will be assessed among the first 21 evaluable subjects. If at least 2/21 achieve CB per modified RECIST 1.1 at 16 weeks, then the trial will proceed to the second stage of recruitment of up to a total of 41 subjects in the evaluable subset of the Full Analysis Set. Otherwise, the trial will be discontinued for lack of efficacy. The trial will test for an unacceptably low CBR of ≤5% versus a CBR more consistent with ≥20%.

Target Accrual: Up to 55 subjects will be enrolled.

Trial Information: www.gtxinc.com
POSITIVE: A study evaluating pregnancy and disease outcome and safety of interrupting endocrine therapy for young women with endocrine responsive breast cancer who desire pregnancy (IBCSG 48-14/BIG 8-13)

Pagani O, Partridge A, Azim Jr HA A, Peccatori FA A, Ruggeri M and Sun Z. International Breast Cancer Study Group, Bern, Switzerland and Dana-Farber Cancer Institute and Alliance for Clinical Trials in Oncology, Boston, MA.

Body: Background
Young breast cancer (BC) patients often face the disease before completing their family planning. The best available retrospective evidence suggests that pregnancy after BC does not negatively impact disease outcome in patients with endocrine sensitive BC and is safe for the offspring. However, given the need for prolonged adjuvant endocrine therapy for 5-10 years, it is not feasible to wait until completion of therapy in most of these women and thus there is a need to explore the safety of temporary interruption of endocrine therapy to allow pregnancy. To date, no definitive prospective study has been conducted in young women desiring future pregnancy.

Trial Design
Young patients with endocrine responsive early BC and pregnancy desire will interrupt endocrine treatment for up to 2 yrs to attempt pregnancy. As resumption of menses and conception depends on many factors, e.g. patient's age and adjuvant treatment received, the 2-yr interruption period is approximate, intended to include treatment wash-out (3 mos) conception (∼3-6 mos), delivery (∼9 mos), breast feeding (∼6 mos). Patients will be strongly advised to resume ET as soon as pregnancy attempts are concluded, and to complete 5-10 yrs ET at the local investigator discretion.

Major Eligibility Criteria
- Histologically-proven stage I-III endocrine-responsive BC.
- Age ≥ 18 and ≤ 42 years at enrollment.
- Adjuvant endocrine therapy (SERM alone, GnRH analogue plus SERM or AI) for ≥18 months but ≤30 months, stopped within 1 month prior to enrollment.
- Patient wishes to become pregnant.
- Premenopausal status at BC diagnosis.

Specific Aim
To assess the risk of BC relapse associated with temporary interruption of ET to permit pregnancy and to evaluate pregnancy success.

Statistical Methods
A true risk of BC recurrence of 2% per year is assumed for patients who do not interrupt endocrine treatment. With 500 patients enrolled in 4.0 yrs and an additional 1.6 yrs of follow up, there will be approximately 1600 patient-yrs of follow up and a median follow up of approximately 3 yrs at the time of the primary analysis, anticipated to occur 5.6 yrs after enrollment of the first patient. If the true risk of BC recurrence is 2% per yr, we anticipate 31 BC recurrences and an estimated 3-yr breast cancer free interval (BCFI) failure of 5.6% (95% CI 4.0% to 7.9%).

Translational Research will investigate different ovarian function parameters; uterine evaluation; and circulating tumor DNA. FFPE tissue of the primary tumor will be collected to integrate different parameters related to biology of BC arising in young women. All material will be banked centrally.

Psycho-oncological Companion Study on fertility concerns, psychological well-being and decisional conflicts is mandatory in the United States and open to interested centers elsewhere.

Accrual: Target: 500; Actual: 4 (31 May 2015)

Contact Information
POSITIVE is conducted and sponsored by the International Breast Cancer Study Group. Alliance for Clinical Trials in Oncology is US sponsor for NCTN network. Contact Trial Coordinators at ibcsg48_positive@fstrf.org.
Title: Randomized phase II trial of CC-486, a DNA methyltransferase inhibitor, in combination with fulvestrant in postmenopausal women with hormone receptor–positive, human epidermal growth factor receptor 2–negative metastatic breast cancer who have progressed on an aromatase inhibitor

Gianni L, Rugo H, Campone M, Hudson M, Chen P, Fandi A and Sachdev JC C. San Raffaele Hospital, Scientific Institute, Milan, Italy; UCSF Comprehensive Cancer Center, San Francisco, CA; Centre Rene Gauducheau, CLCC Nantes Atlantique, Nantes-Saint Herblain, France; Celgene Corporation, Summit, NJ; Celgene Corporation, Summit, NJ; Celgene Corporation, Summit, NJ and Virginia G. Piper Cancer Center, Scottsdale Healthcare, Scottsdale, AR.

Body: Background: Breast cancer is the most common cancer among women in developed countries. The hormone receptor–positive (HR+)/human epidermal growth factor receptor 2–negative (HER2−) subtype comprises approximately 70% of all breast cancers. For patients with HR+ metastatic breast cancer (MBC), sequential endocrine therapies are recommended. Fulvestrant is a selective ER modulator. It is approved in the treatment of HR+ MBC in postmenopausal women with disease progression following antiestrogen therapy. Development of resistance to endocrine therapy poses a major problem in the use of these agents. CC-486 is an orally bioavailable formulation of 5-azacitidine, a cytidine nucleoside analogue that incorporates into DNA and RNA. CC-486 allows for extended dosing and prolonged drug exposure, which may provide the opportunity for incorporation into cycling malignant cells. Epigenetic modifying agents like CC-486 are hypothesized to potentially reverse resistance to hormonal therapy.

Trial Design: This is a phase II, randomized, open-label, 2-arm study. Approximately 92 patients will be enrolled in North America and Europe. Key eligibility criteria include age ≥ 18 years, postmenopausal, metastatic disease, histologically and/or cytologically confirmed estrogen receptor positive (ER+), HER2− (immunohistochemistry [IHC] 0 or 1+ or fluorescence in situ hybridization [FISH], or IHC 2+ and FISH−), refractory to treatment with an aromatase inhibitor (AI) defined as recurrence on or ≤ 12 months after prior AI treatment in the adjuvant setting or disease progression on or ≤ 1 months of prior treatment with an AI in the metastatic setting, and Eastern Cooperative Oncology Group performance status 0 - 1. Measurable disease by Response Evaluation Criteria in Solid Tumors v1.1 is required. Key exclusion criteria include history of or current symptomatic brain metastases, > 1 line of chemotherapy for MBC, prior fulvestrant, or any prior hypomethylating agent. Patients with visceral crisis requiring chemotherapy are also excluded. Patients will be randomized 1:1 to receive CC-486 300 mg on days 1 - 21 of each 28-day cycle plus fulvestrant 500 mg on days 1 and 15 in the first cycle and day 1 in cycles ≥ 2 or fulvestrant 500 mg on days 1 and 15 in the first cycle and day 1 in cycles ≥ 2. The primary endpoint is progression-free survival (PFS). Secondary endpoints include overall response rate, clinical benefit rate, overall survival, duration of response, and safety. Exploratory endpoints include molecular profiling of the tumor for evaluation of predictors of clinical outcome in archival tumor samples, whole blood, and plasma samples. Efficacy will be evaluated every 8 weeks during the first 24 weeks and then every 12 weeks until disease progression, unacceptable toxicity, death, or new therapy. The primary efficacy analysis will be performed after 70 PFS events are documented. The first safety analysis will be conducted after 32 patients have completed ≥ 1 cycle of treatment. The trial is open for enrollment. ClinicalTrials.gov: NCT02374099.
Title: A phase 1 study of RAD1901, a novel, orally available, selective estrogen receptor degrader, for the treatment of ER positive advanced breast cancer


Body: The current NCCN treatment guidelines for ER+ breast cancer involves the use of approved agents such as fulvestrant, tamoxifen and aromatase inhibitors that either inhibit estrogen production or block estrogen receptor binding. While the initial treatment regimens with these selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) is often successful, many women eventually relapse with more aggressive forms of endocrine-resistant disease. To begin to overcome some of the challenges associated with current therapies including exposure limitations and intramuscular administration, we have developed RAD1901, a novel, non-steroidal, orally available SERD. Preclinical studies with RAD1901 have demonstrated potent dose dependent ER degradation consistent with a SERD mechanism of action, as well as potent inhibition of proliferation in vitro in breast cancer cell lines. RAD1901 also demonstrated significant anti-tumor efficacy in vivo, and notably single agent regressions in both MCF7 and a primary patient derived xenograft models harboring an ESR1 mutations.

A phase 1 monotherapy study conducted in healthy postmenopausal female volunteers evaluated forty four subjects treated once daily with RAD1901 with doses ranging from 200 mg/day up to 1000 mg/day for 7 days. All dose levels were generally well tolerated and pharmacokinetic analysis demonstrated plasma exposures consistent with preclinical efficacy in ER+ breast cancer models. Furthermore, 18F-estradiol positron emission tomography (FES-PET) was also performed at baseline and after 7 days of RAD1901 treatment, to evaluate estrogen receptor engagement. Standardized uptake values (SUV) pre- and post-treatment with RAD1901 demonstrated complete attenuation of FES-PET signal in ER+ tissues such as the uterus from the 200 mg/day dose level. Taken together, these results provide strong preclinical and clinical rationale for the development of RAD1901 as a potent and selective oral SERD for the treatment of hormone driven and hormone resistant ER + metastatic breast cancers.

RAD1901-005 is a Phase 1 study currently enrolling ER+ advanced metastatic breast cancer patients. The study consists of two parts: a monotherapy dose escalation followed by a safety expansion at the maximum tolerated dose (MTD). The dose escalation will follow a standard 3+3 design with once daily dosing to establish, safety, tolerability, and PK. Once the MTD for RAD1901 has been established, the safety expansion will further evaluate the safety, tolerability, biomarkers and preliminary efficacy at the recommended phase 2 dose (RP2D) following a continuous once daily schedule. Key inclusion criteria include post-menopausal women aged 18 years or older, with advanced ER positive, HER2 negative breast cancer, who have received ≤ 2 prior chemotherapy regimens in the metastatic setting and > 6 months of prior endocrine therapy. Patient enrollment started in early 2015, and is currently ongoing.

ClinicalTrials.gov identifier: NCT02338349.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-01-11

Title: A phase 2 randomized, double-blind, placebo-controlled trial of radium-223 dichloride with exemestane and everolimus in human epidermal growth factor receptor 2–negative, hormone receptor–positive breast cancer patients with bone metastases

Rugo HS S, Huang L, Petrenciuc O, Zaccarini P and Coleman RE E. UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Bayer HealthCare, Whippany, NJ; Bayer S.p.A., Milan, Italy and University of Sheffield, Weston Park Hospital, Sheffield, United Kingdom.

Body: Background: Radium-223 dichloride (Ra-223), a first-in-class α-emitter with a potent, targeted antitumor effect on bone metastases (mets), was well tolerated and reduced baseline bone biomarker levels in a phase 2 study in metastatic breast cancer (MBC) patients (pts) with bone-dominant disease (Coleman et al. Breast Cancer Res Treat 2014). Adding everolimus (EVE) to exemestane (EXE) significantly improved progression-free survival (PFS) versus EXE alone in human epidermal growth factor receptor 2–negative (HER2-), hormone receptor–positive (HR+) MBC pts with advanced disease. This trial will evaluate efficacy and safety of Ra-223 with EXE and EVE in pts with HER2-, HR+ breast cancer and bone mets (NCT02258451).

Trial design: Pts scheduled to receive EXE (25 mg PO once daily) and EVE (10 mg PO once daily) will be randomized 1:1 to Ra-223 (50 kBq/kg IV) or placebo × 6 cycles q4wk. EXE and EVE treatment (tx) will continue until disease progression, unacceptable toxicity, or the pt can no longer travel to the clinic to receive study medication. Stratification will be by geographic region, previous lines of hormone therapy, and presence of visceral disease. Safety and efficacy will be assessed at each 4-week clinic visit during tx. Long-term safety will be assessed until study termination (ie, pt death, pt loss to follow-up, or pt reaching required number of events).

Main eligibility criteria: Eligible pts are pre- or postmenopausal with estrogen receptor–positive and HER2- bone lesion–related asymptomatic or mildly symptomatic MBC not amenable to cure by surgery or radiotherapy, and with ≥2 bone mets visible on bone scan. Pts must have measurable disease per RECIST v1.1, ≥1 prior line of hormonal therapy in the metastatic setting, and 1-2 skeletal related events before study entry; be on bisphosphonates or denosumab for ≥1 mo before study entry; and have ECOG performance status of 0-1, adequate hematologic, renal, and liver function, and life expectancy ≥6 mo. Pts may not have prior or current need for chemotherapy in the metastatic setting, unresolved spinal cord compression, and prior or current EVE tx.

Specific aims: The primary endpoint is symptomatic skeletal event (SSE)–free survival. Secondary endpoints are overall survival, time to opiate use for cancer pain, time to pain progression, time to cytotoxic chemotherapy, radiologic PFS (rPFS), and acute and long-term safety. Exploratory endpoints include time to first on-study SSE, time to bone alkaline phosphatase (bALP) progression, bALP response at wk 12 and end of tx, bone-specific rPFS, resource utilization, biomarker assessments, and time to visceral mets onset.

Statistical methods: Assuming a one-sided α of 0.1, power of 90%, ∼160 SSEs will be required at the time of analysis. A stratified log-rank test will be used to analyze efficacy (intent-to-treat population). Safety analysis will be descriptive.

Present and target accrual: This trial is now enrolling pts. Target accrual is 311.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-01

Title: Comparison of axillary sentinel lymph node biopsy versus no axillary surgery in patients with early-stage invasive breast cancer and breast-conserving surgery: A randomized prospective surgical trial. The intergroup-sentinel-mamma (INSEMA)-trial

Reimer T, von Minckwitz G, Loibl S, Hildebrandt G, Denkert C, Nekljudova V, Kundt G, Becker D and Gerber B. Breast Center University of Rostock, Rostock, Germany; German Breast Group, Neu-Isenburg, Germany; Radiotherapy University of Rostock, Rostock, Germany; Institute of Pathology Charité, Berlin, Germany and Institute of Biostatistics University of Rostock, Rostock, Germany.

Body: Background:
Currently, axillary surgery for breast cancer is considered as staging procedure that does not seem to influence breast cancer mortality, since the risk of developing metastasis depends mainly on the biological behaviour of the primary. Based on this, the postsurgical therapy should be considered on the basis of biologic tumor characteristics rather than nodal involvement.

Trial design:
The goal of INSEMA is to show that early-stage breast cancer patients with reduced extent of axillary surgery are not inferior regarding invasive disease-free survival (IDFS) outcome. Patients with planned breast-conserving surgery (BCS) will be first randomized (1:4 ratio) to either no axillary surgery or axillary sentinel lymph node biopsy (SLNB). Patients with SLNB and pN+(sn) status will be secondly randomized (1:1 ratio) to either SLNB alone or completion axillary lymph node dissection (ALND) in cases with less than three involved nodes (one or two macrometastases).

Primary objective:
-IDFS after BCS (non-inferiority question)

Inclusion criteria:
-Written informed consent
-Histologically confirmed unilateral primary invasive carcinoma of the breast (core biopsy)
-Age at least 35 years
-Preoperative imaging techniques with estimated tumor size of maximal 5 cm (iT1/iT2 irrespective of hormone sensitivity or HER2 status)
-Clinically and sonographically tumor-free axilla prior to core biopsy
-In cases with cN0 and iN+, a negative core biopsy or fine needle aspiration biopsy of the suspected lymph node is required
-No clinical evidence for distant metastasis (M0)
-Planned breast-conserving surgery (R0 resection) with postoperative external whole-breast irradiation (conventional fractionation or hypofractionation)

Statistics:
Assumptions for first randomization:
-The 5-year IDFS for women with cN0/iN0 axillary lymph nodes and T1/T2 disease is considered to be 88%
-Clinical non-inferiority is defined as the non-SLNB group having a 5-year IDFS of not less than 85% and if the hazard ratio (HR) is less than 1.271 when compared with the SLNB group

The total number of patients in the per-protocol set of the first randomization must be increased from 3,796 to 5,940 (936 events) due to unequal-sample-size design.

Assumptions for second randomization:
-The 5-year IDFS for women with pN+(sn) axillary lymph nodes (1-2 macrometastases) and T1/T2 disease is considered to be 81%
-Clinical non-inferiority is defined as the SLNB alone group having a 5-year IDFS of not less than 76.5% and if the hazard ratio (HR) is less than 1.271 when compared with the completion ALND group

The total number of patients to be included into the per-protocol set for the second randomization will be approximately 1,968.

Finally, the calculated total case number for per-protocol analyses is 6,740 (5,940 German and 800 Austrian patients), the expected total number of randomized patients is 7,095.

Time lines:
-First patient in: September 2015
- Last patient in: August 2019
- Final analysis: End of 2024
Funding by Deutsche Krebshilfe (grant no. 110580).
NRG Oncology/NSABP B-51/RTOG 1304: A phase III clinical 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) will reduce invasive cancer events in patients (pts) with positive axillary (Ax) nodes who are ypN0 after neoadjuvant chemotherapy (NC)

Mamounas EP P, Bandos H, White JR R, Julian TB B, Khan AJ J, Shaitelman SF F, Torres MA A, Vicini FA A, Ganz PA A, McCloskey SA A, Paik S, Gupta N, Li XA, DiCostanzo DJ J, Costantino JP P, Curran Jr WJ J and Wolmark N. NRG Oncology/NSABP, Pittsburgh, PA; UF Health Cancer Center at Orlando Health, Orlando, FL; NRG Oncology and the University of Pittsburgh, Pittsburgh, PA; NRG Oncology/RTOG, Philadelphia, PA; Ohio State University, Columbus, OH; Allegheny Cancer Center at Allegheny General Hospital, Pittsburgh, PA; Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; University of Texas MD Anderson Cancer Center, Houston, TX; Emory Healthcare, Emory Winship Cancer Institute, Atlanta, GA; 21st Century Oncology, Pontiac, MI; University of California at Los Angeles, Los Angeles, CA; Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Korea; Medical College of Wisconsin, Milwaukee, WI and Ohio State University, Wexner Medical Center, Columbus, OH.

**Body: Background:**
This phase III post-NC trial will evaluate if CWRNRT post Mx or whole breast irradiation (WBI) with RNRT after BCS significantly reduces the invasive breast cancer recurrence-free interval (IBC-RFI) rate in pts presenting with positive Ax nodes that are negative after NC. Secondary aims are OS, LRRFI, DRFI, DFS-DCIS, and second primary cancer as well as comparing 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 loco-regional recurrence.

**Methods:**
Clinical T1-3, N1 IBC pts with positive Ax nodes (FNA or core needle biopsy) complete ≥12 wks of NC (anthracycline and/or taxane). HER2-positive pts receive anti-HER2 therapy (tx). After NC BCS or Mx is performed with a sentinel node biopsy (≥3 nodes) and/or Ax dissection with histologically negative nodes. ER/PR and HER-2 neu status before NC is required. Pts receive required systemic tx. Radiation credentialing with a facility questionnaire and a case benchmark is required. Randomization for Mx pts is to no CWRNRT or CWRNRT and for BCS pts to WBI or WBI RNRT.

**Statistics:**
1,636 pts to be enrolled over 5 yrs with 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 in the 5-yr cumulative rate of 4.6%. Intent-to-treat analysis with 3 interim analyses at 43, 86, and 129 events, with a 4th/final analysis at 172 events will occur. Accrual as of 6/4/15 is 143. Pt-reported outcomes focusing on RT effect will be provided by 736 pts before randomization and at 3, 6, 12, and 24 months.

**Contacts:**

**Support:**
U10 CA-2166; -180868, -180822; -189867; Elekta.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-02-03

Title: Magnetic nano-device for identification of the breast sentinel nodes – A novel method

Beitsch PD, Hunt KK, Bold RJ, Gittleman MA, Blair SL, Alvarado MD, and Harmer QJ. Dallas Surgical Group, Dallas, TX; MD Anderson Cancer Center, Houston, TX; University of California at Davis Medical Center, Sacramento, CA; Coordinated Health Breast Care Specialists, Allentown, PA; University of California San Diego Moores Cancer Center, La Jolla, CA; University of California San Francisco, San Francisco, CA and Endomagnetics Inc, Austin, TX.

Body: Breast sentinel node biopsy (SNB) is a well-established procedure that has supplanted traditional axillary dissection for most clinically node-negative breast cancer patients. Techniques to identify the draining lymph nodes include colored dyes and radioactive compounds. The Sentimag system uses a non-radioactive magnetic tracer and a handheld magnetic probe to identify sentinel nodes (SNs). The Sentimag Intraoperative Comparison (SentimagIC) study compares the magnetic technique with the standard combination of radioisotope and isosulfan blue dye.

Methods: SiennaXP is a nano device composed of coated iron oxide nanoparticles designed to optimize lymphatic uptake and SN retention. The Sentimag breast SNB technique involves injection of 2cc of SiennaXP fluid into Sappey's subareolar plexus followed by 5 minutes of breast massage and an additional 15 minutes of time to optimize drainage prior to beginning the procedure. The Sentimag hand held probe is then used to identify a magnetic 'hotspot' through the skin of the axilla. The usual transverse axillary incision is made and the magnetometer is used to identify the SNs. The SentimagIC study involves utilizing the Sentimag technique in combination with the 'standard' techniques of isosulfan blue dye and 99technetium sulfur colloid. All blue, radioactive and magnetic SNs are removed and identified as stained blue (from isosulfan blue dye) or black/brown (from SiennaXP) or not, and both radioactive and magnetic counts are taken ex vivo on each node. Currently there are 6 active sites with a total of 60 patients enrolled.

Trial design: This is a pivotal, prospective, open label, multicenter, paired comparison of the magnetic technique with the standard of care for lymph node localization in patients with breast cancer.

Primary endpoints: The lymph node detection rate with SentiMag / SiennaXP and the detection rate with the standard of care; and the safety of Sentimag / SiennaXP as indicated by adverse events.

Eligibility: Diagnosis of primary breast cancer or pure ductal carcinoma in situ (DCIS); Scheduled for sentinel lymph node biopsy; Clinical negative node status (i.e. T0-3, N0, M0).

Statistical methods: The primary hypothesis is that the magnetic technique is non-inferior to the standard technique. Based on an expected detection rate of 95% for both techniques and a non-inferiority margin of 5%, 140 subjects will be required to show non-inferiority with 85% power.

Discussion: SNB for breast cancer is a robust procedure, able to identify the draining lymph nodes of the breast in essentially all patients. Many techniques have been used including radioactive tracers (utilized on most cases) and colored dyes. SentiMag utilizes a unique nano device that can identify the same draining nodes but without the radioactivity used in most procedures. Radioactive dyes must be handled carefully to minimize radiation exposure to healthcare providers and the patient from the manufacturing process, delivery to facility, injection under a nuclear physician license, and the surgical procedure. This novel technique may supplant radioactive tracers allowing SNs to be removed without the patient/healthcare providers being exposed to radiation or the scheduling inconvenience of pre-procedure injection.
Title: The LORIS trial: A multicentre, randomised phase III trial of standard surgery versus active monitoring in women with newly diagnosed low risk ductal carcinoma in situ

Francis A, Fallowfield L, Bartlett J, Thomas J, Wallis M, Hanby A, Pinder S, Evans A, Billingham L, Brookes C, Dodwell D, Fairbrother P, Gaunt C, Jenkins V, Matthews L, Pirrie S, Reed M, Roberts T, Wilcox M, Young J and Rea D. University Hospital Birmingham NHS Trust, Birmingham, West Midlands, United Kingdom; Sussex Health Outcomes Research & Education in Cancer (SHORE-C) Brighton & Sussex Medical School, Brighton, Sussex, United Kingdom; Ontario Institute for Cancer Research, Toronto, ON, Canada; Western General Hospital, Edinburgh, Scotland, United Kingdom; Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; University of Leeds, Leeds, Yorkshire, United Kingdom; King's College London, London, United Kingdom; Ninewells Hospital and Medical School, Dundee, Scotland, United Kingdom; University of Birmingham, Birmingham, West Midlands, United Kingdom; ICPV, London, United Kingdom and Brighton & Sussex Medical School, Brighton, Sussex, United Kingdom.

Body: Background: The independent review of the UK National Health Service Breast Screening Programme reported (The Lancet, Volume 380, Issue 9855, Pages 1778 - 1786, 17 November 2012) on the benefits & harms of breast screening. It concluded that breast screening saves lives & acknowledged overtreatment. It encouraged randomized trials to elucidate the appropriate treatment of screen-detected ductal carcinoma in situ (DCIS) to gain a better understanding of its natural history. The LORIS trial addresses overtreatment of low & low/Intermediate grade screen detected (low risk) DCIS by randomizing patients to standard surgical treatment or active monitoring.

Trial Design: LORIS is a phase III, multicentre, 2 arm study, with a 2 year feasibility phase, in patients confirmed to have low risk DCIS by central pathology review. Patients are randomised to standard surgery or active monitoring with annual mammography. Patients will be followed up for a minimum of 10 years.

Key Eligibility Criteria:
1) Female 46 years or over.
2) Screen-detected or incidental microcalcification (with no mass lesion clinically or on imaging)
3) Low risk DCIS on large volume vacuum-assisted biopsy, confirmed by central pathology review
4) Patient fit to undergo surgery

Specific Aims: The LORIS Trial aims to establish whether patients with newly diagnosed low risk DCIS can safely avoid surgery without detriment to their wellbeing (psychological and physical) & whether those patients that do require surgery can be identified by pathological and radiological means.

Primary endpoint: Ipsilateral invasive breast cancer free survival rate at 5 years
Secondary endpoints: Overall survival; mastectomy rate; time to mastectomy; time to surgery; patient reported outcomes & health resource utilisation.

A digital image data repository and tissue bank provide a prospective resource for both translational & imaging studies.

Statistical Methods: A total of 932 patients will be randomized to a non-inferiority design to test the null hypothesis that active monitoring of women diagnosed with low risk DCIS is not non-inferior in terms of 5 year ipsilateral invasive breast cancer free survival (iiBCFS) rate compared to treatment with surgery. The iiBCFS rate will be compared across the two arms on a per protocol and intent-to-treat basis, using a 1-sided ($\alpha=0.05$) log-rank test for non-inferiority. The iiBCFS rate is assumed to be 97.5% in the surgery arm giving 80% power to exclude a difference of more than 2.5% in the active monitoring arm at 5 years.

Present Accrual and Target Accrual: 21 UK centres are open & the feasibility phase of the trial is recruiting to target. The web based central pathology review process is functioning well with a one week maximum turn around. A further 40 centres will be opened on completion of the feasibility phase.

Contact: LORIS@trials.bham.ac.uk

This project was funded by the National Institute for Health Research [Health Technology Assessment Programme] (project number 11/36/16)

Department of Health Disclaimer: The views & opinions expressed therein are those of the authors & do not necessarily reflect those of the Health Technology Assessment Programme, NIHR, NHS or the Department of Health.
POSNOC: Positive sentinel node—Adjuvant therapy alone versus adjuvant therapy plus clearance or axillary radiotherapy: A randomised trial looking at axillary treatment in early breast cancer

Goyal A. Royal Derby Hospital, Derby, United Kingdom.

Body: Background
Women with early breast cancer that has spread to 1 or 2 sentinel nodes (SNs) undergo axillary node clearance (ANC) or axillary radiotherapy (ART). The publication of the Z11 trial challenged this practice. There are however concerns about this trial which may limit the interpretation and generalisability of its results. These issues include the proportion of patients with micrometastases, variability in radiotherapy and applicability in patients undergoing mastectomy. Therefore further randomized trials are needed to define the role of axillary treatment in patients with 1 or 2 SNs with macrometastases. The UK-ANZ POSNOC trial is asking this question and will provide a more solid evidence base to inform clinical practice.

Methods
Trial design: A pragmatic, randomized, multicenter, non-inferiority trial.
Interventions: All participants will receive systemic adjuvant therapy according to local guidelines and radiotherapy to breast or chest wall if indicated. Women in the intervention group will receive systemic adjuvant therapy alone, whereas those receiving standard care will receive adjuvant therapy plus ANC or ART.
Study population: Women with unifocal or multifocal invasive breast cancer (≤ 5 cm) undergoing breast conserving surgery or mastectomy, who have 1 or 2 nodes with macrometastases at SN biopsy. The sample size is 1900 women. All participants will be followed up for 5 years.
Primary outcome: axillary recurrence at 5 years.
Secondary outcomes: arm morbidity, quality of life, anxiety, loco-regional recurrence, distant metastasis; time to axillary recurrence, axillary recurrence-free survival, disease-free and overall survival, contralateral breast cancer, non-breast malignancy and economic evaluation.
Key differences from Z11
a) stringent radiotherapy quality assurance program, b) prospective pathology reporting, c) axillary ultrasound is mandatory, d) mastectomy patients are eligible, e) axillary treatment in the standard group may be ANC or ART.

Current enrollment
126 patients.

Further information
Web - www.posnoc.co.uk
Email - posnoc@nottingham.ac.uk
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-03-01

Title: Measuring the Impact of MammaPrint on treatment in breast cancer patients: A prospective registry (IMPaCt)

Hart LL L, Untch S and Stork-Sloots L. Florida Cancer Specialists, Fort Myers, FL and Agendia Inc, Irvine, CA.

Body: Background:
Gene expression profiling in breast cancer offers the potential to improve prognostic accuracy, treatment choice, and health outcomes in women diagnosed with early-stage breast cancer. Numerous gene-profiling assays are now available, which can be applied to a single tumor specimen to provide physicians with a more complete basis for treatment decisions.
• MammaPrint is 70-gene profile to estimate whether patients are at high or low risk of developing metastases within the first 10 years after curative surgery.
• BluePrint is an 80-gene molecular subtyping profile that discriminates between three breast cancer subtypes: Luminal, HER2, and Basal.
• TargetPrint provides a quantitative measurement of estrogen receptor (ER), progesterone receptor (PR), and HER2.

Trial design:
IMPACT is a prospective, case-only, study to measure the impact of MammaPrint on treatment decisions in breast cancer patients. The primary objective will be to measure the impact of MammaPrint on treatment decisions in stage I and II Hormone Receptor (HR)-positive, HER2-negative breast cancer patients. As a secondary objective, the impact of MammaPrint on treatment will be assessed in patients with T1a/bN0/1 (up 1 LN) Triple Negative or HER2-positive breast cancer. Baseline clinical data and physician chemotherapy intention before knowing the MammaPrint result will be entered in CRF 1. After completion of CRF1, the MammaPrint result will be released. CRF2 will be completed after the final treatment decision has been made. This CRF will capture physician chemotherapy intention after the MammaPrint result and the impact of these results.

Eligibility:
The study will include women aged ≥18 years with histologically proven invasive stage I-II, HR-positive, and HER2-negative breast cancer, OR, T1a/bN0/1 (up to 1 node) triple negative or Her2-positive breast cancer who signed informed consent.

Objectives:
Primary objective:
Assess the impact of MammaPrint on chemotherapy + endocrine versus endocrine alone treatment decisions in HR-positive, HER2-negative breast cancer patients. Secondary objectives:
• Assess the impact of MammaPrint on treatment decisions in T1a/b, N0/1 (up to 1 node) triple negative or Her2-positive breast cancer patients
• Assess concordance of TargetPrint ER, PR and HER2 results with locally assessed IHC/FISH ER, PR and HER2
• Compare clinical subtype based on IHC/FISH ER, PR, HER2 and Ki-67 (if available) with BluePrint molecular subtype

Statistical methods:
A sample size of 331 patients is required to detect a 25% overall treatment change (5% significance and 95% power) in stage I and II HR-positive, HER2-negative patients. In addition at least 50 T1a/bN0/1 (up to 1 LN) Triple Negative, and at least 50 T1a/bN0/1 (up to 1 LN) HER2-positive breast cancer patients will be enrolled. A McNemars test will be performed for the comparison of the two proportions of treatment intentions (before and after), both expressed as a percentage.

Accrual: The study did not yet start accrual. First patient visit is expected in August 2015. Approximately 15-20 institutions in the United States will participate.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-03-02

Title: DI study: Decision impact of the NanoString® Technologies Prosigna™ in early breast cancers

Hequet D, Guinebretière J-M, Lerebours F, Roulot A, Callens C, Gentien D, Penault-Llorca F, Zilberman S, Salmon R, Foa C, Berseneff H, Huchon C, Katz G, MacDonald M, Morel P, Bieche I, Dubot C and Rouzier R. Institut Curie-Centre René Huguenin, St Cloud, France; Institut Curie, Paris, France; Centre Jean Perrin, Clermont-Ferrand, France; Tenon Hospital, Paris, France; Private Hospital Les Peupliers, Paris, France; Private Hospital Clairval, Marseille, France; René Dubos Hospital, Pontoise, France; Poissy-St Germain Hospital, Poissy, France; Groupe Général de Santé, Paris, France; NanoString Technologies, Seattle, WA and Equipe d'Accueil 7285, Risk and Safety in Clinical Medicine for Women and Perinatal Health, University Versailles-Saint-Quentin, Montigny-le-Bretonneux, France.

Body: Backgrounds: More than a decade of research, clinical studies, and peer reviewed publications support the value of molecular subtyping based on gene expression analyses to assess prognosis and treatment options for patients with early-stage breast cancer. Therefore, genomic assays are now being introduced to supplement the conventional diagnostic tools. Prosigna is a standardized test that measures the expression levels of 50 classifier genes in formalin-fixed, paraffin-embedded (FFPE) breast tumor tissue samples and provide a subtype classification based on the fundamental biology of individual patient's tumor (referred to as molecular subtyping), as well as a prognostic score (referred to as risk of recurrence (ROR) score) that predicts the probability of cancer recurrence over 10 years.

The primary objective of this study is to assess the extent to which Prosigna affects the medical oncologist's treatment recommendations regarding adjuvant chemotherapy and actual treatments received for patients with early-stage breast cancer. Changes will include hormonal therapy alone, hormonal therapy plus chemotherapy, and changes in types of chemotherapy if chemotherapy was recommended before and after the test. Secondary objectives will be to elicit information on investigators' confidence in the recommendations before and after the test, and by cancer recurrence risk groups, rate of chemotherapy related adverse events stratified by administration of chemotherapy, and patients' decisional conflict status, anxiety levels, and functional status before and after Prosigna results.

Multicentric prospective study. Prosigna will be performed on operative piece for all consecutively postmenopausal women matching the inclusion criteria and having signed an informed consent. Data on patient demographics, disease status, intended cancer-specific postoperative management before and after the test, tests results, investigators and patients' confidence in the treatment and in the test, will be recorded in the inclusion visit, after the tests results and 6 month post-assay.

Inclusion criteria: Postmenopausal patients with resected node-negative, estrogen-receptor-positive, HER2-negative (by the local laboratory) early-stage invasive breast cancer (T1-T2, N0, pN0 (i+), pN1 (micrometastatic), M0), able to give consent, eligible for treatment of breast cancer with adjuvant chemotherapy and with ECOG performance status of 0 or 1.

Statistical methods: The clinical and demographic characteristics of the study sample will be described using mean, median, standard deviation, and range for continuous/ordinal variables and frequency and proportion for categorical variables. Bivariate plots and crosstabs will be performed to inspect bivariate associations between variables. The proportion of patients for whom the physicians' choice of treatment changed from baseline to follow-up will be calculated along with the 95% confidence interval. The change in investigator confidence in treatment recommendations before and after Prosigna results were known will be analyzed by calculating the mean and 95% CI for the question regarding whether a physician is more confidence in treatment recommendation after ordering Prosigna.

47 patients have been included on 200 scheduled.
Women's triple-negative, first-line treatment: Improving outcomes in triple-negative breast cancer using molecular triaging and diagnostic imaging to guide neoadjuvant therapy


BACKGROUND:
In triple negative breast cancer (TNBC), pathologic complete response/residual cancer burden-0 (pCR/RCB-0) or minimal residual disease (RCB-I) following neoadjuvant chemotherapy (NACT) is associated with a good prognosis. This is in contrast to extensive residual disease (RCB-II-III) which carries approximately a 50% chance of recurrence. These patients have a particularly poor prognosis as there are currently no targeted agents to salvage chemoresistant disease. It is important to predict pCR in order to direct responsive disease toward standard NACT and non-responsive disease (NRD) to therapy on clinical trials.

TRIAL DESIGN:
The use of genomic signatures (JAMA, 2011; 305:1873-81) and imaging to predict response to NACT will be validated, and the clinical impact of selecting patients with predicted NRD for targeted therapy on clinical trial will be determined. Patients will undergo primary tumor biopsy for molecular profiling and will be randomized 2:1 to know the results versus not (control). Following that, all patients will receive 4 cycles of anthracycline-based NACT, with imaging used for response assessment. Patients with molecular/imaging criteria for NRD will be offered enrollment on a clinical trial based upon molecular profiling or based upon physician/patient choice (control).

INCLUSION CRITERIA:
Tumor size ≥1.5 cm diameter; TNBC by standard assays; ≥18 years of age; LVEF ≥50%; adequate organ and bone marrow function

EXCLUSION CRITERIA:
Stage IV disease; invasive cancer within 5 years; excisional biopsy of the primary tumor; features that limit response assessment by imaging; unfit for taxane and/or antracycline regimens; prior anthracycline therapy; ≥grade II neuropathy; Zubrod performance status of ≥2; history of serious cardiac events

PRIMARY AIM:
- Prospectively determine the impact of a molecular diagnostic/imaging platform in patients with localized invasive TNBC

SECONDARY AIMS:
- Compare rates of clinical trial enrollment
- Evaluate disease free survival in the experimental arms compared to control standard NACT
- Perform integrated biomarker analyses and identify therapeutic targets for resistant disease

STATISTICAL METHODS:
A maximum of 360 patients will be randomized (2:1) using a group sequential design with one-sided O'Brien-Fleming boundaries, with two equally spaced binding interim tests for futility and superiority and one final test, having an overall Type I error .05 and power .80 to detect an improvement in pCR/RCB-I from 50% to 64%.
Title: Optisoins01: Optimizing the patient-breast cancer care pathway; An observational multicentric prospective study

Héquet D, Baffert S, Hoang HL, Brédart A, Asselain B, Alran S, Berseneff H, Huchon C, Trichot C, Combes A, Alves K, Koskas M, Nguyen T, Roulot A and Rouzier R. Institut Curie-Centre René Huguenin, St Cloud, France; Equipe d’Accueil 7285, Risk and Safety in Clinical Medicine for Women and Perinatal Health, University Versailles-Saint-Quentin, Montigny-le-Bretonneux, France; Institut Curie, Paris, France; René Dubos Hospital, Pontoise, France; Poissy-St Germain Hospital, Poissy, France; Antoine Béclère Hospital, Clamart, France; André Mignot Hospital, Versailles, France; Argenteuil Hospital, Argenteuil, France; Bichat Hospital, Paris, France and Louis Mourier Hospital, Colombes, France.

Body: Background: A care pathway is defined as patient-focused global care that addresses temporal (effective and coordinated management throughout the illness) and spatial issues (treatment is provided near the health territory in or around the patient’s home). Heterogeneity of the care pathways in breast cancer (BC) is presumed but not well evaluated. The OPTISOINS01 study aims to assess every aspect of the care pathway for early BC patients using a temporal and spatial scope.

Trial design: An observational, prospective, multicenter study in a regional health territory (Ile-de-France, France) in different types of structures: university or local hospitals and comprehensive cancer centers. The study consists of three work-packages:
- Cost of pathway
  The aim of this WP is to calculate the overall costs of the early BC pathway at one year from different perspectives (society, health insurance and patient) using a cost-of-illness analysis. Using a bottom-up method, we will assess direct costs, including medical direct costs and nonmedical direct costs (transportation, home modifications, home care services, and social services), and indirect costs (loss of production).
- Patient satisfaction and work reintegration
  Three questionnaires will assess the patients’ satisfaction and possible return to work: the occupational questionnaire for employed women; the questionnaire on the need for supportive care, SCNS-SF34 (‘breast cancer’ module, SCNS-BR8); and the OUTPASSAT-35 questionnaire.
- Quality, coordination and access to innovation
  Quality will be evaluated based on visits and treatment within a set period, whether the setting offers a multidisciplinary consultative framework, the management by nurse coordinators, the use of a personalized care plan, the provision of information via documents about treatments and the provision of supportive care.
  The coordination between structures and caregivers will be evaluated at several levels. Day surgery, home hospitalization and one-stop breast clinic visits will be recorded to assess the patient’s access to innovation.

Inclusion criteria: Histologically confirmed, previously untreated, operable breast cancer women; residence in the Yvelines, Hauts-de-Seine or Val d’Oise departments, Ile-de-France, France.

Exclusion criteria: previous history of breast cancer; metastatic, locally advanced, or inflammatory breast cancer, as defined by the AJCC (7th Edition); unstable over the following 12 months.

Statistical methods: Homogeneous groups of patients will be established based on the patients’ individual medical information, and care pathways will be compared. The endpoints are the costs of care pathways, patients’ satisfaction, work reintegration, readmissions and time lapses between care stages. A multiple correspondence analysis will be conducted with care resource use and socio-demographic and medical characteristics as active variables. The variables that constitute the endpoints will be projected onto a space defined by appropriate axes.

Present accrual and target accrual: 307 patients have been included on 800 scheduled.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-04-02

Title: A non-interventional study to characterize the real world treatment patterns and outcomes of women with ER+, HER2-advanced or metastatic breast cancer in Italy

De Laurentiis M, Mitra D, Bell T, Nuzzo CMA and De Placido S. National Cancer Institute “Fondazione Pascale”, Naples, Italy; Pfizer, Inc; Pfizer, srl, Italy and University of Naples Federico II, Naples, Italy.

Body: Background: Breast cancer is the most common type of cancer in women. There were an estimated 1.67 million new cases of breast cancer worldwide in 2012. Approximately 75% of women have hormone receptor positive (ER positive or PR positive) disease (Buzdar et al., 2009) and most of these patients have HER2-negative disease (Partridge et al., 2014). Although there is evidence via clinical trials on the efficacy of a number of ER+, HER2- breast cancer treatments, real world treatment patterns and related outcomes among this population are poorly characterized. This study aims at examining real world practice patterns and its impact on outcomes to gain a better understanding of the limitations of current treatments, identify specific areas and subpopulations with the greatest unmet need, and demonstrate the economic impact of current treatments. Additionally, this study will provide a longitudinal assessment of the impact of breast cancer on quality of life and work productivity in this subpopulation.

Study design: This study uses a prospective, observational cohort design. Approximately 500 women with ER+, HER2- ABC or mBC within Italy will be enrolled across 50 to 80 sites that represent diverse geographical and treatment settings. All patients will be followed for a minimum of two years (or until patient withdrawal from the study, death, or study discontinuation).

Eligibility criteria: Women who have been diagnosed with ER+, HER2- mBC or locoregionally recurrent ABC not amenable to resection or radiation therapy with curative intent and are initiating their first or second therapy in the ABC/mBC setting will be eligible to participate. Patients could have a de novo diagnosis of ABC or mBC or recur from an earlier stage. Patients participating in any interventional clinical trial that includes investigational products at the time of enrollment will be excluded from the study.

Specific aims: The key measures of interest are demographic and clinical characteristics, treatment patterns, clinical outcomes (e.g. objective response, progression-free survival, and overall survival, disease specific health care resource use, patient reported quality of life (EQ-5D), work productivity (WPAI-SHP), and disease specific symptoms and functioning (FACT-B and FACT-ES).

Statistical methods: The study is descriptive and not designed for formal hypothesis testing. A sample size of 500 patients ensures that the half-width of the 95% CIs on the proportion of patients with objective response at one year is less than 0.06.

Accrual: Patient enrollment is expected to begin in September 2015.

Disclosure: This study is sponsored by Pfizer Inc.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** OT2-04-03

**Title:** An intrinsic subtype-based platform of translational randomized phase II trials of response-guided neoadjuvant therapy in breast cancer (PREDIX)

Foukakis T, Hellström M, Johansson H, Bergh J and Hatschek T. Karolinska Institutet and University Hospital, Stockholm, Sweden.

**Body:**

**Background:** Pathologic complete response (pCR) after neoadjuvant therapy of breast cancer is associated with a better prognosis. The impact of pCR on outcome differs between intrinsic subtypes.

**Trial design:** With the aim to improve treatment on an individual level, the PREDIX platform is designed as a compilation of randomized phase II studies based on functional "intrinsic subgroups" with the intention to direct treatment in relation to response. Four trials will be pursued:

1. Luminal A,N- (ER+ and/or PR+ without node metastases and age >40);
2. Luminal A,N+ (ER+ and/or PR+ with node metastases or age ≤40)/Luminal B (ER+ and high proliferation);
3. HER2 amplified (including HER2 positive luminal B tumors);
4. Triple-negative (this trial is currently in planning phase)

The clinical management is accompanied by repeated core biopsies and blood sampling. Besides mammography and ultrasound, functional imaging with PET-CT, confined to the breast and regional lymph nodes is applied.

**Treatment plan:**

**Trial 1.** Eligible patients are treated with endocrine therapy (ET) for 4 weeks. A biopsy for Ki67 is obtained before start and after 2 weeks. If no decrease of Ki67 is observed, or no objective response (OR) is seen after 4 weeks of treatment, palbociclib is added to ET. Patients with decrease of Ki67/OR are randomized to continuous therapy with or without the addition of palbociclib for 12 weeks, followed by surgery.

**Trial 2.** Patients are randomized to 12 weeks of palbociclib + ET or weekly paclitaxel, followed by switch to the other arm for additional 12 weeks before surgery.

**Trial 3.** Patients are randomized to the combination of pertuzumab + trastuzumab + docetaxel or trastuzumab emtasine for 2 three-weekly cycles. If OR is documented, 4 additional cycles will be administered followed by surgery. Non-responding patients will switch to the opposite arm.

In trials 2 and 3, additional anthracycline-based chemotherapy will be administered postoperatively. Adjuvant ET and radiotherapy will be used according to standard practice.

**Eligibility criteria:** Patients >18y with primary breast cancer >2 cm or node metastases, with adequate performance status and organ function are eligible. Intrinsic subtype defined by immunohistochemistry or, preferably, by genomic profiling using RNA Seq.

**Specific aims:** To evaluate the impact of response-guided treatment on objective response and long-term outcome and to identify tumor characteristics and treatment-related changes of tumor biology predictive of long-term prognosis.

**Statistical methods:** The PREDIX trials are based on current knowledge of molecular characteristics of breast cancer and designed to find the distribution of objective response to primary medical treatment (primary endpoint) and to find predictors of response, disease-free and overall survival, and to evaluate toxicity and quality of life in the two treatment groups (secondary endpoints).

**Present accrual and target accrual:** Present accrual (May 2015) in the HER2 positive trial is 14 patients. Trials 1 and 2 will open in July and September 2015. Target accrual is based on an explorative design and is between 180 to 200 patients in each trial.
**Title:** PRAEGNANT - A prospective academic translational research network for the optimization of the oncological health care quality in the adjuvant and advanced/metastatic setting: Health care research, pharmacogenomics, biomarkers, health economics (NCT02338167)

Fasching PA A, Schneeweiss A, Fehm T, Tesch H, Abenhardt W, Overkamp F, Hadji P, Welslau M, Köhler A, Ortmann O, Ettl J, Becker K, Lüftner D, Geberth M, Grischke E-M, Janni W, Lux MP P, Mueller V, Haeberle L, Belleville E, Wallwiener M, Taran F-A, Wallwiener D and Brucker SY Y. University Hospital of Erlangen, Erlangen, Germany; National Center for Tumor Diseases, Heidelberg, Germany; University Hospital of Duesseldorf, Duesseldorf, Germany; Oncologie Bethanien, Frankfurt/Main, Germany; Onkologie im Elisenhof, Munich, Germany; Onkologie Recklinghausen, Recklinghausen, Germany; Hospital North West, Frankfurt/main, Germany; Hämato-Onkologische Schwerpunktpraxis, Aschaffenburg, Germany; Gemeinschaftspraxis für Hämatologie und Onkologie, Langen, Germany; University Hospital of Regensburg, Regensburg, Germany; Technical University Munich, Munich, Germany; Onkologie Lerchenfeld, Hamburg, Germany; Charité Campus Benjamin Franklin, Berlin, Germany; Praxisklinik am Rosengarten, Mannheim, Germany; University Hospital of of Hamburg-Eppendorf, Hamburg, Germany; University Hospital of Ulm, Ulm, Germany; ClinSol GmbH&Co KG, Wuerzburg, Germany; University Hospital of Heidelberg, Heidelberg, Germany and University Hospital of Tuebingen, Tuebingen, Germany.

**Body:**

**BACKGROUND**

During the last decades the treatment of advanced breast cancer (ABC) patients has improved significantly due to a variety of empirical studies and the implementation and continuous improvement of molecular characterization of patients and tumor characteristics. Nevertheless, many ABC patients are faced with limited prognosis, and their treatment remains a challenge. New targeted therapies complement the well-established treatment options for ABC (anti-endocrine, chemo-, antibody based and bone related therapies), leading to individualized treatment regimes tailored to the needs of special patient sub-populations.

**SPECIFIC AIMS/TRIAL DESIGN**

The PRAEGNANT network study is conducted as an academic, prospective registry and diagnostic translational study, accompanied by biomaterial collection. In the pilot phase, 40 study centers, will document 3500 ABC patients. The primary objective is to discover biomarkers, which predict progression free survival (PFS). Secondary objectives include overall survival (OS), breast cancer specific survival, objective response rate, patient reported outcomes (PRO), description of therapies used in the metastatic setting, therapy adherence, health economics for patients with ABC, incidence of (serious) adverse events. The exploratory objectives comprise correlations of gene alterations and their influence on OS, PFS, side effects and PRO. Exploratory biomarkers are assessed at baseline and at every change of therapy. These include gene expression profiling of the primary tumor and corresponding metastasis, somatic mutations (measured in the tumor and in circulating tumor DNA), germline genetic variations, epigenetic changes and miRNA variations. Furthermore plasma and serum markers will be assessed. If actionable molecular alterations are detected patients are recruited into respective studies if available or treated accordingly.

**ELIGIBILITY CRITERIA:**

Any adult patient (>18 years) with the diagnosis of ABC and who is willing and able to sign the informed consent can be enrolled.

**STATISTICAL METHODS/TARGET ACCRUAL:**

The PRAEGNANT study as a prospective registry and diagnostic translational study aims to identify biomarkers in ABC patients, which may predict PFS. Target accrual for the pilot phase is 3500 patients. Each patient will be documented for up to 36 months with an estimated median PFS for all patients of 10 months.
Title: BRCAsearch: A population based prospective study on screening for BRCA1 and BRCA2 germline mutations in patients with newly diagnosed breast cancer treated in southern Sweden

Nilsson MP P, Borg Å, Henriksson K, Kristoffersson U, Kvist A, Silfverberg B, Törngren T and Loman N. Lund University, Lund, Sweden; Skåne University Hospital, Lund, Sweden; Regional Cancer Centre South, Lund, Sweden and Laboratory Medicine Region Skåne, Lund, Sweden.

Body: The overall purpose of the study BRCAsearch is to evaluate a new method for offering mutation analysis of BRCA1 and BRCA2 to all patients with newly diagnosed breast cancer.

Patients with breast cancer in southern Sweden are offered inclusion in the SCAN-B study at the time of diagnosis pre-surgery. If they consent, a part of the tumor is sent to a lab in Lund, Sweden, for research purposes (RNA sequencing etc.). Patients that are included in the SCAN-B study are eligible for inclusion in BRCAsearch.

Summary of study procedure for BRCAsearch:
1. An envelope with written information is given to the patient at the visit to the surgeon the week after surgery. This envelope contains a written genetic counseling, information about the study, an informed consent form, psychosocial questionnaires and contact information to a genetic counselor and physicians responsible for the study (telephone, e-mail). The patient can contact a genetic counselor for pre-test telephone genetic counseling if she wishes to.
2. BRCA1 and BRCA2 are analyzed.
3. Non-carriers are informed about the test result with a letter. Mutation carriers and VUS (variants of uncertain significance) are telephoned and given a time for an appointment at the Department of Clinical Genetics.
4. Psychosocial self-reported questionnaires are delivered at 3 times over a year.

Inclusion criteria (all):
1. The patient is included in the SCAN-B study.
2. The patient is recently diagnosed with an invasive breast cancer or a ductal cancer in situ.
3. The patient has signed an informed consent form for BRCAsearch.

Exclusion criteria (any of):
1. The patient is unable to understand the written information in Swedish.
2. The patient is a psychological state, due to chronic or temporary reasons, where one could suspect that information about the study substantially could be detrimental to the psychological well-being.

Primary outcome measures:
- Prevalence of BRCA1/2 mutations in an unselected breast cancer cohort in southern Sweden
- Uptake of genetic testing
- Proportion of the mutation carriers that do not fulfill current criteria for genetic testing

Secondary outcome measures:
- How many of the patients that contact us for questions and what type of questions they have
- How uptake of genetic testing varies with patient, treatment, and tumor characteristics
- The patients' attitudes towards the method used for identifying mutation carriers
- The economic cost per QALY (quality-adjusted life year)
- Psychosocial comparisons between mutation carriers and non-carriers

Targeted accrual is 500 patients included in the study, which is expected to be reached by the end of 2016.

The study enrolls patients from two hospitals in southern Sweden:
1. Helsingborg Hospital (study start: February 2, 2015)
2. Kristianstad Hospital (study start: March 2, 2015).
Characterization of the oral, gut, and breast tissue microbiomes in women with invasive breast cancer, ductal carcinoma in situ (DCIS), or no history of breast cancer

Campbell M, O'Meara T and Esserman LJ J. University of California, San Francisco, San Francisco, CA.

Background: We now know that there is an interplay between our bodies and the bacteria that we carry, both in health and in disease. Inflammatory diseases associated with disruptions in microbial equilibria, such as periodontal disease and ulcerative colitis, as well as chronic antibiotic use, have been associated with increased risk for cancer development. In a previous small pilot study, we observed differences in the oral microbiomes of women with breast cancer and healthy women. This presentation describes an ongoing study in which we aim to validate our previous findings in a larger cohort of women, as well as extend the microbiomes studied to include the gut and local breast tissue microbiota.

Trial design and eligibility criteria: Women with invasive breast cancer or with ductal carcinoma in situ (DCIS) who have not received prior therapy for their disease are eligible for enrollment. A cohort of healthy women will also be enrolled. Sample collection kits containing cheek and stool swab materials are distributed to patients in the clinic, and breast tumor tissue is collected at the time of surgery.

Specific aims: 1. To characterize and compare oral and gut microbial diversity from women with early stage invasive breast cancer, women with DCIS, and healthy women; and 2. To characterize the microbiota associated with breast tumor tissue and compare this to healthy breast tissue.

Methods: DNA from the three sites is isolated, the bacterial 16S rRNA gene is PCR amplified and sequenced, and the bacterial species present are enumerated. Oral, gut, and breast tissue microbial diversity at the genus and species levels will be assessed using the Shannon diversity index. Student’s t-test will be used to compare the mean diversity index values. Evaluation of microbial diversity and clinical variables will be determined using Spearman or Pearson correlations depending on the distribution of the data. We will also compare oral microbial diversity and clinical variables across racial and ethnic groups to determine if differences in the microbiomes may explain disparities in incidence across these groups. Classification and regression trees (CART) will be employed to develop rule sets based on the abundance of oral bacteria that discriminate breast cancer vs. DCIS vs. healthy controls.

Present accrual: As of June, 2015, 32 women with early-stage invasive cancer, 8 women with DCIS, and 9 healthy women received sample collection kits. Breast tumor tissue has been collected from 11 invasive breast cancer cases and 2 DCIS cases.

Target accrual: For the oral and gut microbiome analyses: 100 women with early-stage invasive breast cancer, 100 women with ductal carcinoma in situ (DCIS), and 100 healthy women with no history of breast cancer. For the breast tissue microbiome analyses: 20 women with invasive breast cancer, 10 patients with DCIS, and 10 reduction mammoplasty cases from healthy women.
Body: Over the past decade, genomic characterization of tumors has shed enormous light on the molecular underpinnings of cancer. These discoveries have led to the development of novel therapies and preventive measures that have already revolutionized cancer care. Despite this progress, the genomics of metastatic breast cancer (MBC), one of the leading causes of cancer death in the U.S., remains poorly understood.

The challenge in studying tumor samples from patients with MBC has been that the tumors from most patients are not available for research, largely because the vast majority of patients are cared for in community settings where genomics studies are not typically conducted. To address this, we have launched a nationwide study, The Metastatic Breast Cancer Project, which seeks to empower patients to accelerate cancer research through sharing their samples and clinical information. We have developed an outreach program in collaboration with MBC advocacy organizations to connect MBC patients around the country with genomics research performed at the Broad Institute, allowing them to participate regardless of where they live.

Working with MBC patients and advocates, we designed a website (www.mbcproject.org) with an online questionnaire that allows patients with MBC to provide information about themselves and their cancer. Based on their answers, patients are offered an electronic consent form that explains the risks and benefits of the study and asks for permission to obtain a portion of their stored tumor tissue, a saliva sample, and copies of their medical records. For patients who consent, our clinical research team contacts their physicians and obtains copies of their medical records, which are reviewed to confirm eligibility. Enrolled patients are sent a saliva kit and asked to mail back a saliva sample, which is used to extract germline DNA. The clinical research team also contacts the patient's pathology department and requests a portion of the tumor to be sent to the Broad Institute for genomic analysis. Whole exome and transcriptome sequencing is performed on tumor and germline DNA. Sequencing data are linked to de-identified clinical information, and the resulting data are used to identify drivers of tumorigenesis, mechanisms of response and resistance to therapies, and diagnostic, prognostic, and therapeutic biomarkers. The database of clinically annotated genomic information will be shared with the NIH and the cancer research community. Study updates and discoveries are shared at regular intervals with all patients who complete the initial questionnaire.

This direct-to-patient approach should be particularly enabling for the identification of patients with rare phenotypes or clinical behavior. For this reason, the first cohorts being studied are patients with extraordinary responses to therapies and patients who present with de novo MBC. Additional cohorts will be added in the future, including young women with MBC and patients with drug-resistant MBC. This project seeks to establish a patient-researcher partnership to accelerate genomic discoveries and improve outcomes in MBC, and may ultimately serve as a means to build a new clinical and translational research model for all patients with cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT2-05-04

Title: The Talazoparib Beyond BRCA (TBB) trial: A phase II clinical trial of talazoparib (BMN 673) in BRCA1 and BRCA2 wild-type patients with (i) advanced triple-negative breast cancer (TNBC) and homologous recombination deficiency (HRD) as assessed by myriad genetics HRD assay, and (ii) advanced HER2-negative breast cancer (BC) with either a germline or somatic mutation in homologous recombination (HR) pathway genes

Afghahi A, Chang P-J, Ford JM M and Telli ML L.  Stanford University Medical Center, Palo Alto, CA.

Body: Background: Poly-ADP-ribose polymerase (PARP) inhibition induces synthetic lethality in tumor cells bearing deleterious mutations in the genes BRCA1/2. Talazoparib (BMN 673) is a novel, dual-mechanism PARP inhibitor that potently inhibits the PARP enzyme and effectively traps PARP on DNA. Talazoparib has shown promising single-agent anti-tumor efficacy in several BRCA1/2 mutation-associated advanced cancers. The efficacy of PARP inhibition in BRCA1/2 wild-type TNBC with HR defects and in breast tumors with mutations in other non-BRCA1/2 HR pathway genes is currently unknown.

Trial Design & Eligibility: This Phase 2 trial (TBB) explores the activity of single agent talazoparib in BRCA1/2 wild-type BC patients using an optimal Simon two-stage design. Eligible subjects will be assigned to one of two parallel cohorts: 1) Cohort A: Subjects (n=29) with advanced TNBC with underlying HR defects as assessed by the HRD assay and, 2) Cohort B: Subjects (n=29) with advanced HER2-negative BC with a somatic or germline deleterious mutation in a non-BRCA1/2 HR pathway gene. Gene mutations of interest are: PTEN, PALB2, CHEK2, ATM, NBN, BARD1, BRIP1, RAD50, RAD51C, RAD51D, MRE11, ATR, Fanconi anemia complementation group of genes (FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL). Other key eligibility criteria include metastatic disease treated with at least one prior line of chemotherapy, no deleterious BRCA1/2 mutation, and no prior platinum exposure. In cohort A, TNBC patients must have a HRD score of ≥ 42. Eligible patients will receive oral talazoparib (1.0 mg/day, 28-day cycles) until disease progression or unacceptable toxicity.

Endpoints & Statistical Plan: The primary endpoint is objective response rate (ORR). Secondary endpoints include clinical benefit rate (CBR), progression free survival (PFS), and safety. In this study, we have set a null hypothesis of ≤ 5% objective response rate and alternative response rate of ≥ 20% based on RECIST 1.1. Interim analysis will be performed after accrual of 10 patients in each cohort. If at least one out of the 10 patients responds, then we will accrue 19 additional patients for a total of 29 patients in each cohort. At least four patients have to respond out of the 29 patients in each cohort to declare statistical significance at a one-sided 5% level with 80% power or better. This trial is enrolling patients at Stanford University in California (NCT 02401347).
Title: Fluorescence navigation system using indocyanine green (ICG) instead of radioisotope for sentinel lymph node (SLN) biopsy in early breast cancer

Benson JR R, Pitsinis V, Provenzano E and Wishart GC C. Cambridge Breast Unit, Addenbrooke's Hospital, Cambridge, United Kingdom and Cambridge Breast Clinic, Mediterraneo Hospital, Athens, Attica, Greece.

Body: Background: Dual localisation methods with blue dye and radioisotope are commonly employed for SLN identification but potential drawbacks include allergic reactions, staining of cutaneous/surgical breast tissue, radiation exposure and mandatory licencing. A majority of studies have reported near 100% identification rates using the fluorescent tracer ICG in combination with standard tracer agents. A feasibility study (ICG-10) has confirmed high sensitivity of ICG fluorescence mapping for SLN detection in early breast cancer with 95% of nodes both blue and fluorescent. This follow-on study has specifically evaluated a combination of ICG with blue dye for SLN localization.

Methods: As an observational cohort study, 50 consecutive patients (49 female; 1 male) with core biopsy proven unilateral invasive (37 cases) or non-invasive (13 cases) breast cancer underwent SLN biopsy with blue dye and ICG. All patients were clinically and sonographically node negative. Axillary surgery (SLN biopsy) followed neoadjuvant chemotherapy in 5 patients (10%). The median patient age was 48 years and for primary surgical patients median tumor size was 19mm. Patients received a dual peri-areolar/intradermal injection of blue dye [2ml 2.5% Patent Blue] and ICG [2mls 0.5%] after induction of anaesthesia. The number of sentinel nodes for each patient was recorded numerically and whether blue, fluorescent or both. Subcutaneous lymphatics were visualised with a photodynamic eye camera and nodal and procedural detection rates calculated for ICG alone and in combination with blue dye.

Results: Final analysis was performed on a total of 87 nodes retrieved from 50 patients with an average nodal count of 1.8 per patient (range 1 – 4). Eighty-four nodes were blue and fluorescent and 3 nodes fluorescent only with no harvesting of non-blue, non-fluorescent nodes. At least one transcutaneous lymphatic channel was visible in all cases. Nodal detection rates for ICG alone and combined with blue dye were 100% (87/87) and 96% (84/87) respectively. Metastases (>0.2mm) were present in 18 nodes which were all blue and fluorescent and a total of 10 patients had at least one positive node (node positivity rate = 20%). The procedural detection rate was 96% (48/50) for blue dye and 100% (50/50) for ICG with 2 patients having fluorescent only nodes which were deemed sentinel (4%). No serious adverse reactions were noted.

Conclusion: ICG fluorescence navigation system permits real-time visualization of lymphatic tissues and provides an additional dimension to SLN biopsy using methodology which is sensitive, valuable and safe. These results confirm accuracy of ICG fluorescence for SLN identification with nodal sensitivity of 96% for a combination of blue dye and ICG. With further refinements of the technique, use of ICG as a sole tracer may be possible agent without concerns about excessive nodal yield but improved patient convenience and costs.
Title: A comparison of significance with indocyanine green fluorescence imaging method and computed
tomography-lymphography in sentinel lymph node identification for early breast cancer patients

Abe H, Yamasaki K, Teramoto A, Yoneda K, Ogawa M, Kawasaki M and Kameyama M.  Breast Center, Bell Land General
Hospital, Sakai, Osaka, Japan and  Bell 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. The combination of the radioisotope and dye-staining methods is the most accurate
way to identify sentinel lymph nodes. We had reported feasibility and safety of a new technique of SLN identification using
fluorescence imaging of indocyanine green (ICG) injection without any need for training. Recently, SLN identification using
computed tomography-lymphography (CTLG) has been reported in Japan. This study investigated the comparison of significance
with fluorescence imaging method and CTLG.

Patients and method: Between January 2013 and May 2015, 213 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 CT. We performed an
intradermal injection in the periareolar area, using 4 ml of contrast agent with 1 ml of local anesthetic. The contrasted lymph flow
and SLN were identified in reconstructed three-dimensional imaging. The SLN spot was indicated by CT laser light navigator
system. During the operation, fluorescence images were obtained using the fluorescence imaging system, Photodynamic Eye
(pde-neo, Hamamatsu Photonics Co., Japan). After 5 mg / 0.3ml ICG was injected intradermally in to the periareolar skin,
lymphatic drainage was observed with fluorescence images. SLN biopsy was performed referring to the point by axillary
compression technique by plastic device.

Results: The median age of the 213 patients was 59 (range 28 – 87) years old. CTLG and fluorescence imaging was safely
performed in all patients. CTLG could visualize lymphatic flow and accurately identify SLN in 189 (89 %) and 196 (92 %) cases,
respectively, whereas fluorescence imaging identified successfully lymphatic flow and SLN in all patients. Fluorescence imaging
with axillary compression technique was visually easy to identify the location of SLN on the axillary skin even in obese patients.
Lymphatic flows of CTLG were completely consistent with those of fluorescence imaging. The number of SLN identified by CTLG
was significantly lower than that by fluorescence imaging (1.1 vs. 1.6, p<0.01). Twenty seven patients (13%) were found to have
lymph node metastases pathologically, and 7 of them had micrometastases of lymph node. In case of partial enhancement of
SLN with CTLG, the rate of positive metastasis was significant higher compared to the cases of whole enhancement (p<0.01),
however, even if enhancement is poor, about 70 % cases without metastasis are present.

Conclusion: Both of fluorescence imaging and CTLG revealed easy and effective to detect SLN. The fluorescence imaging with
fluorescence mapping showed strong fluorescence from all parts of the surgical field, which hindered identification of SLNs, and
fluorescence imaging was more high detection rate of SLN.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-01-03

**Title:** Optimal concentration of indocyanine green in near-infrared fluorescence guided sentinel lymph node biopsy in breast cancer

Paik H-J, Yi HW, Park S, Ryu JM, Nam SJ, Lee JE, Kil WH, Lee SK, Bae SY and Kim SW. Division of Breast and Endocrine Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

**Body:**

**Background:** Near-infrared (NIR) fluorescence-guided sentinel lymph node biopsy (SLNB) using indocyanine green (ICG) has been successfully applied in various kinds of cancers, especially in breast cancer. Optimizing the concentration of ICG without using human serum albumin in natural lymphatic system is still required.

**Methods:** 25mg of ICG was diluted with distilled water (DW) or normal saline (NS). Dilution concentrations used for measurement were 0.25µg/mL, 0.5µg/mL, 2.5µg/mL, 5.0µg/mL, 25µg/mL, and 250µg/mL. The brightness of fluorescence was measured by an image system called Visual Navigator (SH System, Korea). We used graphic software (GIMP, version 2.8.14, The GIMP Team) to measure the brightness. We assumed that drained serous lymphatics from a breast cancer patient can reflect a natural lymphatic condition injected with ICG in the operation field. Drained lymphatics were mixed to reduce the concentration of ICG by half that was diluted with DW or NS. We also measured the brightness of fluorescence diluted with DW or NS that was mixed with drained lymphatics. After this screening test, we subdivided the range which brightness was measured as 100 and adjusted the intensity of illumination for more specific results.

**Results:** Highest brightness values were measured as 100 between 2.5µg/mL and 25µg/mL concentration of ICG with DW dilution in screening settings, and the values of brightness were measured as 100 between 2.5µg/mL and 5.0µg/mL concentration of ICG with NS dilution. When drained lymphatics were mixed together, the values of brightness were measured as 100 between 2.5µg/mL and 25µg/mL concentration of ICG with DW or NS dilution. After subdividing the ICG concentration to 2.5µg/mL, 3.13µg/mL, 5.0µg/mL, 6.25µg/mL, 12.5µg/mL and 25µg/mL with adjusting the intensity of illumination, the highest brightness value was measured as 76 in 5.0µg/mL concentration of ICG with DW dilution. When diluting with NS, the highest brightness value was measured as 61 in 5.0µg/mL ICG concentration. After mixing with drained lymphatics, the highest brightness value was both 79 in 5.0µg/mL concentration of ICG with DW and NS dilution.

**Conclusions:** This study showed a practically optimal ICG concentration range in fluorescence guided SLNB. The optimal range of ICG concentration is from 3.13µg/mL to 6.25µg/mL with DW or NS dilution. Although 5.0µg/mL met the best result, adjustment for individual settings may be considered.
Title: Improved sentinel lymph node detection with the use of superparamagnetic iron oxide tracer after neoadjuvant treatment in breast cancer patients

Rubio IT Teresa, Esgueva-Colmenarejo A, Diaz-Botero S, Peg V and Espinosa-Bravo M. Breast Surgical Oncology. Hospital Universitario Vall de Hebron, Barcelona, Spain and Hospital Universitario Vall de Hebron, Barcelona, Spain.

Body: Background. The use of superparamagnetic iron oxide (SPIO) tracer for sentinel node biopsy (SLN) has shown non inferiority compared with the radioisotope technique in early breast cancer. In the neoadjuvant setting (NAC) identification rates with dual technique (blue dye and Tc 99) have been reported to be from 80% to 95%. SLN biopsy false negative (FN) rates after NAC have decreased with the use of dual tracer (radioisotope/ blue dye) and the excision of > 2 SLNs. This study was designed to evaluate the outcome of SLN after NAC using a dual tracer (SPIO and Tc99) versus SLN after NAC with radioisotope (Tc 99) alone.

Material and Methods. After NAC, 188 patients underwent SLN with Tc99 (group Tc99) and 92 patients underwent a SLN with dual technique (group SPIO/Tc99). Inclusion criteria were patients T1-3 N0-1 before NAC and all patients had clinically and /or ultrasound negative axilla before the SLN procedure. Patients were injected subareolar with Tc99 the day before surgery and with the SPIO intraoperatively. SLN was excised if it was radioactive, magnetic or palpable. Patients signed an inform consent. Patients who were N0 pre and postNAC did not undergo an axillary lymph node dissection (ALND) (except for the initial validation patients) , while patients who were N1 pre NAC and N0 post NAC underwent SLN and ALND as part of the protocol.

Results. Mean age of patients between groups were similar, 53.18 (range, 24-90 years old) in the Tc99 group and 52.53 (range, 23-82 years old) in the SPIO/Tc99. Identification rates were 95.72% and 97.8 % in the Tc99 and the SPIO/Tc99 group respectively (p = 0.21). In the Tc99 group, 58 (30.8%) patients were cN1 pre NAC while 13 (14%) in the SPIO/Tc99 (p =0.01). Rates of positive SLN after NAC were 32% in the Tc99 group while 20% in the SPIO/Tc99 group. False negative rates were 4.19% in the Tc99 group (1/50) and 8.3% in the SPIO/Tc99 group (1/13) (p=0.62). There was a statistically significant difference in the median number of SLNs between techniques, being 2.04 in the Tc99 and 2.60 in the SPIO/Tc99 group (p = 0.001). When the mean number of SLNs and the identification rates were differentiated between cN1 and cN0, the statistically significant differences remained unchanged.

Conclusions. The use of dual technique (SPIO and Tc99) for the detection of SLN after NAC increases the identification rates over 97%, better than the reported studies in the NAC setting with Tc99 and blue dye. Although in our study increasing the number of SLNs excised has not decreased the false negative rates, rates of FN are below 10% in both groups. Larger number of cN1 patients is needed in the SPIO/Tc99 technique to validate these initial results. The dual technique with SPIO and Tc99 may optimize the success of SLN after NAC in breast cancer patients.
Title: The use of Tc-99 tilmanocept in sentinel lymph node biopsy after neoadjuvant chemotherapy in clinically node-negative patients with breast cancer

Unkart JT T and Wallace AM M. University of California, San Diego.

Body: Background: Neoadjuvant chemotherapy (NAT) has been shown to induce fibrosis and inflammation that alters lymphatic drainage of axillary lymph nodes in breast cancer. Technetium-99 Tilmanocept (TcTM), a CD206-macrophage receptor targeted radiopharmaceutical, is a small agent with recent FDA-approval for lymphatic mapping. No prior studies have investigated the use of TcTM in the neoadjuvant setting. The aim of this study was to compare the identification rate, node-positivity rate, and number of total nodes evaluated in sentinel lymph node (SLN) biopsy with TcTM and vital blue dye (VBD) in clinically node-negative patients receiving NAT vs. initial surgery.

Methods: A retrospective review was conducted on patients undergoing SLN biopsy with TcTM and VBD from May 2013- May 2015 at UCSD. Patients with a history of prior SLN biopsy or axillary lymph node dissection were excluded. Patients undergoing neoadjuvant chemotherapy or receiving > 3 months of neoadjuvant endocrine therapy were grouped and compared to patients undergoing initial surgical treatment. The SLN identification and node-positivity rates were compared with the X2 test. To compare the number of SLNs evaluated between groups, a zero-truncated negative binomial (ZTNB) count model was constructed to assess the effect of NAT and other covariates on the SLN count. Covariates included age, body mass index (BMI), gender, surgeon, mastectomy vs. lumpectomy, node positivity, pathologist, T-stage, and receptor status. A p-value < 0.05 was used for statistical significance.

Results: Of the 417 total SLN cases identified, 72 (17.2%) cases were in patients who had received NAT (61- chemo, 11- endocrine). The SLN identification rate was 100% in both groups (p= 1.0). Overall, there were 68 (16.3%) cases of SLN-positivity, 14 (19.4%) in the NAT group versus 54 (15.7%) in the non-NAT group (p= 0.54). The median number of identified nodes was 3 in both groups. In the ZTNB count model, age, surgeon and evaluating pathologist were significant predictors of the total number of SLN evaluated. The use of NAT did not significantly affect the number SLNs evaluated. Incident rate ratios, confidence intervals and p-values are reported in the attached table.

Sentinel Lymph Node Count Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>IRR</th>
<th>95% CI LL</th>
<th>95% CI UL</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age per 5 years</td>
<td>0.96</td>
<td>0.93</td>
<td>0.99</td>
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<tr>
<td>Surgeon #2</td>
<td>1.23</td>
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<td>NAT</td>
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<td>0.92</td>
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<tr>
<td>Pathologist #2</td>
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<td>0.57</td>
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<td>0.005</td>
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<tr>
<td>Pathologist #3</td>
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<td>1.22</td>
<td>0.90</td>
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<tr>
<td>Pathologist #4</td>
<td>0.93</td>
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<td>0.70</td>
</tr>
</tbody>
</table>

Discussion: Prior studies have indicated that NAT may induce fibrosis and inflammation that may obscure lymphatic mapping procedures. For SLN biopsy with TcTM in VBD in our study, the use of NAT did not change the identification rate or node-positivity rate. Additionally, when controlling for covariates, the use of NAT did not change the total number of SLNs evaluated. While NAT might induce fibrosis and inflammation, SLN biopsy with TcTM and VBD is technically successful in clinically node-negative patients undergoing neoadjuvant chemotherapy.
Sentinel lymph node biopsy after NACT: Results of a validation study in large/locally advanced breast cancer patients

Background: Sentinel lymph node biopsy (SLNB) is the current standard of care for surgical staging of clinically node negative axilla (N0) early breast cancer patients undergoing primary surgery. SLN- identification rate (IR) of 90% and SLN- false negative rate (FNR) of 10% are considered minimum acceptable indices for SLNB. Its role in staging axillae in patients undergoing post-NACT surgery is somewhat unclear. In India, and most low-and-middle income countries, large operable breast cancers (LOBC) and locally advanced breast cancers (LABC) constitute a large proportion of breast cancer patients treated. These patients are usually are treated with NACT, followed by surgery and radiation therapy. In a prospective validation SLNB study, we investigated the accuracy of SLNB in staging post-NACT N0 axilla in a patient cohort that were LOBC or LABC at the time of initial presentation.

Methods: Hundred consenting non-inflammatory LOBC/LABC patients (mean age 49.3+8.6; index stage T3,N0-1=21; T4b,N0-1=33; T1-3,N2a=24; T4b,N2a=22) who were N0 after NACT at time of surgery (Breast conservation surgery in 19, Mastectomy in 81) were included. Majority had Infiltrating ductal carcinoma (n=87), and grade II/III tumors (n=93); 45 were hormone receptor positive (+), 29 had HR negative (-) HER2(+); and 26 had triple negative breast cancer on IHC sub-typing. Commonest NACT regimen used was Anthracycline followed by taxanes in 83. SLNB was performed using low-cost methylene-blue and 99mTc-Antimony-colloid, which were produced in-house using well standardized protocols, with clearance of the institutional ethics committee. Irrespective of the SLN histology, a complete axillary dissection (ALND) was carried out in all. SLN-IR and SLN-FNR were calculated, comparing the histological status of the SLN and the ALND specimen. Factors predicting non-identified SLN and false negative SLN were evaluated in uni-variate and multi-variate analysis.

Results: With a combination of methylene blue dye and radiopharmaceutical, the SLN-IR was 81%. Mean number of SLN removed was 2.4+/-1.02. Mean number of nodes removed at ALND was 13.3+/-2.2. SLN-IR varied significantly (p<0.05) per index stage, and were- 90.4% in T3,N0-1; 84.4% in T4b,N0-1; 83.3% in T1-3,N2a; and 63.6% in T4bN2a. The FNR was 17.3% for the whole cohort. FNR varied significantly (p<0.05) per index stage, and were- 8.3% in T3,N0-1; 14.9% in T4b,N0-1; 22.2% in T1-3,N2a; and 30% in T4bN2a. Factors found predictive of non-identified SLN were tumor stage T4b, nodal stage N2a, extra-nodal spread, and LVI. Factors found predictive of FNR SLN were tumor stage T4b, nodal stage N2a, and extra-nodal spread.

Conclusions: Considering SLN-IR of 90% and SLN-FNR of 10% as acceptable standards, SLNB in post-NACT N0 patients undergoing surgery was not found robust in staging the axilla, with the exception of patients with index stage T3,N0-1 who had SLN-IR of 90.4% and SLN-FNR of 8.3%. Patients with (pre-NACT) skin involvement(T4b), matted axillary nodes(N2a) and LVI are fraught with high-risk of non-identification and false-negative SLNB.
Title: Second axillary sentinel lymph node biopsy for breast tumor recurrence: Instituto Alexander Fleming experience in Buenos Aires, Argentina


Body: Sentinel Lymph Node Biopsy (SLNB) is the standard technique for axillary staging of patients with operable breast cancer and a clinically negative axilla because it avoids unwarranted axillary dissection and consequently reduces postoperative morbidity.

Purpose: The aim of this study is to determinate the feasibility and accuracy of the second SLNB for patients with ipsilateral breast cancer recurrences with clinically negative axilla, who were treated previously with breast surgery and study of the axilla.

Methods: Retrospective review of the database of the Instituto Alexander Fleming. Between October 2009 and October 2014, 1029 patients with diagnosis of breast cancer required surgery. The study included 26 patients with the diagnosis of operable local breast cancer recurrence, who had previously undergone axillary surgery either as SLNB, sampling or axillary lymph node dissection (ALND). They subsequently underwent additional breast surgery and a second SLNB.

Results: The mean age of the ipsilateral breast cancer recurrences was 59.23 years (range: 32-87) and the most common histologic subtype was invasive ductal carcinoma in 22 patients (84.6%) and 4 patients (15.4%) with invasive lobular carcinoma. 4 patients (15.4%) had previously ALND or sampling and 22 patients (84.6%) SLNB. The identification rate of the second SLNB was 92.31%. Only 2 patients were not identify, one patient with a previous axillary sampling and another one with previous SLNB. In those patients the ALND was performed and the axilla was negative. Lymphoscintigraphy failed to identify any SLN in 6 patients (23%), 2 patients which were not identify and 4 patients only detected with patent blue. The average number of nodes removed at second SLNB was 1.8 (range: 1-5). Second SLNBs were negative in 21 patients (80.8%), and macrometastasis disease was identified in 2 patients and complete ALND was performed.

In 1 patients additional extra-axillary aberrant drainages was observed in the contralateral axilla and interpectoral, and other 3 patients had aberrant drainage in the contralateral axilla (1 patient), internal mammary regions (1 patient), and interpectoral (1 patient). Aberrant drainage pathways were not routinely dissected. Only those accessible during surgery were removed.

The median time between first surgery and ipsilateral breast tumor recurrence was 7.19 years (range: 1-22). The disease free survival (DFS) was 9.16 years (range: 2.25-24).

Conclusion: In the present serie we show a high identification rate of 92.31% in the second SLNB, comparable with other international series published in the literature (range: 51-97%). A second SLNB should be considered for patients with ipsilateral breast tumor recurrence who underwent conservative surgery and have clinically negative axilla. The procedure is technically feasible and accurate for selected patients, and avoids unnecessary ALND. Extra-axillary sentinel lymph node localization rates are higher than for primary SLNB but the clinical significance and management of extra-axillary nodes needs to be clarified.
Title: Sentinel lymph node biopsy alone after neoadjuvant chemotherapy in patients with cytologically proven node-positive breast cancer

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Body:

Background
The purpose of this study was to identify the feasibility and accuracy of sentinel lymph node biopsy (SLNB) after neoadjuvant chemotherapy (NAC) in patients with axillary lymph node (ALN) metastasis at diagnosis.

Methods
This is a retrospective study of 332 patients who were diagnosed with invasive breast cancer and ALN metastasis and treated with NAC followed by curative surgery at Samsung Medical Center between January 2007 and December 2013. Patients were classified into five groups according to surgical procedure for the ALNs and pathologic results; group 1, patients with negative SLN status and no further dissection was performed; group 2, patients with negative SLN status undergoing further axillary lymph node dissection (ALND); group 3, patients with positive or undetected SLNs undergoing further ALND; group 4, patients without residual axillary metastasis undergoing complete ALND; and group 5, patients with pathologic nodal positive disease undergoing ALND.

Results
Sentinel lymph nodes identification rate after NAC was 99.1% and false negative rate was 24.1%. The median number of retrieved SLNs was 4 (range, 1–10). There was no difference in the overall survival among the groups (p=0.06). There was no significant difference in the disease-free survival rate between the SLNB only and complete ALN dissection groups who revealed a pathologic complete node response (79.6% versus 80.5%) and the rate of axillary recurrence demonstrated no significant differences among the groups. (p=0.225) There was a statistical difference of recurrence between group 1 versus 2, and group 1 versus 4 in hormone receptor-negative patients. (p=0.027)

Conclusion
SLNB after NAC in breast cancer patients with initial ALN metastasis may help identify downstaging to negative nodal status and thereby reduce the surgical morbidity by avoiding standard ALN dissection.
A phase I clinical trial of VST-1001 (dilute fluorescein) in lymphatic mapping and sentinel lymph node localization in clinically node negative breast cancer

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Background: Combined use of a radiocolloid and a vital blue dye is recommended for accurate lymphatic mapping and sentinel lymph node (SLN) identification. However, vital blue dyes can cause tattooing, skin necrosis and allergic reactions. Hence, there is a great need for new lymphatic mapping agents. Here we describe the novel use of VST-1001 (dilute fluorescein) and direct visualization devices in lymphatic mapping and SLN identification.

Methods: A prospective, non randomized, single arm, open label, single dose, dose-finding Phase I clinical trial in patients (pts) with high-grade DCIS and clinical stage I/II breast cancer eligible for SLN biopsy was performed. All pts had SLN localization with technetium-99m-sulfur colloid (Tc99mSC) and intraoperative lymphatic mapping with VST-1001 injected peritumorally, periareolarly, and/or intradermally. SLN radioactivity was identified with a gamma probe, and VST-1001 fluorescence was induced by light emitting diodes and detected as a yellowish-green fluorescence in the visible light range with notch filter spectacles. The primary endpoints were safety, ability of VST 1001 to localize lymph nodes, and the optimal dose of VST\textregistered-1001. SLN concordance of Tc99mSC radioactivity and VST-1001 fluorescence was also assessed.

Results: Fifteen women with a median age of 60 yrs (range, 43-80) were enrolled. In cohort 1, 5 pts received 0.01% VST-1001. All patients had at least 1 SLN that was fluorescent and radioactive. A total of 22 SLNs were identified, many with faint fluorescence. Per protocol, the dose of VST-1001 was increased, and in cohort 2, 10 patients received 0.1% VST-1001. All 10 (100%) pts in cohort 2 had at least 1 SLN that was fluorescent and radioactive. Of a total of 24 SLNs identified, 20 (83%) were fluorescent and radioactive, 2 (8%) were radioactive only, and 2 (8%) were fluorescent only. Four SLNs in 3 patients contained micrometastatic breast cancer; all 4 SLNs were radioactive and fluorescent. There were no adverse events related to VST-1001. A Phase II clinical trial is currently accruing.

Conclusions: VST-1001 safely localized lymph nodes in breast cancer. VST-1001 was able to localize lymph nodes that were not radioactive and had a high concordance with Tc99mSC. VST-1001 may be a novel alternative to vital blue dyes in lymphatic mapping and lymph node localization.
Title: Sentinel lymph node biopsy after neoadjuvant chemotherapy for patients with clinically node-positive breast cancer: A single institution retrospective evaluation


Body: Background: In patients with clinically node-negative breast cancer sentinel lymph node biopsy (SLNB) offers accurate staging information with considerably less morbidity than a full axillary lymph node dissection (ALND). However, for clinically node-positive (cN1) patients who undergo neoadjuvant chemotherapy SLNB is thought to have a high false-negative rate and not suitable for this population. We sought to evaluate the false-negative rate (FNR) of SLNB following chemotherapy in patients initially presenting with cN1 breast cancer at a single institution.

Methods: Patients undergoing neoadjuvant chemotherapy diagnosed with cN1 breast cancer between October 2004 and February 2014 were identified from the University of California, San Francisco cancer registry. All patients underwent single agent mapping with Tc99 and SLNB followed by completion axillary lymph node dissection (ALND). Pathologic complete response, number of sentinel nodes removed and FNR were calculated.

Results: Of the 80 patients who underwent SLNB and ALND, 43 had residual metastatic disease in the nodes producing a nodal pCR of 46.25% (95%CI. 35.0%-57.8%). In 14 patients, cancer was not identified in the SLNs but was discovered in the lymph nodes retrieved by ALND, resulting in an overall FNR of 32.6% (95% CI, 19.1%-48.5%). 49 patients had only 1 SLN removed. Of the patients with only 1 SLN removed, a false-negative SLN was identified in 9 of the 21 patients with a positive node for a FNR of 42.9% (95% CI, 21.8%-66.0%). Of the patient with more than 1 SLN removed, 5 of 18 patients with a positive node had a false-negative SLN yielding a FNR of 27.8% (95% CI, 9.7%-53.5%). Only 14 patients had more than two SLNs excised.

Conclusion: Recent studies including the French GANEA 2 and ACOSOG Z1071 trials demonstrated a significant decrease in FNR when more than 1 SLN was excised. In this retrospective study however a single SLN was sampled from most patients. The FNR from this study was more than three times the generally accepted threshold of 10%. This substantial FNR further supports the need to remove more than 1 SLN during surgery in order to accurately assess nodal disease. Furthermore the implementation of a dual mapping technique would likely facilitate this process.
Title: Sentinel lymph node (SLN) localization is highly successful after neoadjuvant chemotherapy (NCT) for breast cancer


Body: Background: Recent multi-center trial results are concerning for the ability to identify SLNs after NCT. SLN localization was shown to be less successful (80%) after NCT when compared with no NCT (99%) (SENTINA), and the SLN identification rate in Z1071 in which all patients received NCT was 93%.

Purpose: To examine the effect of NCT, patient and disease characteristics, imaging and surgical technique on SLN localization rates in breast cancer patients undergoing chemotherapy.

Methods: Retrospective, single institution study was performed on patients who underwent surgery for breast cancer from January 2008 to December 2013. All patients who underwent SLN biopsy and either adjuvant chemotherapy (ACT) or NCT, were included. All patients underwent lymphoscintigraphy, and SLN biopsy was performed with the definitive breast surgery.

Results: 68 patients underwent NCT, and 133 underwent ACT. Our SLN localization rate was 198/201 (98.5%) overall; 98.6% (67 of 68) with NCT and 97.7% (130/133) with ACT (p=1.0). Compared with the NCT group, the ACT patients were significantly older, white, with more ER/PR positive tumors. The NCT group had more positive nodes on preop imaging (64% v. 20%, p<0.001), FNA (82% v. 22%, p<0.001), and a lower use of blue dye (37% v. 61%, p=0.05) but there were no differences in the number of SLN removed (1.43 v. 1.33 p=0.32), or nodes that were positive on intraoperative evaluation (30 v. 33%, p=0.75). Comparing the patients who had successful and failed SLN localization, there were no differences in demographics, tumor type, Stage, prior breast surgery, preoperative node positivity on imaging or FNA or timing of chemotherapy.

Conclusion: In this single institution series, SLN non-localization was a rare event and not associated with NCT. We were unable to identify any patient or disease characteristics, imaging or surgical techniques associated with SLN non-localization. The etiology of the lower SLN identification rates with NCT in multi-institutional trials remains to be elucidated.
Title: PET/CT might not improve the accuracy of sentinel lymph nodes biopsy and clip-containing nodes dissection to identify candidates for preserving axilla after neoadjuvant chemotherapy in HER2 positive breast cancer


Body: Background: The increasing proportion of axillary lymph nodes pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC) is particularly marked among HER2 overexpression patients which indicates the necessity to tailor the conventional axillary lymphnodes dissection (ALND) to a less invasive process. We designed this study to investigate the optimal process to avoid ALND in HER2 positive patients obtaining axillary pCR after neoadjuvant systemic treatment.

Material and methods: This prospective study enrolled locally advanced breast cancer patients with positive axillary lymph nodes validated by fine needle biopsy. All the patients launched PET/CT scanings before NAC and after 2/3 cycles treatment respectively and the SUVmax of involved LNs was recorded. Patients enrolled place a metal clip to mark the involved nodes since last December. After NAC, patients accepted sentinel lymph node biopsy (SLNB) as well as dissection of clip-containing LNs and ALND was performed eventually to all study population. Receiver operating characteristic (ROC) curve was performed to analyze results and selecting cut off values.

Results: There were 100 patients enrolled including 37 HER2 positive patients receiving final surgery. Here we analyzed the HER2-pos patients only. The overall axillary LNs pCR rate has obtained 86.5% (32/37) among HER2 positive patients after NAC. Of 37 patients, 31 accepted NAC plus trastuzumab, leaving the axillary LNs pCR rate as 87.1% (27/31). We analyzed the decreasing rate between the second SUVmax and the baseline (the formula is $\Delta$ SUVmax = (SUVmax-2-SUVmax-baseline/SUVmax-baseline). The area under the curve (AUC) was only 0.419, which revealed that $\Delta$ SUVmax might provide little guidance to assist us in finding the potential patients who could avoid ALND through NAC. 13 patients in our study accepted SLNB and 9 of them underwent SLNB plus clip-containing LN dissection. The SLNs detection rate was 84.6% (11/13) and the average number of detected SLNs was 2.1. All the marked LNs were removed during the operation through X-ray or ultrasound location just before surgery, making the detection rate of clip-containing LNs 100%. One exception was observed when the pathological outcome showed both negative SLN and marked nodes but another positive lymph nodes. Overall, the accuracy of SLNB and clip-containing LNs dissection was 81.8% (9/11) and 90.9% (10/11) respectively.

Conclusion: HER2 positive patients were the latent candidates to avoid ALND after NAC. PET/CT might have little value in screening the candidates, whereas SLNB plus clip-containing LNs dissection would be the optimal process in screening candidates to preserve their axilla. This study is still ongoing and it will provide more information about the other molecular subtypes in the near future.
Risk factors of non-sentinel lymph node metastasis in breast cancer patients with metastatic sentinel lymph node

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Objective To study the factors influencing the non-sentinel lymph node (NSLN) status and to assess Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram performance in predicting SLN metastases in a sentinel lymph node (SLN) positive Chinese breast cancer population. Methods Data were collected from breast cancer patients who were diagnosed with pathological positive sentinel lymph node and received further axillary lymph node dissection (ALND) in Shanghai Ruijin Hospital from January 2011 to August 2014. Use MSKCC nomogram to calculate each patient's NSLN metastasis risk score. The receiver operator characteristic curve (ROC curve) and the area under the ROC curve (AUC) was used to assess the predictive accuracy of the model. Results Among the 1147 patients who received sentinel biopsy in our center, 150 SLN positive patients who received ALND were enrolled in this study. By univariate analysis, multifocal breast cancer (P = 0.017), SLN+/SLN ratio (P = 0.010) and axillary lymphadenopathy displayed by ultrasound (P = 0.005) are the influencing factors of NSLN metastases. By multivariate analysis, multifocal breast cancer (OR 7.25, 95% CI 1.73–30.43, P = 0.007), SLN+/SLN ratio ≥0.5 (OR 2.564, 95% CI 1.22–5.39, P = 0.013) and axillary lymphadenopathy displayed by ultrasound (OR 2.471, 95% CI 1.18–5.19, P = 0.017) are the independent influencing factors of NSLN metastases. The AUC of MSKCC nomogram in this population is 0.677. Conclusion For breast cancer patients with positive sentinel lymph node, multifocality, SLN+/SLN ratio and axillary lymphadenopathy displayed by ultrasound is related to NSLN metastasis. MSKCC has low accuracy in predicting NSLN status of this population.
Title: A nomogram to predict the likelihood of axillary non-sentinel lymph node metastases in sentinel lymph node positive primary breast cancer patients

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Body: Background:
Completion of axillary lymph node dissection (ALND) was long lasting standard in patients with non-metastatic primary breast cancer (PBC) and axillary sentinel lymph node (SLN) involvement. However, this approach is currently questioned since recent prospective trials have found that some SLN-positive women with PBC might not benefit from subsequent ALND. Nomograms that estimate the probability of further non-sentinel lymph node (NSLN) metastases could guide clinical decisions.

Methods:
We compared the predictability of three different nomograms (MSKCC, Stanford University, Cambridge) on the NSLN status with an own logistic regression model in a training and validation set. SLN-positive women who underwent primary surgery including ALND at Tuebingen University, Germany, between 06/2005 - 12/2009 (training set) and 01/2010 - 02/2012 (validation set) were available for this analysis. The area under the receiver operating characteristics (ROC) curve was calculated for each nomogram.

Results:
295 and 175 patients were included into the training and validation set, respectively. Of these 118 (40%) and 57 (33%) patients were NSLN-positive. Variables within our model were tumor size, proportion of positive SLNs, size of SLN metastases, lymphangiosis carcinomatosa, multicentricity and extracapsular invasion. The respective area under the ROC curve values for the MSKCC / Stanford University / Cambridge / own regression model were 0.73 / 0.70 / 0.63 / 0.75 in the training set and 0.73 / 0.66 / 0.52 / 0.73 in the validation set.

Conclusion:
The predictability of our logistic regression model was comparable to the MSKCC nomogram and superior to the Stanford University and Cambridge nomogram. Although a training and a validation set was used, further testing on an independent patient population is warranted.
Body: Objective
To assess differences in axillary infiltration (global, sentinel, and non-sentinel lymph nodes) according to breast cancer molecular subtype.

Material and methods
Patients with infiltrating breast carcinoma diagnosed in the years 2011 to 2014 in our institution were included. Sentinel lymph node biopsy (SLNB) was performed in all patients staged N0 by clinical examination and axillary ultrasound. One-step nucleic acid amplification (OSNA) was performed in cases positive for cytokeratin-19. Complete lymphadenectomy was done in patients with axillary macrometastasis and, until February 2012, also in those with micrometastasis in the SLNB. Immunohistochemistry-based St. Gallen 2013 criteria were used to assign the molecular subtype (hormonal receptors, ki67 and HER2).

Results
Overall 720 patients were included, 53.9% of whom had axillary infiltration (75.8% macrometastases). Axillary infiltration was most common in the HER2 subtype -82.1% (91.3% macrometastases) and least common in the basal subtype -42.7%.

Global and Sentinel node

<table>
<thead>
<tr>
<th>FENOTYPE</th>
<th>LUMINAL A</th>
<th>LUMINAL B</th>
<th>LUM B HER2</th>
<th>HER 2</th>
<th>BASAL</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axilla</td>
<td>312</td>
<td>255</td>
<td>50</td>
<td>28</td>
<td>75</td>
<td>720</td>
</tr>
<tr>
<td>Negative</td>
<td>160 (51.3%)</td>
<td>103 (40.4%)</td>
<td>21 (42.0%)</td>
<td>5 (17.9%)</td>
<td>43 (57.7%)</td>
<td>332</td>
</tr>
<tr>
<td>Positive</td>
<td>152 (48.7%)</td>
<td>152 (59.6%)</td>
<td>29 (58.0%)</td>
<td>23 (82.1%)</td>
<td>32 (42.3%)</td>
<td>388</td>
</tr>
<tr>
<td>Macrom.</td>
<td>112 (73.7%)</td>
<td>115 (75.7%)</td>
<td>23 (79.6%)</td>
<td>21 (91.3%)</td>
<td>23 (71.9%)</td>
<td>294</td>
</tr>
<tr>
<td>Microm</td>
<td>40 (26.3%)</td>
<td>37 (24.3%)</td>
<td>6 (20.3%)</td>
<td>2 (8.7%)</td>
<td>9 (28.1%)</td>
<td>94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FENOTYPE</th>
<th>LUMINAL A</th>
<th>LUMINAL B</th>
<th>LUM B HER2</th>
<th>HER 2</th>
<th>BASAL</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sentinel Node</td>
<td>234</td>
<td>158</td>
<td>17</td>
<td>7</td>
<td>34</td>
<td>450</td>
</tr>
<tr>
<td>Negative</td>
<td>152 (65%)</td>
<td>100 (63.3%)</td>
<td>12 (70.6)</td>
<td>6 (85.7%)</td>
<td>22 (64.7%)</td>
<td>292</td>
</tr>
<tr>
<td>Positive</td>
<td>82 (35%)</td>
<td>58 (36.7%)</td>
<td>5 (29.4)</td>
<td>1 (14.3%)</td>
<td>12 (35.3%)</td>
<td>158</td>
</tr>
<tr>
<td>Macrom</td>
<td>44 (53.7%)</td>
<td>26 (44.8%)</td>
<td>2 (40%)</td>
<td>0 (0.0%)</td>
<td>4 (33.3%)</td>
<td>76</td>
</tr>
<tr>
<td>Microm</td>
<td>38 (43.3%)</td>
<td>32 (55.2%)</td>
<td>3 (60%)</td>
<td>1 (100%)</td>
<td>8 (66.7%)</td>
<td>82</td>
</tr>
</tbody>
</table>

On the contrary, the HER2 subtype had the lowest risk of axillary infiltration in patients subjected to SLNB (14.3%). No differences were seen in the other subtypes subjected to SLNB.

90 patients underwent complete lymphadenectomy. Positive non-sentinel lymph nodes were most commonly seen in luminal A tumors (30%).
Non Sentinel Nodes

<table>
<thead>
<tr>
<th>FENOTYPE</th>
<th>LUMINAL A</th>
<th>LUMINAL B</th>
<th>LUM B HER2</th>
<th>HER 2</th>
<th>BASAL</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non SN</td>
<td>50</td>
<td>33</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>Negative</td>
<td>35 (70%)</td>
<td>27 (81.8%)</td>
<td>2 (100%)</td>
<td>0</td>
<td>4 (80%)</td>
<td>68</td>
</tr>
<tr>
<td>Positive</td>
<td>15 (30%)</td>
<td>6 (18.2%)</td>
<td>0</td>
<td>0</td>
<td>1 (20%)</td>
<td>22</td>
</tr>
<tr>
<td>Macrom.</td>
<td>14 (93.3%)</td>
<td>5 (83%)</td>
<td>0</td>
<td>0</td>
<td>1 (100%)</td>
<td>20</td>
</tr>
<tr>
<td>Microm</td>
<td>1 (6.7%)</td>
<td>1 (16.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Conclusions

1. Significant differences in axillary infiltration were seen according to molecular subtypes.
2. Most HER2+ tumors had N+ disease detected by ultrasound. However, in those HER2+ with negative ultrasound, the SLNB was usually negative. If positive, non-sentinel lymph nodes were most commonly negative.
3. Half of luminal A tumors had N+ disease detected by ultrasound. In the other half, there was an increased risk of positive SLNB and non-sentinel lymph nodes with regard to other subtypes.
AIM
Evaluate within a pilot setup feasibility and safety of minimally invasive needle-biopsy of sentinel nodes guided by SPECT/US as compared to surgical removal while defining optimal needle for follow-up trial.

METHOD AND MATERIALS
As pretrial test phase of the MinimalSNB study, 38 breast cancer patients (6 centers) were taken a needle-biopsy of their sentinel lymph nodes (SLNs) under guidance of SPECT/US (SentiGuide by SurgicEye, Munich, DE). All patients were indicated for a surgical SLN biopsy which was performed immediately after the needle-biopsy. For the test phase, 4 different biopsy systems were tested: HistoCore 14G (BIP, Tuerkenfeld, DE), elite 10G and 13G (Mammotome, Cincinnati, OH, US) and CASSI II 10G (Scion Medical Technologies, Boston, MA, US). Histopathological examination (H&E, step-sectioning) of needle-biopsies and surgically removed SLNs were compared.

RESULTS
No single complication was reported. Occasionally, small hematomas could be found close to the SLN during surgery. Duration of complete procedure (imaging, needle placement, biopsy) took in average 17min. A learning curve was observed in duration (average after 5 biopsies 12min). 1-14 samples were taken of each SLN (average 5 samples). Final pathological examination of material harvested with both methods matched in 34 cases (33 negatives, 1 positive). The needle biopsy failed to detect metastases in 2 pN1 SLNs. In 1 case, the surgically resected tissue did not contain lymph nodes and the needle biopsy remained the only information on nodal status. In 1 case a metastasis found in needle-biopsy motivated a second reading of an originally negative SLN which resulted in the upstaging of the patient. In both cases a metastases was missed by needle-biopsy, the retrieved lymph tissue was minimal (1x 14G sample, 1x 10G sample tangential to node).

CONCLUSION
SPECT/US showed to be a valid method for percutaneous detection of SLNs and needle-guidance. Sampling SLNs with a needle seems safe and feasible. However it requires proper training and user experienced with axillary needle-biopsies. Retrieving more tissue (more cores and larger lumen needles) improves diagnostic power of needle-biopsy. These considerations will be taken within the upcoming MinimalSNB trial.

CLINICAL RELEVANCE
Sentinel lymph biopsy today is a surgical diagnostic procedure with a nonzero morbidity. Moving it out of the operating theatre to a needle-based intervention has a huge impact on the burden of this procedure for the patient as well as relevant improvements in logistics, workflow and radiation burden.
Purpose: To describe the ultrasound technique for tattooing axillary lymph nodes (ALNs) after lymph node (LN) biopsy in patients with breast cancer.

Background: Preoperative evaluation of metastatic disease within ALNs in patients with newly diagnosed breast cancer has significant prognostic value and is quickly becoming routine, particularly in the neoadjuvant setting. A recent study showed tattooed LNs are visible intraoperatively and on histological evaluation months following the tattooing procedure. These results suggest that LN tattooing can obviate the need for additional localization procedures during axillary staging, such as wire localization. Given the increasing use of preoperative ALN biopsy, a robust technique to insure proper LN tattoo marking is proposed.

Methods and Technique: Tattooing was performed under real-time US guidance using a 5-cm long 21-gauge hypodermic needle attached to a 1 mL tuberculin syringe containing 1 mL carbon suspension tattoo ink (SPOT™, GI-supply Inc).

Imaging was performed with the patient in a supine oblique position with the patient's arm over their head. The anatomically anterior and lateral aspects of the node and perinodal fat were marked with ink. The only regions of the LN not targeted for ink tattooing were the hilum and the posterior cortex and perinodal fat. At least 0.5 mL of ink was used.

Results: Optimal technique for intraoperative visualization was determined to be tattooing the anatomically anterior and lateral aspects of the LN cortex and the adjacent perinodal fat using at least 0.5 mL of ink.

Tattooed LNs which had undergone biopsy and tattooing months prior to surgery were visible intraoperatively and on histological evaluation. Factors contributing to less optimal visualization of the tattooed lymph node included: using less than 0.5 mL of ink, tattooing only the superficial cortex and not the perinodal fat, and tattooing a portion of the LN that was not visible with the patient in the operative position.

Discussion: The most easily accessed portion of the LN during the US procedure may not be the portion of the LN most easily seen intraoperatively. Locating and tattooing the anatomically anterior and lateral aspects of the LN, regardless of the patient position and orientation of the ultrasound probe, is the primary challenge. Doing so will maximize the likelihood that the tattoo ink will be visible by the surgeon when the patient is in a supine position with the arm abducted 90 degrees using an axillary incision. Tattooing using less than 0.5 mL resulted in suboptimal visualization. Using a larger volume of ink may be judged necessary for larger LNs, very fatty axillae, and for deeply seated nodes.

Other reports in the medical literature suggest cutaneous tattooing can result in ink within ALNs. In patients with ipsilateral cutaneous tattoos, an alternative method of marking any biopsied LNs should be considered to avoid false positives associated with prior migration of the cutaneous tattoo ink to the LN.

Tattooing of ALNs under ultrasound guidance is a straightforward technique which can be performed at the time of initial biopsy and obviates the need for future preoperative wire localization of the LN.
Title: Validation of a novel diagnostic kit using the semi-dry dot-blot method for detecting metastatic lymph nodes in breast cancer

Otsubo R, Hirakawa H, Oikawa M, Shibata K, Tanaka A, Matsumoto M, Yano H and Nagayasu T. Nagasaki University Hospital, Nagasaki, Japan; Chiba Aiyuukai Memorial Hospital, Chiba, Japan; Oikawa Hospital, Fukuoka, Japan and Japanese Red Cross Nagasaki Genbaku Hospital, Nagasaki, Japan.

Body: Background: The semi-dry dot-blot (SDB) method is a diagnostic procedure for detecting lymph node (LN) metastases. The metastases are visualized by the presence of cytokeratin (CK) with lavage fluid of sectioned LNs by anti-pancytokeratin antibody, based on the theory that epithelial components such as CK are not found in normal LNs. We previously reported 93.3% sensitivity, 96.9% specificity, and 96.6% accuracy for this method in detecting metastasis in sentinel LNs, compared with permanent pathological diagnosis in breast cancer. In this study, we evaluated a novel kit that applies the SDB method using the newly developed anti-CK19 antibody for diagnosing LN metastases in breast cancer.

Methods: We obtained 141 LNs dissected from 81 breast cancer patients from July 2013 to April 2015 at Nagasaki University Hospital and the Japanese Red Cross Nagasaki Genbaku Hospital, including 33 dissected axillary LNs and 108 sentinel LNs, which were sliced at 2-mm intervals and washed with phosphate-buffered saline. The suspended cells in the lavage fluid of sliced LNs were centrifuged to collect the cell pellet and lysed with lysis buffer to extract protein. This extracted protein was used with the kit to diagnose LN metastasis. The washed LNs were blindly diagnosed by pathologists using hematoxylin and eosin (H&E) stain. Diagnoses based on the kit were compared with their H&E counterparts.

Results: Of the 141 LNs, 57 were assessed as positive and 84 as negative by permanent pathological examination with H&E. Use of the kit resulted in correct diagnoses in 46 of the 57 pathologically positive cases and all of pathologically negative cases. Sensitivity, specificity and accuracy of the kit in detecting LN metastases were 80.7% (95% confidence interval [95% CI]: 75.6–80.7%), 100% (95% CI: 96.5–100%), and 92.2% (95% CI: 88.1–92.2%), respectively. In 11 false-negative cases, there were 9 micrometastases; therefore, sensitivity was 95.5% (95% CI: 90.1–95.5%) in cases of macrometastases. Diagnosis was achieved in approximately 20 min using the kit, reducing the diagnostic time by half compared with the original SDB method. The cost of this kit was within 8 USD, and we are currently developing an improved kit for the detection of smaller metastases.

Conclusions: The kit in our study is accurate, quick, and cost-effective in diagnosing LN metastases without the loss of LN tissue. Its sensitivity in detecting macrometastases is excellent, which is important in clinical practice.
Title: Upper lateral boundaries of the axillary dissection. Result of the SENTIBRAS protocol: Multicentric protocol using axillary reverse mapping in breast cancer patients requiring axillary dissection

Nos C, Bonnier P, Clough K, Lasry S, Le Bouedec G, Flipo B, Classe J-M, Missiana M-C, Doridot V, Giard S, Martel P, Charles-Nelson A and Ngô C. Hôpital Européen Georges Pompidou APHP, Paris, France; Hôpital Privé Beauregard, Marseille, France; Institut du Sein, Paris, France; Institut Curie, Saint Cloud, France; Centre Jean Perrin, Clermont-Ferrand, France; Centre Antoine Lacassagne, Nice, France; Institut de Cancérologie de l'Ouest René Gauducheau, Saint-Herblain, France; Centre Hospitalier Princesse Grace, Monaco, Monaco; Centre République, Clermont-Ferrand, France; Department of Breast Surgery, Centre Oscar Lambret, Lille, France; Institut Claudius Regaud, Toulouse, France and Clinical Research and Epidemiology Unit, Hôpital Européen Geroges Pompidou APHP, Paris, France.

Body: Background: Two thirds of node-positive breast cancer patients have limited pN1 disease and could benefit from a less extensive axillary lymph node dissection (ALND). Recent controversies about management of the axilla lead to a binary decision between sentinel node biopsy (SNB) or ALND. It might be useful, for selected patients, to have a third intermediate option. Patients and methods: From 2009 to 2012, a selection of 172 breast cancers patients requiring an ALND were prospectively enrolled in the French Sentibras Protocol of Axillary Reverse Mapping (ARM) in 11 centers. All patients had breast carcinoma with features that excluded a sentinel node biopsy (SNB) according to French guidelines. We used clinical examination and imaging to select patients with limited axillary disease, N1 or small N2. Clinical N3 patients or patients N2 with 4 or more suspicious LNs on MRI, PET-CT or CT were excluded. Among the 172 patients, 33 (19.2%) patients required an ALND after a positive SNB, 68 (39.5%) patients received neo-adjuvant chemotherapy (NAC) and 71 (42.3%) patients were clinically N+ or had multicentric tumors or tumor greater than 5 cm. Radioisotope was injected in the ipsilateral hand the day prior to surgery. The usual technique for ALND was not modified. Removed lymph nodes were classified into three groups: non radioactive nodes, radioactive nodes draining the upper limb located in the upper outer part of the axilla, above the second intercostal brachial nerve and lateral to the lateral thoracic vein (we called them the SENTIBRAS nodes) and radioactive nodes located in other parts of the axilla. The main objective was: feasibility of identification of the "Sentibras" nodes. Secondary objectives were: metastatic involvement of the "Sentibras" nodes and the lymphedema rate.

Results: The "Sentibras nodes" were identified in 92 % of cases (158/172). Mean number of removed nodes was 14.6 [4-32]. Mean number of radioactive nodes was 4.8 [1-14]. Mean number of SENTIBRAS nodes was 1.8 [1-8]. The global rate of metastatic nodes was 72%. 9.4% of the SENTIBRAS nodes were metastatic. In the SENTIBRAS group, metastatic rate was 4.8% in pN1 patients versus 33% in the pN2/N3 patients (p<0.05). The rate of positive nodes in the SENTIBRAS group was 7.4% in patients undergoing ALND for a positive SNB, 8.8% in case of primary ALND and 21.4% after NAC. After 24 months of median follow up, progression-free survival was 85%. 28 % of patients have a lymphedema and up to 50% of patients complain about their arm.

Conclusion: The ARM technique reliably identifies the "Sentibras nodes". These nodes can also easily be identified using knowledge of axillary anatomy. In selected patients, N1 or N2 < 4 identified by pre-operative imaging and without neo-adjuvant chemotherapy, a less morbid selective ALND sparing the SENTIBRAS nodes could be performed.
Title: The management of axillary lymph nodes guided by ultrasound in patients with early breast cancer: A systematic review, a retrospective analysis of 1200 patients and a classification proposal


Body: Background: Axillary ultrasound is a widely accepted preoperative lymph node staging method with relatively high accuracy and cost-effectiveness. Nevertheless, there is no classification system to guide the management of lymph nodes like the Breast Imaging Reporting and Data System (BI-RADS), which provides a standardized classification for breast imaging findings.

Method: An ultrasound classification system was developed by a committee including breast surgeons, physicians and ultrasound specialists. We performed a systematic literature search regarding the diagnostic accuracy of various ultrasonographic appearance including size and morphology changes in lymph nodes. Based on their positive predictive value (PPV), a preliminary recommendation for the categorization of each finding was provided. Cases with detailed ultrasonographic description of lymph nodes during 2012 to 2014 at our institution were reviewed to assess the malignancy predictive value of this novel classification system.

Results: A final assessment system including 5 categories (category 0, 3, 4, 5, 6) was created on consensus. The linking of assessment categories with concordant management recommendations was recommend to further enhance sound medical practice.

Concordance Between Assessment Categories and Management Recommendations

<table>
<thead>
<tr>
<th>Category</th>
<th>Assessment</th>
<th>Management Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Incomplete (Prior breast or axillary surgery, infection)</td>
<td>Ultrasound unreliable</td>
</tr>
<tr>
<td>3</td>
<td>Probably Benign</td>
<td>SLNB* (Short-interval follow-up optional in selected patients (Evidence not sufficient))</td>
</tr>
<tr>
<td>4</td>
<td>Suspicious</td>
<td>Needle biopsy* when feasible/SLNB* (if needle biopsy not feasible or with negative result)</td>
</tr>
<tr>
<td>5</td>
<td>Highly suspicious of metastasis</td>
<td>Definitive axillary surgery (with or without needle biopsy) when clinically appropriate</td>
</tr>
<tr>
<td>6</td>
<td>Proven metastasis</td>
<td>Definitive axillary surgery when clinically appropriate</td>
</tr>
</tbody>
</table>

SLNB, Sentinel lymph node biopsy; *, Subsequent definitive surgery choice depends on biopsy results.

Meta-analyses revealed that the PPV of a rounded shape, cortical thickening and hypoechoogenicity were 46%, 58% and 93% relatively. We concluded that nodes with a rounded shape or cortical thickening were assessed as category 4, and nodes with hypoechoogenicity were assessed as category 5. Nodes with thin cortex or no visible nodes were categorized as category 3. The axillary ultrasound records of 1200 patients were reviewed and retrospective assessments on the diagnosis were performed, linking to definitive histological results of axillary lymph nodes. The PPV of category 3, 4, 5 were 8.9%, 53% and 93.5% relatively.

Conclusion: Categorized axillary lymph node assessment linking to concordant management recommendations is feasible in current practice. Prospective validation should be carried out in future.
Title: Is lymph node ratio (LNR) having additional contribution for predict prognosis on pathologic lymph node staging in node-positive breast cancer patients?

Kaplan MA, Odabasi H, Ozdemir N, Harputluoglu H, Aliustaoglu M, Berk V, Gunaydin Y, Uncu D, Elkiran T, Aydin D and Isikdogan A. Dicle University, Diyarbakir, Turkey; Kartal Research and Education Hospital, Istanbul, Turkey; Numune Research and Education Hospital, Ankara, Turkey; Inonu University, Malatya, Turkey; Erciyes University, Kayseri, Turkey and Gazi University, Ankara, Turkey.

Body: PURPOSE:
The aim of the study was to determine whether LNR have additional contribution on pathologic lymph node staging.

METHODS:
To examine the prognostic value of LNR examined the original histopathological reports of 2049 node-positive breast cancer patients treated in the references centers of the Turkey. The LNR was defined as the number of positive lymph nodes (LN) over the total number of LNs removed. The LNR cutoffs were defined as low-risk, 0.01-0.20; intermediate-risk, 0.21-0.65; and high-risk, LNR >0.65.

RESULTS:
The median follow-up was 11.8 years. Median Disease free survival (DFS) was 191.8, 110.6 and 78.2 months in patients with pN1, pN2 and pN3 tumor, respectively (p<0.001). Median DFS was 191.9, 106.4 and 78.1 months in patients with LNR low, intermediate and high risk tumor, respectively (p<0.001). Median DFS was not reached and 200.1 months in patients with pN2 and LNR low risk patients, pN1 and LNR high risk patients, respectively (p=0.254).

CONCLUSIONS:
LNR is an important prognostic parameter for DFS and might provide potentially more information than pN-stage in patients with pN1/LNR high risk and pN2/LNR low risk.
Title: Sentinel lymph node metastases in breast cancer: A contributor to distant metastases?

Pereira ER R, Kedrin D, Jones D, Beech E, Taghian A G and Padera TP. Massachusetts General Hospital, Boston, MA and Harvard Medical School, Boston, MA.

Body: Breast cancer metastasis remains a major cause of mortality in patients. Although significant progress has been made in understanding the mechanisms of this complex process, the findings have yet to be translated into improved survival rates in patients with metastatic disease. The presence of lymph node metastasis in most breast cancer patients is associated with tumor aggressiveness, poorer prognosis and often results in the need for systemic therapy. However, whether tumor cells in the lymph node exit and contribute to distant metastases remains controversial. To track the fate of tumor cells that metastasized to the lymph node, we engineered breast tumor cell lines to express Dendra2, a photo-convertible protein that fluoresces green in the native state and on light activation converts to red fluorescence. Using a novel chronic lymph node window that allows time-lapse imaging over a period of 10 days, we were able to track the movement of cancer cells in the dynamic microenvironment using high-resolution multi-photon microscopy. Our studies show that tumor cells entering the lymph node through the afferent lymphatic vessel proliferate in the sub-capsular sinus and later begin to invade the lymph node parenchyma. Importantly, by photo-conversion of tumor cells in the lymph node, we are able to track the tumor cells that escaped the lymph node and entered the blood circulation. The circulating tumor cells that transited through the lymph node were viable and proliferated in vitro. Our data indicate that metastatic breast cancer cells can exit the node and potentially colonize distant organs.
Title: uPA and PAI-1 expression in breast cancer

Park YL, Kim EY, Hyun K, Byun W and Park CH. Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

Body: To evaluate expression of uPA and PAI-1 as reliable prognostic markers in breast cancer, we analyzed the demographic and clinicopathological parameters of 214 patients with breast cancer, diagnosed and treated from 2006 to 2010 at Kangbuk Sam Sung Hospital. We used immunohistochemistry as a detection method. In univariate analyses, age at diagnosis, history of hormone replacement therapy, radiation therapy, skin/chest wall invasion, Paget disease, lymphovascular invasion, ER positivity and triple negative subtype had statistically significant influences on patient prognoses (P<0.05). The DCIS group had higher PAI-1 expression levels than did the IDC group (94% and 63%, respectively) (P=0.012). A positive correlation was established between uPA and PAI-1 (HR= 2.608, P=0.002). Lymph node metastasis was more frequent in the group with high uPA levels, compared to the group with low uPA levels. This finding was statistically significant (P=0.001). In conclusion, it is possible that PAI-1 could play some role in tumor progression in the early stages of breast cancer, such as DCIS. The demonstration of a statistical correlation between lymph node metastasis and uPA/PAI-1 levels determined by immunohistochemical evaluation, suggests that this method could be used as a prognostic factor in patients with breast cancer.
Targeting the tumor microenvironment: A phase II study of copper-depletion using tetrathiomolybdate (TM) in patients (pts) with breast cancer (BC) at high risk for recurrence


Background: Bone marrow derived VEGFR2+ endothelial progenitor cells (EPCs) and copper-dependent pathways, including lysyl oxidase (LOX), are critical components to remodeling the tumor microenvironment and establishing the pre-metastatic niche. In preclinical models, it has been well established that copper depletion (CD) inhibits tumor progression. We hypothesized that TM-associated CD would reduce EPCs and other copper dependent processes in the pre-metastatic niche in BC pts at high risk for relapse. We investigated the relationship between CD and its effect on EPCs and other components of the tumor microenvironment including LOX, an enzyme critical for cross-linkage of collagen and priming the pre-metastatic niche.

Methods: In this single arm, phase II study, BC pts at high risk for recurrence, defined as node+ triple negative (TN), stage 3 and 4 with no evidence of disease (NED) were enrolled on a trial of CD with TM. Pts received oral TM to maintain ceruloplasmin (Cp) between 5-17 mg/dl for 2 years on the primary study. The primary endpoint was change in EPCs measured by flow cytometry before and during treatment with TM. Secondary endpoints included tolerability, safety and effect of copper depletion on other markers including LOX, quantified by ELISA.

Results: We enrolled 75 pts. The study treatment duration was 24 cycles (each cycle is 28 days). Over 2200 cycles have been administered. The median age is 51 (range 29-66). 45 pts have Stage 2/3 BC and 30 are Stage 4 NED. TNBC pts represent 48%, and 40% of pts are Stage 4 NED. Median Cp level decreased from 28 at baseline to 15.5 (p<0.0001) after one cycle. Copper depletion was most efficient in TNBC, with 91% achieving a target CP within 4 weeks. TM was well tolerated and the only grade 3/4 toxicities were reversible neutropenia (3.2%) and anemia (0.0005%). CD was associated with a significant decrease in EPCs (p=0.0014) and LOX (p<0.001). At a median follow-up of 5.4 years, the PFS for all 75 pts from the start of TM treatment was 71%, including a PFS of 90% for all stage 2/3 pts with TNBC. The overall survival of all patients enrolled in the trial is 86%. Relapse after two years is a rare event. Conclusions: TM is safe, well tolerated and appears to affect multiple copper dependent biologic processes in the tumor microenvironment known to be important for tumor progression. This seems to be most striking in TNBC. We believe, further phase III trials in a high risk for relapse population are warranted.
Title: Intracranial PDX models of breast cancer metastasis

Nedergaard MK, Wick MJ J, Papadopoulos K, Tolcher AW W, Kjaer A and Nielsen CH H. Minerva Imaging, Copenhagen, Denmark; Rigshospitalet, University of Copenhagen, Copenhagen, Denmark and South Texas Accelerated Research Therapeutics (START), San Antonio, TX.

Body: Background: Overexpression of the human epidermal growth factor receptor 2 (HER2) in breast cancer is an independent factor for development of brain metastases. Up to 37% of patients with HER2 positive disease relapse intracranially despite control of extra-cranial metastatic disease. Inability of anti-cancer agents to cross an intact blood-brain barrier (BBB) is a possible explanation for the increased incidence of brain metastases. Subcutaneous (SQ) patient-derived xenograft (PDX) models are increasingly used for efficacy studies in drug development. However, orthotopic PDX models may confer a translational advantage as the patient tumor microenvironment is more closely mimicked. Especially when targeting brain tumors, the major impact of the BBB on drug bioavailability must be taken into consideration. The aim of this study was therefore to develop a panel of intracranial PDX models of breast cancer brain metastases for pre-clinical efficacy studies of new anticancer drugs.

Methods: SQ tumors from three different HER2 positive PDX breast cancer models designated ST340, ST1339 and ST1616B were enzymatically digested and used for intracranial stereotactic injection in nude mice. Contrast-enhanced T1- and T2-weighted Magnetic Resonance Imaging (MRI) were used to determine tumor take. Intracranial tumor growth was monitored using MRI and positron emission tomography (PET) in conjunction with the amino acid radio tracer ^18^F-FET.

Results: MRI confirmed tumor take in one model as early as 2 weeks after intracranial implantation. Increased ^18^F-FET uptake was detected in all models. MRI could be effectively used to monitor tumor growth and the corresponding ^18^F-FET PET images demonstrated increased ^18^F-FET uptake over time.

Conclusion: Three different HER2 positive intracranial PDX breast metastases models were established from low passage SQ PDX models. We suggest, that using these intracranial PDX models of brain metastases, new drugs for advanced breast cancer can be evaluated in preclinical models that more closely mimic the microenvironment and the BBB in patients. In addition, translational imaging techniques can be evaluated during preclinical testing and the potential of tracers like ^18^F-FET as imaging biomarkers of therapeutic response can be assessed. Together, the established SQ and orthotopic PDX models of breast cancer and brain metastases can be used as a relevant translational platform for testing of new drugs.
Title: Identification of molecular pathways to define the intake rate of patient-derived hormone receptor positive (HR+) breast cancer xenografts (PDXs) in NOD/SCID/interleukin-2 receptor gamma chain null (NSG) mice


Body: Background and Purpose: Despite recent progress in our endocrine therapy of hormone receptor positive (HR+) breast cancers, a significant number of patients with primary breast cancer continue to relapse, and those with stage IV disease face a median overall survival of ~ 3.5 years. Primary or acquired resistance to anti-estrogen-based therapies is an overarching challenge. To guide our treatment selection, there is an essential need to improve our understanding of the biology of HR+ breast tumors responsive to and those resist to anti-estrogens or aromatase inhibitors (AIs). The application of patient-derived xenografts (PDXs) in preclinical studies has begun to open the door to mimicking human disease on the research bench. However, HR+ breast cancer PDXs are difficult to establish. Although preclinical data from DeRose et al [Nat. Med. 2011: 17:1514-1520] indicate that the rate of engraftment serves as an independent predictor for poor outcome, the question which has not yet been adequately addressed is: "why some tumors can grow in mice, and some don't, even when their clinical, pathological stage and subtype (i.e. ER positivity) are same?" Here, we hypothesize that the molecular characteristics of patient HR+ tumors are key determinants to the tumor intake rate in NOD/SCID/interleukin-2 receptor gamma chain null (NSG) mice. Hence, reverse phase protein array (RPPA) analysis has been performed using human patient tumors to identify driver-pathways that impact tumor intake in NSG mice.

Results and Discussion: We compared the protein expression profile of six HR+ patient tumors (four HR+ and two HR+ HER2+), which were successfully engrafted into NSG mice and established as PDX models, with the patient tumors which we were unable to establish as PDX. Of 90 patient HR+ tumors which failed to transplant, 21 tumors were picked to match the tumor type (all of them were invasive ductal carcinoma or its metastases), clinical stage and pathological grade of engrafted tumors [Table 1]. In addition to patient tumors, six established HR+ PDXs were also submitted for analysis. Quantified expressions of 272 cancer-related proteins and phospho-proteins by RPPA have been performed on these specimens. Pathways identified as predictors of intake rate of PDXs in NSG mice, and tissues from paired PDX from mice with different passages, will be evaluated for the protein expression changes to elucidate the passage effects and generate therapeutic models based on protein expression and tumor growth.

Table 1. Characteristics of the patient tumors which were successfully established as PDX models

<table>
<thead>
<tr>
<th>ER</th>
<th>PgR</th>
<th>HER2</th>
<th>Age</th>
<th>Patient ethnicity</th>
<th>Clinical stage</th>
<th>Nottingham histologic score</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>63</td>
<td>Hispanic</td>
<td>3</td>
<td>III</td>
<td>Breast tumor</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>71</td>
<td>Hispanic</td>
<td>2</td>
<td>III</td>
<td>Breast tumor</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>52</td>
<td>African-American</td>
<td>4</td>
<td>N/A</td>
<td>Brain mets</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>63</td>
<td>Caucasian</td>
<td>4</td>
<td>N/A</td>
<td>Chest wall mets</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>34</td>
<td>Caucasian</td>
<td>2</td>
<td>II</td>
<td>Breast tumor</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>72</td>
<td>Caucasian</td>
<td>4</td>
<td>III</td>
<td>Chest wall mets</td>
</tr>
</tbody>
</table>

mets: metastases
Title: A mouse model of sporadic breast tumor with a conditional P53 mutation

Zhang Y, Xiong S, Pant V, El-Naggar A and Lozano G. UT MD Anderson Cancer Center, Houston, TX.

Body: Missense mutations in the tumor suppressor gene p53 are present in more than 50% of human tumors. In particular, the arginine-to-histidine mutation at codon 175 (p53R175H) has been found in more than 4% of human breast cancers. p53 mutations fall into two general categories: germline mutations that are associated with hereditary tumors, and somatic mutations that cause sporadic tumors. Mouse models for cancers induced by p53 germline mutations have been established and characterized. However, hitherto there are no accurate animal models for sporadic tumors induced by p53 somatic missense mutations, although this mechanism of inactivating p53 occurs in a large fraction of human cancers.

We have generated a mouse allele, p53WM, that carries coding sequences for both wild type (WT) and R172H mutant p53 (corresponding to R175H in humans) at the p53 endogenous locus. The coding sequence for WT p53 is flanked by loxP sites and therefore can be deleted by Cre recombinase. In the absence of Cre, following radiation, the p53WM/+ mice have the similar ability as the p53 WT mice to activate p53, as demonstrated by transcriptional activation of p53 targets and induction of apoptosis. In addition, like WT mouse embryonic fibroblasts (MEFs), the p53WM/+ MEFs also underwent cell cycle arrest following radiation.

After crossing p53WM/+ mice with Zp3-Cre mice which express the Cre recombinase in oocytes, the coding sequence for WT p53 was removed, and the mutant p53R172H allele was regenerated as determined by sequencing, western blot analysis and real time reverse transcription PCR.

To investigate whether a somatic p53R172H mutation in mammary epithelium can induce breast tumors, adenoviruses expressing Cre (Ad-Cre) were injected into the mammary duct of p53WM/+ mice. Limiting the adenovirus dose allowed us to induce the p53R172H mutation in 1 of 20, 100 or 1000 cells, generating a mutant p53 surrounded by normal cells which more accurately mimics the clinical situation of sporadic tumors. Meanwhile, p53WM/+; K14-Cre and p53WM/+; WAP-Cre mice have been generated, in which p53R172H mutation are induced through Cre transgene specifically in the mammary epithelial cells. Lastly, to examine how somatic p53 mutation cooperates with an oncogene in mammary tumor induction, MMTV-neu; p53WM/+ mice were also subjected to the mammary intraductal Ad-Cre injection. We are currently monitoring mice and will further investigate the molecular mechanisms of tumorigenesis by studying the loss of heterozygosity, and the co-evolution of tumor epithelial cells and their microenvironment. This mouse model is more likely to share the underlying molecular pathology with human sporadic tumors. Therefore, it will be more predictive of human responses to drugs, and thus a more valuable tool in preclinical testing.
The estrogen receptor (ER or ESR1) drives proliferation and growth of luminal type breast cancers. Endocrine therapies are highly effective in a majority of these cancers types; however, disease progression eventually occurs due to acquired resistance resulting in hormone-independent breast cancer. One mechanism of resistance is acquired mutations at codons 537 and 538 in the ligand binding domain of the receptor which are found in ∼12% of pretreated, ER+ patients. These mutations result in constitutive ER activation and hormone-independent progressive disease. Although ESR1 mutated breast cancers are insensitive to endocrine therapy, novel agents targeting these mutations may be effective; however few preclinical models of ESR1-mutant breast cancer are available for preclinical analysis. To this end we have established two patient derived xenograft (PDX) models harboring mutations at codon 537; ST941 with ESR1Y537S and ST1799 with ESR1Y537C. We have characterized these models using genomic analysis and compared with analysis from paired or serially collected clinical tissue and blood. Dependence on estradiol for model growth and sensitivity to endocrine therapies were also evaluated and compared with a hormone-dependent breast cancer models. The ESR1 mutations were not present in primary clinical tissue while several additional mutations were identified including in TP53 and PIK3CA genes. In vivo the ESR1 mutant models grew in the presence or absence of exogenous estradiol and demonstrated reduced sensitivity to endocrine therapies compared with hormone-dependent breast cancer models. ST941 treated with tamoxifen, letrozole or fulvestrant reported moderate tumor growth inhibition versus control while tumor stasis or regressions were reported in MCF-7 and hormone-dependent PDX breast models. Overall we have established and characterized two models of ESR1 mutant breast cancer which can be utilized for development of targeted therapies.
Establishment and evaluation of ER+ breast cancer models using an optimized methodology for exogenous hormone delivery


Body: Preclinical in vivo models of estrogen receptor positive (ER+) breast cancer rely on exogenous supplementation of hormones for growth. This requirement leads to animal toxicity and mortality over time, limiting development and drug testing in these types of models. Efficacy of test agents, particularly endocrine therapies, may also be altered in these models due to excessive hormone exposure, highlighting the need to improve methods for the establishment and testing of ER+ breast models. We have developed an alternative method of hormone supplementation in ER+ breast cancer models and optimized this method for testing of endocrine therapies. Using two cell-based breast models, we demonstrated improved breast tumor take and time to tumor volume endpoint while reducing animal toxicity and mortality associated with standard hormone supplementation. Subsequent studies identified the lowest effective dose (LED) of supplement for hormone dependent model growth with a preclinically relevant time to tumor volume endpoint. Activity of endocrine therapies including tamoxifen, letrozole, fulvestrant and exemestane were compared at the standard and LED hormone concentrations. In these studies tamoxifen treatment resulted in tumor regressions which was not appreciably improved using the LED dose of supplement. However letrozole activity was improved in the LED study suggesting hormone supplementation can impact activity of some agents. Using this process we also generated a panel of ER+ patient-derived xenograft (PDX) models, including two novel hormone therapy responsive models from chemo-naïve or hormone therapy pretreated patients, designated ST986 and ST2177, respectively. This improved method of hormone supplementation diminishes the adverse effects of standard hormone supplementation and provides utility for development of anticancer therapies in ER+ breast models.
Title: Manual and digital detection of HER2 status of 2721 circulating tumour cells in patients with metastatic breast cancer

Brouwer A, van de Wiel M, Peeters B, van Dam P-J, Vermeulen P, Peeters M, Van Laere S, Peeters D and Dirix L. Center for Oncological Research (CORE), University of Antwerp, Antwerp, Belgium; GZA Hospitals Sint-Augustinus, Antwerp, Belgium; Antwerp University Hospital, Antwerp, Belgium; GZA Hospitals Sint-Augustinus, Antwerp, Belgium and Antwerp University Hospital, Antwerp, Belgium.

Body: Introduction:
In metastatic breast cancer (MBC), discordant expression levels of the human epidermal growth factor receptor 2 (HER2) have been noted between primary tumours (PT) and matched metastatic lesions (Meta). Therefore reassessment of this predictive marker at time of metastatic disease might help to optimize treatment. Circulating tumour cells (CTCs) offer the potential to provide a repeatedly accessible source of tumour cells for the real-time assessment of actual tumour characteristics.

Here we report on a retrospective study analysing over two and a half thousand CTCs in order to evaluate the inter-observer variability when using the semi-quantitative scale (0-3+) described by Riethdorf in 2010. Furthermore we designed a digital scoring system using 13 parameters selected by ImageJ. HER2 status in CTCs was compared to PT and/or Meta of patients with MBC.

Materials and methods:
65 patients starting first or second line systemic therapy for MBC and harbouring more than 5 CTC/7.5 mL blood were selected. HER2 status of 2721 CTCs was determined by immunofluorescence using the CellSearch system. HER2 status of the solid lesions was determined by IHC or FISH. Inter-observer variability was calculated using the Kendalls tau tests. 284 CTCs were analysed with the digital scoring system using ImageJ 'plot profile' and 'analyse particles'. Selected parameters comprise cell size, mean and maximum intensity of the cell and its surrounding, and both ratio's and differences of the aforementioned. Dissimilarity matrix was calculated using Pearson Correlation-Distance.

Results:
Of 2721 CTCs, 1485 cells (55%) were scored 0+ and 2263 cells (83%) were found to be HER2- (0+ or 1+) by both observers. 458 cells (17%) were scored HER2+ (2+ or 3+) by at least one of the observers, however only 175 (6%) by both observers.

Inter-observer variability was 0.703, but when omitting the usually undebatable 0+ cells, this variability showed to be 0.278.

<table>
<thead>
<tr>
<th>HER2- (0+/1+)</th>
<th>HER2+ (2+/3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2263 (83%)</td>
<td>131 (4.8%)</td>
</tr>
<tr>
<td>152 (5.6%)</td>
<td>175 (6.4%)</td>
</tr>
</tbody>
</table>

24 of 65 patients had at least 80% 0+ CTCs (≥96% HER2- cells). Of these patients, 5 were HER2+ based on their PT. Oppositely, 10 and 20 patients harboured at least 40% and 10% HER2+ CTCs respectively. From these 20 patients only 10 were diagnosed with HER2+ disease on PT or Meta and 1 was shifted form HER2- PT to HER2+ Meta.

The digital scoring system was able to identify four groups with different HER2 expression levels. When comparing the identified clusters with the manually scored cells the two moderately related clusters showed to contain almost only 2+ and 3+ CTCs. A very isolated cluster contained almost solely 0+ CTCs.

Discussion:
The manual scoring system showed to be feasible, however we noticed that there are some discrepancies regarding the scoring of 1+ to 3+ cells. The digital scoring is able to predict the outcome and can by itself cluster CTCs into 4 groups. It strongly distinguishes between the HER2+ and HER2- cells. HER2+ status can change during disease progression, both with gain and
loss of HER2 positivity. This can be monitored using CTCs.
Title: Invasive lobular carcinoma cell lines utilize WNT4 signaling to mediate estrogen-induced growth

Sikora MJ J and Oesterreich S. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Body: Invasive lobular carcinoma (ILC) is a histological subtype of breast cancer representing 10-15% of newly diagnosed breast tumors. Over 90% of ILC are estrogen receptor (ER)-positive, however, endocrine response and estrogen signaling are not well understood in ILC. Retrospective analyses suggest that ILC patients treated with endocrine therapy have poorer outcomes than invasive ductal carcinoma (IDC) patients, and that ILC patients may not benefit from adjuvant tamoxifen. Based on these observations, we hypothesize that ER regulates unique signaling pathways in ILC cells that control growth and endocrine response.

To identify putative targets that regulate endocrine response in ILC, we assessed genome-wide ER-mediated gene expression and ER genomic binding in the ILC cell lines MDA MB 134VI (MM134) and SUM44PE (SUM44). Among ILC-specific estrogen-regulated genes, the most strongly induced was the secreted ligand WNT4. Additionally, we identified an ILC-specific ER binding site (ERBS) at WNT4, suggesting that WNT4 is directly controlled by ER in ILC cells. Direct ER-regulation of WNT4 is in contrast to control of WNT4 by progesterone receptor (PR) during mammary gland development; however, further ER ChIP experiments suggest that ILC cells place WNT4 under ER control via unique use of the WNT4 ERBS. Thus, ILC cells may hijack a tightly regulated developmental program to drive estrogen-regulated cell phenotypes.

Based on the role of WNT4 in mammary gland growth and expansion, we hypothesized that WNT4 is required for estrogen-induced growth in ILC cells. To test this, we used siRNA to knock down WNT4 in ILC and IDC cell lines. Using either of two siRNAs, WNT4 knockdown completely blocked estrogen-induced growth in ILC cells, but not IDC cells. Consistent with this, WNT4 knockdown abrogated estrogen-regulation of a subset of ER-target genes in MM134 cells, suggesting that these genes are downstream of WNT4 signaling. Wnt signaling typically acts via the canonical, β-catenin-dependent pathway; however, we observed that β-catenin is dysfunctional in ILC cells, and WNT4 cannot activate β-catenin signaling in cancer cell lines. This suggests that WNT4 regulates estrogen-induced growth in ILC cells via a novel non-canonical signaling pathway.

Clinical observations suggest that ER regulates unique downstream pathways in ILC. We identified WNT4 as a putative downstream effector of ER signaling in ILC, as WNT4 is strongly-induced and directly regulated by ER specifically in ILC cells. Further, WNT4 is necessary for estrogen-induced growth in ILC cells, and likely signals via a non-canonical signaling pathway. Targeting WNT4 signaling represents a novel approach to modulate endocrine response specifically for ILC patients. Future studies will focus on characterizing the signaling pathway controlled by WNT4 in order to identify putative therapeutic targets.
Title: Investigating the therapeutic use of estrogen receptor β agonists in breast cancer

Samayoa C, Krishnegowda NK K, Vadlamudi R and Tekmal RR R. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: In breast cancer, estrogen receptor status reflects the biology of the tumor and is used to determine the likelihood of responding to endocrine therapy. 70% of all breast cancers express Estrogen Receptor α, which is indicative of estrogen dependence for growth. Current therapies aim to lower estrogen levels, or inhibit estrogen receptor signaling to ultimately prevent recurrence. However, only two-thirds of ERα-positive respond to endocrine therapy. The Estrogen Receptors are ligand-activated transcription factors with similar structures that slightly differ in their DNA binding sites and ligand binding domains. Estrogen Receptor α has been shown to induce the proliferation of mammary gland cells, whereas Estrogen Receptor β exhibits tumor suppressive properties. The objective of this study was to investigate the utility of selective ERβ agonists as a treatment for breast cancer. Using different breast cancer models, we investigated the effects of ERβ agonist treatment on growth, migration, apoptosis, cell cycle distribution and gene expression. In this study, we demonstrate that treatment with ERβ agonist results in inhibition of cell growth and migration. Additionally, treatment also impacts cell cycle distribution and affects key proteins involved in cell cycle regulation. We also demonstrate an increase in ERβ mRNA and protein levels upon treatment. This shift in the ERβ to ERα ratio, represents a viable targeting strategy in the treatment of breast cancer. To determine if our finding translate in-vivo, we employed xenograft and syngeneic mouse models. Our in-vivo studies demonstrated reduced tumor volumes in mice treated with ERβ agonists in combination with conventional therapy, confirming the growth inhibitory activity of ERβ agonists. Selective activation of ERβ, through the use of ERβ agonists has enabled us to exploit its tumor suppressive function and investigate the utility as a treatment for breast cancer. This study suggest that activation of ERβ signaling is a valuable strategy to inhibit breast cancer growth and progression.
Title: Estrogen receptor β5 increases aggressiveness of the triple negative breast cancer cell line SUM159

Faria M, Tin-U C, Dey P, Gustafsson J-A and Strom AM M. University of Houston, Houston, TX; Karolinska Institutet, Huddinge, Sweden and MD Anderson, Houston, TX.

Body: Recent clinical studies are indicating that the estrogen receptor β variant β5 (ERβ5) expression correlates to worse prognosis. We wanted to know if expression of ERβ5 is changing the growth behavior of the triple negative cell line SUM159. Estrogen receptor β5 is highly similar to estrogen receptor β1 except for a truncated C-terminus making the remaining ligand binding domain incapable of binding to estrogen. In addition a 4 amino acid unique peptide is added to the C-terminal end. Stably expressing ERβ5 using a transposon integrated tetracycline regulated expression system we find that expression of ERβ5 increases proliferation of the triple negative SUM159 cells especially in reduced serum condition compared to control cells. Since SUM159 have been shown to depend on autocrine stimulation for growth we are suggesting that expression of ERβ5 is affecting the production of autocrine growth factors.
Title: Prognostic effect of semiquantitative measurement of PgR in luminal type breast cancer

Kim MK and Han W. Kangwon National University Hospital, Chuncheon, Republic of Korea and Seoul National University Hospital, Seoul, Republic of Korea.

Body: Background: The identification of prognostic factors for hormone receptor(HR)-positive and human epidermal growth factor receptor 2(HER2)-negative breast cancer is an important issue for the accurate determination of the indications for chemotherapy. The progesterone receptor(PgR) is a downstream molecule induced by transcriptional activation of estrogen signaling. In recent report, quantitatively low PgR expression was significantly associated with a poorer prognosis for patients treated with anastrozole and/or tamoxifen. These findings seem to suggest that the clinically used Ki-67-based IHC classification of luminal subtypes is inaccurate and that semiquantitative measurement of PgR expression can improve the IHC-based classification.

Method: 1327 consecutive patients with ER and/or PgR-positive HER2-negative invasive breast cancer treated with surgery and adjuvant treatment at the Seoul National University Hospital from January 2009 to December 2011 were reviewed. All patients had been pathologically diagnosed with breast cancer, and patients who had received chemotherapy or endocrine therapy before surgery were included. ER, PgR, HER2, and Ki-67 were determined from surgically resected, formalin-fixed, paraffin-embedded tumor tissue. The RFS and OS in Kaplan-Meier plots for the PgR high and low expression groups was compared using the log-rank test. Univariate and multivariate analyses of DFS and OS in relation to various factors were performed with a Cox proportional hazards model, which yielded the HR and 95% of CI for each variable.

Results: Based on pathology data of semiquantitative measurement of the percentage of PgR-positive cells, patients were separated into two groups with PgR percentage cutoff 20%. Among 1372 patients, 665(48.5%) showed high PgR expression, 707(51.5%) showed low PgR expression. PgR expression was relatively high in younger age(<50 years, p<0.001). Higher PgR expression was related to low Ki-67 expression(<10%, p=0.003), and low cancer stage(p<0.001). The DFS and OS of patients in the high PgR subset were significantly better than the low PgR subset only in patients under 50 years old.(p=0.005 and 0.037) Cox analysis showed that low PgR expression(HR, 3.06; 95% CI, 1.44-6.47) and negative ER expression(HR, 19.68; 95% CI, 5.89-65.80) were significant independent risk factors for DFS. Low PgR expression(HR, 5.66; 95% CI 1.62-19.74) and high Ki-67 expression(HR 1.06; 95% CI 1.01-1.17) were significant independent risk factors for OS.

Conclusion: We were able to demonstrate that high PgR expression is associated with a better prognosis for luminal type breast cancer. The semiquantitative determination of PgR expression might thus improve the accuracy of the IHC-based classification of luminal A and luminal B breast cancer, especially for premenopausal patients.
Title: Expression of genes for peptide/protein hormones and their receptors in breast carcinomas as biomarkers predicting risk of recurrence

Wittliff JL L, Daniels MW W and Brock GN N. University of Louisville, Louisville, KY.

Body: Background: Several reports have suggested expression of certain peptide/protein hormones in breast cancer cells appear to be related to clinical behavior. We have taken a global approach by evaluating relationships between genes for these hormones and their cognate receptor proteins as independent predictors of risk of recurrence.

Methods: Expression of genes for 55 peptide/protein hormones (the ligands) and 73 of their cognate receptor proteins were measured by microarray analyses of LCM-procured carcinoma cells from 247 breast carcinoma biopsies. Using an IRB-approved biorepository and comprehensive database, total RNA was extracted from carcinoma cells to determine expression levels of 22,000 genes. Univariate and multivariate Cox regressions with interaction were determined using expression values of each ligand and its cognate receptor with an interaction term.

Results: When pairs of hormone ligand and cognate receptor genes were evaluated by multivariate Cox regression with interaction, the following sets of genes were identified that predicted risk of breast cancer recurrence (INS/IGF2R, HAMP/SLC40A1, POMC/MC4R, GH1/GHR and VIP/VIPR2) and OS (CORT/SSTR5, VIP/VIPR2 and GHRH/GHRHR, based on unadjusted p-value for interaction term < 0.05). Since expression in situ of both hormone and receptor are necessary to elicit endocrine action, a unique alternative approach using the minimum expression value between ligand and receptor for each patient was applied. These results revealed that the complex of HAMP/SLC40A1 showed significance in both the interaction and minimum models, and was further investigated by splitting the ligand and receptor into low (below 1st quartile) and high (above 1st quartile) expression groups. The group consisting of high expression for both ligand and receptor was contrasted with the other three groups (low expression for ligand, receptor, or both). The higher expression group exhibited a better DFS (hazard ratio (HR) = 0.56 95% CI 0.37-0.84, p=0.004) and OS (HR = 0.59 95% CI 0.37-0.93, p=0.022).

Conclusion: As a result of determining gene expression directly on pure populations of breast carcinoma cells procured by LCM, we have demonstrated the many lesions synthesize mRNA species for a wide variety of peptide and protein hormones as well as for their cognate receptor proteins. Using clinical follow-up that extended as much as 12 years, univariate and multivariate Cox regression analyses with and without interaction models revealed a number of noteworthy candidates of hormone-receptor complexes that predicted risk of breast cancer recurrence as well as overall survival. Collectively, our results suggests that many breast carcinomas exhibit considerable endocrine autonomy for controlling progression, which warrants investigation of the protein products of the gene candidates in isolated populations of breast carcinoma cells.
Title: Molecular analysis of cancer tissue, circulating tumor cells (CTC) and cell-free plasma tumor DNA (ptDNA) suggests variable mechanisms of resistance to endocrine therapy (ET) in estrogen receptor (ER) positive metastatic breast cancer (MBC)


Body: Introduction: Fifty percent of ER positive MBC patients do not benefit from ET. Potential mechanisms of resistance to ET in this patient population include absence of ER expression by deletion or suppression, alteration in ER signaling pathway genes, or upregulation of multiple growth factor receptor pathways. We hypothesized that genotyping and phenotyping of CTC combined with genomic analysis of ptDNA will provide important insights into the multiple mechanisms of ET resistance and that a set of blood tests might serve as a "liquid biopsy" abrogating the need for tissue specimens.

Methods: Twenty-four patients providing informed consent were enrolled into the Mi CTC-ONCOSEQ study, a companion trial to Mi-ONCOSEQ (the Michigan Oncology Sequencing Program). Seven of these patients (5 with ER immunohistochemistry (IHC) positive and 2 with ER negative cancers) who had available archived primary and metastatic cancer tissue, a research metastatic biopsy for genomic analysis, and who had ≥5 CTC/7.5 ml whole blood (WB) characterized for ER protein (CTC-ER) are the focus of this report. All the patients were ET refractory. None of them was progressing on fulvestrant at the time of study entry. CTC enumeration and phenotyping was performed with CellSearch®. Circulating ptDNA was analyzed by droplet digital polymerase chain reaction (ddPCR). ER status from archived tissue was obtained from chart review. ER mRNA expression was determined in the research biopsy of metastatic tissue by using quantitative RNA sequencing. Mutational status of ER gene, ESR1, was determined by Next-gen Sequencing using the Illumina HiSeq 2500 platform.

Results: The 2 control patients with triple negative breast cancer had negative CTC-ER. Discordance between CTC-ER and tissue ER by IHC was observed (Table). Two of the 5 ER positive patients retained CTC-ER positivity (39% and 19% of the CTC). One of them (#7) harbored an ESR1 mutation in the research biopsy tissue and in ptDNA, whereas the other (#14) had wild type (WT) ESR1. CTC-ER protein levels in patients #12, 17 and 24 were negative. All had WT ESR1 in the research biopsy tissue. Of note, patient #12 had WT ESR1 in the research biopsy, but an ESR1 mutation was detected in her ptDNA.

<table>
<thead>
<tr>
<th>Pt#</th>
<th>CTC-ER</th>
<th>Tissue-ER</th>
<th>ESR1 status in research biopsy</th>
<th>ESR1 status in ptDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N° CTC/7.5ml WB</td>
<td>% CTC-ER</td>
<td>Primary by IHC</td>
<td>Met by IHC</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>39%</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>16</td>
<td>19%</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>0%</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>16</td>
<td>0%</td>
<td>+</td>
<td>weakly+</td>
</tr>
<tr>
<td>24</td>
<td>275</td>
<td>0%</td>
<td>weakly+</td>
<td>weakly+</td>
</tr>
</tbody>
</table>

Conclusions: These exploratory data suggest heterogeneous mechanisms of resistance to ET in patients with previously determined ER-positive MBC, including ESR1 mutations in ER positive cases (seen in 2 patients) and loss of ER expression (seen in CTC of 3 patients). In contrast, other cancers continue to express WT ESR1, and therefore must have developed
alternative mechanisms of resistance. At least 2 of these mechanisms can be detected and monitored with complementary circulating assays: CTC and ptDNA. Further investigations are needed to understand the heterogeneous mechanisms of resistance to ET.
Body: Background
About 65% of breast cancers express the estrogen receptor α and the mainstay of treatment are therapies that result in estrogen receptor modulation (selective estrogen receptor modulators, SERMs) or estrogen deprivation (aromatase inhibitors, AIs). Even though endocrine therapy has resulted in reduced recurrence and mortality, a significant portion of patients relapse with metastatic disease and subsequently progress while on therapy for advanced disease (endocrine resistance). Recent evidence showed that activating hot spot mutations in the ligand binding domain of the ERα (ESR1) are acquired on treatment (frequency of 20%) and can drive resistance to endocrine therapy, especially AIs. ESR1 mutations can be detected by evaluation of circulating tumor DNA (ctDNA), a method where circulating DNA fragments with tumor-specific sequence alterations are identified in the blood of patients.

Methods
This is a retrospective evaluation of 9 patients with hormone receptor positive (HR+) metastatic breast cancer (MBC) who had progressed on multiple lines of endocrine therapy (ET) and were found to have ESR1 mutations in ctDNA. Patients had blood drawn for ctDNA analysis either at progression to serve as a baseline before starting a new regimen or to monitor response to ongoing treatment. Guardant360™ (Guardant Health) involves ctDNA isolation from plasma using a Qiagen circulating nucleic acid kit, then a panel of 68 gene mutations associated with solid tumors as reported in the COSMIC database sequenced using single-molecule digital sequencing technology.

Results
All of the patients had MBC and were luminal subtype except for one HER2+, and most had invasive ductal carcinoma although 2 patients were invasive lobular carcinoma (22%). Most patients had both bone and visceral involvement (78%), only two patients had bone only metastasis. The patients were generally heavily pretreated with an average of 3 lines of ETs and 6 lines of therapy altogether (chemotherapy + ET). Duration on endocrine therapy ranged from 23 months to 7 years (mean 4.3 years). All patients were found to have ESR1 mutations on ctDNA, the range of percentage of mutant allele was 0.28-23.76%. Three patients had tissue sent for NGS and none of the tissue samples had an ESR1 mutation detected although they were biopsied at various time points in treatment. One of those patients had two ESR1 mutations in ctDNA, which were not detected on tissue sent for NGS one year prior, and had not been on ET for several years. One patient with abdominal carcinomatosis from lobular carcinoma who had been on ET therapies for 6 years was found to have 4 distinct ESR1 mutations in a single blood draw, suggesting sub-clonal evolution of resistance. One patient also had 5 circulating tumor cells, all of which had ESR1 mutations detected when circulating tumor cells were individually sequenced.

Conclusions
ctDNA is a sensitive test for detection of ESR1 in HR+ MBC patients, with the advantage of being a blood based assay which lends itself to serial analysis. In this patient population ctDNA can be a helpful tool to predict response to ET and predict treatment failure.
Title: Estrogen receptor alpha reactivation for the treatment of anti-estrogen-resistant breast cancer

Hosford SR R, Kaufman PA A and Miller TW W. Dartmouth College, Lebanon, NH and Dartmouth Hitchcock Medical Center, Lebanon, NH.

Body: Adjuvant anti-estrogen therapies that antagonize ER transcriptional activity have improved outcome in many patients, yet resistance to anti-estrogen therapies is common, resulting in disease recurrence in 1/3 of patients within 15 years of follow-up. However, prior to the introduction of tamoxifen, estrogens were used for treatment of breast cancer with response rates similar to those obtained by anti-estrogens in the advanced setting. Similarly, withdrawal of anti-estrogen therapy has shown anti-tumor effects, indicating that reactivation of ER may elicit therapeutic benefit.

MCF-7 cells with long-term (>1 yr) acquired resistance to the selective ER downregulator fulvestrant (fulv; MCF-7/FR) retain ER expression and harbor ESR1 (ER) gene amplification. Upon withdrawal of fulv, these cells re-engage ER as demonstrated by increased luciferase transcriptional reporter activity and re-expression of proteins encoded by ER-inducible genes. Following fulv withdrawal, MCF-7/FR cells show drastically decreased proliferation and increased apoptosis that are temporally correlated with ER reactivation. Protein levels of the anti-senescence protein FoxM1 decline following ∼12 d of fulv withdrawal, paralleled by increased staining for senescence-associated β-galactosidase. Transcriptomic analyses confirmed that fulv withdrawal progressively induces gene expression patterns indicative of stress and senescence. Similar effects were observed in long-term estrogen-deprived (LTED) MCF-7 cells treated with 17b-estradiol. Prospective studies characterizing the development of acquired anti-estrogen resistance have demonstrated the MCF-7 cells at 9 months of fulv resistance do not respond to fulv withdrawal, contrasting the long-term (>1 yr) MCF-7/FR cells. Additionally, withdrawal of fulv from T47D/FR, ZR75-1/FR, or HCC-1428/FR cells did not induce cell death or re-engage ER activity, confirming that ER reactivation is required for anti-cancer effects.

Ongoing studies are characterizing the temporal changes in ER transcriptional activity during 1) development of acquired anti-estrogen resistance, and 2) 17b-estradiol treatment of mice bearing WHIM16 patient-derived xenografts (regress in response to 17b-estradiol) to elucidate the mechanism underlying sensitivity of anti-estrogen resistant cells to ER reactivation. While estrogen therapies have shown clinical efficacy for decades, biomarkers to identify patients with tumors likely to respond to estrogen remain undefined. We are conducting a Phase II clinical trial [Pre-emptive OscilLation of ER activitY levels through alternation of estradiol/anti-estrogen therapies prior to disease progression in ER+/HER2- metastatic breast cancer (POLLY); NCT02188745] that will use tumor biopsy tissues to identify baseline and pharmacodynamic biomarkers that predict response to 17b-estradiol therapy.
Title: Estrogen receptor α-regulation of nucleocytoplasmic transport pathways as modulators of breast cancer therapy effectiveness

Madak-Erdogan Z, Chen-Zhao Y and Wrobel K. University of Illinois, Urbana-Champaign, Urbana, IL.

Body: Estrogen Receptor α (ERα), a member of the large superfamily of nuclear receptors, exerts profound effects on gene expression, cellular response programs, and phenotypic properties of estrogen target cells. Because of these broad and important actions, ERα is considered the single most important predictor of breast cancer prognosis and is the target of endocrine therapies. The importance of kinases in cancer biology is well known, as increased kinase activity through phosphorylation, mutations or increased expression is often observed in clinical samples and is associated with a poorer prognosis. It is believed that, in therapy-resistant breast cancers, control of cellular physiology switches from ERα nuclear-initiated pathways to extranuclear-activated protein kinase pathways, which enable these cells to adopt a more aggressive phenotype. However, the mechanisms underlying the interplay between ERα and protein kinase pathways in cancer, and the processes by which ERα influences these pathways are poorly understood.

Our aim in this study was to elucidate how ERα modulated extranuclear-initiated kinase signaling through alteration of the subcellular localization of ERK5. We have previously shown that upon estradiol treatment ERα elicits nuclear localization of ERK5 and Cofilin in ERα-positive breast cancer cells. This event diminishes ERK5 and Cofilin localization to regions of high actin remodeling in the cytoplasm, thereby possibly accounting for the reduced invasiveness and metastatic potential that is characteristic of many ERα-positive vs. ERα-negative breast cancer cells (PMID: 24505128). In our studies involving an endocrine-resistance cell model, we have found that ERK5 and Cofilin become localized to the cytoplasm as resistance progresses. Using several publicly available tumor gene expression databases, we have identified a signature of genes regulating nucleocytoplasmic transport that are differentially upregulated in more aggressive tumors. Additionally, using RPPA data from TCGA, we find that high expression of the signature genes correlates with higher phosphorylation of key signaling molecules like TAZ, indicative of their mislocalization in invasive breast carcinomas. Low expression of signature genes would successfully predict those patients that would respond to endocrine therapy with Luminal B type tumors that are generally more aggressive and harder to manage clinically. We verified our findings in cell line models by modulating levels and activities of these proteins by overexpression, knock-down and use of small molecule inhibitors. These findings provide insight into how ERα regulates extranuclear initiated kinase signaling by modulating nuclear transport of key kinases in breast cancer. They also suggest that therapeutic targeting of nucleocytoplasmic transport machinery in breast cancer might decrease aggressiveness of breast cancers and increase the efficiency of endocrine therapies by sequestering factors in their proper subcellular localizations where they would contribute to effective anti-estrogenic actions of endocrine targeting agents.
Targeting tumor re-wiring by triple blockade of mTORC1, ERBB and ER signaling pathways in endocrine resistant breast cancer


AIM To target tumor re-wiring by combined mTORC1 inhibition plus hormonal treatment with or without co-blockade of ERBB signaling in endocrine resistant models of human breast cancer (BC).

BACKGROUND Around 80% of BCs are estrogen receptor positive (ER+). Endocrine therapies target estrogenic stimulation of tumor growth but resistance remains problematic. Several strategies have shown that resistance often depends on the acquisition of enhanced cross-talk between ER and growth-factor pathways, allowing the disease to circumvent the need for steroid hormones. We have previously reported the antiproliferative effects of the combination of everolimus (RAD001-mTORC1 inhibitor) with endocrine therapy in resistance models, but potential routes of escape from treatment via ERBB2/3 signaling were observed. We hypothesised that combined targeting of three signaling pathways, namely ER, ERBB and mTORC1 may provide enhanced anti-tumor activity.

METHODS ER+ BC cell lines (MCF7, SUM44 and HCC1428) adapted to long term estrogen-deprivation (LTED) which model relapse on an aromatase inhibitor, along with their wild-type (wt) cell lines were treated with neratinib, a pan-ERBB tyrosine kinase inhibitor, in combination with RAD001 ± estradiol (E2), tamoxifen or fulvestrant. End points included proliferation, cell signaling, cell cycle and effect on ER-mediated transactivation and recruitment by ChIP.

RESULTS All cell lines showed a concentration-dependent decrease in proliferation in response to RAD001 (IC50 0.6-50nM in absence of E2 and 1-10nM in presence of E2). A wide range of IC50 values (300-1000nM) was observed with neratinib treatment in the presence of E2. However, in the absence of E2, wt cell lines showed IC50 values in excess of 1800nM with hormetic dose response curves, in which lower concentrations induced cell proliferation. In contrast, LTED IC50 values ranged between 400-900nM. Combination of either agent with endocrine therapy caused a concentration dependent decrease in proliferation in all wt cell lines and their LTED derivatives, but the maximum effect was observed when a triple combination of RAD001, neratinib and ER-blockade was used. Expression of pS6 was dramatically suppressed by RAD001 ± neratinib in all cell lines tested, whilst neratinib caused a cell line specific reduction in expression of ERBB family proteins. Upregulation of pAKT was observed in all cell lines following treatment with RAD001, indicating both inhibitors were effectively suppressing their respective targets. Combination of RAD001 with neratinib suppressed the upregulation of pAKT and significantly reduced cell cycle progression. In the absence of E2, RAD001 caused a reduction in ER-mediated transcription and decrease in recruitment of ER and the CREB-binding protein (CBP) to the TFF1 promoter. In contrast, neratinib induced a marked increase in ER-recruitment and concomitant rise in ER-mediated transactivation, which was reduced by the addition of RAD001.

CONCLUSION Targeting tumor re-wiring by triple blockade of ERBB, ER and mTORC1 signaling pathways significantly reduces cell proliferation supporting the potential combination in patients who have relapsed on endocrine therapy and retain a functional ER.
Evolution of genomic alterations on endocrine therapy and mTOR inhibition in estrogen receptor (ER)-positive breast cancer

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Background: Endocrine therapy (ET) and mTOR inhibition are important treatment strategies in ER-positive breast cancer, but no specific genomic alterations reliably predict benefit. Because tumors are tested pretreatment, we hypothesized that this may not capture tumor interaction with therapy.

Methods: We studied tumors from protocol NCT00570921 using fulvestrant and everolimus for metastatic ER-positive breast cancer after aromatase inhibitor (AI) failure. DNA from FFPE tumor tissue was subjected to next-generation genomic profiling using the FoundationOne® assay and alterations were then compared between available paired samples: primary/metastatic, before/after everolimus, and upon progression on everolimus.

Results: The most common alterations encountered were in the PI3K/AKT/mTOR pathway with increased frequency of PIK3CA in endocrine-sensitive disease but no specific association with everolimus benefit (Massarweh et al, ASCO 2015). One patient with lobular carcinoma relapse on ET and a new contralateral primary, had PIK3CA, CDH1, and MAP3K1 in both tumors with no new alterations detected. Her disease was everolimus sensitive. Another patient with PIK3CA at baseline acquired a CTNNA1 mutation upon relapse with no everolimus benefit. Interestingly, one patient with liver metastasis and complete response to everolimus lasting 3 years had no known alterations reported in the primary tumor but had a PIK3CA mutation in one of two simultaneous biopsies of separate liver lesions. Another patient with liver metastasis and a GATA3 mutation at baseline had response to everolimus lasting 18 months, then developed a PIK3R2_c.1936A>T mutation on progression reported as a variant of unknown significance (VUS). Another patient with metastatic lobular carcinoma to skin and bone had PIK3CA, CDH1, and ERBB2 mutations at baseline, and acquired KRAS and MCL1 amplification on two sequential skin biopsies in the first month on everolimus. She remained on therapy for 1 year. One patient with locally advanced disease and de novo bone metastasis had TP53 and GATA3 mutations at baseline with resistance to multiple chemotherapy and endocrine treatments. Upon progression on AI, her tumor acquired PDGFRA and SMAD4, detected on day 1 biopsy of everolimus treatment. Repeat biopsy on day 28 revealed loss of PDGFRA and SMAD4 with emergence of PIK3CA and MLL2 mutations and loss of STK11. After 1 year on everolimus her tumor progressed and repeat breast biopsy revealed loss of the PIK3CA, STK11, and MLL2 events, with appearance of AKT1 and NF1 mutations. Interestingly, her tumor also acquired ESR1_c.1607T>G, MTOR_c.6104C>T, and NSD1_c.5938G>A mutations, all classified as VUS but were not previously encountered in her course. Further analysis and biologic relevance of these changes will be presented.

Conclusion: This small study suggests that ER-positive breast cancer is a dynamic disease with genomic evolution on endocrine therapy and mTOR inhibition. In some patients, this change occurs early on therapy, possibly through clonal selection, but may also be related to tumor heterogeneity. The significance of this change is not fully understood, but study of early on-treatment biopsies may help us better understand tumor response to therapy.
Title: RAD1901, a novel oral, selective estrogen receptor degrader (SERD) with single agent efficacy in an ER+ primary patient derived ESR1 mutant xenograft model


Body: Despite advances in the treatment of metastatic breast cancer, many women eventually relapse with more aggressive forms of endocrine-resistant disease. Mutations in the ESR1 gene encoding the estrogen receptor (ER) have recently emerged as a potential mechanism for the development of clinical resistance to conventional anti-estrogen therapies, such as fulvestrant. To overcome some of the pharmacokinetic limitations and intramuscular administration challenges associated with fulvestrant endocrine therapy and to combat the development of resistance, there is a significant need for the development of more durable and more effective ER-targeted therapies. Here, we begin to describe and characterize the preclinical efficacy of RAD1901, a novel, orally bioavailable small-molecule SERD, with significant therapeutic potential for treatment of breast cancer. RAD1901 selectively binds to and degrades the ER and is a potent antagonist of ER-positive breast cancer cell proliferation. Importantly, RAD1901 demonstrated profound tumor growth inhibition in MCF-7 xenograft models when compared to fulvestrant and tamoxifen. Importantly, RAD1901 also demonstrated marked single agent efficacy in a primary patient-derived xenograft (PDx) model harboring the ESR1 Y537S mutation indicating the utility of this SERD against clinically relevant ER mutants. Further biochemical binding studies and co-crystallization experiments of RAD1901 bound to the ER further confirms the ability of RAD1901 to bind to both mutant and wild type forms of the ER. RAD1901 is currently undergoing clinical testing in postmenopausal women with ER-positive advanced breast cancer.
**Title:** High prevalence of splicing variant AR-V7 in triple negative breast carcinoma


**Body:** The androgen receptor (AR) and its pathway have been implicated in tumorigenesis and progression of breast cancer. Anti-androgen therapy has shown efficacy in the metastatic breast cancer and numerous clinical trials are underway to study efficacy in various clinical settings. 15 splicing variants of AR (AR-Vs) have been described in prostate cancer. Structurally, AR-Vs have insertions of cryptic exons downstream of the exons that encode the DNA-binding domain or deletions of the exons encoding the ligand-binding domain, resulting in a disrupted AR open reading frame and expression of ligand-binding-domain-truncated AR proteins. In prostate cancer, some of the AR-Vs especially AR-V7 are associated with aggressive disease and resistance to anti-AR therapy. The AR-V prevalence in human breast cancer specimens has hitherto not been studied. We aimed at studying the expression of AR-Vs in breast cancer specimens and present the data on AR-V1, AR-V7, AR-V8, and AR-V567, in AR-positive triple negative (TNBC) and ER+/Her2- breast cancer.

**Design:** 98 cases of TNBC, 40 cases of ER+/Her2 breast cancer and 17 cases with reduction mammoplasty were abstracted from NYULMC pathology database and screened for AR expression by immunohistochemistry (IHC). IHC for AR was performed using antibody clone N-20 (Santa Cruz) at 1:100 dilution. Normal breast tissue was used as internal control and 10% nuclear staining was used for categorizing a tumor as AR positive. A subset of cases which over-expressed AR were macrodissected from formalin fixed paraffin embedded sections with total RNA extracted by using the PureLink® FFPE RNA Isolation Kit (Invitrogen). Reverse-transcription was performed by using the SuperScript® III Reverse Transcriptase Kit (Invitrogen). AR-V expression was presented as cycle number difference to housekeeping gene (delta CT) for real-time PCR or as absolute copy number for digital PCR.

**Results:** AR+ TNBC and AR+/ER+ cases ranged from stage 1A to IIIA. IHC for AR showed > 10% staining in 27 of 98 TNBC cases and in 39 of 40 ER+/Her2 cases.

<table>
<thead>
<tr>
<th>AR Positive Prevalence in TNBC and ER+ Breast Carcinoma</th>
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<tr>
<td></td>
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<tr>
<td>TNBC</td>
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<tr>
<td>ER+/Her2-</td>
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AR-V7 was expressed in 11 of 13 AR+/TNBC cases (p < 0.05); AR-V1 and AR-V4 were expressed in 4; ARV8 and ARv567es were expressed in 3 and 2 cases respectively. In 25 AR+/ER+/Her2- cases AR-V7 was expressed in 14; AR-V4 in 9; ARV567 in 5, AR-V1 in 3 cases.

**AR Spliced Variant Incidence in TNBC, ER+ and Benign breast tissue**

<table>
<thead>
<tr>
<th></th>
<th>Benign (N=17)</th>
<th>ER+/AR+ (N=25)</th>
<th>TNBC+/AR+ (N=13)</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>AR-V1</td>
<td>1</td>
<td>3(12%)</td>
<td>4(25%)</td>
<td>0.145</td>
</tr>
<tr>
<td>AR-V4</td>
<td>1</td>
<td>9(36%)</td>
<td>4(25%)</td>
<td>0.35</td>
</tr>
<tr>
<td>AR-V7</td>
<td>8(47%)</td>
<td>14(56%)</td>
<td>11(85%)</td>
<td>0.021</td>
</tr>
<tr>
<td>AR-V8</td>
<td>0</td>
<td>1(4%)</td>
<td>3(19%)</td>
<td>0.474</td>
</tr>
<tr>
<td>AR-V567</td>
<td>1</td>
<td>5(20%)</td>
<td>2(12.5%)</td>
<td>0.092</td>
</tr>
</tbody>
</table>
Conclusion: We report expression of various spliced variants in TNBC, ER+/Her2- breast cancer. A statistically significant expression of AR-V7 is seen in TNBC. Since AR-V7 predicts for poor prognosis and lack of response to anti-AR therapy in prostate cancer, AR-V7 expression may be a useful biomarker to analyze response data in ongoing breast cancer clinical trials.
Title: Exploring bazedoxifene and palbociclib as potential therapeutic strategies for overcoming ESR1-mediated endocrine resistance


INTRODUCTION
Despite effective endocrine treatments, endocrine resistance remains a major clinical challenge. We and other groups have recently detected ligand-binding domain ESR1 mutations in metastatic estrogen receptor positive (ER+) breast cancers. Our preclinical studies showed that these mutations confer constitutive activity and relative resistance to tamoxifen and fulvestrant. In this study we sought to investigate therapeutic strategies to overcome resistance rendered by the ESR1 mutations. Since our previous studies showed relative resistance to tamoxifen or fulvestrant, we hypothesized that bazedoxifene, a high affinity third generation SERM/SERD could overcome resistance driven by the ER mutant resistance. Additionally, we hypothesized that inhibiting cyclin D1, a key ER transcriptional target gene and cell cycle regulator, is a second potential therapeutic strategy to circumvent resistance rendered by mutant ER. Therefore in this study we tested the effects of bazedoxifene, palbociclib and their combination on cell proliferation in the presence and absence of the ESR1 mutations.

METHODS
For this study we established doxycycline inducible MCF7 cell lines expressing the ER-LBD mutations (Y537S, Y537N and D538G) and WT-ER as control. Cell proliferation response to bazedoxifene, tamoxifen, fulvestrant, palbociclib and the bazedoxifene-palbociclib combination was evaluated.

RESULTS
Cells harboring mutant ER were relatively resistant to tamoxifen and fulvestrant, as expected, and remained sensitive to single agent bazedoxifene and palbociclib. The combination of bazedoxifene and palbociclib was found to be superior to the single agents and exhibits synergistic activity.

CONCLUSION
The combination of bazedoxifene and palbociclib inhibits mutant ER cell growth and other cell models of endocrine resistance and is a potential therapeutic combination.
Title: Non-coding RNAs derived from near the ESR1 gene acts as a transcriptional regulator during estrogen deprivation adaptation of ER positive breast cancer cells

Fujiwara S, Saitoh N, Tomita S, Abdalla MO Osama, Iwase H and Nakao M. Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan and Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan.

Body: Endocrine therapies that blocks estrogen production are effective for estrogen receptor (ER)-positive breast cancer. However, endocrine therapy treated patients eventually experience relapse after a long period of estrogen deprivation. The mechanism underlying acquisition of estrogen independent growth by ER positive breast cancer cells remains unclear. To understand such molecular mechanism, we used a cell model LTED (long term estrogen deprivation) which MCF7 cells were cultured under estrogen deprivation for 4-10 months. In LTED cells, we found that ER encoded gene ESR1 was up-regulated and ER overproduction was essential for estrogen-independent cell growth. We also revealed that RNA transcriptions of the ESR1 and several neighbor genes were co-induced from both coding and non-coding regions in LTED cells, using RNA-sequence. These highly transcribed regions were corresponded to active histone modifications and transcription factor bindings according to publically available genome-wide analyses data. Fluorescence in situ hybridization (FISH) analyses indicated that RNA from the chromatin domain region nearby ESR1 were co-localized and made foci in nucleus. We found non-coding regions that are particularly highly transcribed. FISH analyses indicated that RNAs from these regions might interact with the parental ESR1 gene locus. Recent studies have shown that non-coding RNAs are involved in transcriptional regulation and chromatin regulation. To understand the role of the non-coding RNA, we have generated MCF7 cells lines that lack the non-coding site, using CRISPR/CAS9 system. We found that mRNA transcription of multiple genes including ESR1 were impaired by the deletion. These findings suggested that these non-coding RNAs may be involved in chromatin regulation of the chromatin domain nearby ESR1.

In this study, we found non-coding RNAs that control transcription of chromatin domain genes in ER positive breast cancer cells. Such non-coding RNA mediated transcriptional regulation might be critical for endocrine therapy resistance adaptation.
Title: The evolution of the estrogen receptor (ER) complex conformation governs estrogen-induced apoptosis in antihormone-resistant breast cancer cells

Maximov PY Y, Sengupta S, Fernandes DJ J, Fan P, Curpan RF F, Rajan SS S, Greene GL L and Jordan VC. The University of Texas, MD Anderson Cancer Center, Houston, TX; Georgetown Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC; The institute of Chemistry, Romanian Academy, Timisoara, Romania and The University of Chicago, the Ben May Department of Cancer Research, Chicago, IL.

Body: Over the past decade new insights have been gained into the acquired resistance to tamoxifen and the Aromatase Inhibitors (AIs) with the discovery of the new biology of estrogen-induced apoptosis. However, it has also been learned that estrogens can be classified into planar class I and angular class II estrogens. Using model systems of long-term estrogen-deprived breast cancer cells in vitro (MCF-7:5C), it was previously shown that class I estrogens cause immediate apoptosis over a 3-4 day period. Paradoxically, class II estrogens actually block apoptosis caused by planar class I estrogens. To gain insight into this paradox we have successfully crystallized an angular class II triphenylethylene (TPE) estrogen bound to the ligand binding domain (LBD) of the ER and derived a new conformation for the TPE:ER complex (code 3Q97 in the PDB). Surprisingly, Helix 12 seals the LBD with the class II estrogen, but not the same conformation is observed with the planar class I estrogen 17β-estradiol (E2). There would seem to be no reason why the 3Q97 complex would not cause immediate apoptosis. To address this issue we have used Western blot analysis for protein and qRT-PCR for mRNA levels for the ER. ER parameters were monitored for up to 72 hours and results compared and contrasted between E2, the Class II estrogens, 4-hydroxytamoxifen (4OHT) and endoxifen (Endox). ER protein and mRNA levels with 4OHT or Endox accumulated and remained high throughout the study period. In contrast, the planar estrogen E2 produced a rapid decline in the protein and mRNA levels for the ER complex. The angular class II estrogens initially produced an accumulation of the ER protein complex, which decreased with time. Using a chromatin immunoprecipitation (ChIP) technique we demonstrated that the class II estrogens recruit only half of the ER to the estrogen-responsive genes promoters (TFF1 And BREB1) and less than half co-activator binding compared to E2. The TPEs were only partial agonists compared to planar estrogen. These results explain why the Class II estrogens induce delayed apoptosis. We conclude that, for the first time, we have observed the binding of a ligand to a receptor that changes conformation against time and evolves from an antagonist to an agonist conformation to trigger apoptosis. These observations have current relevance to the decryption of the protective effects of estrogen alone therapy against breast cancer incidence in the Women's Health Initiative (WHI) trial. This work was supported by the Susan G. Komen for the Cure Foundation award SAC100009.
Combination of anti-progranulin (GP88/PGRN) antibody and letrozole inhibits tumor formation of letrozole resistant breast cancer cell lines

Serrero G, Dong J, Yue B, Hicks D and Hayashi J. A&G Pharmaceutical Inc., Columbia, MD and Precision Antibody, Columbia, MD.

The 88 kDa glycoprotein GP88 (Progranulin, PCDGF, acrogranin) is the largest member of the granulin/epithelin family of growth modulators identified as a driver of tumorigenesis. GP88 (PGRN) was also shown to be overexpressed in invasive ductal carcinoma (IDC) whereas it was negative in benign tumors and normal mammary epithelial tissue, thereby establishing GP88 as a therapeutic and diagnostic target in breast cancer (BC). Our laboratory has developed validated tools to measure GP88 in tumor biopsies and biological fluids as well as blocking its action. We showed that GP88 was secreted and detected in the serum of BC patients at an increased level when compared to healthy subjects. Pathological studies with 530 cases of ER+ IDC with clinical outcomes showed that GP88 tumor expression was an independent prognostic indicator of recurrence in early stage BC patients. Training study followed by an independent validation study demonstrated that high GP88 tissue expression (GP88 3+) was associated with a 4-fold increase in risk of recurrence at 5 years. A neutralizing anti-GP88 antibody AG1 was expressed in a high yield CHO cell line was developed. The present study examined the effect of AG1 in letrozole resistant cell line AGLetR developed by long term selection in letrozole supplemented medium. This cell line showed decreased letrozole responsiveness in vivo and therefore constituted an excellent model for investigating letrozole resistance in vitro as well as in vivo. Here we report the results of studies investigating the effect of various doses of AG1 on LetR tumor development in combination with letrozole for AGLetR. We show that treatment with AG1 (10 mg/kg i.p.) in combination with letrozole was efficient to maintain long term responsiveness and inhibit tumor growth. Letrozole alone (5mg/kg) was unable to inhibit tumor growth and showed a doubling of tumor volume. Interestingly, long term combination treatment lead to tumor regression and inhibited tumor growth. These data suggest that inhibiting GP88 could provide a novel and alternative therapeutic strategy for patients with resistance to anti-estrogen therapy, being tamoxifen or letrozole.

This work is supported by 2R44CA124179, HHSN 261201200060C, and HHSN2612014400C from NCI.
Title: Estrogen-independent growth of ESR1-mutant breast cancer cells requires CDK4/6

Luo F, Le X, Jeselsohn R, Brown M and Garraway L. Dana Farber Cancer Institute, Harvard Medical School, Boston, MA; Broad Institute, Cambridge, MA and Beth Israel Deaconess Medical Center, Boston, MA.

Body: A recent breakthrough in cancer treatment has been the development of highly specific cyclin-dependent kinase 4/6 (CDK4/6) inhibitors. CDK4/6 inhibitors, such as palbociclib, prevent cell cycle progression from growth phase (G1) to the DNA synthesis (S) phase of the cell cycle. Palbociclib, however, is only approved for first-line treatment of patients with estrogen receptor positive (ER+) breast cancer in combination with an aromatase inhibitor, letrozole. Approximately 20% of metastatic breast cancer patients, harbor an activating mutation in the ligand-binding domain (LBD) of the estrogen receptor gene, ESR1, which confers resistance to estrogen deprivation. It is unclear whether tumors harboring these endocrine treatment-associated mutations will also respond to CDK4/6 inhibition. We have found that estrogen-independent growth of ESR1-mutant breast cancer cells requires CDK4/6. Thus, ESR1-mutant breast cancer cells are sensitive to CDK4/6 inhibition. Mechanistically, ERS1 LBD-mutant cells upregulate key mediators of the G1-S cell cycle transition. Furthermore, the sensitivity of ESR1-mutant breast cancer cells to CDK4/6 inhibition requires RB1. While cells expressing RB1 respond to CDK4/6 inhibition, knockout of RB1 via CRISPRs enables ESR1-mutant breast cancer cells to resist combined estrogen depletion and CDK4/6 inhibition. Our studies suggest that tumors harboring ESR1 LBD mutations and an intact RB1 will respond to combined CDK4/6 inhibition and estrogen deprivation.
Title: A neoadjuvant window trial of endocrine response in women with invasive lobular carcinoma


Body: Background:
Patients with invasive lobular carcinoma (ILC) would be expected to have favorable outcomes compared to patients with invasive ductal carcinoma (IDC) given that ILC is more often hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-negative, of lower grade, and displays decreased proliferation markers. Based on our preclinical studies showing differential hormone response in HR+ ILC vs. IDC and on recent studies suggesting differences in endocrine treatment response between patients with ILC vs. IDC, we designed a biomarker-driven, neoadjuvant window trial for newly diagnosed women with HR+, HER2-negative ILC. We hypothesize that Ki67 will be reduced by 85% in the fulvestrant arm compared with 60% and 75% reduction in the tamoxifen and anastrozole arms, respectively, and that Ki67 reduction will correlate with alterations in expression of ER and ER-regulated genes. Differential Ki67 effect will serve as a surrogate for outcome of patients with ILC on endocrine therapy.

Trial Design: This multicenter study (NCT02206984) will enroll 150 women with HR+ and HER2-negative ILC. A mandatory research breast tumor biopsy will be performed at baseline. Fifty patients will be randomized to each of three open-label treatment arms for 21 days: fulvestrant (two 250 mg IM injections on both day 1 and day 14), anastrozole (1mg orally daily), or tamoxifen (20 mg orally daily). Biomarkers of response will be assessed on baseline and post-treatment tumor tissue. Patients will proceed to definitive surgery on day 21 after study drug exposure, or they will undergo a second research breast core biopsy if further neoadjuvant treatment is planned.

Eligibility Criteria: Eligible patients include postmenopausal women with newly diagnosed, HR+, HER2-negative ILC (excluding pleomorphic subtype) measuring ≥ 1cm, with adequate organ function, ECOG PS ≥ 2, and agreeable to baseline research breast tumor biopsy.

Specific Aims: The primary endpoint is percent change from baseline to post-treatment Ki67 values in ILC tissue after 21 days of endocrine treatment. Comparisons across study arms will be made using a general linear model adjusting for institutional effect, with 80% power estimated for pairwise comparisons of \( \log_2 \) (% staining) between treatment arms, allowing for 10% attrition. Secondary endpoints include post-therapy Ki67, and change in ER and PR protein expression by IHC. Finally, planned correlative studies include evaluation of gene expression, epigenetic markers, and DNA sequence variants in ILC tissues in an effort to identify biomarkers of endocrine response and putative drivers of endocrine resistance in ILC.

Target Accrual: This study will be open to enrollment by August 2015 at the University of Pittsburgh. Additional sites will be opened through the Translational Breast Cancer Research Consortium (TBCRC). We anticipate an accrual rate of 8 patients per month.

(Funding from Susan G. Komen® and AstraZeneca).
Title: Spatial and temporal genomic heterogeneity of estrogen receptor and clinical impact in a patient with advanced breast cancer

Rizel S, Dvir A and Soussan-Gutman L. Davidoff Center, Rabin Medical Center, Petah Tikva, Israel and Teva Pharmaceutical Industries Ltd, Shoham, Israel.

Body: Endocrine therapy targeting Estrogen receptor alpha (ERα) is a key therapeutic strategy for hormone-driven breast cancer. Resistance to endocrine therapy may be intrinsic, arising from different clones or acquired by evolution influenced by selective pressure conditioned by therapy. Emerging evidence points to the role of acquired ERα mutations in driving resistance, when detected in the metastases but were absent from primary tumor.

Here we studied tumor genomic evolution in a patient who developed two distinct recurrent consequences. The first recurrence was located at the lung and developed 14 years from diagnosis. In the adjuvant setting, the patient was treated with chemotherapy and had no endocrine therapy incorporated. On initial recurrence, the patient was treated with aromatase-inhibitor which resulted in complete response and the site is free of tumor now for 5.7 years. However, 2 years into endocrine therapy with aromatase-inhibitor the patient developed a single hepatic metastasis and treatment was changed to fulvestrant for 8 months with slow ongoing progression. The patient had partial hepatectomy, cholecystectomy and metastatectomy 3 months after fulvestrant was stopped. Histology and immunohistochemical stains confirmed breast origin, ER +2, 100%, PR +3, 100%.

The endocrine therapy resistant hepatic lesion was sequenced by hybrid capture Next Generation Sequencing (NGS) and ERα (D538G) mutation was detected along with PIK3CA and GATA3 typical for hormone+ breast cancer. We have studied the primary tumor by same NGS method and detected only the PIK3CA and GATA3 mutations. In the lung lesions, responsive for endocrine therapy when sequenced by NGS the ERα (D538G) resistant mutation was absent.

Clinical data for treatment of ERα (D538G) mutation driving endocrine resistance is lacking. The patient could not tolerate tamoxifen and failed treatments with aromatase-inhibitor and chemotherapy, losing 14 kg in weight. Treatment with megestrol acetate 160 mg was initiated and patient achieved partial response confirmed by hepatic MRI and an improved performance status which is now ongoing for 8 months.

This case represents the first evidence of heterogeneous response to endocrine therapy explained by presence/absence of ERα (D538G) resistant mutation along with evidence for an active treatment in not an uncommon scenario.
Mechanisms of resistance to CDK4/6 inhibition in ER+ breast cancer


Estrogen receptor positive breast cancers show frequent deregulation of Cyclin D-CDK4/6 signaling. Combining CDK4/6 inhibitors with hormonal therapies produces clinical benefit, supporting drug approval of the first CDK4/6 inhibitor, Palbociclib, in combination with Letrozole or Fulvestrant. Due to the adaptive nature of cancers, development of drug resistance is common and resistance to CDK4/6 inhibitors can be expected. To understand potential mechanisms of resistance to palbociclib, ER+ breast cancer cells were made resistant to Palbociclib in vitro. Characterization of resistance models derived from T47D and MCF7 cell lines revealed different mechanisms of resistance to Palbociclib. Resistant T47D revealed loss of functional Rb through a large 3 prime genomic deletion of RB1. Palbociclib failed to inhibit proliferation of these cells confirming Rb inactivation as the primary mechanism of Palbociclib activity.

Palbociclib-resistant MCF7 cells retained Rb mRNA and protein expression suggesting direct loss of Rb function was not the mechanism of resistance. RNAseq and CNV analysis of several resistant MCF7 lines revealed distinguishing features of the resistant lines including up-regulation of E2F, TGFβ, WNT and NF-κB transcriptional networks. Also, proteomic analysis showed that prominent features of all the resistant MCF7 cells included elevated Myc expression as well as increased abundance of Rb, phosphorylated Rb, E2F1 and its downstream targets Cyclin E1, Cyclin A2 among others. Palbociclib treatment of resistant MCF7 lines showed that CDK4/6 inhibition still modulated phosphorylated Rb and E2F1 levels, but repressed levels were higher than compared to basal levels of vehicle treated parental MCF7 cells. Given the precedence for Cyclin E/CDK2 activity to redundantly regulate Rb function at the G1 checkpoint, Cyclin E1 expression was inhibited using shRNA. Results show that knockdown of Cyclin E1 protein in Palbociclib-resistant cells restores sensitivity to Palbociclib.

Palbociclib-resistant MCF7 transcriptomes also exhibited significantly decreased expression of Estrogen Receptor target genes. Resistant cells proved unresponsive to the ER antagonist Fulvestrant, suggesting a loss of dependence on ER signaling. Cyclin E1 dysregulation is also implicated in resistance to hormonal therapy and Cyclin E1 knockdown resensitized Palbociclib-resistant MCF7 cells to Fulvestrant treatment. The observations of cross resistance to CDK4/6 inhibitors and hormonal therapy highlight the co-dependence of Cyclin D-CDK4/6 and ER signaling in ER+ breast cancer. Continued studies are focused on describing the alterations driving increased Cyclin E1 expression in response to CDK4/6 inhibition and strategies to circumvent development of resistance via Cyclin E/CDK2 signaling.
Title: Characterization of resistance to the selective CDK4/6 inhibitor palbociclib in ER positive breast cancer


Body: Background: Dysregulation of the cyclin D-CDK4/6-Rb axis occurs in a substantial proportion of ER-positive (ER+) breast cancers and has been linked with endocrine resistance. Adding the CDK4/6 inhibitor palbociclib to endocrine treatment has led to a substantial improvement of the outcome of patients with ER+ metastatic breast cancer. However, with the increasing clinical use, acquired resistance to palbociclib is merging as a new major clinical challenge.

Methods: The ER+ cell lines T47D and MCF7 have been shown to be highly sensitive to treatment with palbociclib. Using long-term co-culture with increasing doses of Palbociclib, we generated MCF7 and T47D cell line clones with acquired resistance to Palbociclib. Three distinct resistant clones were selected for each cell line showing an IC50 shift from sensitive to resistant of approximately 300nM to 3μM for MCF7 and 400nM to 3.5μM for T47D, respectively. Resistant cell lines were characterized using RNA sequencing and mass spectrometry-based phosphoproteomics. Effects on selected target proteins (eg pAKT, pS6, pRB, RB or Cyclin D1) were confirmed using Western Blots. To modify resistance to palbociclib, a targeted in vitro drug-screen was performed using a range of inhibitors of the PI3K/AKT/mTOR and MEK pathways.

Results: Western blot analysis of resistant cell lines demonstrated sustained down-regulation of Rb and phospho-Rb in response to palbociclib, which was reversible after discontinuation of palbociclib. Mass spectrometry identified >6,000 peptides across parental and resistant cells corresponding to 4,757 phospho-peptides and 5,337 phosphorylation sites. Pathway analysis suggested increased activity in the P31K/AKT/mTOR pathway in resistant clones (including Akt1, p90S6K and mTOR), as well as changes in p53 and apoptotic regulation (e.g. phosphorylation of BAD). In addition, resistant clones showed multiple phosphorylation changes in the Rho/Rac pathway, suggesting changes in cytoskeletal organisation and a more invasive phenotype. Targeted drug screening showed a variable pattern across resistant clones with increased sensitivity to co-treatment of palbociclib with AKT inhibitors, PI3K alpha/delta inhibitors and/or MEK inhibitors in selected resistant clones, whereas pan-PI3K or PI3K beta/delta inhibitors showed limited efficacy in the selected clones.

Conclusions: Phosphoproteomic analysis of palbociclib-resistant ER+ breast cancer cell lines demonstrated up-regulation of PI3K/AKT/mTOR and anti-apoptotic pathways. Resistant cell lines were sensitive to inhibition of PI3K/AKT/mTOR and/or MEK pathways with distinct patterns of activity across resistant clones suggesting that co-treatment of CDK4/6 inhibitors and PI3K/AKT and/or MEK inhibitors warrants further investigation as potential new therapeutic strategies in palbociclib resistance.
Title: Downregulation of histone H2A and H2B pathways is associated with anthracycline sensitivity in breast cancer


Body: Background: Meta-analyses performed by the Early Breast Cancer Trialists Collaborative Group demonstrated a significant increase in disease free and overall survival through the addition of anthracyclines to polychemotherapy. Anthracyclines have, however, significant toxicities including cardiotoxicity and leukaemia. It is, therefore, imperative to identify those patients who will benefit from adjuvant anthracycline treatment; other patients could then be spared unnecessary toxicities and be considered for alternative adjuvant therapy. Several markers that may predict anthracycline benefit have been explored in patient cohorts (HER2, TOP2A, Ch17CEP and TIMP1) with limited success.

Methods: To identify markers that are clinically-relevant, we generated MDA-MB-231, MCF7, SKBR3 and ZR-75-1 breast cancer cell lines sensitive and resistant to epirubicin to identify pathways contributing to anthracycline resistance. A complementary approach including gene expression analyses to identify molecular pathways involved in resistance, and small-molecule inhibitors to reverse resistance were performed. RNA was extracted from patients in the BR9601 adjuvant trial evaluating the addition of epirubicin (E) to CMF and analysed through Nanostring technology. Log-rank analyses explored the predictive values of the signatures 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, ER status and grade, and treatment by marker interactions.

Results: Gene expression analysis identified upregulation of a histone gene module in all four cell lines which was validated by qRT-PCR. Histone deacetylase small-molecule inhibitors reversed resistance and were cytotoxic for epirubicin-resistant cell lines, with IC50's ranging from 0.1-3.69µM, confirming that histone pathways are associated with epirubicin resistance. Gene expression analysis of the 18-gene histone module in the BR9601 clinical cohort revealed that patients whose tumour had low expression had an increased DRFS (HR: 0.35, 95%CI 0.17-0.73, p=0.005) when treated with E-CMF compared with patients treated with CMF alone. Conversely, there was no apparent benefit of E-CMF vs CMF in patients with high histone module expression (HR: 0.96, 95%CI 0.58-1.59, p=0.87). After multivariate analysis and adjustment for HER2 status, nodal status, age, grade and ER status, the treatment by marker interaction was 0.35 (95%CI 0.13-0.96, p=0.042) for DRFS.

Conclusion: Histone gene expression was an independent predictor of anthracycline benefit in terms of DRFS. In vitro data demonstrated that resistance could be reversed with histone deacetylase small-molecule inhibitors. The histone signature identified could be a potential theranostic candidate for patients with early breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-06-04

Title: Investigating clonal dynamics in triple negative breast cancer chemoresistance

Echeverria GV V, Seth S, Moulder S, Symmans W, Chang J, Cai S, Heffernan T and Piwnica-Worms H. M.D. Anderson Cancer Center, Houston, TX and University of Texas Health Science Center, Houston, TX.

Body: Approximately 50% of triple-negative breast cancer (TNBC) patients have extensive residual disease following neoadjuvant chemotherapy (NAC). These patients have a four-fold increase in mortality risk and an increased risk of distant metastases within three years (1). Understanding the molecular basis of resistance to NAC is expected to provide opportunities to better treat patients in the primary setting. Extensive intratumoral subclonal heterogeneity has been well documented in primary, treatment-naïve TNBC (2). Subclonal populations harboring distinct molecular profiles may confound targeted therapy strategies, yet the functional impact of subclonal heterogeneity in TNBC resistance to therapy is unknown. We are implementing DNA barcoding to quantitatively track changes in subclonal architecture pre- and post-treatment in patient-derived xenograft (PDX) models of TNBC in order to design novel combination therapies. Such barcoding strategies have been used to monitor clonal dynamics in breast cancer PDXs with great sensitivity (3).

We have established an orthotopic PDX from a treatment-naïve TNBC patient (PIM1, procured from a patient later found to have chemoresistant disease). In order to model chemoresistance, we treated PIM1 with Adriamycin and cyclophosphamide (AC), standard of care NAC for TNBC patients, which resulted in partial response but left residual disease. To characterize subclonal dynamics in response to NAC, we transduced freshly isolated PIM1 cells with a lentiviral library expressing 25 million unique DNA barcodes (Cellecta) using conditions to ensure each transduced cell contained a single unique barcode. Transduced cells were selected with puromycin, then orthotopically implanted into immuno-compromised mice. High-throughput barcode sequencing revealed reproducible maintenance of greater than 60,000 unique barcodes in PDX tumors. Comparison of barcode distribution in tumors treated with vehicle or NAC will reveal whether NAC selects for a subpopulation of cells during the development of resistance. Future directions will include whole-exome and RNA sequencing to characterize genomic changes associated with alterations in barcode distribution in response to NAC treatment. Our ultimate goal is to identify novel combination therapies to eliminate subclones that contribute to chemoresistance in primary TNBC.

References
Title: Importance of TGFβ-SMAD3 axis in resistance to anti-HER2 drugs

Shimoda M, Chihara Y, Kagara N, Naoi Y, Shimomura A, Shimazu K, Kim SJ and Noguchi S. Osaka University Graduate School of Medicine, Suita, Osaka, Japan.

Body: **Aim:** The aim of this study was to elucidate the role of transforming growth factor β (TGFβ) in the resistance of HER2-positive breast cancer cells to anti-HER2 drugs including trastuzumab and lapatinib.

**Methods:** A HER2-positive breast cancer cell line, SKBR3, was cultured in the presence or absence of TGFβ for 14 days. Subsequently, TGFβ-treated cells were cultured for seven days with or without the anti-HER2 drugs. Sensitivity to trastuzumab and lapatinib was estimated by the WST-8 cell viability assay or absolute cell counts using In Cell Analyzer (GE Healthcare). Proportion of CD44+ CD24– breast cancer stem cells was estimated by flow cytometry of cells immunostained with anti-CD44 and anti-CD24 antibodies. For clinical study, 33 patients with HER2-positive breast cancer receiving neoadjuvant paclitaxel plus trastuzumab in our institution were analyzed. Among the cases, 27 biopsy samples obtained before any treatment from 27 patients who completed 12 cycles of weekly paclitaxel and trastuzumab were subjected to CD24 immunohistochemistry.

**Results:** SKBR3 cells cultured with TGFβ for 14 days exhibited decreased sensitivity to both trastuzumab and lapatinib. Time course study revealed that continuous stimulation for 14 days with TGFβ was required for the resistance to anti-HER2 drugs. Activation of SMAD3, a downstream target molecule of TGFβ, was enhanced over time, judged by the increase in phosphorylation and in nuclear translocation. During 14 day culture with TGFβ, proportion of CD44+ CD24– cells were dramatically increased, and mammosphere formation, another marker of breast cancer stem cells, was significantly enhanced compared to cells treated without TGFβ. Among four HER2-positive breast cancer cell lines, only SKBR3 cells showed increased proportion of CD44+ CD24– cells and resistance to the anti-HER2 drugs, while other two cell lines exhibited epithelial-mesenchymal transition (EMT) in response to TGFβ. To explore the possibility of targeting TGFβ-SMAD3 axis to overcome resistance to anti-HER2 therapy, we used SIS3, a specific inhibitor of SMAD3. Importantly, SIS3 completely restored the sensitivity to both trastuzumab and lapatinib of TGFβ-treated SKBR3 cells, with the decrease in the proportion of CD44+ CD24– cells. These in vitro results suggest that CD24 downregulation can be a surrogate marker of resistance to anti-HER2 therapy. To establish this, we evaluated the CD24 expression in tumor samples of breast cancer patients who received paclitaxel plus trastuzumab in the neoadjuvant setting. Weak CD24 expression in tumor cells in biopsy samples obtained before any treatment was significantly correlated with poorer response to the drugs.

**Conclusion:** These data clearly indicate the importance of TGFβ-SMAD3 axis in the acquired resistance to anti-HER2 drugs. Moreover, resistance to anti-HER2 therapy is associated with the property of breast cancer stem cells rather than EMT. Targeting TGFβ-SMAD3 axis warrants further investigation for overcoming resistance to anti-HER2 therapeutics.
Title: Whole exome analysis of HER-2 positive human breast cancers: Molecular mechanisms underlying response to neoadjuvant therapy with trastuzumab

La Ferla M, Aretini P, Scatena C, Menicagli M, Lessi F, Franceschi S, Cantini L, Bevilacqua G, Naccarato AG Giuseppe, Fontana A and Mazzanti CM Maria. Pisa Science Foundation - ONLUS, Pisa, Italy; Division of Pathology, University Hospital of Pisa, Pisa, Italy and University Medical Oncology Unit II, Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy.

Body: Background: HER2 receptor is overexpressed in 20–30 % of BC and it has been chosen to be an effective therapeutic target. Trastuzumab (Herceptin), a humanized monoclonal antibody, has been approved by FDA to be anti-HER2 therapy, but the mechanism by which Trastuzumab exerts its antitumor activity is not fully understood. Despite that almost 50% of HER2 Positive BC patients do not respond to Trastuzumab based therapy or become resistant during the therapy. An understanding of Trastuzumab resistance mechanisms would be a helpful tool in the development of rational drug combinations to circumvent resistance and allow better selection of patients likely to respond. The aim of this study is the correlation of the mutational profile of HER-2 positive patients with complete response and partial response to the neoadjuvant therapy with Trastuzumab and evaluation of possible molecular factors predictive of response.

Materials and Methods: We performed, so far, whole-exome DNA sequencing (Ion Proton system) to analyze primary FFPE biopsy and primary surgical removed tumors of HER2 positive BC patients specifically selected for different response to the neoadjuvant therapy with trastuzumab and all ER and PR negative: 3 Full Responders (FR) with a complete disappearance of the tumor mass after the therapy and 2 Partial Responders (PR) with a partial shrinkage of the tumor mass, which is then removed by surgery. More patient samples are in the process to be analyzed by whole exome analysis for a total of 20 patients (10 each group).

Results: We identified gene mutations in SYNE2, ANKRD44, CEP350, OR6C74, STRN3 and GIN1 genes present in the biopsy of PR patients and their presence rise in the post therapy samples. Interestingly these mutations rise in the post therapy samples while are nearly absent in the FR patients. On the other side we found some variants present in MACC1 and MAPK1 genes present in the FR samples and absent in the PR samples. Thanks to literature analysis we found that some of these variants are already associated with Trastuzumab resistance and sensitivity.

Conclusions: We found that some genes have a reduction/disappearance or an increase of the mutant allele after treatment. Our findings indicate a possible involvement of a combination of gene mutations associated with resistance to the Trastuzumab therapy for the PR patients. The final gold is the creation of a gene panel implicated in resistance and/or in the complete response to Trastuzumab. Moreover other genes which resulted differentially expressed between PR and FR HER2+ patients are in the process of being investigated. However functional studies, as well as association studies in a larger patient dataset are needed to explore our hypothesis.
Title: Changes in the tumor microenvironment develop acquired resistance to pegylated liposomal doxorubicin in breast cancer mouse model

Kai M, Liu YT, Saito Y, Ferrari M and Yokoi K. Houston Methodist Research Institute, Houston, TX.

Body: Pegylated liposomal doxorubicin (PLD) is one of the most widely used nanotherapeutics for the treatment of advanced/metastatic breast cancer. PLD accumulates in tumors utilizing so-called the enhanced permeation and retention (EPR) effect. Nevertheless, therapeutic efficacy and long term survival remain variable due to the development of acquired resistance. Elucidating the mechanisms of acquired resistance to PLD shall help developing new strategies to improve therapeutic outcome. It has been largely overlooked that the transport of therapeutics across biological barriers can significantly affect the efficacy of cancer therapies. Previously, we showed that the transport of PLD to tumors depends both on the tumor type and organ site. This effect is controlled by the extent to which endothelial cells (ECs) are covered by the collagen type IV (Col4) in the basement membrane, which in turn is influenced by the levels of MMP-9. Here, we have developed 4T1 tumor model which develops acquired resistance to PLD and spontaneous lung metastases. Our objective is to elucidate the resistant mechanism by evaluating the changes in the transport of PLD to the sensitive and resistant/metastatic tumors.

BALB/c mice bearing 4T1 cancers were treated with PLD intravenously when tumor volumes reached a size of approximately 100-200 mm$^3$. Tumor volumes in all mice decreased after initial PLD injections (sensitive). However, tumors started to grow again after 20 days and didn't respond to the second/third injections (resistant). Furthermore, 13 out of 14 mice developed spontaneous lung metastases. To elucidate the mechanisms of the resistance, mice bearing sensitive or resistant tumors were sacrificed after 24 hours of PLD injection, respectively. PLD accumulation in tumors was evaluated by imaging fluorescence of doxorubicin. Immunofluorescence staining was performed to evaluate the expression of ECs, Col4, MMP-9, and efflux pump associated p-glycoprotein (P-gp) in the primary tumors, and the expression of ECs and Col4 were also evaluated in lung metastases.

PLD accumulation was significantly decreased in the resistant tumors compared to the sensitive tumors, although P-gp expression was not increased in the resistant tumors. The amount of ECs and Col4 increased in the resistant tumors. Interestingly, ECs were covered more tightly by Col4 in the resistant tumors as compared with the sensitive tumors, which could decrease the EPR effect in the tumors. MMP-9 expression decreased in the resistant tumors, suggesting less degradation of Col4 in the basement membrane. Coverage of ECs by Col4 was similar between the metastatic lung tumors and uninvolved lung tissue as well as the resistant primary tumors, indicating the EPR effect is not increased in the metastatic tumors.

In summary, ratio of ECs covered by Col4 is higher in the resistant/metastatic tumors as compared to that in the sensitive primary tumors. This structural change in the tumor microenvironment, impeding the sufficient PLD transport to the tumors after the initial PLD therapy, can be a cause of acquired resistance/development of lung metastasis. These changes should be taken into account to develop strategies for overcoming the acquired resistance and metastasis.
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Publication Number: P3-06-08

Title: Abstract Withdrawn

Body:
Leptin is a potent adipokine that plays an important role in the progression of breast cancer and interferes with the action of tamoxifen. We investigated the molecular mechanism underlying the effect of leptin on tamoxifen resistance in breast cancer cells that express leptin receptor (ObRb), and evaluated the impact of ObRb suppression on tamoxifen treatment in MCF-7 and tamoxifen-resistant (TAM-R) cells. Leptin-induced signaling pathway activation was examined by qRT-PCR and western blotting. Chromatin immunoprecipitation assays were performed to further examine the binding of estrogen receptor (ER) α on the promoter of cyclin D1 (CCND1) gene. The effects of combined ObRb knockdown and tamoxifen treatment were evaluated in MCF-7 and TAM-R cells. We found the enhanced proliferation effects induced by leptin were related to extracellular signal-regulated kinase (ERK) 1/2 and signal transducers and activators of transcription (STAT) 3 signaling pathway activation and CCND1 upregulation. Leptin enhanced CCND1 gene transcription by inducing the binding of ERα to the promoter of CCND1 gene. ObRb knockdown significantly enhanced the inhibitory effects of tamoxifen on TAM-R cell proliferation and survival. This study suggested that Long-term endocrine therapy facilitates leptin and ObRb overexpression in breast cancer cells, which attenuates the inhibitory effect of tamoxifen by activating both the ERK1/2 and STAT3 signaling pathways and upregulating CCND1 gene expression. Combination therapy involving ObRb knockdown and tamoxifen treatment may be an alternative therapeutic option for tamoxifen-resistant breast cancer.
**Title:** t-Darpp enhances protein kinase A signaling through direct effects on PKA holoenzyme and indirectly influences cell metabolism, proliferation and apoptosis

**Body:** Trastuzumab has dramatically improved the adjuvant therapy of Her2-positive breast cancer. However, acquired resistance to trastuzumab frequently occurs. Breast cancer cells in vitro selected for trastuzumab resistance exhibit over-expression of t-Darpp, a truncated form of the 32 kDa dopamine and cAMP-regulated phosphoprotein (Darpp-32). t-Darpp over-expression has previously been demonstrated to confer trastuzumab resistance and to result in increased protein kinase A (PKA) activity and signaling. Phosphorylation of Darpp-32 at threonine 75 has been shown to promote inhibition (not activation) of PKA by a yet unknown mechanism. T75 phosphorylation on t-Darpp is required for its trastuzumab resistance activity but the role of T75 phosphorylation in PKA activation by t-Darpp is not known and the molecular mechanism by which t-Darpp enhances PKA activity has not been reported. We used cells stably over-expressing either wild type t-Darpp or a T75A mutant of t-Darpp. Using proximity ligation assays, we found that t-Darpp binds directly to the RI regulatory subunit of PKA leading to 75% less RI subunit bound to the catalytic subunit of PKA (PKAc), compared to parental cells. In contrast, the t-Darpp-T75A mutant does not bind RI and those cells have only 34% less RI bound to PKAc compared to parental. These results suggest a direct effect of t-Darpp on PKA activation by binding the regulatory subunit RI and thereby preventing RI from inhibiting PKAc. The T75 phosphorylation site appears to be required for this activity. Using enzymatic and caspase activity assays, we also show that cells over-expressing t-Darpp, but not t-Darpp-T75A, have increased proliferation, up to 4-fold more cellular ATP, and diminished apoptotic response to etoposide, relative to parental cell controls. So far, this data suggests a dual mode of t-Darpp action: modulation of cell metabolism (possibly via effects on ATP production) and direct effects on PKA holoenzyme. Ultimately, pro-apoptotic response is diminished. Further studies will determine whether PKA activation by t-Darpp is responsible for, or separately influenced by, the metabolic effects of t-Darpp.
Title: Understanding resistance mechanisms of PIK3α inhibition in breast cancer

Le X, Luo F, Treacy D, Wulf G and Garraway L. Dana-Farber Cancer Institute, Boston, MA and Beth Israel Deaconess Medical Center, Boston, MA.

Body: Breast cancer is the most common non-cutaneous cancer in women worldwide. PIK3CA is the most frequently mutated oncogene in all invasive breast cancers, with about one third of cases harboring activating mutations. Small molecule therapeutics targeting PI3K pathway has been developed and investigated for a decade. Everolimus (an mTOR inhibitor) is now successfully used in conjunction with hormonal therapy in patients with metastatic hormone-receptor positive breast cancers. Inhibitors of PI3Kα have shown promise as a single agent as well as in combination with hormonal therapies. The high rate of primary and acquired resistance; however, limits clinical usage of this class of drugs. There is an urgent need to understand the resistance mechanisms of PI3K inhibition and develop strategies to overcome the resistance in breast cancer. We took a genome-scale functional screening approach to identify resistance mechanisms to PI3K inhibition in breast cancer. We performed a whole-genome lentiviral open-reading frame (ORF) overexpression screen in a breast cancer cell line (T47D) in the presence of a PI3Kα inhibitor. We screened a total of 15,590 ORFs and identified 75 genes whose overexpression confers resistance to PI3K inhibition. Once we validate the hits, we plan to determine the mechanisms by which the genes confer resistance. We also plan to perform RNA-sequencing in patient samples with PI3Kα resistance to evaluate the clinical relevance of these genes and pathways.
Title: Are specific interactions of proteasome with Hsp72 responsible for resistance of triple negative breast cancer cells to proteasome inhibitors?

Gaczynska M and Osmulski PA A. The University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Proteasome, the essential giant protease, is the hub of the ubiquitin-proteasome pathway critical for maintaining intracellular proteostasis. Inhibitors of the proteasome cause overload of cells with non-degraded protein debris and activate proapoptotic signaling. The effects are especially dramatic in fast metabolizing cancer cells; hence the proteasome inhibitors are used as anti-cancer drugs. There are two FDA-approved drugs directly blocking the proteasome activity, bortezomib and carfilzomib, and several others are in trials. So far, the high effectiveness of these drugs is limited to multiple myeloma and other hematological malignancies. Unfortunately, breast and other solid cancers are much more resistant to apoptosis triggered by attenuation of the ubiquitin-proteasome pathway than majority of blood cancers. The relatively low effectiveness of proteasome inhibitors has been noted even for cancers that have been identified in genome-wide screens as addicted to the proteasome activity, such as triple negative breast cancers. The source of the resistance, noted in cell culture, animal studies and clinical trials, remains unknown. Here we propose that, at least in part, the resistance can be tracked down to protein-protein interactions between the drug target, the proteasome, and a cytosolic heat shock protein, Hsp72 (inducible Hsp70). Indeed, high levels of Hsp72 have been observed in many cancers, including breast, and generally correlate with poor prognosis. The intracellular roles of the ubiquitous chaperone are undoubtedly very diverse, however we postulate a novel molecular action: direct protection of the proteasome from competitive inhibitors of its enzymatic activity. The hypothesis has been inspired by our recent work exploring the very effective ubiquitin-proteasome pathway in the naked mole rats. These small African rodents do not develop cancers and maintain unusually long lifespan and health-span. Interestingly, the proteasome in naked mole rats is exceptionally resistant to inhibition. We discovered that the resistance is bestowed by a cytosolic protein factor containing Hsp72 and Hsp40 chaperones (Biochim. Biophys. Acta-MBD 1842; 2060-2072; 2014). We partially purified the factor and tested its in vitro effectiveness with human proteasomes. The molecular mechanism beneficial for naked mole rats may be also protective for human cancer cells. We used pilifithrin µ, a specific small molecule inhibitor blocking the interactions of Hsp72 with other proteins, to test the hypothesis. We prepared lysates from cultured triple negative breast cancer cells MDA-MB-231. Such lysates are rich in proteasome activity and preserve interactions of the proteasomes with natural protein ligands. Interestingly, upon the treatment with pilifithrin µ proteasomes in lysates maintained their high activity while becoming significantly more sensitive to inhibition with bortezomib. The studies are in progress to assess the identity of a putative chaperone-based proteasome resistance factor in breast cancer cells.
Title: Overexpression of miR-200a confers chemoresistance by antagonizing TP53INP1 and YAP1 in human breast cancer

Yu S-J, Yang L, Hong Q and Shao Z-M. Breast Cancer Institute, Cancer Hospital, Shanghai Medical College, Fudan University, Shanghai, China.

Body: Introduction:
Emerging evidence suggests molecular and phenotypic association between treatment resistance and epithelial–mesenchymal transition (EMT) in cancer. Compared with the well-defined molecular events of miR-200a in EMT, the role of miR-200a in therapy resistance remains to be elucidated.

Methods:
Breast cancer cells transfected with mimic or inhibitor for miR-200a was assayed for chemoresistance in vitro. miR-200a expression was assessed by quantitative real-time PCR (qRT-PCR) in breast cancer patients treated with preoperative chemotherapy. Luciferase assays, cell proliferation assay were performed to identify the targets of miR-200a and the mechanism by which it promotes treatment resistance. Survival analysis was used to evaluate the prognosis value of miR-200a.

Results:
In this study, our results showed ectopic expression of miR-200a promotes chemoresistance in breast cancer cell lines to several chemotherapeutic agents, whereas inhibition of miR-200a enhances gemcitabine chemosensitivity in resistance cancer cells. Furthermore, overexpression of miR-200a was closely associated with poor response to preoperative chemotherapy and poor prognosis in breast cancer patients. Furthermore, knockdown of YAP1 and TP53INP1 phenocopied the effects of miR-200a overexpression, and confirmed that TP53INP1 is a novel target of miR-200a. Remarkably, TP53INP1 expression is inversely correlated with miR-200a expression in Breast cancer cell lines. Taken together, these clinical and experimental results demonstrate that miR-200a is a determinant of chemoresistance of breast cancer.

Conclusions:
Upregulated miR-200a enhances treatment resistance via antagonizing TP53INP1 and YAP1 in breast cancer.
DSS1 depletion is a promising strategy increasing chemosensitivity possibly independent of BRCA2 expression

Gondo N, Rezano A, Kuzushima K, Iwata H and Kuwahara K. Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan; Aichi Cancer Center Hospital, Nagoya, Aichi, Japan and Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia.

Background
DSS1 (deleted in split-hand/split-foot malformation 1) was originally identified as a BRCA2-associated protein, and its downregulation results in the degradation of BRCA2. Some reports demonstrated that BRCA2 overexpression was correlated with histopathological grade III in sporadic breast cancers, implicating the involvement of BRCA2 overexpression in the proliferation rate of breast cancer cells. Because DSS1 is a stabilizer of BRCA2, we investigated whether altered expression of DSS1 was associated with malignant advancement of sporadic breast cancers. By comparison of DSS1 mRNA level, we reported that the high DSS1 expression groups in breast cancer patients showed worse prognosis in relapse-free survival; however, DSS1 expression per se was not correlated with other clinical parameters including cellular proliferation or tumor grade. Therefore, we hypothesized that breast cancer cells highly expressing DSS1 might be resistant to anti-cancer drugs, and compared chemosensitivity in overexpression or underexpression of DSS1 in breast cancer cells.

Methods
We established MCF7 overexpressing DSS1 (MCF7/DSS1) by retroviral transfection. DSS1 or BRCA2 knockdown in MCF7 was performed using siRNA transfection. The susceptibility to the cytotoxic chemotherapy such as doxorubicin and paclitaxel in breast cancer cells was analyzed by flow cytometry to detect apoptosis.

Results
MCF7/DSS1 showed more resistant to cytotoxic drugs compared with GFP-control MCF7 transfectants (MCF7/GFP). The percentages of apoptotic cells in MCF7/DSS1 and MCF7/GFP treated by doxorubicin were 40.2% and 12.0%, respectively. Conversely, depletion of DSS1 in breast cancer cells resulted in enhanced chemosensitivity compared to control cells. Although DSS1 knockdown induced the downregulation of BRCA2, BRCA2 depletion itself did not show such enhancement of chemosensitivity.

Conclusion
Consistent with the cohort study of sporadic breast cancers, we demonstrated that high expression of DSS1 increased resistance of breast cancer cells to cytotoxic chemotherapy in vitro. Conversely, DSS1 knockdown increased the susceptibility to these drugs in spite that BRCA2 depletion did not affect chemosensitivity. These results indicate that DSS1 could be a molecular target to increase chemosensitivity, which is independent of BRCA2 expression.
Title: Notch3 as a predictor of GSI sensitivity in distinct subtypes of triple negative breast cancer

Shah D and Osipo C. Loyola University Chicago, Maywood, IL.

Body: Breast cancer is the second leading cause of cancer-associated death in women. Triple negative breast cancer (TNBC) accounts for 10-15% of breast cancer, lacks expression of ER, PR, and HER2 gene amplification. Due to lack of targeted therapy, TNBC has the worst prognosis. TNBC is a heterogeneous disease with six distinct subtypes. Current treatment includes combination of surgery, radiation therapy and chemotherapy. Notch signaling is increased in TNBC and predicts for worst overall outcome. The goal of the study is to identify novel Notch-biomarkers that predict sensitivity of subtypes of TNBC to Notch inhibition in both bulk and cancer stem cells.

Methods: Two subtypes of TNBC, Mesenchymal stem-like MDA-MB-231 and basal like MDA-MB-468 cells were used. Notch signaling was evaluated using both RNA-sequencing and quantitative real time PCR (q-RTPCR). Cancer stem cell markers were evaluated using Aldefluor assay, CD44 high/ CD24 low expression, and mammosphere forming assay. To study the effect of chemotherapy and Notch, MDA-MB-231 and MDA-MB-468 cells were treated with carboplatin or a pan Notch inhibitor- gamma secretase inhibitor (GSI) or combination of both and cell viability was measured using the trypan blue exclusion test.

Results: The RNA-seq and RT-PCR results showed that Notch3 is differentially expressed in the two cell lines. Notch3 was knocked down using siRNA and its effect on cell viability was assessed after GSI or carboplatin or combination treatment. MDA-MB-468 cells had higher Notch receptors and activity, most notably Notch3. MDA-MB-468 cells had high levels of Aldefluor whereas MDA-MB-231 cells showed higher levels of CD44 High/CD24 Low expression. MDA-MB-468 cells had higher mammosphere forming efficiency (MFE) compared to MDA-MB-231. Notch inhibition decreased MFE of MDA-MB-468 whereas it increased MFE of MDA-MB-231 cells. MDA-MB-468 cells have lower IC50 for carboplatin or GSI compared to MDA-MB-231 cells, as measured by cell viability assay. However, combination treatment lowered IC50 for GSI in MDA-MB-231 cells as compared to MDA-MB-468 cells. Since Notch3 levels were strikingly different between the two cell lines, we hypothesized that Notch3 levels are necessary for GSI sensitivity. Carboplatin treatment increased Notch3 in MDA-MB-231 cells and the increase in Notch3 could be the reason for lower IC50 of GSI during combination treatment as increased Notch3 would provide the substrate for gamma-secretase. In MDA-MB-468 cells, Notch3 knockdown significantly reduced cell viability and was similar to GSI, suggesting that GSI acts through Notch3. Furthermore, Notch3 knockdown and carboplatin treatment reduced viability comparable to carboplatin and GSI treatment, reinforcing that the GSI acts through Notch3 in TNBC. Using the Kaplan-Meier Plotter, high Notch3 predicted poor recurrence free survival post chemotherapy in patients with ER-, HER2-, basal breast cancer.

Conclusions: GSI acts through Notch3 in two TNBC subtypes and combination of chemotherapy with Notch inhibition results in a better outcome as compared to either drug alone. Future experiments would elucidate the role of Notch3 inhibition in targeting cancer stem cells post chemotherapy treatment in different subtypes of TNBC.
Title: Prognostic role and impact of multi-clonal ER and PgR expression in ductal carcinoma in situ: Results from the UK/ANZ DCIS trial

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Background:
Majority of studies investigating prognostic role of ER and PgR expression in ductal carcinoma in situ (DCIS) have failed to show a significant relationship with recurrence. Branched evolutionary tumour growth and resulting intratumour heterogeneity is now increasingly acknowledged in a variety of cancers. DCIS offers the opportunity to investigate relevance of clonal populations in different ducts that may have evolved differently and may impact outcome differently. We investigated prognostic role of ER and PgR expression in DCIS and impact of clonal variation in ER/PgR expression using material from UK/ANZ DCIS trial.

Methods:
Formalin-fixed paraffin embedded tissues (FFPETs) were collected from patients enrolled in the UK/ANZ DCIS trial, a randomised 2X2 factorial design trial investigating role of tamoxifen, radiotherapy or both as adjuvant treatment in DCIS. A nested case-control design was used; cases (patients with recurrence) were matched by treatment arm and age to 2 controls each (no recurrence).

ER and PgR expression was evaluated on whole sections by immunohistochemistry using 1D5, PGR636 antibodies and EnVision™ FLEX + detection system (Dako). Assays were scored by Allred method (positive if expression >1%) and clonal method. Clonal method documented presence of ducts with complete lack of ER or PgR expression in otherwise ER or PgR positive (Allred method) DCIS. Analyses categorising such multi-clonal DCIS as ER or PgR positive as per current practice (standard) and categorising these as ER or PgR negative DCIS (clonal method) were performed.

Results:
Of 540 samples (180 cases, 360 controls), ER and PgR status was evaluable in 504 and 498 patients respectively. ER expression was absent (ER-) in 148 (29.4%), 356 (70.6%) were ER positive (ER+) with 39 (7.7%) of these displaying multi-clonal expression (ERmulti-clonal). PgR expression was absent in 163 (32.7%), 335 (67.3%) were PgR positive with 84 (16.9%) of these displaying multi-clonal expression.

ER- DCIS showed increased risk (Table) of in situ ipsilateral breast event (DCIS-IBE) and invasive ipsilateral breast event (I-IBE) (borderline significance). Prognostic discrimination was higher when ER status was determined by clonal method (ΔX² 1.57 for I-IBE and 9.05 for DCIS-IBE). Increases in the risk of I-IBE and DCIS-IBE were similar for ER- and ERmulti-clonal DCIS.

Table

<table>
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<th>Endpoint</th>
<th>Comparison</th>
<th>Scoring Method</th>
<th>Hazard Ratio (95%CI)</th>
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<td>I-IBE</td>
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<tr>
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<td>ERmulti-clonal vs. ER+*</td>
<td>Clonal</td>
<td>1.93 (0.74-5.03)</td>
<td>0.1759</td>
</tr>
<tr>
<td>DCIS-IBE</td>
<td>ER- vs. ER+</td>
<td>Standard</td>
<td>2.58 (1.68-3.94)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Lack of PgR expression was only associated with increased risk of DCIS-IBE and PgR status did not add to the prognostic information provided by ER status.

Conclusions:
ER expression is prognostic for recurrence in DCIS. ER+ DCIS with distinct ER- clones has a recurrence risk similar to ER- DCIS. ER scoring should take clonality of expression into account.
Title: Prognostic and predictive relevance of HER2 status in ductal carcinoma in situ: Results from the UK/ANZ DCIS trial

Thorat MA A, Wagner S, Jones LJ J, Levey PM M, Bulka K, Hoff R, Sangale Z, Flake II DD D, Bundred NJ J, Fentiman IS S, Forbes JF F, Lanchbury JS S and Cuzick J. Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, United Kingdom; Centre for Tumour Biology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom; Blizard Institute Core Pathology, Blizard Institute of Cell and Molecular Science, Queen Mary University of London, London, United Kingdom; School of Medicine and Public Health, The University of Newcastle, Australia, Callaghan, New South Wales, Australia; Institute of Cancer Sciences, The University of Manchester, Manchester, United Kingdom; Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom and Myriad Genetics, Inc., Salt Lake City, UT.

Body: Background:
As compared to invasive breast cancer (IBC), HER2 is much more frequently overexpressed in ductal carcinoma in situ (DCIS). Unlike IBC, the prognostic significance of HER2 overexpression remains to be established in DCIS and large studies to investigate its predictive role are lacking. We investigated the prognostic and predictive relevance of HER2 protein and ERBB2 mRNA expression in DCIS using material from UK/ANZ DCIS trial.

Methods:
Formalin-fixed paraffin embedded tissues (FFPETs) were collected from patients enrolled in the UK/ANZ DCIS trial, a randomised 2X2 factorial design trial investigating role of tamoxifen, radiotherapy or both as adjuvant treatment in DCIS. ERBB2 mRNA expression was evaluated by reverse transcription followed by PCR on customized Taqman low-density arrays. ERBB2 mRNA expression was analysed as a continuous variable and also as a binary variable using a cut-off to reproduce HER2 expression distribution similar to that observed with immunohistochemistry (IHC). HER2 protein expression was evaluated by IHC using HercepTest™ and scored as per ASCO-CAP 2013 recommendations; HER2 equivocal (IHC2+) were grouped with HER2 negative (IHC 0 or 1+) for main analyses. Additional analyses using binary ERBB2 mRNA expression as a reflex test for HER2 IHC2+ were also performed.

Results:
HER2 protein expression was evaluable in 713 (181 events) of 755 available samples (DCIS absent or lost during assay in 42). ERBB2 mRNA expression was evaluable in 521 (134 events) of 704 available samples (DCIS absent or insufficient RNA in 51, assay failure in 132). Both results were available in 508 cases (130 events). Increase in ERBB2 mRNA expression (median 0.62; range 0.07-36.76) was associated with increased risk of in situ ipsilateral breast event (DCIS-IBE) [Hazard ratio (HR) = 1.07; 95% Confidence Interval (95%CI) 1.04-1.10; p < 0.0001] but not with increased risk of invasive ipsilateral breast event (I-IBE) [HR = 1.03; 95%CI 0.97-1.10; p = 0.3209]. HER2 positivity by IHC was similarly associated with increased risk of DCIS-IBE [HR = 2.90; 95%CI 1.91-4.40; p < 0.0001] but not with increased risk of I-IBE [HR = 1.40; 95%CI 0.81-2.42; p = 0.2313]. Reclassification of HER2 IHC2+ cases using binary ERBB2 mRNA expression (46 as negative, 16 as positive; 18 expression data unavailable) further improved prognostic discrimination of HER2 IHC [ΔΧ² (1d.f.) 5.51; p = 0.0189] for any recurrence. The effect of radiotherapy (RT) for reducing I-IBE was greater in HER2 positive (by ERBB2 mRNA expression) cases [HR = 0.24; 95%CI 0.07-0.83; p = 0.0237] as compared with HER2 negative cases [HR = 0.60; 95%CI 0.23-1.55; p = 0.2925]. Kaplan-Meier estimates of 10-year I-IBE rates with and without RT were 4.5% (2.5%-1.4%) and 15.8% (9.6%-25.3%) in HER2 positive DCIS; rates in HER negative DCIS were 5.2% (2.1%-2.4%) and 7.3% (4.3%-12.2%) respectively. The differential benefit of RT by HER2 status was also seen for reduction in DCIS-IBE.

Conclusions:
HER2 overexpression is associated with increased risk of DCIS-IBE but not of I-IBE. HER2 status is predictive of radiotherapy response with larger reductions in both I-IBE and DCIS-IBE seen in HER2 positive DCIS.
**Title:** PIK3CA mutations predict resistance to trastuzumab/pertuzumab and nab-paclitaxel in primary HER2-positive breast cancer – Massive parallel sequencing analysis of 293 pretherapeutic core biopsies of the GeparSepto study

Loibl S, Budczies J, Weichert W, Furlanetto J, Stenzinger A, Pfarr N, von Minckwitz G, Jackisch C, Schneeweiss A, Fasching P, Schmatloch S, Aktas B, Nekljudova V, Weber K, Untch M and Denkert C. German Breast Group, Neu-Isenburg, Germany; Charite University of Berlin, Berlin, Germany; University Hospital Heidelberg, Heidelberg, Germany; Sana Klinikum Offenbach, Offenbach, Germany; University Hospital Erlangen, Erlangen, Germany; Elisabeth Krankenhaus Kassel, Kassel, Germany; University Hospital Sen, Essen, Germany and Helios Kliniken Berlin-Buch, Berlin, Germany.

**Body: Background:** Phosphatidylinositol 3-kinase mutations (PIK3CA) are common in breast cancer (BC). Mutations are predominantly found in hot-spots located in the helical and kinase domains (exons 9 and 20). We recently demonstrated that PIK3CA mutations predict lower pathological complete response (pCR) to double blockade with trastuzumab/lapatinib in HER2+ve primary BC.

**Methods:** We evaluated PIK3CA mutations in 293/403 HER2+ve tumors of participants of the neoadjuvant GeparSepto (G7) study (Untch et al. SABCS 2014). The G7 study investigated the effect of exchanging paclitaxel for nab-paclitaxel prior to EC. All patients received trastuzumab and pertuzumab. The G7 study showed a significantly higher pCR rate in patients receiving nab-paclitaxel. HER2, hormone receptors (HR), Ki67 and tumor infiltrating lymphocytes (TILs) were centrally assessed prior to randomization. PIK3CA mutations in exons 9 and 20 were evaluated in formalin-fixed, paraffin embedded core biopsies taken before therapy using deep targeted massive parallel sequencing with a minimum coverage of 500 and a mean coverage of 6520 and 6346 per amplicon, (exon9 and exon 20). Only non-synonymous mutations in the coding region that were called at variant allele frequency ≥10% were taken into consideration. Only cases with a tumor cell content of ≥20% were included.

**Results:** In the G7 study, 396 patients with HER2+ve BC have been randomized from 06/2012 to 01/2014 and started treatment. From these 293 could be sequenced. Median age in the analyzed cohort was 49 years (range 22-75); most tumors were cT1-2 (89.9%); cN0 (54.4%); ductal invasive (88.7%), grade 3 (53.9%), HR+ve (69.6%), Ki67>20% (69.3%), LPBC-negative (83.2%). Overall, 22.2% of the tumors were found to have a PIK3CA mutation, 20.1% in HR+ve and 27.0% in HR-ve. Overall, the pCR rate was significantly lower in the PIK3CA mutant tumors compared to the wild type (wt) group (47.7% vs. 66.7%; p=0.009). This effect was seen both in the HR+ve (43.9% vs. 61.3%; p=0.052) and the HR-ve population (54.2% vs. 80.0%; p=0.029). There was also a significant difference in pCR according to PIK3CA mutation status dependant on the taxane. In the nab-paclitaxel group, pCR rates were significantly lower in patients with PIK3CA mutations compared to those without PIK3CA mutations (38.7% vs. 72.0%; p=0.001), whereas in the paclitaxel group, there was no significant difference between patients with and without a PIK3CA mutation (55.9% vs. 60.9%; p=0.690). The respective interaction could be demonstrated in univariate (p=0.039) as well as multivariate regression analysis (p=0.010) after adjusting for known baseline factors.

**Conclusion:** Patients with PIK3CA mutantHER2+ve BC have a significantly lower pCR rate compared to patients with wt tumors. In contrast to the results with double anti-HER2 blockade consisting of trastuzumab/lapatinib, the effect was evident irrespective of the HR status. In addition, PIK3CA mutation status was significantly associated with higher pCR following nab-paclitaxel.

The project has partly been funded within the EU-FP7 project RESPONSIFY No 278659 and the German Cancer Consortium (DKTK).
Title: Intrinsic subtype and therapeutic response among early stage HER2-positive breast tumors from the North Central cancer treatment group (Alliance) N9831 trial

Body: Importance: 20-25% of patients with early stage HER2-positive breast cancer develop tumor relapse after adjuvant trastuzumab. Identification of such patients is a key goal for clinical management decisions.

Objective: To assess molecular heterogeneity among early stage HER2-positive patients using the Prosigna™ algorithm, to define intrinsic subtypes, and to determine the clinical significance of such heterogeneity.

Design: The NanoString® platform was used to measure the abundance of the PAM50 subtype signature transcripts. Samples from the NCCTG (Alliance) N9831 trial were analyzed using the Prosigna™ algorithm to define intrinsic subtype and risk scores. Subtypes were evaluated for recurrence-free survival following chemotherapy with or without trastuzumab.

Setting: Samples were obtained from a multi-center randomized phase III trial of chemotherapy versus chemotherapy plus trastuzumab.

Participants: All tumors were centrally evaluated for HER2 positivity, defined as IHC 3+ and/or FISH >2.0; 1392 patients were evaluated for molecular subtype.

Intervention(s): Patients received adjuvant chemotherapy (doxorubicin plus cyclophosphamide followed by paclitaxel) (n=484) or chemotherapy plus trastuzumab (n=908).

Main Outcome Measure(s): The primary outcome was recurrence-free survival as a function of subtype and treatment.

Results: Patients with HER2-positive tumors with HER2-enriched features comprised about 70% of the sample cohort, and these individuals received significant benefit from adjuvant trastuzumab (HR=0.68, 95%CI: 0.52, 0.89, p=0.005), as did the relatively fewer patients (291/1392) with Luminal-type tumors (HR=0.52, 95%CI: 0.32, 0.85, p=0.01). The sample cohort contained a small number of patients with tumors having Basal-like features (97/1392), and the data suggest that these individuals may have received less benefit from trastuzumab, beyond that received from chemotherapy alone (HR=1.06, 95%CI:0.53,2.13, p=0.87).

Conclusions: The majority of HER2-positive tumors are classified as HER2-enriched or Luminal using the Prosigna algorithm, and patients with such tumors benefit from adjuvant trastuzumab. About 10% of HER2-positive tumors exhibit Basal-like genomic features, and such tumors appear to recur at fairly similar frequency irrespective of treatment with chemotherapy or chemotherapy plus trastuzumab. Patients with HER2-positive/Basal-like tumors may represent a cohort that should be considered for enrollment in trials to evaluate emerging novel HER2-targeted agents, other targeted therapies, or combinations of both approaches.

Support provided in part by CA129949 and CA15083.
**Title:** Non-amplification ERBB2 genomic alterations in 5,605 cases of refractory and metastatic breast cancer: An emerging opportunity for anti-HER2 targeted therapies


**Body:** Background: Non-amplification ERBB2 alterations (ERBB2 mut) in advanced/metastatic breast cancer (mBC) are not detected by IHC or FISH, but when detected by DNA sequencing assays can lead to clinical responses to anti-HER2 targeted therapy. We queried a database of more than 43,000 clinical cases to uncover the frequency, type and associated genomic alterations (GA) in mBC driven by ERBB2 mut and highlight clinical responses to small molecule drug and antibody-based anti-HER2 therapeutics.

Methods: DNA was extracted from 40 microns of FFPE sections from 5,605 mBC. Comprehensive genomic profiling (CGP) was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay of up to 315 genes to a mean coverage depth of >600X. The results were analyzed for base substitutions, short insertions and deletions, selected rearrangements, and copy number changes.

Results: 698 (12.5%) of 5,605 mBC featured ERBB2 alterations. 596 (10.6%) featured ERBB2 amplifications and 137 (2.4%) featured ERBB2mut. 35 (0.6%) of total mBC had both ERBB2amp and ERBB2mut, which accounted for 5.0% of all ERBB2 altered mBC. The 137 ERBB2mut mBC cases had a median age of 61 years (range 29 to 93 years) and were sequenced to a mean depth of 600X. Samples utilized for CGP included 52 (38%) from the patient's primary BC and 85 (62%) from metastatic sites including bone/soft tissue/skin (12%), liver (20%), LN (14%), serous cavities (6%), lung (4%) and miscellaneous sites (6%). 71 (52%) mBC were submitted as carcinoma NOS, 44 (32%) as IDC, 22 (16%) as ILC and 1 (1%) as mucinous mBC. Of the 137 ERBB2mut cases, 8 featured more than 1 ERBB2 mut. There were 124 (85%) ERBB2 kinase domain mutations and 15 (10%) extra-cellular domain ERBB2mut. The most common genes co-altered in ERBB2mut mBC were TP53 (49%), PIK3CA (42%), CDH1 (37%), MYC (17%), and CCND1 (16%). The enrichment of ERBB2mut in CDH1 mut mBR was significant (p=0.0006) and associated with relapsed lobular mBC. Multiple case examples of kinase domain and extra-cellular domain ERBB2mut mBC responding to a variety of anti-HER2 targeted therapies will be presented.

Conclusions: In this large series of 5,605 mBC, 20% of the total ERBB2 alterations were non-amplification ERBB2mut not detectable by standard of care IHC and FISH slide-based HER2 tests. Given the demonstration of ERBB2mut driven mBC responsive to anti-HER2 targeted therapies in this study, expansion of clinical trials designed to detect these ERBB2mut cases with CGP and optimize the targeted therapies for these patients is strongly recommended.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-06

Title: Objective measurement of HER2 (ERBB2) intracellular and extracellular domain spatial co-localization stratifies benefit from adjuvant trastuzumab

Carvajal-Hausdorf DE, Toki M, Schalper KA A, Pusztai L, Pysrri A, Kalogeras KT T, Kotoula V, Fountzilas G and Rimm DL L. Yale University, New Haven, CT; Yale University, New Haven, CT; Attikon University Hospital, Athens, Greece; Aristotle University of Thessaloniki, Thessaloniki, Greece and Aristotle University of Thessaloniki, Thessaloniki, Greece.

Body: Background: The ASCO/CAP guidelines consider chromogen-based immunohistochemistry (IHC) as the primary assay to determine HER2 status in breast cancer. U. S. Food and Drugs Administration (FDA) approved HER2 antibody assays target the protein's intracellular domain (ICD). Studies suggest that quantitative, domain-specific measurement of HER2 might predict benefit from trastuzumab therapy, further classifying traditional HER2-positive breast cancer. Here we define a method of simultaneous, objective measurement of HER2 ICD and extracellular (ECD) domains, and determine its effect on trastuzumab benefit in the adjuvant setting.

Methods: We measured co-expression of HER2 ICD and ECD using a proximity ligation assay (PLA) and quantitative immunofluorescence (QIF) in a HER2 standardization tissue microarray (TMA) with CLIA-lab defined HER2 status. Previously validated, standardized HER2 antibodies were used to detect ICD and ECD (CB11 and SP3, respectively). We determined the relationship between HER2 PLA scores, HER2 clinical status and domain-specific scores. Finally, we measured HER2 ICD/ECD PLA in 180 patients from a clinical trial of adjuvant chemotherapy followed by trastuzumab (HeCOG 10/05). Median cut-point was used to stratify patients according to HER2 PLA scores. Cut-points for HER2 ICD and ECD were obtained using Joinpoint software. All statistical tests were two-sided.

Results: In the standardization TMA, HER2 PLA levels were associated to HER2 CLIA status (P<0.0001). There was a good correlation between HER2 PLA scores and HER2 ICD and ECD (R²=0.57 and R²=0.54, respectively). In trastuzumab-treated patients from HeCOG 10/05, a similarly good correlation was observed between HER2 PLA scores and HER2 ICD and ECD (R²=0.41 and R²=0.3, respectively). In univariate analysis, HER2 PLA-low status was associated with ER-positive status (P=0.005). There was no association with age, histological grade, tumor size, lymph node status and TNM stage. Although all tumors were HER2-positive, HER2 PLA-high status was significantly associated with longer 5-year disease-free survival (DFS) (log-rank P=0.036, HR=0.32, 95% CI: 0.132-0.935). HER2 PLA status was superior to ICD status (log-rank P=0.67) and numerically comparable to ECD status (log-rank P=0.049, HR=0.31, 95% CI: 0.144-0.997) to predict benefit from adjuvant trastuzumab, as previously published by our group. HER2 PLA high status was independent predictor of better outcome in a Cox proportional hazards model including age, histological grade, ER status, tumor size, lymph node status and TNM stage.

Discussion: Using an objective, quantitative HER2 assay for synchronous, domain-specific measurement, we stratified benefit from adjuvant trastuzumab treatment in patients from a prospective cohort. Our results further support the concept that benefit from HER2 ECD-targeted therapies might be modulated by the presence of truncated HER2 protein variants and that tyrosine kinase inhibitors (ICD-directed) may be advantageous for a subset of HER2-positive patients. Furthermore, this technique that uses two antibodies has the potential to increase both sensitivity and specificity of the IHC assay to predict response to HER2 pathway inhibitors.
Title: The updated ASCO/CAP guidelines for HER2 testing create more uncertainty for clinicians

Chen J, Klein P and Shao T. Mount Sinai Beth Israel Medical Center, NY, NY.

Body: Background: Accurate assessment of the Human Epidermal Growth Factor Receptor 2 (HER2) status has been an integral part of clinical decision making in treatments of breast cancer. In 2007, American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) published a series of guidelines on how to determine the status of HER2. The guidelines were updated in 2013 with the goal of reducing the numbers of false negative cases. The new guidelines are based on a combination of HER2:CEP17 ratio and average HER2 copy number. We sought to assess the overall effect of the new guidelines.

Methods: We retrospectively identified all cases of invasive breast cancer with HER2 testing done in 2014 from the pathology database of Mount Sinai Beth Israel, Mount Sinai St. Luke's and Roosevelt Hospitals. Our pathology department guideline is to perform initial testing for HER2 with immunohistochemistry (IHC) by the HercepTest (Dako) method. Those with IHC of 2+ would be followed by reflex HER2 dual probe FISH. The HER2:CEP 17 ratio and average HER2 copy number were then reviewed for each IHC 2+ case using the 2013 guidelines. These cases were then rescored using the 2007 guidelines. All equivocal cases as determined by the new 2013 guidelines (HER2:CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals/cell) were further evaluated to determine whether repeat HER2 testing was performed as suggested by the new guidelines and whether HER2 directed therapy was recommended for patient.

Results: Among 853 cases identified in the database, 337 were IHC 2+. Using 2007 guidelines, 27/337 cases (8.0%) were amplified (HER2:CEP 17 ratio >2.2), 6 (1.8%) were equivocal (HER2:CEP 17 ratio 1.8-2.2), and 305 cases (90.2%) were non-amplified (HER2:CEP 17 ratio <1.8). Using the 2013 guidelines, 29/337 cases (8.6%) were amplified (HER2:CEP 17 ratio ≥2 or HER2 copy number ≥6), 23 (6.8%) were equivocal (HER2:CEP17 ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0), and 284 (84.3%) were non-amplified (HER2:CEP 17 ratio <2 with an average HER2 copy number <4.0). The new guidelines resulted in change in HER2 status in 24 cases (7.1%): 2 cases changed from equivocal to amplified, 1 case changed from equivocal to non-amplified, but 20 cases changed from non-amplified to equivocal. Of the 23 equivocal cases determined using the 2013 guidelines, only 13 cases had repeat HER2 analysis. On repeat HER2 testing, one case was found to be HER2 amplified, 4 cases were non-amplified, and 8 cases remained equivocal. Only one equivocal case received HER2 directed treatment.

Conclusion: The 2013 ASCO/CAP guidelines for HER2 assessment identified a slightly increased number of patients eligible for HER2 directed therapy, but also resulted in a significant increase in the number of equivocal cases. The new guidelines appear to have generated more uncertainty for the clinician due to the rise in equivocal cases. Further studies are needed to determine whether patients with equivocal HER2 status would benefit from HER2 directed therapy.
**Title:** Quantitative HER family proteins assessment as prognostic and predictive biomarkers in the EGF30008 clinical trial


**Body:**

**Background**

Combined targeted strategy with letrozole (Le) and lapatinib (La) improves progression-free survival (PFS) in patients with metastatic breast cancer (MBC) co-expressing hormone receptor-positive (HR+) and HER2+ but not in HR+/HER2-negative (HER-) disease (Johnston J Clin Oncol 2009). However, among HER2+ tumors, quantitative levels of HER2 are heterogeneous with a broad dynamic range corresponding to approximately 163.7 to 17446.7 amol/µg as previously reported (Nuciforo SABCS 2014). In addition, within HER2- tumors, quantitative measurement of HER family proteins may identify those patients most likely to benefit from the addition of La to Le. In this retrospective study, we tested the prognostic and predictive ability of HER proteins quantification in clinically HER2+ tumor samples from the EGF30008 study.

**Methods**

Formalin-fixed paraffin-embedded primary tumor tissues sections from HER2+ MBC population were used. After laser microdissection, tissue lysates were prepared for selected reaction monitoring mass spectrometry (SRM-MS) analysis. Absolute quantitation was accomplished through simultaneous detection of endogenous target and synthetic labeled heavy peptide identical to analytical targets (EGFR, HER2, HER3). HER2 protein levels were correlated with PAM50 molecular subtypes, ERBB2 and ESR1 genes by nCounter. PFS and overall survival (OS) were analyzed by Kaplan–Meier and log-rank test. To determine whether HER2 protein levels were predictive of La benefit, we tested the interaction term of HER2 protein as a continuous variable by treatment arm in a Cox model.

**Results**

Within the HER2+ study cohort (n=219), 107 had an available tumor block; 84 cases had sufficient material for HER expression measurement by SRM-MS. Average HER2 levels were 2321.1 amol/µg (median, 817.6). HER2 levels were lower in Le+La (n=43; mean, 1761 amol/µg) compared to Le (n=41; mean, 2908 amol/µg) arms, although the difference was non-significant (p=0.108). No expression of EGFR and HER3 was observed. HER2 protein levels were significantly different among PAM50 subtypes with HER2-enriched (HER2E) tumors showing the highest expression followed by Basal-like, Luminal A, Luminal B, and Normal-like (p<0.001). A correlation between HER2 protein, ERBB2 (r=0.5, p<0.001) and ESR1 (r=-0.5, p=0.001) gene expression was found. In patients with disease that expresses HER2 protein levels above the median a trend towards worse PFS (2.9 vs 7.7 months, p=0.092) and OS (21 vs 39 months, p=0.071) were observed. A statistically significant interaction was observed between HER2 protein levels and La treatment for both PFS (p=0.049) and OS (p<0.001). HER2+ tumors with lower expression of HER2 benefited more from La than those with higher expression.

**Conclusions**

Levels of HER2 protein in HER2+ MBC are extremely heterogeneous. An association between HER2 protein and gene expression by nCounter was observed. HER2E tumors by PAM50 showed the highest levels of HER2 protein. Within the group of HER2+ MBC by standard IHC/FISH, tumors with high HER2 protein had a statistically non-significant worse outcome and do not seem to benefit from La. Further validation of these findings is warranted.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-07-09

**Title:** Quantitative p95HER2 protein expression is predictive of trastuzumab response in HER2-positive metastatic breast cancer

Sperinde J, Bachmeier B, Weidler JM M, Lie Y, Chenna A, Winslow J, Engel J, Schubert-Fritschle G, Sommerhoff C, Petropoulos C, Bates M, Huang W and Nerlich A. Monogram Biosciences, Integrated Oncology, LabCorp, South San Francisco, CA; Institute of Laboratory Medicine, Ludwig-Maximilians-University, Munich, Germany; Formerly Monogram Biosciences, South San Francisco, CA; Munich Cancer Registry (MCR) of the Munich Tumour Centre, Institute of Medical Informatics, Biometry and Epidemiology (IBE), University Hospital of Munich, Ludwig-Maximilians-University, Munich, Germany; Institute of Laboratory Medicine, Ludwig-Maximilians-University, Munich, Germany and Munich Municipal Hospital, Munich, Germany.

**Body: Background:** Expression of p95HER2 (p95), a truncated form of the HER2 receptor that lacks the trastuzumab binding site but retains kinase activity, has been reported as a prognostic biomarker for poor outcome in trastuzumab-treated HER2-positive metastatic breast cancer (MBC). However, the ability of p95 to predict trastuzumab benefit has not been demonstrated due to the difficulty in obtaining the appropriate control group, namely HER2+ MBC patients not treated with trastuzumab. In the current study, the predictive value of p95 expression was tested in a cohort comprised of HER2-positive MBC patients treated before the availability of trastuzumab and trastuzumab-treated HER2-positive MBC patients.

**Methods:** The current cohort was derived from 206 HER2-positive MBC patients in the Munich Cancer Registry with a median follow up of 64 months. Cases were divided between those that received trastuzumab (n=115) and those that were treated before the availability of trastuzumab (n=91). Quantitative p95 protein expression was measured in formalin-fixed paraffin-embedded samples using the p95 VeraTag® assay (Monogram Biosciences), which is specific for the active M611 form of p95. Quantitative total HER2 protein expression was measured using the HERmark® assay (Monogram Biosciences). p95 and HERmark cutoffs were pre-specified (Duchnowska, Clin Cancer Res, 20:2805, 2014 and Huang, Am J Clin Pathol, 134:303, 2010). Analyses with p95 were restricted to samples with confirmed HER2 overexpression by HERmark. All hazard ratios (HR) were stratified by estrogen receptor status and grade.

**Results:** Consistent with previous training (Sperinde, Clin Cancer Res, 16:4226, 2010) and validation (Duchnowska, Clin Cancer Res, 20:2805, 2014) datasets, subjects treated with trastuzumab experienced a shorter time to progression (TTP) when p95 expression levels were above the cutoff versus below the cutoff (HR = 3.8, p = 0.019). However, only a trend was observed between p95 expression levels and overall survival (HR = 2.2, p = 0.20), possibly due to a lower frequency of events and relatively small sample size. The predictive value of p95 was assessed by determining the benefit of adding trastuzumab to chemotherapy treatment in subsets below and above the p95 cutoff. As expected, patients with p95 below the cutoff experienced significant benefit in TTP from adding trastuzumab (HR = 0.13, p<0.001), whereas patients with p95 above the cutoff experienced less benefit (HR = 0.70, p=0.47). p95 expression level was predictive of trastuzumab response with an interaction p-value of 0.015. The results for OS were similar, however trastuzumab benefit was less distinct between the two groups (interaction p = 0.18); HR = 0.23, p = 0.0013 below the p95 cutoff versus HR = 0.50, p = 0.14 above the p95 cutoff.

**Conclusions:** In this dataset, quantitative p95 expression was predictive of trastuzumab treatment benefit in MBC. Patients with high p95 expression may be particularly good candidates for dual HER2 blockade, as reported in the NeoALTTO trial (Scaltriti, Clin Cancer Res, 21:569, 2015), or other additional therapies.
Title: The association between the expression of progesterone receptor and clinical benefit of adjuvant trastuzumab in estrogen receptor-positive and HER2-positive breast cancer patients

Lee HW, Ahn SG, Park JT, Yang BS, Park S, Jeong J and Kim SI. Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea and Yonsei University College of Medicine, Seoul, Korea.

Body: 

**Background:** Previous studies have shown that progesterone receptor (PR) status has a prognostic value in hormone receptor-positive breast cancer. In this study, we evaluated the clinical significance of PR status in estrogen receptor (ER)-positive and HER2-positive breast cancer.

**Methods:** We retrospectively analyzed the data of ER+ and HER2+ breast cancer patients who underwent surgery at Gangnam Severance hospital and Severance hospital from 2002 to 2012. We excluded patients who had a history of previous cancer, received neoadjuvant chemotherapy, did not received adjuvant chemotherapy, and had contralateral breast cancer or metastasis at diagnosis. A total of 346 patients were identified. Among them, 155 patients (44.8%) received adjuvant trastuzumab.

**Results:** At a median follow-up of 59 months, median disease-free survival (DFS) and overall survival (OS) were 56 and 59 months, respectively. The DFS and OS showed no difference according to PR status in overall patients. Then, these patients were categorized into two groups: ER+/HER2+/PR+ and ER+/HER2+/PR-. In ER+/HER2+/PR+ patient, there was no difference of DFS or OS according to trastuzumab use. In ER+/HER2+/PR- patients, DFS was significantly better in patients who received adjuvant trastuzumab treatment compared to those who did not (p=0.009). We also analyzed influence of PR status on treatment outcome between patients who received adjuvant trastuzumab and those who did not. In patients who received adjuvant trastuzumab, there was no difference of DFS or OS according to PR status. However, in patients who did not receive adjuvant trastuzumab, ER+/HER2+/PR- patients showed worse DFS than ER+/HER2+/PR+ patients (p=0.006).

**Conclusions:** In patients with ER+/HER2+ breast cancer, we found that a prognostic value of PR only retained in those who did not receive adjuvant trastuzumab. Our findings suggest that the use of adjuvant trastuzumab may offer less clinical benefit for the patients with ER+/HER2+/PR+ breast cancer.
Title: High activation of PI3K pathway defined by PIK3CA mutation, PTEN, and INPP4B expression are associated with trastuzumab efficacy in HER2-positive breast cancer

Sueta A, Yamamoto Y, Takeshita T, Yamamoto-Ibusuki M and Iwase H. Kumamoto University Graduate School of Medical Science, Kumamoto, Japan.

Body: Background
Aberrations of phosphoinositide-3-kinase (PI3K) pathway are extensively found in many human cancers through several mechanisms, including mutation or amplification of PIK3CA and loss of phosphatase and tensin homolog (PTEN) and inositol polyphosphate 4-phosphatase-II(INPP4B). In breast cancer, a number of studies have suggested the putative mechanism of resistance to trastuzumab therapy in terms of PI3K pathway activation. We aimed to evaluate the predictive relevance of these biomarkers to trastuzumab efficacy in HER2-positive disease.

Patients and Methods
A total of 43 breast cancer patients with HER2-positive who received both neoadjuvant treatment and surgery at Kumamoto University Hospital between 2004 and 2012 were selected. The regimens of chemotherapy included anthracycline or taxane-containing drugs in combination with trastuzumab. Using pretreatment tumor tissues, PIK3CA mutations (E542K, E545K, and H1047R) were analyzed by direct dideoxynucleotide sequencing and digital PCR methods. Additionally, the expressions of PTEN, pAkt, and INPP4B were assessed by immunohistochemistry (IHC).

Results
The overall pathological complete response (pCR) rate was 60%. Direct sequencing detected PIK3CA mutations in 21% of all patients, whereas digital PCR detected them in 26 % when the cutoff point of the mutation was set at 1%. In some cases, it was difficult to differentiate mutant DNA from artifact by direct sequencing, but we could identify the mutation clearly using digital PCR. We found the correlation between the proportion of the PIK3CA mutation and the pCR rate; the pCR rates in the patients with PIK3CA mutations with cut-off of 1%, 10% and 20% were 55%, 29%, and 0%, respectively.

There were no significant correlations of clinicopathological features with PIK3CA mutations, copy number status, PTEN, and pAkt expression. Low INPP4B expression was associated with larger tumor size (P = 0.035), and higher nuclear grade (P = 0.031) compared to high expression.

We evaluated the contribution of biomarkers related to the PI3K pathway to the prediction of pCR by logistic regression models. In multivariate analysis, activation of the PI3K pathway due to either PIK3CA mutation or low PTEN expression were related to poorer response to trastuzumab (OR of predictive pCR was 0.11, P = 0.041). Similarly, high activation defined as PIK3CA mutation or low expression of PTEN or INPP4B tend to have lower pCR (OR was 0.14, P = 0.064).

Conclusions
1. Digital PCR has potential to complement the direct sequencing data, leading to more accurate measurement of the mutation frequency.
2. Our findings provide additional support for the recently published studies regarding activating mutation in PIK3CA in HER2-positive breast cancer, and further suggest that integrated biomarkers of PIK3CA mutation, PTEN, INPP4B are stronger predictors of trastuzumab response than either one alone.
**Title:** Homologous recombination deficiency (HRD) as a predictive biomarker of response to neoadjuvant platinum-based therapy in patients with triple negative breast cancer (TNBC): A pooled analysis

Telli ML L, McMillan A, Ford JM M, Richardson AL L, Silver DP P, Isakoff SJ J, Kaklamani VG G, Gradishar W, Stearns V, Connolly RM M, Loibl S, Elkin EP P, Timms K, Hartman A-R and von Minckwitz G. Stanford University School of Medicine, Stanford, CA; Dana-Farber Cancer Institute, Boston, MA; Massachusetts General Hospital Cancer Center, Boston, MA; Harvard Medical School, Boston, MA; UT Medicine Cancer Therapy and Research Center, San Antonio, TX; Northwestern University, Chicago, IL; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD, Germany; German Breast Group, Neu-Isenburg, Germany and Myriad Genetics, Inc., Salt Lake City, UT.

**Body:**

**Background:** TNBC patients with homologous recombination (HR) deficient tumors have significantly higher pathologic complete response (pCR, ypT0/is ypN0) rates when treated with platinum-based chemotherapy regimens than TNBC patients whose tumors are HR non-deficient. We performed a pooled analysis of 5 phase II studies that included patients with TNBC treated with neoadjuvant platinum-based chemotherapy to better estimate the pCR rates amongst HR deficient and HR non-deficient tumors.

**Methods:** Patients with TNBC and known HR deficiency status from the following clinical trials were available for analysis: PrECOG 0105 (N=72), NCT00148694/NCT00580333 (N=50), NCT01372579 (N=26), TBCRC 008 (N=18). Neoadjuvant chemotherapy regimens included 1) carboplatin, gemcitabine, iniparib, 2) cisplatin with or without bevacizumab 3) carboplatin, eribulin, 4) carboplatin, nab-paclitaxel, with or without vorinostat. HR deficiency status was defined as a high HRD score (42 or higher) and/or presence of a BRCA1/2 tumor mutation (tBRCA). Logistic regression models were used to adjust for study effects. The addition of data from the TNBC platinum-treated arm of GeparSixto (n=101) to this pooled analysis will be available at the time of presentation bringing the total sample size to 267.

**Results:** pCR was achieved in 51 patients (31%) and 104 patients (63%) were HR deficient (31 with high HRD score and tBRCA mutation, 67 with high HRD score only, and 6 with tBRCA mutation only). Patients with HR deficient tumors were more likely to achieve a pCR than those with HR non-deficient tumors: 44% vs. 8% (p<0.01). When adjusting for study effects in separate logistic regression models, patients with HR deficient tumors (OR=12.5), with tBRCA mutations irrespective of HRD score (OR=2.3), or with high HRD scores (OR=14.6) were more likely to have a pCR (see Table).

<table>
<thead>
<tr>
<th>Model #</th>
<th>Predictor Variable</th>
<th>Population (n)</th>
<th>Adjusted Odds Ratio</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HR Deficiency</td>
<td>All (166)</td>
<td>12.5</td>
<td>4.2</td>
<td>37.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>tBRCA mutation</td>
<td>All (166)</td>
<td>2.3</td>
<td>1.02</td>
<td>5.1</td>
<td>0.045</td>
</tr>
<tr>
<td>3</td>
<td>High HRD score</td>
<td>tBRCA non-mutants (124)</td>
<td>14.6</td>
<td>4.2</td>
<td>50.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Conclusion:** HR deficiency status is a robust predictor of pCR across different neoadjuvant platinum-based regimens. This pooled analysis suggests that HRD can be used to identify TNBC patients with a high probability of obtaining pCR with a platinum-based neoadjuvant chemotherapy regimen.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-13

Title: Homologous recombination deficiency (HRD) as a predictive biomarker of response to preoperative systemic therapy (PST) in TBCRC008 comprising a platinum in HER2-negative primary operable breast cancer

Connolly R, Elkin E, Timms K, Goetz M, Boughey J, Zhang Z, Walsh B, Carpenter J, Storniolo A, Watkins S, Gabrielson E, Hartman A-R and Stearns V. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; Myriad Genetics Inc, Salt Lake City, UT; Mayo Clinic, Rochester, MN; University of Alabama at Birmingham, Birmingham, AL; Indiana University, Indianapolis, IN and Anne Arundel Medical Center, Annapolis, MD.

Body: Background:
Biomarkers to predict response to platinum-based therapy in early breast cancer are needed. HRD is a promising predictor of response to DNA damaging agents, such as platinums. We hypothesized that HRD (high HRD score $\geq 42$ and/or tumor BRCA [tBRCA] mutation) would predict pathological complete response (pCR) in patients with HER2-negative early breast cancer treated with PST comprising a platinum, regardless of estrogen receptor (ER) status.

Methods:
TBCRC008 was a multicenter placebo-controlled trial that investigated pCR (no invasive cancer in breast/axilla) following 12 weeks of preoperative carboplatin and albumin-bound paclitaxel with or without vorinostat in patients with ER-positive or triple-negative breast cancer (TNBC) (Connolly RM. JNM 2015). The pCR rate was similar in both arms (vorinostat 25.8%, placebo 29%). We performed an exploratory biomarker study correlating baseline tumor biopsy HRD status with pCR. The analysis population included all patients with available HRD and pCR data. We compared the proportion of patients with pCR by HRD status using Fisher's exact test. A subset analysis compared pCR proportions by high ($\geq 42$) vs. low (<42) HRD score in those patients who did not have tBRCA mutation. A logistic regression model included HRD status, ER status, treatment arm, tumor grade and use of additional anthracycline-based non-study chemotherapy prior to definitive surgery.

Results:
HRD status and pCR data were available for 48/62 patients (30 ER-positive, 18 TNBC). Of these, 46% of tumors were HR deficient (n=22/48, 33% ER-positive [10/30], 67% TNBC [12/18]). We observed a significantly higher pCR rate in patients with HR deficiency vs not (50% vs 7.7%, $p=0.002$) in the overall population. A similar trend was observed in ER-positive (30% vs 5%, $p=0.095$) and TNBC (66.7% vs 16.7%, $p=0.13$) patients. There was no significant difference when analyzed by treatment arm (vorinostat vs placebo). In a subgroup analysis (n=40) of patients without tBRCA (25 ER-positive, 15 TNBC), a significantly higher pCR rate was observed in those with high vs low HRD score (64.3% vs 7.7%, $p <0.001$). After adjusting for ER status, randomized treatment, use of AC treatment, and tumor grade, patients whose tumors exhibited HR deficiency had a greater than 6 fold increase in pCR compared to those without HR deficiency (adjusted odds ratio = 6.76, 95% CI = 0.85-53.99, $p=.072$).

Conclusion:
This is the first study to evaluate the predictive role of HRD status in patients with ER-positive, HER2-negative breast cancer treated with platinum-based therapy. Our results also support prior observations that HRD status is a promising predictive biomarker of response to platinum agents in TNBC. Further evaluation of this question is warranted in both TNBC and ER-positive breast cancer.
**Title:** Prosigna® intrinsic subtyping predicts response to neoadjuvant combination therapy in study that includes herceptin within HER2+ (IHC) patients


**Body:**

**Background:** The role of the HER2-enriched (HER2E) subtype determined by the Prosigna Assay in the neoadjuvant setting has remained largely uncharacterized. In this study, we examine whether Prosigna can identify a subgroup of HER2+ patients for whom combination neoadjuvant therapy that includes trastuzumab (Herceptin) is associated with a greater likelihood of pathological complete response (pCR).

**Methods:** In this single-arm retrospective analysis, 75 patients determined to be HER2+ by IHC were treated with a neoadjuvant regimen (NAC) consisting of 8-12 cycles of anthracyclines and taxanes as well as Herceptin. The Prosigna Assay was performed on the NanoString nCounter® Dx Analysis System at HU Virgende la Victoria de Málaga/CIMES-UMA. pCR was used as the endpoint for this study and was determined using the Miller & Payne scoring criteria.

**Results:** Mean patient age for this study population was 49 (±11.1yr) and all patients were determined to be HER2+ by IHC. The overall pCR rate in this patient population was 46.2%. Of the 75 patient samples analyzed for this study, 59 (78.6%) were HER2E, 4 (5.3%) were Luminal A and 12 (16.1%) were Luminal B, as identified by the Prosigna Assay. Of the 16 tumors classified as Luminal (A or B) by Prosigna within this HER2+ population, only 2 (12.5%) responders were observed. Categorical analysis revealed that Prosigna subtype predicted response to a NAC regimen combined with Herceptin (Odds ratio [Her2E vs. non-Her2E]=6.4, p=0.023). Further analysis of the Her2E subtype revealed that tumors with profile expression that correlated well with the prototypical Her2E centroid were significantly more likely to respond to combination NAC and Herceptin (Odds ratio [Unit increase of 1 in Her2E correlation]=88.2, p=0.004).

**Conclusions:** The results of this study indicate that HER2+ patients with greater correlations to the HER2E subtype have an increased likelihood of response to combination neoadjuvant regimens that included HER2-targeted therapy.
**Publication Number:** P3-07-15

**Title:** Prosigna® subtype correlation is a strong predictor of response to neoadjuvant chemotherapy (NAC) in early breast cancer (EBC) study


**Body: Background:** Prosigna's ROR score was demonstrated as a strong predictor of response to NAC in a representative cohort of EBC patients including HR+/HER2- N0-N1 patients. Given that the ROR score is partially derived from the correlation of the tumor's expression profile to that of the four prototypical intrinsic subtypes, we determined the relative strength of the association between each subtype correlation and the likelihood of response to NAC. **Methods:** We analyzed 294 FFPE breast cancer samples from pts treated with NAC (anthracyclines and taxanes) in a multi-center Spanish cohort. The Prosigna Assay was performed on the NanoString nCounter® Dx Analysis System at HU Virgen de la Victoria de Málaga/CIMES-UMA. Pathologic complete response (pCR) was used as the primary endpoint for this study and was determined using the Miller and Payne scoring criteria. **Results:** Mean patient age in this population was 50 (±11yr). Apart from targeted therapy, all patients received a standard neoadjuvant treatment regimen consisting of 8-12 cycles of anthracyclines and taxanes. 58% of patients were HR+/HER2- while 24% were classified as HER2+ and 18% were TNBC patients. Of the 311 pts samples previously tested, subtype correlation data was available for 294. Overall subtype concordance between IHC and Prosigna was 72% (K=0.66). The overall pCR rate in this population was 24.9%. Prosigna subtype breakdown in the full study population was 60 Luminal A, 118 Luminal B, 69 HER2-enriched and 47 Basal-like with response rates of 7.2%, 7.2%, 46.2% and 57.4%, respectively. We found that in all study populations, subtype correlation was a strong predictor of response to NAC. Tumors with expression profiles that correlated well with the Luminal prototypical centroids were found to be largely unresponsive to NAC (Luminal A Odds ratio=0.074 per unit increase, p<0.0001; Luminal B Odds ratio=0.059 per unit increase, p<0.0001). Conversely, higher HER2E and Basal-like correlations were associated with increased probability of response (HER2E Odds ratio=11.2 per unit increase, p<0.0001; Basal-like Odds ratio=9.0 per unit increase, p<0.0001). Increased proliferation-score (p-score) was also associated with increased probability of response to NAC (Odds ratio=3.43 per unit increase, p<0.0001). **Conclusions:** In a representative cohort of breast cancer patients, both the magnitude of the subtype correlation to the prototypical centroids as well as p-score, as determined by the Prosigna Assay, were strong predictors of response to NAC. This data underlines the importance of molecular testing for optimal systemic therapy indications in EBC.

Comparing surrogates of oncotype Dx recurrence scores in invasive ductal carcinoma: How complicated does it have to be?

Robertson SJ J, Petkiewicz SL L, Arnaout A, Clemons M, Gravel DH H and Pond GR R. EORLA, Ottawa, ON, Canada; University of Ottawa, Ottawa, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada and McMaster University, Hamilton, ON, Canada.

**Body: Background:** The Oncotype DX Recurrence Score (ODX) is commonly used to estimate recurrence risk and chemotherapy benefit in ER positive, node negative breast cancer but is associated with significant cost. The Magee score (MS), a free online calculation using numerical values of commonly assessed pathological features (ER, PR, Her2/neu, and Ki-67), has been validated as an ODX surrogate. This includes the MS using classic H scores (HMagee) or Surrogate H scores derived from total % positive cells and average intensity or our use of Surrogate H scores derived from Allred scores (SMagee). Also Gage et al (2015) used a simple algorithm based on tumor grade, PR >1% and ER>20%. Here we compare three methods of predicting ODX scores.

**Design:** 61 patients from The Ottawa Hospital with ER positive HER2/neu negative invasive ductal carcinoma (IDC), known ODX were assessed. Classic H scores (CH) were assessed using Image analysis. All cases had Ki67 (1000 cell hot spot score), tumor size, grade, and Allred scores available. Surrogate H scores (SH) were derived (average reported intensity x midpoint of reported Allred positive score range or absolute percentage if between 1-10%). The three MS were calculated with CH giving classic MS (HMagee) and with SH giving surrogate scores (SMagee). Each case was also categorized using rules published by Gage (2015): LOW predicted if Low Grade and PR positive (>1%), HIGH predicted if High Grade or Low ER (<20%). Agreement with classic ODX categories of low (<18), intermediate (18-30) and high risk (≥31) was assessed.

**Results:** ODX included 35 low, 20 intermediate and 6 high risk (Mean 17.4, sd=10). There was a good correlation between corresponding HMagee and SMagee 1-3(0.820, 0.73, 0.84). Concentrating on the theoretically best MS1 that includes Ki67 and all clinical variables (Table 1); HMagee1 predicted 32 cases as low grade with 78% accuracy and SMagee1 predicted 26 low grade with 92.3% accuracy. Of note, all intermediate ODX predicted low by MS were ODX 18-20. None of the MS falsely predicted high risk in low risk ODX. The one case of high ODX which MS predicted low (false negative) is controversial as an Allred ER&PR 8 tumor gave low ODX PR score. Using SMagee1 in this population would safely leave 32 (52.5%) in the intermediate category. Of note, all intermediate ODX predicted low by MS were borderline ODX 18-20. The Gage algorithm in our population is less useful with higher discordance rate and left 69% in the intermediate category.

<table>
<thead>
<tr>
<th>Test</th>
<th>Magee Risk</th>
<th>N</th>
<th>ODX Low</th>
<th>ODX Intermediate</th>
<th>ODX High</th>
<th>%Discordant</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMagee1</td>
<td>Low (&lt;18)</td>
<td>32</td>
<td>25/78%</td>
<td>7/22%</td>
<td>0</td>
<td>7/22%</td>
</tr>
<tr>
<td></td>
<td>Moderate (≥18-30)</td>
<td>28</td>
<td>10/36%</td>
<td>15/54%</td>
<td>3/11%</td>
<td>13/46%</td>
</tr>
<tr>
<td></td>
<td>High (≥31)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1/1.6%</td>
<td>0</td>
</tr>
<tr>
<td>SMagee1</td>
<td>Low (&lt;18)</td>
<td>26</td>
<td>24/92%</td>
<td>1/4%</td>
<td>1/4%</td>
<td>2/8%</td>
</tr>
<tr>
<td></td>
<td>Moderate (≥18-30)</td>
<td>32</td>
<td>11/33%</td>
<td>19/58%</td>
<td>3/9%</td>
<td>14/44%</td>
</tr>
<tr>
<td></td>
<td>High (≥31)</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2/100%</td>
<td>0</td>
</tr>
<tr>
<td>Gage</td>
<td>Low</td>
<td>10</td>
<td>8/80%</td>
<td>1/10%</td>
<td>1/10%</td>
<td>2/20%</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>42</td>
<td>25/60%</td>
<td>16/38%</td>
<td>1/2.4%</td>
<td>26/62%</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>9</td>
<td>2/22%</td>
<td>3/33%</td>
<td>4/44%</td>
<td>5/56%</td>
</tr>
</tbody>
</table>
Conclusions: In IDC, while simpler algorithms without proliferation markers do not perform as well; SMagee1 based on Allred, performs at least as well in prediction of ODX as an MS based on classic H score and can potentially save considerable time and money. In our hands the simpler Gage algorithm does not perform as well.
Body: Oncotype DX (OD) 21-gene breast cancer (BC) assay is used for testing of estrogen receptor positive (ER+) early stage BC and provides a low, intermediate or high 10-year risk recurrence score (LRS, IRS, HRS) for BC. Scores are used as guidelines to predict the probability of successfully adding adjuvant chemotherapy (AC) to endocrine therapy (ET) to reduce the risk of BC recurrence. In retrospective analysis of 2 clinical trials, patients (pts) with HRS benefited from addition of AC to ET, no benefits were shown for LRS, and benefits of AC for IRS were not clear. The OD assay has been utilized since 2004, but data on the impact of using assay results in clinical practice across the US are lacking. The TAILORx and RxPONDER prospective clinical trials which are using OD scores are ongoing (results will be available in 2017 and 2022, respectively) and may help clinicians better understand the predictive capabilities of the IRS OD score.

We analyzed the current impact of IRS OD score in a retrospective observational study of the National Cancer Data Base (NCDB) (which represents 75% of the US population) from 2010-2012. This time period encompasses the beginning of required recording of molecular assays and the latest data released by NCDB in 4/2015. Demographic and clinical variables of all pts with IRS results were analyzed using frequency statistics, chi-square and logistic regression analysis.

Data from 24260/27995 pts with IRS and documented AC information was analyzed. 11520=47.4% pts received AC. Age ranged from 20-90 (mean 58.4, median 59 years); 99.2% were ER+ and females, 2.7% were HER2+; 6143=25.3% had T1a, T1b or T3 tumors; 19791=81.5% were N0, 3684=15.1% N1; 17950=73.6% had ≥G2 tumors and 3389=14% had lymphvascular invasion (LVI).

Selected demographic and clinical characteristics of patients (pts) with -/+ chemotherapy (AC)

<table>
<thead>
<tr>
<th></th>
<th>AC No</th>
<th>AC Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td># Pts</td>
<td>12740=52.6%</td>
<td>11520=47.4%**</td>
</tr>
<tr>
<td>Mean age</td>
<td>61.2</td>
<td>55.3*</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>11328=46.9%</td>
<td>9973=41.1%</td>
</tr>
<tr>
<td>Black</td>
<td>868=3.5%</td>
<td>966=3.9%*</td>
</tr>
<tr>
<td>Other</td>
<td>431=1.7%</td>
<td>491=2%</td>
</tr>
<tr>
<td>Unknown</td>
<td>113=0.4%</td>
<td>90=0.3%</td>
</tr>
<tr>
<td>Insurance (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>7181=29.6%</td>
<td>8227=33.9%</td>
</tr>
<tr>
<td>Medicaid</td>
<td>550=2.2%</td>
<td>646=2.6%</td>
</tr>
<tr>
<td>Medicare</td>
<td>4605=18.9%</td>
<td>2201=9%*</td>
</tr>
<tr>
<td>No I</td>
<td>182=0.7%</td>
<td>197=0.8%</td>
</tr>
<tr>
<td>Tumor size mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>487=2%</td>
<td>238=1%</td>
</tr>
<tr>
<td>6-10</td>
<td>3162=13%</td>
<td>1890=7.8%</td>
</tr>
<tr>
<td>11-50</td>
<td>8874=36.5%</td>
<td>9060=37.3%*</td>
</tr>
<tr>
<td>&gt;50</td>
<td>132=0.5%</td>
<td>234=1%*</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>3450=14.2%</td>
<td>1760=7.2%</td>
</tr>
</tbody>
</table>
IRS OD result had poor PPV for the administration of AC (47.4%). AC administration was significantly associated with larger tumors, LN+, LVI, high tumor grade, higher TNM stage, younger age and black race (p<.001), but not with facility type (community/comprehensive/academic-research program), income, or education (p>0.05). Medicare pts were less likely to receive AC than ones with private insurance (p<.001).

Our data analysis reveals that additional guidelines for selection of pts for OD testing and new algorithms for AC administration in the IRS subsets are needed. The authors of this abstract solicit a call to action from interested parties on behalf of the patients affected by this conundrum.

Orucevic A, Heidel RE E and Bell JL L. The University of Tennessee Medical Center, Knoxville, TN.

Oncotype DX (OD), initially used for early stage estrogen receptor-positive (ER+), node-negative (LN-) invasive breast cancers (BC) and later also for ER+ and 1-3 LN+BC, provides a low, intermediate or high 10-year risk recurrence score (LRS, IRS or HRS) for BC. Based on the score, the addition of adjuvant chemotherapy (AC) to endocrine therapy is recommended for HRS; no benefit for LRS, and unclear benefits for IRS.

The test has been in use since 2004. Payment for OD was approved by the Centers for Medicare & Medicaid Services in 2006. Patient selection for OD use remains unclear, and data on utilization and impact of the assay in clinical practice across the US are lacking.

The utilization and impact of OD across the US is presented in a retrospective observational study of National Cancer Data Base (NCDB) patients (represents 75% of the US population) from 2010-2012. This time period encompasses the beginning of required recording of molecular assays and the latest data released by NCDB in 4/2015. Demographic and clinical variables of all patients that had OD results were analyzed using frequency statistics, chi-square and logistic regression analysis.

513080 patients had BC; 406525 were ER+. 86409 patients with known OD results were analyzed (23.7% of ER+). Patients age range from 19-90 (mean 58.8, median 59 years); 99.1% were females.

Demographic and clinicopathologic characteristics of OD tested patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>OD 86409=100%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>18-40</td>
<td>3429=4%</td>
</tr>
<tr>
<td>41-64</td>
<td>55313=64%</td>
</tr>
<tr>
<td>65-90+</td>
<td>27667=32%</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>75749=87.7%</td>
</tr>
<tr>
<td>Black</td>
<td>6616=7.7%</td>
</tr>
<tr>
<td>Other</td>
<td>4044=4.6%</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>73901=85.5%</td>
</tr>
<tr>
<td>1</td>
<td>10643=12.3%</td>
</tr>
<tr>
<td>≥2</td>
<td>1865=2.2%</td>
</tr>
<tr>
<td><strong>Median Income Quartiles 2008-2012</strong></td>
<td>Missing 634=0.7%</td>
</tr>
<tr>
<td>&lt;$38000</td>
<td>10430=12.1%</td>
</tr>
<tr>
<td>$38000-47999</td>
<td>17346=20.1%</td>
</tr>
<tr>
<td>$48000-62999</td>
<td>23273=26.9%</td>
</tr>
<tr>
<td>$&gt;63000</td>
<td>34726=40.2%</td>
</tr>
<tr>
<td><strong>US geographic location</strong></td>
<td></td>
</tr>
<tr>
<td>CT MA ME NH RI VT</td>
<td>5404=6.3%</td>
</tr>
<tr>
<td>NJ NY PA</td>
<td>15781=18.3%</td>
</tr>
<tr>
<td>DC DE FL GA MD NC SC VA WV</td>
<td>18984=22%</td>
</tr>
<tr>
<td>IL IN MI OH WI</td>
<td>16542=19.1%</td>
</tr>
<tr>
<td>AL KY MS TN</td>
<td>4704=5.4%</td>
</tr>
<tr>
<td>IA KS MN MO ND NE SD</td>
<td>7256=8.4%</td>
</tr>
<tr>
<td>AR LA OK TX</td>
<td>4342=5%</td>
</tr>
<tr>
<td>AZ CO ID MT NM NV UT WY</td>
<td>4757=5.5%</td>
</tr>
<tr>
<td>AK CA HI OR WA</td>
<td>8639=10%</td>
</tr>
</tbody>
</table>

**Urban vs rural**

| Urban | Missing 2264=2.6% |
| Rural | 4503=5.2% |

**ER**

| Missing 190=0.2% |
| ER+ 85018=98.4% |
| ER- 1181=1.4% |
| ER borderline 20=0.0002% |

**Cancer stage**

| Unknown 626=0.7% |
| I 58614=67.8% |
| II 25907=30% |
| III 1134=1.3% |
| IV 128=0.2% |

**Tumor size (mm)**

| Missing 723=0.8% |
| 1-5 2825=3.3% |
| 6-10 17997=20.8% |
| 11-50 63509=73.5% |
| 51-900 1355=1.6% |

**LN+**

| Missing 2147=2.4% |
| 0 70165=81.2% |
| 1 10322=12% |
| 2-3 3113=3.6% |
| 4-39 662=0.8% |

**Histologic grade**

| Unknown 4178=4.8% |
| G1 23142=26.8% |
| G2 44451=51.4% |
| G3&G4 14638=17% |

57.9% of tests followed National Comprehensive Network (NCCN)-defined intermediate risk guidelines (ER+/LN- tumors >1cm), while 15.5% of tests included N1 disease and 25.7% included T1a, T1b and T3 tumors. LRS had 90.1% negative predictive value (NPV) for no AC administration. IRS had 47.4% positive predictive value (PPV) and HRS had 88.9% PPV for AC administration.

**OD score and chemotherapy administration**

<table>
<thead>
<tr>
<th>OD score</th>
<th>Chemotherapy</th>
<th>Chemotherapy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>LRS</td>
<td>42600=90.1%</td>
<td>4014=9.9%</td>
<td>46614</td>
</tr>
<tr>
<td>IRS</td>
<td>12740=52.6%</td>
<td>11520=47.4%</td>
<td>7591</td>
</tr>
<tr>
<td>HRS</td>
<td>840=11.1%</td>
<td>6751=88.9%</td>
<td>78465</td>
</tr>
</tbody>
</table>
The OD is obtained in almost ¼ of ER+BC patients across the US. Its application across geographic and racial groups is fair. Factors influencing patient selection for OD test and AC administration upon obtaining IRS results require further study.
Title: Progesterone receptor positivity is an independent predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer

Fohlin H, Nordenskjöld A, Fornander T, Löfdahl B, Skoog L and Stål O. Linköping University, Linköping, Sweden; Regional Cancer Center South East Sweden, Linköping, Sweden; Institute of Clinical Sciences, Sahlgrenska Academy, Sahlgrenska University Hospital, Gothenburg, Sweden; Southern Älvsborg Hospital, Borås, Sweden; Karolinska University Hospital, Stockholm, Sweden; Unilabs, St Göran Hospital, Stockholm, Sweden and Karolinska University Hospital, Karolinska Institute, Södersjukhuset, Stockholm, Sweden.

Body: Introduction: The expression of estrogen receptor (ER) and progesterone receptor (PgR) predicts the response to endocrine therapy of breast cancer. Nearly all PgR positive tumors are also ER positive. The independent predictive information of PgR has been questioned after an overview by the EBCTCG. However, the studies in the overview were performed before modern PgR immunohistochemistry (IHC) was developed.

Purpose: We aim to investigate the independent predictive value of PgR determined by IHC in ER positive tumors.

Materials and methods: Between 1976 and 1990 the Stockholm Breast Cancer Study Group conducted a randomized trial comparing adjuvant tamoxifen versus control. We evaluated 618 patients with ER-positive "low-risk" breast cancer (tumor size ≤ 30 mm and lymph node-negative) for whom PgR was determined by immunohistochemistry. The median follow-up was 17 years. Hazard ratios (HRs) and 95 % confidence intervals (CIs) were estimated using the Cox’s proportional hazards model.

Results: Patients with ER+/PgR+ tumors receiving tamoxifen had a reduced recurrence risk compared with those who were not treated with tamoxifen (HR= 0.40, 95% CI 0.27 – 0.59, p< 0.001). For patients with ER+/PgR- tumors the difference between tamoxifen vs. no tamoxifen treatment was not statistically significant (HR= 0.88, 95% CI 0.51 – 1.52, p= 0.65). P for interaction between the groups was 0.02.

<table>
<thead>
<tr>
<th>Tam vs. control</th>
<th>PgR (IHC)</th>
<th>HR (95% CI)</th>
<th>P value</th>
<th>P for interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence-free survival</td>
<td>≥ 10 %</td>
<td>0.40 (0.27 - 0.59)</td>
<td>&lt; 0.001</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>&lt; 10 %</td>
<td>0.88 (0.51 - 1.52)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Distant recurrence-free survival</td>
<td>≥ 10 %</td>
<td>0.41 (0.25 - 0.65)</td>
<td>&lt; 0.001</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>&lt; 10 %</td>
<td>0.80 (0.44 - 1.47)</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Breast-cancer specific survival</td>
<td>≥ 10 %</td>
<td>0.35 (0.21 - 0.60)</td>
<td>&lt; 0.001</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>&lt; 10 %</td>
<td>0.70 (0.37 - 1.33)</td>
<td>0.28</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: Our results indicate that the PgR expression adds predictive value to the ER expression regarding benefit from tamoxifen treatment.
**Title:** A validated test for neoadjuvant clinical response to endocrine therapy in breast cancer that estimates accurately recurrence-free and overall survival


**Body:**

**Background:** Aromatase inhibitors (AIs) have an established role in the treatment of estrogen receptor alpha positive post-menopausal breast cancer. Recently we have developed and validated a microarray-derived 4-gene test (Edinburgh EndoResponse4) to predict response to AIs in the neoadjuvant setting. We have also demonstrated the translational potential of this test in predicting accurately clinical response when mRNA is measured for these genes by polymerase chain reaction (PCR) or the gene protein is measured by immunohistochemistry (IHC). There is a major clinical need for biomarkers to predict which patients are likely to recur on adjuvant endocrine therapy so alternative or additional treatments can be provided to reduce recurrence and improve outcome. The aim of this study was to determine if Endoresponse4 and IHC of these gene proteins could do this.

**Methods:** The original microarray assay used pre- and on-treatment (14-days) biopsies from 73 post-menopausal women with ER-rich breast cancer receiving 3 months of neoadjuvant letrozole prior to surgery with 10 years follow-up after adjuvant letrozole. Matched formalin-fixed paraffin embedded (FFPE) tissue sections from 42 of these patients were used for IHC and antibodies were optimised against 3 of the 4 proteins (where validated antibodies were available) using Envision technology. The ability of our test to estimate recurrence-free (RFS) and breast cancer specific overall survival (OS) using both PCR and IHC was then tested in a unique validation cohort of 140 post-menopausal women with ER-rich breast cancer treated with 2 weeks of neoadjuvant letrozole or anastrozole prior to surgery followed by adjuvant endocrine therapy and 10 years of follow up.

**Results:** Within our training cohort (n=73) using Kaplan-Meier analysis our 4-gene test predicted neoadjuvant clinical response and demonstrated a significant association with both RFS (P=0.029) and OS (P=0.009). This approach predicts outcomes within 2-weeks rather than 4-months of treatment required in other studies such as P024. Using IHC in the training cohort (n=42), two gene markers in combination (IL6ST at diagnosis and MCM4 after 2-weeks treatment) predicted both RFS (P=0.017) and OS (P=0.009) with great accuracy. The 140 patient group is being analysed and the findings are so far are consistent with the initial training cohort and indicate a significant association with outcomes.

**Conclusion:**
- A 4 gene model with clinical potential has been developed and validated to predict response to neoadjuvant aromatase inhibitors.
- This 4 gene model predicts for response and also predicts relapse free and overall survival.
- Proteins encoded by 2 of these 4 genes measured by IHC in an initial test set of 42 patients predict accurately both EFS and OS.
- A validation cohort (n=140) with over 10-years of follow-up will be available at SABCS 2015 to determine if this 2 biomarker test can predict outcome on adjuvant endocrine therapy.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-21

Title: Non-alcoholic fatty liver disease induced by selective estrogen receptor modulators is a protective factor for breast cancer survival


Body: Purpose: Non-alcoholic fatty liver disease (NAFLD) induced by selective estrogen receptor modulators (SERMs) treatment may be related to treatment efficacy for the antagonism of circulating estrogens. We conducted a retrospective study to investigate the relationship between survival outcomes and NAFLD in breast cancer patients treated with tamoxifen or toremifene.

Patients and methods: 785 eligible patients received tamoxifen or toremifene in Sun Yat-sen university cancer center from January 2005 to December 2009 were included in our study. All patients have at least one abdominal ultrasonography measurement at baseline and every year's follow-up. Patients who diagnosed NAFLD by ultrasonography during three-year's follow-up were classified into NAFLD cohort, others were classified into no-NAFLD cohort. Univariate and multivariate Cox regression was utilized to analyse the relationship between NAFLD and disease-free survival (DFS) and overall survival (OS).

Results: 158 patients had reported NAFLD in the first 3 years' follow-up. Patients who developed NAFLD had better DFS and OS than those not developing NAFLD. The 5-years DFS was 91.56% and 85.01% at NAFLD and no-NAFLD cohort (univariate hazard ratio [HR]: 0.59 [95%CI 0.37-0.96], P=0.034), respectively. The 5-years OS was 96.64% and 93.31% at NAFLD and no-NAFLD cohort (univariate HR: 0.39 [95%CI 0.16-0.99], P=0.047), respectively. Multivariate analysis revealed that NAFLD is an independent prognostic factor on DFS in breast cancer patients with SERMs treated. Women treated with SERMs experienced NAFLD in the first three-year's follow-up had a reduced risk of DFS of 42% (multivariate HR: 0.58; [95%CI 0.36-0.95], P=0.032) compared with patients without NAFLD.

Conclusion: NAFLD induced by SERMs seems to be associated with better survival outcomes and may therefore be helpful in predicting treatment responses in breast cancer patients treated with SERMs.
Title: RS1008805 polymorphism in CYP19A1 gene is related to the efficacy of hormone therapy in early breast cancer

Wang X, Shao X, Zheng Y, Shi L and Huang Y. Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China.

Body: Purpose It has been suggested that genetic polymorphisms in CYP19A1 gene were related to aromatase activity as well as circulating steroid hormone levels in postmenopausal women. Therefore, it is biologically reasonable that CYP19A1 rs1008805 (A/G) polymorphism may be associated with clinical outcome for hormone therapy.

Methods Genotyping for CYP19A1 polymorphism rs1008805 was performed on 287 women with HR-positive early breast cancer. Associations were evaluated between CYP19A1 rs1008805 genotypes and disease-free survival (DFS).

Results Based on the analysis of the whole cohort, no significant differences were observed between rs1008805 genotypes and DFS, 5-year DFS rate. However, in postmenopausal women, rs1008805 genotypes were significantly associated with DFS and 5-years DFS rate (AA versus AG versus GG: 89.2 months versus 58.2 versus 32.7 months; 55.9% versus 47.8% versus 0%; P = 0.019). In addition, when the population was subgrouped into two cohorts, women carrying GG variant have a poorer DFS, 5-years DFS rate (GG versus AA or AG: 32.7 months versus 70.6 months; 0% versus 52.1%; HR, 3.613; 95% CI, 1.380-9.457; P = 0.005). Furthermore, being adjusted by patients features in multivariate analyses, GG genotype remained an independent prognostic factor for DFS (HR, 3.439; 95% CI, 1.251-9.456; P = 0.017). However, there was no significant differences in DFS and 5-years DFS rate between women harbor the minor allele and those with the homozygous common allele (AG or GG versus AA: 52.4 months versus 89.2 months; 41.0% versus 52.9%; HR, 1.288; 95% CI, 0.705-2.353; P = 0.408). In addition, there were no differences between rs1008805 polymorphisms and DFS among premenopausal women.

Conclusions The homozygous minor allele (GG) of CYP19A1 rs1008805 is significantly associated with worse clinical outcome of hormone therapy in postmenopausal HR-positive early breast cancer patients. If confirmed, genotyping for CYP19A1 polymorphisms rs1008805 may provide predictive information for better selection of endocrine treatment.
Title: CYP2D6 genotype and breast cancer recurrence in tamoxifen treated patients: An evaluation of the importance of loss-of-heterozygosity

Ahern TP, Hertz DL, Damkier P, Ejlertsen B, Hamilton-Dutoit SJ, Rae JM, Regan MM, Thompson AM, Lash TL and Cronin-Fenton DP. University of Vermont College of Medicine, Burlington, VT; University of Michigan College of Pharmacy, Ann Arbor, MI; Odense University Hospital, Odense, Denmark; Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; Aarhus University Hospital, Aarhus, Denmark; University of Michigan Medical Center, Ann Arbor, MI; Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; University of Texas MD Anderson Cancer Center, Houston, TX; Emory University, Atlanta, GA and Aarhus University, Aarhus, Denmark.

Body: Background: Tamoxifen therapy for estrogen receptor positive (ER+) breast cancer reduces recurrence risk by about half. Steady-state concentrations of endoxifen, a potent anti-estrogenic tamoxifen metabolite, are reduced in women whose CYP2D6 genotypes confer poor enzyme function. Many studies have measured associations between genetically impaired CYP2D6 function and tamoxifen resistance. It has been suggested that the subset of studies using DNA extracted from tumor-infiltrated tissue may have been susceptible to genotyping error induced by loss of heterozygosity (LOH); the putative non-differential genotype misclassification may have biased these studies’ estimates toward the null. We reviewed the clinical epidemiology studies conducted to date to assess the importance of loss-of-heterozygosity (LOH) at the CYP2D6 locus and its implications for assessing tamoxifen effectiveness.

Methods: We searched for the terms "tamoxifen" and "CYP2D6" in PubMed, including all papers and abstracts through 31 May 2015 on the association of CYP2D6 gene variants and the risk of breast cancer recurrence or mortality. We used a quantitative bias analysis (QBA) to evaluate the importance of genotype misclassification in studies that extracted DNA from tumor-infiltrated tissue. We conducted a random effects meta-analysis to evaluate all studies simultaneously, and within groups according to whether DNA was derived from tumor-infiltrated tissue or non-neoplastic tissue.

Results: Thirty-one studies investigated CYP2D6 genotype and breast cancer recurrence, yielding relative effect estimates ranging from 0.08 to 14. DNA was extracted from blood or non-neoplastic tissue in 21 of these 31 studies (68%), and from tumor-infiltrated tissue in the remaining 10 (32%). Our analysis of the association between variant/variant genotype compared with wildtype/wildtype genotype included 21 of the 31 studies. Sixteen (76%) of these 21 studies extracted DNA from blood or non-neoplastic tissue and five (24%) extracted DNA from tumor-infiltrated tissue. Genotype misclassification parameters for the QBA were estimated from six concordance studies. There was little difference between the effect estimates (EE) and 95% confidence/simulation intervals (95% CI/SI) before and after QBA (EE=1.71, 95%CI=1.24, 2.36, and 1.80 95%SI=1.28, 2.54, respectively). Studies using non-neoplastic DNA had higher variance than those based on tumor-infiltrated tissue DNA, half reported implausibly high EE, and many were susceptible to design and analysis errors that would bias estimates of association away from the null.

Conclusions: We found little relative bias in the summary estimates of association, either overall or when limited to the tumor-infiltrated tissue DNA studies. Three guideline panels, based on robust evidence, recommend against CYP2D6 genotype-guided tamoxifen therapy. Alternatives for optimizing the effectiveness of tamoxifen therapy, such as assuring adherence and persistence, are more likely to achieve clinically important benefits.
Title: The role of glucocorticoid receptor (GR) expression in predicting pathological complete response (pCR) to neoadjuvant chemotherapy in triple-negative breast cancer (TNBC)


Body: Background
Up to 40% of breast cancers have moderate to strong expression of GR, and activation of GR is associated with poor prognosis in ER-negative breast cancer. GR activation in breast cancer cells initiates anti-apoptotic signaling, contributing to chemotherapy resistance. We hypothesize that GR highly expressing TNBCs will have a suboptimal response to neoadjuvant chemotherapy.

Methods
We identified patients with triple-negative breast cancer (TNBC) who received neoadjuvant chemotherapy at the University of Chicago between 2002 and 2014 under IRB approved protocols. Those patients for whom pre-treatment and post-treatment tissue was available were included in this study. Unstained sections of formalin-fixed paraffin-embedded primary tumor were obtained. Percentage tumor cell GR expression was determined via immunohistochemical (IHC) examination using two different antibodies: the anti-GR rabbit monoclonal XP antibody (Cell Signaling, D8H2, 1:500 dilution) and the polyclonal rabbit anti-GR antibody (1:80). Staining was performed according to methods previously published by Belova et al. TNBCs were considered GR positive if greater than 10% of cancer cells stained moderately to strongly positive for GR.

Results
Fifty paired tissue samples were identified and pre- and post-treatment tissue was stained for GR using two anti-GR antibodies as above. Of these 50 cases, 80% had moderate to strong expression of GR using the XP antibody; results with the polyclonal ab will be compared. Percentage GR expression did not change in the setting of treatment. Work to correlate GR expression using the monoclonal versus the polyclonal antibody and clinicopathological features is ongoing.

Conclusions
To our knowledge, this is the first study attempting to evaluate percentage GR expression as a biomarker of response to neoadjuvant chemotherapy in TNBC. If higher GR expression correlates with a lower pCR rate in TNBC as hypothesized, then GR blockade in conjunction with chemotherapy may overcome chemotherapy resistance and lead to improved response to treatment in patients with GR expressing TNBCs.
Title: Improved clinical outcomes on enzalutamide observed in patients with PREDICT AR+ triple-negative breast cancer: prognosis or prediction?

Miller K, Krop I, Schwartzberg L, Abramson V, Garcia-Estevez L, Eakle J, Nanda R, Yardley D, Ademuyiwa F, Chan S, Crown J, Danso M, Gelmon K, Ma L, Martinez-Janez N, Gradishar W, Steinberg J, Tudor IC, Uppal H, Paton VE E, Parker J, Hudis CA and Traina TA A. Indiana University School of Medicine, Indianapolis, IN; Dana-Farber Cancer Institute, Boston, MA; The University of Tennessee Health Science Center, Memphis, TN; Vanderbilt Ingram Cancer Center, Nashville, TN; Comprehensive Cancer Center Clara Campal, Madrid, Spain; Florida Cancer Specialists, Sarasota, FL; The University of Chicago Medicine, Chicago, IL; Tennessee Oncology PLCC, Nashville, TN; Washington University School of Medicine, St. Louis, MO; Brigham and Women's Hospital, Boston, MA; All Ireland Co-operative Oncology Research Group, Dublin, Ireland; Virginia Oncology, Norfolk, VA; BC Cancer Agency, Vancouver, BC, Canada; Rocky Mountain Cancer Centers, Lakewood, CO; Hospital de Madrid Norte Sanchinarro, Madrid, Spain; Northwestern University Feinberg School of Medicine, Chicago, IL; Astellas Pharma Global Development, Inc., Northbrook, IL; Medivation, Inc., San Francisco, CA; UNC-Chapel Hill, Chapel Hill, NC and Memorial Sloan Kettering Cancer Center, NY, NY.

Body: Background: A novel genomic signature that identifies androgen receptor (AR)-driven disease (PREDICT AR) is being developed for its ability to select patients (pts) who may benefit from enzalutamide (ENZA), a potent AR inhibitor. In a phase 2 study of triple-negative breast cancer (TNBC) pts treated with ENZA (NCT01889238) half of pts had PREDICT AR+ TNBC, with improved clinical outcomes in this sub-population. Because pts with hormonally driven (estrogen-receptor/progesterone-receptor[ER/PR]+) BC are thought to have a better prognosis, we investigated whether the outcomes observed in pts with PREDICT AR+ TNBC could be explained by prognosis alone.

Methods: PREDICT AR was assessed on advanced TNBC from 118 ENZA-treated pts. Because the duration of treatment (Tx) typically decreases with each subsequent Tx, exploratory analyses included median progression-free survival (mPFS) on ENZA vs duration of Tx prior to ENZA (used as a PFS surrogate) in pts who had received ≤1 prior Tx (data cutoff 24Mar15). Baseline characteristics by PREDICT AR status were also evaluated. An independent non-ENZA-Tx TNBC data set (after neo/adjuvant Tx) was probed for PREDICT AR status and clinical outcomes.

Results: ENZA-Tx pts with PREDICT AR+ TNBC (n=56; 47%) were older (66 years vs 52 years), had a longer disease-free interval (DFI) (37 vs 20 months), more bone involvement (57% vs 24%), were more likely to have had prior ER/PR+ BC (30% vs 8%), and a longer median duration on the first-line Tx (29 vs 19 weeks[wks]) vs pts with PREDICT AR- TNBC (n=62;53%).

Table 1. mPFS from ENZA-Tx pts and duration of prior Tx by PREDICT AR status and line of Tx.

<table>
<thead>
<tr>
<th>PREDICT AR Status</th>
<th>Tx prior to ENZA</th>
<th>mPFS on ENZA (wks)</th>
<th>Median duration of prior Tx (wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREDICT AR+ (n=26)</td>
<td>No prior Tx (n=10)</td>
<td>32</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1 prior Tx (n=16)</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>PREDICT AR- (n=36)</td>
<td>No prior Tx (n=11)</td>
<td>8</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>1 prior Tx (n=25)</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

The prevalence of PREDICT AR+ primary TNBC from the non-ENZA-Tx dataset was 51% (n=182). DFI after neo/adjuvant therapy was not statistically different (p=0.605) between pts with PREDICT AR+ vs PREDICT AR- TNBC, and pathologic complete response rates were 23% vs 38% in PREDICT AR+ vs PREDICT AR- TNBC, respectively.

Conclusion: mPFS in pts with PREDICT AR+ TNBC who received first-line ENZA was longer than first-line Tx duration in those who received prior Tx (32 vs 29 weeks). In pts with PREDICT AR+ TNBC who received second-line ENZA, mPFS was longer than the median duration of their first-line Tx (40 vs 34 weeks). This trend was not observed in pts with PREDICT AR- TNBC. Key demographic differences did not explain these outcomes, suggesting benefit from ENZA. Duration of first-line non-ENZA-Tx was shorter in pts with PREDICT AR- TNBC vs pts with PREDICT AR+ TNBC who received second-line ENZA (17 vs 34 weeks),
suggesting a better prognosis in pts with PREDICT AR+ advanced TNBC; however, PREDICT AR status was not associated with DFI following neo/adjuvant treatment for primary TNBC. PREDICT AR may identify a hormonally driven subset of pts with advanced TNBC who have a better prognosis and who may benefit from ENZA.

Title: Biomarker comparison between androgen receptor – Positive-triple-negative breast cancer (AR+ TNBC) and quadruple-negative breast cancer (QNBC)

Xiu J, Obeid E, Gatalica Z, Reddy S, Goldstein LJ J, Link J and Waisman J. Caris Life Sciences, Phoenix, AZ; Breastlink Medical Group, Orange, CA; City of Hope Medical Oncology, Duarte, CA and Fox Chase Cancer Center, Philadelphia, PA.

Body: Background: Quadruple-negative breast cancer (QNBC) is a subgroup of triple-negative breast cancer (TNBC) that lacks androgen receptor (AR) expression. While TNBC patients with AR expression have shown a promising response to AR-targeted therapies, QNBC patients' treatment options remain limited, with no targeted therapy. We investigated the biomarker profiles of large cohorts of AR+TNBC and QNBC to identify their molecular differences.

Method: TNBC tumors (defined as negative by IHC for ER, PR, Her2 and ISH for Her2) referred to Caris Life Sciences (Phoenix, AZ) between 2009 and 2015 were evaluated by board-certified pathologists with a combination of immunohistochemistry (AR, cKIT, cMET, EGFR, ER, ERCC1, Her2, MGMT, PD-1, PD-L1, PGP, PR, PTEN, RRM1, SPARC, TLE3, TOPO2A, TOPO1, TS and TUBB3), fluorescent/chromogenic in-situ hybridization (cMET, EGFR, Her2, TOP2A), and sequencing (Next-generation and Sanger). Tumors evaluated included a mix of primary tumors and metastases. QNBC tumors were defined as TNBC tumors that showed negative AR expression (<10% of cells staining).

Results: Among 2,071 TNBC tumors identified, 1,952 tumors had AR IHC performed, out of which 1,612 (83%) were QNBC and 340 (17%) were AR+ TNBC tumors. Tumor expression of PD-L1 (Ab: SP142, Spring Bioscience/130021, R&D Systems, cutoff used: 2+, 5%) was significantly higher in QNBC compared to AR+TNBC tumors (18% vs. 8%, p=0.01), while PD-1 (Ab: NAT105, Ventana) expression on tumor-infiltrating lymphocytes was comparable between the two cohorts (60% vs. 62%). QNBC tumors were significantly more likely to express proteins of cKIT (26% vs. 15%, p=0.01), EGFR (69% vs. 56%, p=0.03), TS (49% vs. 33%, p<0.0001) and TOPO2A (85% vs. 65%, p<0.0001) compared to AR+TNBC. TLE3 expression was significantly higher in AR+TNBC cohorts (48% vs. 32%, p<0.0001). Sequencing reveals that QNBC tumors carried significantly higher mutation rate of TP53 (71% vs. 55%, p<0.0001) while AR+TNBC tumors showed significantly higher mutation rates of PIK3CA (42% vs. 12%, p<0.0001), AKT1 (13% vs. 1%, p<0.0001) as well as ERBB2 (5% vs. 1%, p=0.0003).

Conclusion: Biomarker comparisons between two molecular subgroups of the TNBC tumors confirm the molecular heterogeneity of this aggressive type of breast cancer. Our biomarker results suggest that for AR+TNBC tumors, future clinical trial design can consider fluoropyrimidines, taxanes, and agents targeting PI3K/AKT/mTOR pathway as well as pan-HER inhibitors, and those agents may be combined with anti-androgen therapies. On the other hand, clinical trials for immune checkpoint inhibitors, TOP2A inhibitors, as well as agents that target cKIT and EGFR should be considered for QNBC tumors. Our findings highlight the molecular differences that should be considered in the design of future clinical trial strategies, warranting further investigation for improving targeted therapy and outcomes in TNBC.
**Title:** Distinct biomarker features in triple-negative breast cancer metastases to the brain, liver and bone

Xiu J, Gatalica Z, Reddy S, Waisman J and Link J. Caris Life Sciences, Phoenix, AZ; City of Hope Medical Oncology, Duarte, CA and Breastlink Medical Group, Orange, CA.

**Body:**

**Background:** Triple-negative breast cancer (TNBC) is characterized by its aggressive nature and accounts for a disproportionate number of metastatic disease cases and breast cancer-related deaths. Despite recent improvements, TNBC patients who develop metastatic diseases have limited treatment options. We investigated biomarkers from brain, liver and bone metastases collected from TNBC patients to identify therapeutic options and to examine molecular differences between the metastatic sites.

**Method:** Triple-negative breast cancer tumors referred to Caris Life Sciences (Phoenix, AZ) between 2009 and 2015 were tested with a combination of immunohistochemistry, fluorescent/chromogenic in-situ hybridization and sequencing (Next-generation and Sanger).

**Result:** 1570 TNBC tumors were analyzed, including 1297 tumors taken from breast, 54 from brain, 172 from liver and 47 from bone. Select biomarker frequencies of protein overexpression (IHC), gene amplification (ISH) and mutations (SEQ) are summarized in Table 1. Brain metastases showed the highest protein expression of TOP2A and PDL1; liver metastases showed the highest expression of AR and SPARC, as well as the highest mutation rate of PIK3CA. Bone metastases showed the lowest expression of TS, RRM1 and ERCC1. BRCA1 and BRCA2 mutation rates ranged from 0-11% in various specimen sites.

<table>
<thead>
<tr>
<th>Biomarker and Method</th>
<th>Breast Metastases (%)</th>
<th>Brain Metastases (%)</th>
<th>Liver Metastases (%)</th>
<th>Bone Metastases (%)</th>
<th>p value £</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP2A IHC</td>
<td>76</td>
<td>100§</td>
<td>73</td>
<td>39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PDL1 IHC</td>
<td>15</td>
<td>40§</td>
<td>8</td>
<td>17</td>
<td>0.03</td>
</tr>
<tr>
<td>AR IHC</td>
<td>15</td>
<td>10</td>
<td>36§</td>
<td>26</td>
<td>0.0005</td>
</tr>
<tr>
<td>SPARC IHC</td>
<td>17</td>
<td>30</td>
<td>40§</td>
<td>15</td>
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</tr>
<tr>
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<td>5.3</td>
<td>29§</td>
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<tr>
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<td>54</td>
<td>24</td>
<td>15§</td>
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</tr>
<tr>
<td>RRM1 IHC†</td>
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<td>43</td>
<td>32</td>
<td>16§</td>
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</tr>
<tr>
<td>ERCC1 IHC†</td>
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<td>48</td>
<td>16§</td>
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<td>BRCA1 SEQ</td>
<td>7</td>
<td>0</td>
<td>8</td>
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<td>ns</td>
</tr>
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<td>11</td>
<td>11</td>
<td>4</td>
<td>n/a</td>
<td>ns</td>
</tr>
</tbody>
</table>

§: the group with the highest frequency of actionable results; £: p values are calculated from comparing the group with the highest frequency with the lowest frequency using two tailed Fisher-Exact test, further detailed statistical analysis will be presented; †: low levels predict response to associated drugs; Ns: non-significant, i.e., p >0.05; n/a: data not available due to low N

**Conclusion:** Distinct biomarker features identified in different metastatic sites in TNBC present the rationale to investigate differential treatment strategies. Based on biomarker results, etoposide, immune-modulatory agents may seem promising for brain metastases; anti-androgen therapies and nab-paclitaxel may be promising in treating liver metastases; while fluoropyrimidines, gemcitabine and platinum may be considered for TNBC patients with bone metastases.

Body: Background: The BRCA1-like copy number (CN) profile can be used as a biomarker to predict which stage III breast cancer patients benefit from myeloablative, DNA double-strand-break (DSB)-inducing chemotherapy. In addition, a BRCA1-like gene expression classifier, derived from the BRCA1-like CN profile, can predict which patient group will achieve an increased pathological complete remission rate on a standard neoadjuvant regimen complemented with carboplatin/veliparib. A non-myeloablative, dose-dense schedule of epirubicin, paclitaxel and cyclophosphamide (ETC) is used in the clinic to treat stage III patients. Since ETC contains an intensified dose of DNA DSB-inducing cyclophosphamide, we tested the BRCA1-like CN profile as a predictive biomarker for ETC benefit in the GAIN trial.

Methods: The GAIN trial was a prospective, multi-center, non-blinded, randomized phase III trial. Eligibility comprised histologically confirmed invasive breast cancer with at least one positive axillary or internal mammary lymph node and no signs of distant metastases. The allocated adjuvant treatment was intensified chemotherapy with sequential E (150 mg/m2), T (225 mg/m2) and C (2500, after amendment 2000 mg/m2) each 3 cycles every 2 weeks (ETC) or concurrent E (112.5 mg/m2) and C (600 mg/m2) for 4 cycles every 2 weeks followed by 10 cycles of weekly T (67.5 mg/m2) combined with 4 cycles of capecitabine (2000mg/m2) on day 1-14 in a 3-weekly cycle (EC-TX). Only the triple negative patients were used for these analyses. For samples with good quality DNA extracted from formalin-fixed paraffin-embedded tumor tissue, a library was prepared and sequenced on an Illumina HiSeq2000 platform. Copy number estimates were extracted from the sequence data by normalizing GC-content and mappability corrected read counts to the average read count. These CN profiles were classified as either BRCA1-like or non-BRCA1-like using a previously established shrunken centroid classifier with an established cut-off. Disease-free survival (DFS) and overall survival (OS) were estimated using the Kaplan-Meier method, the difference in survival was analysed using log-rank tests. Multivariate analyses were done by generating Cox regression models with important prognostic factors.

Results: Out of 424 triple negative patients, 166 patients with available tissue and a tumour cell content of at least 60% were analysed for BRCA1-like status and included in the analyses (classified as BRCA1-like: n=122). Based on clinicopathological characteristics, these patients were similar to the total group of triple negative participants. In the BRCA1-like patients, there was no significant difference in DFS or OS between ETC and EC-TX (log-rank tests n.s.). In accordance, Cox regression models confirmed these findings.

Conclusion: In contrast to the predictive value of the BRCA1-like profile in myeloablative chemotherapy, it could not predict survival benefit for a non-myeloablative, non-platinum-/veliparib-containing regimen in this study population. An intensified dose of cyclophosphamide resulted in similar outcomes in BRCA1-like patients as addition of capecitabine to standard chemotherapy. These results help to define the appropriate application of the BRCA1-like profile as a predictive biomarker.
Title: Role of germline BRCA status and tumor homologous recombination (HR) deficiency in response to neoadjuvant weekly paclitaxel followed by anthracycline-based chemotherapy


Body: Background: Both HR deficiency and BRCA mutation status predict response to platinum-based therapy and BRCA mutation status predicts docetaxel resistance. However, the association of either biomarker with response to the individual elements of either AC or taxanes (T) is unknown since T is commonly given concomitantly with or after anthracyclines (A). We evaluated the association of HRD and BRCA mutation status with response to neoadjuvant weekly T followed by AC or (F)EC in high-risk breast cancer.

Methods: We studied 140 high risk Stage I-III breast cancer patients (pts), enrolled in the breast cancer genome guided therapy study (BEAUTY), obtaining biopsies for DNA/RNA sequencing and MRI imaging to assess response to neoadjuvant weekly T (+trastuzumab+-/pertuzumab for HER2+ disease) followed by AC or (F)EC. Germline BRCA status and HR status of tumor samples (Myriad laboratories) were obtained. HR deficient tumor was defined as HRD score ≥42 or BRCA mutation. MRI response by changes in tumor size after 12 weeks of T was classified by WHO criteria. pCR was defined as ypT0/Tis ypN0. Both MRI response after T and pCR (after T and AC) were examined in terms of germline BRCA mutation (gBRCAmut vs. gBRCAwt) and tumor HR deficiency.

Results: Of 140 pts enrolled, 8 withdrew consent and 2 carboplatin treated pts were excluded. Germline data were available for 124/130 pts. 12 patients had BRCA deleterious germline mutations (4 BRCA1, 8 BRCA2). MRI partial (PR)/complete response (CR) rate to T was 47.3% (95% CI: 37.8-57.0%) in the BRCAwt group and 66.7% (95% CI: 34.9-90.1%) in the BRCAmut group. No MRI CR’s were observed in BRCA1 mut pts. In contrast, pCR rate was 50% in the 12 gBRCAmut pts (95% CI: 21.1-78.9%) and 31.3% in the 112 gBRCAwt pts (95% CI: 22.8-40.7%). HR deficiency status has thus far been determined for 74 pts: 26 pts have HD deficient tumors: 18 TNBC, 5 Luminal B, 2 ER-/HER2+; and 1 ER+/HER2+. Determination of HR deficiency is ongoing and will be reported for the full cohort in terms of 12 week MRI response to T and pCR to T+AC.

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>HR deficient yes (%)</th>
<th>no (%)</th>
<th>TBD (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0/11</td>
<td>2/11 (18.2)</td>
<td>9/11 (81.8)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>5/37 (13.5)</td>
<td>13/37 (35.1)</td>
<td>19/37 (51.3)</td>
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<tr>
<td>Luminal NOS</td>
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<td>1/2 (50)</td>
<td>1/2 (50)</td>
</tr>
<tr>
<td>ER+/Her2+</td>
<td>1/17 (5.8)</td>
<td>14/17 (82.4)</td>
<td>2/17 (11.8)</td>
</tr>
<tr>
<td>ER-/Her2+</td>
<td>2/20 (10)</td>
<td>11/20 (55)</td>
<td>7/20 (35)</td>
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<tr>
<td>Triple Negative</td>
<td>18/43 (41.9)</td>
<td>6/43 (18.6)</td>
<td>17/43 (39.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>germline BRCA status</th>
<th>MRI partial response after T (%)</th>
<th>MRI complete response after T (%)</th>
<th>pCR after T&amp;AC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>1/4 (25)</td>
<td>0/4</td>
<td>2/4 (50)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>5/8 (62.5)</td>
<td>2/8 (25)</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>BRCAwt</td>
<td>35/112 (31.3)</td>
<td>18/112 (16.1)</td>
<td>35/112 (31.3)</td>
</tr>
</tbody>
</table>

Conclusion: In the setting of neoadjuvant weekly T followed by AC, pCR rates were non-significantly higher in pts with BRCA1 mutations. While we observed no overall association between BRCA mutation status and response rates to taxanes; nearly all
MRI responses to taxanes (partial and complete) were observed in the BRCA2 group. Prospective studies are needed to validate these findings and to determine whether BRCA status can be used to select therapy. HR deficiency is uncommon in luminal A and HER2+, frequent in TNBC, and the association of HRD with both MRI response to taxanes and pCR will be reported at the meeting.
Molecular profiling comparison of BRCA1/2-mutated and BRCA1/2 non-mutated triple-negative breast cancer (TNBC)


**Body:** Background: Triple-negative breast cancer (TNBC) is one type of breast cancer that remains challenging because of its aggressive nature and the lack of effective targeted therapy for it. Molecular profiling has revealed different subtypes, indicating a potential for promising targeted therapy such as androgen blockade and PARP inhibition in some TNBCs. The purpose of this study is to identify differences in BRCA1/2 mutated and non-mutated TNBC to shed light on potential therapeutic options in both subtypes, utilizing a multiplatform approach.

**Methods:** A cohort of 386 triple-negative breast cancer specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and gene amplification (fluorescence or chromogenic in situ hybridization [FISH or CISH]). Primary and metastatic specimens were evaluated. Tumor specimens with any BRCA1 and/or BRCA2 mutation (i.e. pathogenic or variant of unknown significance) were categorized as "BRCA1/2-mutated", while all others were considered "BRCA1/2 non-mutated".

**Results:** In our cohort, 16.3% (63/386) of specimens were BRCA1/2-mutated while 83.7% (323/386) had no BRCA1/2 alteration detected. Amongst the highest rates of protein expression in BRCA1/2-mutated and non-mutated specimens were biomarkers like TOPO1 (63.5% and 63.4%), EGFR (65.2% and 67.4%), and the immune checkpoint biomarker, PD-1 (65.1% and 61.9%), with non-statistically significant differences. Differences noted between BRCA1/2-mutated and non-mutated specimens were detected by IHC in AR (11.1% versus 22.0%, p=0.0585) and PTEN (47.6% versus 59.6%, p=0.0941), with both trending but not achieving statistical significance. The highest overall mutation rate in both BRCA1/2-mutated and non-mutated were TP53 (80.6% and 73.1%, p=0.2659). Differences were also noted between BRCA1/2-mutated and non-mutated specimens by NGS in APC (6.3% versus 1.9%, p = 0.0644) and PIK3CA (11.1% versus 25.8%, p = 0.0137), with PIK3CA being statistically significant.

**Conclusion:** Multiplatform tumor profiling identified differences in molecular profiles between BRCA1/2 mutated and BRCA1/2 non-mutated TNBC. Our findings raise the possibility for future investigation of potential combination therapeutic targeted therapy. Increased AR overexpression in BRCA1/2 non-mutated specimens is consistent with reports from other institutions. Further studies utilizing tumor profiling to elucidate the biological differences in in TNBC subtypes are warranted, to optimally include patients on clinical trials with specific targeted therapy and possibly improve treatment options.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-31

Title: Immune activation signatures identify a subset of ER+ breast cancers with increased pathologic complete response to neoadjuvant chemotherapy


Body: Background: Proliferation is the strongest predictor of response to neoadjuvant chemotherapy in estrogen receptor-positive (ER+) breast cancer. Evidence of immune activation has also been associated with improved response to neoadjuvant chemotherapy in breast cancer. We hypothesized that immune signatures may be associated with response independent of proliferation in ER+ breast cancers.

Approach: We compiled microarray expression data from breast cancer biopsies obtained prior to neoadjuvant chemotherapy on 465 ER-positive/HER2-negative patients by reported pathologic receptor status. We evaluated the association of 118 published gene expression signatures with response to neoadjuvant chemotherapy, based on study-defined pathologic complete response (pCR) versus residual disease (RD).

Results: Overall, 42 of 118 signatures were significantly associated with response to neoadjuvant chemotherapy in ER+ breast cancer (FDR-corrected p<0.05, simple logistic regression). Of those signatures that achieved significance, 52% (22/42) of signatures were proliferation-associated based on correlation to the 11-gene PAM50 proliferation index (Pearson's R^2>0.30, p<1e-10). Among signatures that were NOT proliferation-associated, 50% (10/20) were immune-related. Using unsupervised hierarchical clustering of all 118 signatures, these ten immune signatures formed a distinct cluster. Of the 10 signatures, nine were designed to reflect "immune activation" and were highly correlated with each other in ER+ tumors (R^2>0.4, p<0.001). The mean of each of these nine signatures was significantly higher in patients with pCR versus RD (FDR-corrected p<0.05, t-test). Patients with higher "immune activation" signatures had increased likelihood of pCR within multiple subgroups of ER+ breast cancer, including luminal B and non-luminal PAM50 subgroups, as well as intermediate- and high-proliferation ER+ breast cancers. For luminal A or low-proliferation breast cancers, "immune activation" signatures were not significantly associated with response, though very few patients achieved pCR in these two subgroups.

Conclusions: Gene expression signatures associated with "immune activation" identify a subset of ER+ breast cancers with higher rates of pCR to neoadjuvant chemotherapy. These "immune activation" signatures appear to be proliferation-independent and may provide additional predictive information to existing gene expression-based approaches for ER+ breast cancer.
Title: Tumour infiltrating lymphocyte (TIL) and chemokine gene signature predicts for benefit of anthracycline-containing chemotherapy in breast cancer patients

Braunstein M, Yao C, Lyttle N, Liao L, Boutros PC C, Twelves CJ J, Bartlett JMS MS and Spears M. Ontario Institute for Cancer Research, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Leeds Institute of Cancer and Pathology and Cancer Research UK Centre, St James' University Hospital, Leeds, United Kingdom; University of Toronto, Toronto, ON, Canada and Edinburgh Cancer Research Centre, Western General Hospital, Edinburgh, United Kingdom.

Body: Background: The contribution of immune cells has long been appreciated in tumour development and disease progression; however, their translational potential as cancer-associated prognostic and predictive markers was only recently recognized. High densities of tumour-infiltrating lymphocytes (TILs) correlate with improved clinical outcome in breast cancer; whether TILs also predict anthracycline benefit in all, or only a particular subgroup, of breast cancer patients remains largely unknown. Furthermore, since identification of TILs is generally based on H&E staining, it has not previously been possible to evaluate relative contribution of distinct T-cell types, and B cells, to patient outcome.

Methods: We assessed 290 patient samples from the BR9601 adjuvant breast cancer trial for the capacity of TIL contexture to predict for anthracycline (E-CMF) benefit over CMF. We immunoprofiled patient samples on the Nanostring platform to gain insight into the impact of lymphocyte populations predicting for anthracycline benefit. Our immunoprofiling panel included 38 genes representing TIL-gene signatures and chemokines that may be responsible for recruiting TILs to the tumour site.

Results: The analyses revealed two important findings. First, refinement of the 38-gene panel resulted in the generation of a novel 9-gene signature that includes cytotoxic T lymphocytes (CTL) and chemokine genes. Low CTL gene expression correlated with ER+ expression while high expression correlated with ER- expression (p<0.0001), consistent with the notion that high TIL densities are predominantly observed in non-luminal breast cancers. Second, in an univariate Cox regression analysis, this 9-gene signature was a predictive biomarker of anthracycline benefit with respect to breast-cancer specific OS (HR: 0.371, 95%CI 0.158-0.868, p=0.022) and DRFS (HR: 0.395, 95%CI 0.172-0.907, p=0.028); this effect was no longer significant after adjustment for other prognostic factors (OS HR: 0.437, 95%CI 0.166-1.150, p=0.094; DRFS HR: 0.488, 95%CI 0.185-1.287, p=0.147).

Conclusion: This study highlights the significance of assessing the entire tumour since TILs, tumour and stromal cells collectively engage in a complex interplay that contributes to disease development and progression. Importantly, it reveals that not only CTLs but also chemokines may be clinically relevant and should be validated as potential biomarkers of anthracycline benefit and as therapeutic targets.
**Title:** Prediction of the treatment response to neoadjuvant chemotherapy in breast cancer by subtypes using tumor-infiltrating lymphocytes

Kashiwagi S, Asano Y, Goto W, Morisaki T, Noda S, Takashima T, Onoda N and Hirakawa K. Osaka City University Graduate School of Medicine, Osaka, Japan.

**Body:** Purpose: Monitoring the host immunological response to cancer in the microenvironment of the interaction between tumor and the body plays an important role in predicting treatment response and outcomes. Recent interest has focused on the morphological evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer (BC) and on the evidence showing their clinical relevance. Meanwhile, no consensus has yet been reached on standard methods for pathological evaluation of TILs. Therefore, methods of evaluation have differed in reports to date showing the clinical relevance of TILs. An International Working Group (2014) announced recommendations for evaluating TILs in an effort to improve consistency and reproducibility. In this study, the clinical validity and utility of TILs in NAC were investigated based on this recommendation with a stratified analysis by BC subtypes. Changes in TILs after recurrence, which have seldom been reported to date, are also discussed.

Experimental Design: TILs was evaluated in 177 patients with breast cancer treated with NAC and subsequent curative surgery. All patients received a standardized protocol of NAC consisting of four courses of FEC100 (500 mg/m² fluorouracil, 100 mg/m² epirubicin, and 500 mg/m² cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m² paclitaxel administered weekly. Forty-five patients had HER2-positive breast cancer and were additionally administered weekly (2 mg/kg) or tri-weekly (6 mg/kg) trastuzumab during paclitaxel treatment. The correlation between TILs evaluated according to the standard method, and prognosis, including the efficacy of NAC, was investigated retrospectively.

Results: In the 96 high-TIL group, compared to the 81 low-TIL group, triple-negative breast cancer (TNBC) (p < 0.001) and HER2-enriched (p = 0.040) were significantly more frequent, and the pathological complete response (pCR) rate were significantly higher (p = 0.003). On multivariate analysis also demonstrated that high-TIL status was an independent factor to indicate significantly more favorable prognosis of the patients compared with low-TIL status (p = 0.036, HR = 0.45). Among the 61 TNBC and the 36 HER2-enriched patients, the pCR rate was significantly higher in the high-TIL group than in the low-TIL group (p = 0.013) (p = 0.014). On multivariate analysis also showed that high-TIL status was an independent factor to predict the favorable prognosis (p = 0.023, HR = 0.24) (p = 0.036, HR = 0.13). Biopsy specimens from local recurrence after successful NAC frequently showed decreased TILs.

Conclusion: TILs may be a biomarker to predict treatment response to NAC in patients with TNBC and HER2-enriched subtypes of BC. A decrease in TILs may also be associated with tumor recurrence.
Title: Predictive role of stromal tumor infiltrating lymphocytes (TILs) in patients with metastatic HER2-positive breast cancer (BC) treated with trastuzumab

Yoon JA, Yoo C, Lee HJ, Kim K-P, Kim J, Ahn J-H, Jung KH, Gong G and Kim S-B. Asan Medical Center, University of Ulsan College of Medicine, Song-pa gu, Korea and Asan Medical Center, University of Ulsan College of Medicine, Song-pa gu, Korea.

Body: Background: Prognostic significance of stromal TILs in metastatic BC has been suggested in various BC subtypes. However, predictive role of stromal TILs for the efficacy of trastuzumab has not been established in patients with HER2-positive BC. This study was performed to evaluate whether the stromal TILs are associated with the efficacy of trastuzumab in patients with metastatic HER2-positive BC.

Method: Between June 2006 and March 2013, a total of 60 women with recurrent or metastatic HER2-positive BC treated with trastuzumab were included in this retrospective analysis. In these patients, trastuzumab was administered either as single agent or combination with taxanes. Stromal TILs were assessed using immunohistochemistry in surgical specimen (n=39, 65%) and biopsy specimen of metastatic lesion (n=21, 35%) by the academic pathologist (HJL). Primary endpoint of this study was progression-free survival (PFS), and secondary endpoints were response rate and overall survival (OS).

Result: Median age was 54 year old (range, 36-76), and all patients had invasive ductal carcinoma. Hormone receptor was positive in 34 patients (57%) and 18 patients (30%) initially presented with metastatic disease. Nine patients (15%) received cytotoxic chemotherapy without trastuzumab before the administration of trastuzumab. Patients were grouped according to the TILs (< 10% [n=50] and >10% [n=10]), and there was no significant difference in age (p=0.68), histologic grade (p=1.00), metastatic sites (p>0.05), and number of lines of chemotherapy before the administration of trastuzumab (p=0.33) among patients with low and high stromal TILs. High TILs were more common in hormone receptor (HR)-negative tumor compared with HR–positive tumor (31% vs 6%; p=0.02). In overall, median PFS and OS were 15.0 months (95% CI, 9.7-20.2) and 35.0 months (95% CI, 29.8-40.2), respectively. Median PFS in patients with high stromal TILs were numerically longer than that in those with low TILs (22.0 months [95% CI, 9.6-34.4] vs 14.0 months [95% CI, 9.6-18.4]; p=0.057). There was no difference in response rates (p=0.43) and OS (p=0.94) according to the stromal TILs. FcR genotype was not significantly correlated with objective response rate, PFS and OS.

Conclusion: This study suggests that the stromal TILs might be associated with the clinical outcomes of HER2-targeted therapy in patients with metastatic HER2-positive BC. Our finding should be validated in future studies based on a large sample size.

Keywords: Breast cancer, tumor infiltrating lymphocyte. Trastuzumab, HER2.
Title: Tumour immune subtyping as a predictive biomarker for adjuvant endocrine therapy in early breast cancer


Body: Adjuvant endocrine therapy is one of the mainstays in the treatment of breast cancer. Approximately 75% of all patients is treated with either tamoxifen or an aromatase inhibitor (AI), without any predictive biomarkers besides the estrogen receptor. It is known, that the immune system plays a pivotal role in the treatment of breast cancer. Earlier, subtyping the local immune response has been shown to predict therapy efficacy for chemotherapy. The current study was performed to discover predictive relations between the local immune response and survival in two different cohorts treated with adjuvant endocrine therapy. Tumour tissue was collected from a cohort of 236 Dutch post-menopausal patients from the Intergroup Exemestane Study (IES-trial) who were randomized between 5 years of tamoxifen and 2.5 years of tamoxifen followed by 2.5 years of an AI (switch-scheme). The tumour tissue was stained for infiltration of CD8+ cytotoxic T-cells, FoxP3+ regulatory T-cells and CD68+ macrophages and the expression of different HLA-markers (classical HLA-I, HLA-E, HLA-G). These results were combined into three immune profiles (strong, intermediate, weak), and correlated to survival. Similar analyses were performed on a cohort of 2596 patients participating in the international TEAM trial, who were randomized between 2.5 years of tamoxifen followed by 2.5 years of an AI (switch scheme) and 5 years of AI.

In the IES-cohort, patients with a strong local immune response had a significant better recurrence free survival when treated with 5 years of tamoxifen (5-year RFS 89.5%) compared to the switch scheme (5-year RFS 76.5%) (p=0.003). In patients with a weak local immune response, RFS was improved for patients treated with the switch scheme (5-year RFS 100%) compared to tamoxifen monotherapy (5-year RFS 75.6%) (P=0.003). Similar trends were observed for overall survival, although not statistically significant (p-values of 0.051 and 0.109 respectively). In the TEAM-cohort, no statistical significant differences were observed between both treatments in either immunological subtype (strong immune subtype: 5-year RFS 87.2% for 5-years AI versus 82.8% for the switch scheme (p=0.495), weak immune subtype: 85.9% for 5-year of AI vs 82.2% for the switch scheme (p=0.343)).

This study is the first to show combined immunological markers as a predictive marker for endocrine therapy. A strong immune response predicts a benefit for tamoxifen monotherapy, whereas a weak response predicts a benefit for AI-containing therapy. Although the exact mechanism of this effect is not known, we hypothesize that it might be contributed to immunomodulatory capacities of AIs. If the effect observed in the IES-cohort is indeed contributed to the effect of AIs, it would be expected that there is no difference in the TEAM-cohort between both arms since they both contain AIs, which was indeed observed. Future studies will be directed towards validation of these findings in an independent cohort.
Title: Immune related gene expression signatures predict benefit of letrozole over tamoxifen in BIG 1-98

Willis S, Gray KP, Regan MM, Rae JM, Kammler R, Young B, Ditzel HJ, Lyng MB, Colleoni M, Viale G and Leyland-Jones B. Avera Cancer Institute, Sioux Falls, SD; IBCSG Statistical Center, Boston, MA; University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; International Breast Cancer Study Group Coordinating Center Pathology Office, Bern, Switzerland; Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark; Breast Center, Kantonsspital, St. Gallen, Switzerland and European Institute of Oncology, Milan, Italy.

Body: Background: The prognostic significance of increased levels of CD8+ tumor infiltrating lymphocytes (TILs) in ER- breast cancer has been described. We sought to identify possible immune-related biomarkers for predicting benefit from letrozol (LET) or tamoxifen (TAM) for recurrence in ER+ breast cancer.

Patient and Methods: We used Illumina DASL Assay to measure gene expression in FFPE primary breast cancers from a subset of postmenopausal patients enrolled in the BIG 1-98 randomized phase 3 trial comparing 5 years LET (n=344) vs TAM (n=381) as adjuvant endocrine therapy. Gene sets (n=1910) that represent cell states and perturbations within the immune system from the Human Immunology Project Consortium were used in an exploratory analysis to identify possible predictive signatures.

Results: We identified five distinct gene signatures from previously reported laboratory experiments associated with immune cell differentiation that are highly predictive of benefit (reduced breast cancer recurrence risk) of LET over TAM, each with gene signature p-values <1E-5 and signature-by-treatment interaction p<1E-6. The signatures predict a similar pattern that patients at low-risk score benefit from LET and patients with high-risk score appear to have an advantage with TAM after 5 years. The gene signatures originate as a result of being differentially expressed in the following previously reported experiments. [RAP2A EEF2K TRAF3IP2 GPR37L1 DDX54] down regulated comparing TLR3 and TLR9 agonists in dendritic cells. [RPA1 DUSP4 NUDT18 ZFYVE28] up regulated in comparison of T follicular helper versus Th17 cells. [MAPK15 CCR4 SORCS2 RAMP1 SH3PXD2A] up regulated in regulatory T cell versus CD4+ T cells. [NDUFA6 GIMAP1 CPNE3 ST3GAL6 CCDC88A] down regulated in comparison of untreated CD8+ dendritic cells versus treated with IFNG. [GPN1 COX17 CUL2 CDSA] down regulated in naïve vs stimulated CD8 T cells after 48 hours. We further investigated the signatures using Hungarian Academy of Sciences (HAS) cohort (Gyorffy B 2010), which is a collection of smaller published affymetrix cohorts combined into a larger ER+, TAM treated cohort (n=700). One signature was not tested because two genes were not present in the affymetrix cohort. Three of the remaining four signatures gave informative prognostic results in the HAS cohort, and the signature associated with differentiation of CD8+ dendritic cells was highly prognostic with HR=0.36 (0.26-0.49) p=1E-11.

Conclusion: The role of selective estrogen receptor modulators on immune response has been well described, where TAM has been shown to prevent differentiation and activation of dendritic cells (Naibandian 2005). Similarly, it has been shown that MET inhibitors negatively regulate neutrophils suggesting that anti-MET drugs in cancer could impact immune response (Finisquerra 2015). These findings suggest that if TAM is a negative regulator of immune response why in the ATAC clinical trial, the combination therapy of anastrozole plus TAM were not significantly different from TAM alone were anastrozole was superior. With the increasing importance of understanding the role of immune response on outcome and the use of combination therapies the assessment of TILs in the neoadjuvant setting will be critical for guiding therapy.
Title: Quantitative analysis of T cell and macrophage immune markers in Her2-positive breast cancer


Body: Purpose/Objectives:

Her2-neu positive breast cancers have a good overall prognosis with the advent of Her2-directed therapies such as trastuzumab. However, despite the increased efficacy with Her-2-directed therapies 20-30% of patients still have local and/or distant failure despite being Her-2 amplified on pathology. The etiology of this local failure remains unknown. Recent evidence has suggested that there may be immune factors that contribute to the progression of breast cancer and the response to therapy so we undertook a study to examine the relationship between immune-based markers and traditional pathologic and clinical markers of outcome.

Materials/Methods:

Paraffin-embedded sections were generation and clinical records were reviewed for 88 patients, age ≥ 18 years, with pathologically-proven Her2-neu+ breast cancer who were treated at a single institution from 01/2001 to 12/2013. Single-color immunohistochemical staining was performed for CK5/6, CK14 and EGFR and scored by a breast pathologist. Adjacent sections were also then stained for CD45, CD4, CD8 and CD68 using a multi-color immunohistochemical approach. Slides were scanned using the Vectra Automated Quantitative Pathology Imaging System and analyzed using an in-house algorithm to quantitate the number of immune cells within the tumor, tumor margin and within 2 mm outside the tumor. We then compared the level of CK5/6, CK14, EGFR with the number of immune cells. The number of different immune cells were also analyzed with respect to other clinical parameters including age, tumor size, nodal status, hormone receptor status, time to progression, progression-free survival and overall survival.

Results:

At a median follow-up of 66.5 months, 20 (22.7%) patients had progressed. We found that the number of CD45+ leukocytes at the margin correlates with the expression of CK5/6 (p = 0.015) which predicted for local failure. Further, we found that the ratio of CD8 to CD4 cells within the tumor and margin highly correlates with the expression of the hormone receptors (p = 0.01).

Conclusions:

Our preliminary results suggest that immune markers may be important predictors of a basal-like phenotype as defined by CK5/6 expression in Her2+ breast cancers which itself correlated with significantly higher local failure. Further higher CD8 to CD4 ratios were highly correlated with hormone receptor expression, particularly PR expression suggesting that in the Her2+ population the more favorable prognosis for the "triple-positive" subtype of Her2+ cancers may be in part due to a more favorable immune microenvironment.
Title: Increment of neutrophil/lymphocyte ratio (NLR) can be one of the useful predictive markers for the metastatic breast cancer (MBC) with first line hormonal therapy (HT)

Miyamoto T, Fujisawa T, Morishita A, Yanagita Y and Kuwano H. Gunma Cancer Center, Ota, Gunma, Japan and Graduate School of Medicine, Gunma University, Maebashi, Gunma, Japan.

Body: [Introduction] Cancer microenvironment formed by the immune and inflammatory cells is noticed to be one of the factors for tumor growth, invasion or metastasis. To figure out the systemic inflammatory environment, the neutrophil / lymphocyte ratio (NLR) is a useful method and a simple indicator. We have some reports that NLR can predict the prognosis in many malignancies, including breast cancer, as I reported in SABCS 2014, however, NLR cannot be a predictive marker in major cancers, because the usage with anti-cancer agents will make NLR status out of order. In hormonal receptor positive metastatic breast cancer (HR+ve MBC), we choose hormonal therapy (HT) for the first line, mainly. HTs have little influence for NLR, therefore, the change of NLR, increment or decrement, can reflect the systemic inflammatory status and be a useful predictive marker for the HR+ve MBC with HTs.

[Purpose] I defined dNLR as the difference between NLR at the 2-3 months after HT and before HT. To evaluate the dNLR affects the Overall Survival (OS) and Progression Free Survival (PFS) of the patients of MBC or not.

[Patients] From 2003 to 2013, we have 299 MBC patients in our hospital. Out of them, 134 patients had HTs as first line, included 18 Stage4 patients at the first visit. Median Disease free survival (DFS) is 1497-day, the median OS after the recurrence is 1472-day. Average value of the NLR before HT is 2.62. The reasons of MBC are bone metastases (mets), pleural and pulmonary mets, liver mets, lymph node mets, central nervous system (CNS) mets, unresectable metastatic chest wall recurrence, or other.

[Results] By univariate analysis, DFS < 1000 (p<0.01) and liver mets (p<0.05) made a contribution to poor OS. dNLR>=0.5 (p<0.05), liver mets (p<0.05) and 2 or more organs involvements (p < 0.05) made a contribution to poor PFS. With multivariate analysis, for OS, only DFS < 1000 was an independent prognostic factor. And for PFS, only dNLR>=0.5 was an independent predictive factor. Poor OS factors by univariate analysis had no influence for PFS as well as poor PFS factors did not reflect the OS.

[Discussion] The dNLR was a predictive marker for HR+ve MBC which we can easily and simply examine by blood sample. Now we cannot reveal the relationship with dNLR and OS, however, this fact suggests that we can decide to stop the first line HT and select the second line therapy using with not only images or tumor makers but also dNLR. This can certainly contribute for good OS. It remains some questions between dNLR and other examinations, for example, which can detect the patient status earlier and more accurate? First, we need to accumulate further retrospective cases and plan the prospective study to make sure of the adequate treatment divided by dNLR.

[Conclusion] The dNLR>=0.5 is one of the independent predictive markers for HR+ve MBC with first line HT.
**Body: BACKGROUND:** Currently available therapeutic armamentarium for locally advanced and/or metastatic breast cancer (LA/MBC) allows an increasing tailored approach for each of the major tumor immunophenotypes. Nevertheless, there is scarce information about how these subgroups fare and how the alternative therapeutic approaches are actually being used during the disease course. CASCADE is an epidemiological, retrospective, and multicenter study aimed to retrieve demographic and clinical information from a representative cohort of LA/MBC patients treated within the Spanish National Healthcare System.

**MATERIALS AND METHODS:** Several strategies were used to identify patients diagnosed with LA/MBC for the first time between 01/2007 and 12/2008 in 13 Spanish public hospitals covering nearly 5'000'000 inhabitants (>10% of the national population) and followed throughout their metastatic lifetime until death, lost to follow-up, or until December 2013. Data collected included demographical and clinical information for each line of treatment. Descriptive statistics were applied to analyze the information.

**RESULTS:** We identified 443 LA/MBC patients. Median age at diagnosis was 59 years (CI95%: 49.5 - 71.6). Significant differences in dropout rates per line of treatment were found according to the tumor intrinsic immunophenotype. Patients reaching a 4\textsuperscript{th} line were: whole study population 38.4%, HER2-/HR+ 42.8%, HER2+/HR- 41.5%, HER2+/HR+ 39.5%, and Triple-negative 31.9%. Median Overall survival (OS) and per line Progression Free Survival (PFS) for each line of treatment by tumor subtype were:

<table>
<thead>
<tr>
<th>Treatment line</th>
<th>Subtype (%)</th>
<th>OS (months)</th>
<th>PFS (months)</th>
<th>PFS (months)</th>
<th>PFS (months)</th>
<th>PFS (months)</th>
<th>PFS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Population</td>
<td>All</td>
<td>33.5</td>
<td>7.2</td>
<td>5.9</td>
<td>4.3</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>HER2-/HR+</td>
<td>43.8</td>
<td>38.6</td>
<td>8.8</td>
<td>5.8</td>
<td>4.4</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>HER2+/HR-</td>
<td>12.0</td>
<td>36.3</td>
<td>7.4</td>
<td>6.7</td>
<td>4.3</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>HER2+/HR+</td>
<td>17.2</td>
<td>34.4</td>
<td>11.2</td>
<td>7.9</td>
<td>4.9</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>Triple-negative</td>
<td>16.3</td>
<td>19.0</td>
<td>4.0</td>
<td>3.5</td>
<td>2.4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**Percent use of the four major pharmacological families per line of LA/MBC treatment was:**

<table>
<thead>
<tr>
<th>Treatment line</th>
<th>1L</th>
<th>2L</th>
<th>3L</th>
<th>4L</th>
<th>5L</th>
<th>6L</th>
<th>7L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>75.4</td>
<td>63.0</td>
<td>75.9</td>
<td>79.4</td>
<td>87.9</td>
<td>76.1</td>
<td>78.6</td>
</tr>
</tbody>
</table>
**CONCLUSION**: Our study identifies differences in OS and PFS among BC immunophenotypes, with Triple-negatives faring the poorest. Among therapeutic families, chemotherapy clearly prevails along the disease lifetime, with hormone therapy being primarily used during the initial lines of treatment.
Pharmacogenetic study of exemestane and everolimus in metastatic breast cancer patients progressing on prior non-steroidal aromatase inhibitors


Background: Activation of the mTOR pathway has been observed in patients (pts) with metastatic breast cancer (MBC) progressing on endocrine therapy. BOLERO-2 trial demonstrated a significant increase in progression free survival (PFS) obtained with everolimus (EVE) and exemestane (EXE) versus EXE alone in pts refractory to a prior non-steroidal aromatase inhibitors (NSAI). However, there is large interindividual variability in toxicity and efficacy profiles of EXE-EVE treatment. Single Nucleotide Polymorphisms (SNPs) have been proposed to explain some of these differences. The objective of this study was to perform a pharmacogenetic analysis to identify SNPs associated with EVE toxicity and activity.

Methods: This is a prospective unicentric clinical trial for postmenopausal pts with hormone receptor-positive, HER2 negative, MBC progressing on prior NSAI, treated with EXE-EVE.

Blood samples were obtained from all pts, and 12 SNPs in key genes involved in EXE pharmacokinetics (ABCB1, CYP2C8, CYP3A4, CYP3A5) and pharmacodynamics (AKT1, AKT2, FGFR4, PHLPP2, PIK3R1, RAPTOR) were genotyped. EVE pharmacokinetics data was available for a subset of 37 pts. The association between the SNPs and EVE pharmacokinetic parameters were analyzed using U-Mann-Whitney test. A selection of clinically relevant toxicities (non-infectious pneumonitis (NIP), mucositis, hyperglycemia and hematological) was analyzed using binary logistic regression. Cox regression was used to analyze the association of SNPs with time to treatment modifications (reduction or interruption) due to toxicity, PFS and OS.

Results: 90 patients with median age of 62 yrs were included. At data cut off, a total of 77 pts had discontinued treatment. Major reasons for discontinuation were: disease progression (80%), adverse events (17%) and death (2%). Number of prior chemotherapy lines: 0; 50%, 1;18%, 2;10%, ≥3; 22%. 76% of pts experienced at least one adverse event related to EVE. CYP3A4 rs35599367 (CYP3A4*22 allele) heterozygous pts had higher EVE concentration in blood compared to wild type pts (P=0.019), in agreement with previous data.

Regarding EVE toxicities, ABCB1 SNP rs1045642 showed a higher risk of mucositis (HR=2.3, 95%CI=1.1-4.8, P=0.031; multivariable analysis), and PIK3R1 rs10515074 variant was inversely associated with hyperglycemia (HR=0.24, 95%CI=0.1-0.8, P=0.016; multivariable analysis). RAPTOR rs9906827 protected for NIP (HR=0.38, 95%CI=0.2-0.9, P=0.024; multivariable analysis). When analyzing the time to treatment modification due to any toxicity, we observed a trend for FGFR4 rs351855 and RAPTOR rs9906827 (HR=0.59, 95%CI=0.3-1.0, P=0.06 and HR=0.71, 95%CI=0.5-1.1, P=0.12; respectively, in multivariable analysis).

Regarding activity, RAPTOR rs9906827 was also associated with longer PFS (dominant model HR=0.48, 95%CI=0.3-0.8, P=0.006; multivariable analysis). No SNP was significantly associated with OS.

Conclusions: We found altered EVE pharmacokinetics for CYP3A4*22 carriers and protection for NIP and longer PFS for RAPTOR rs9906827. This study supports prospective trials for genetic testing prior to EXE-EVE therapy to stratify patients according to toxicity and efficacy risk profiles.
Title: Genomic alterations indicative of a luminal A subtype associate with clinical benefit to buparlisib and letrozole in endocrine-resistant ER+/HER2– metastatic breast cancer

Balko JM M, Hicks M, Berger MF F, Solit DB B, Bouvier N, Sanders ME E, Estrada MV V, Won H, Cantley LC C, Mayer IA A and Arteaga CL L. Vanderbilt University; Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College.

Body: Background: Activation of the phosphoinositide-3-kinase (PI3K) pathway has been associated with resistance to endocrine therapy in estrogen receptor-positive (ER+) breast cancer. Recently, we reported a Stand Up 2 Cancer (SU2C) Phase Ib trial of buparlisib, an oral, reversible, pan-PI3K inhibitor in combination with the aromatase inhibitor letrozole in patients with metastatic ER+/HER2– breast cancer (n=51) who had previously progressed on endocrine therapy. In this study, 31% of patients demonstrated clinical benefit (CR, PR and SD ≥6 months; Mayer et al. JCO 2014). Clinical activity was observed in patients with PIK3CA-wild type (PIK3CA-WT) and PIK3CA-mutant (PIK3CA-MUT) tumors. We performed targeted next-generation sequencing (tNGS) to identify somatic alterations associated with clinical benefit to the combination therapy.

Methods: tNGS was performed using Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) in DNA extracted from tumors of 33 study patients (22 samples from archived primary/untreated and 11 from metastatic biopsies). Detected alterations were tested for association with clinical benefit (Fisher's exact test) and progression-free survival (PFS; log-rank test). For PFS analysis, patients were censored if they discontinued buparlisib for toxicity.

Results: The most commonly detected alterations occurred in PIK3CA (36%), TP53 (30%), MAP3K1 (27%), GATA3 (24%), CCND1 (24%), CDH1 (21%) and PTEN (21%). Additional alterations of note included FGFR1 amplification (15%), MYC amplification (12%), ESR1 mutations (6%) and ERBB2 mutations (6%). Probable inactivating mutations occurring in MAP3K1 (MAP3K1-MUT) were significantly associated with improved clinical benefit, regardless of other mutations (6/9 patients, 67%, P=0.044). PIK3CA-MUT tumors trended toward greater clinical benefit (7/12, 58%, P=0.067). Patients with coexisting PIK3CA-MUT and MAP3K1-MUT tumors derived the largest clinical benefit (5/7, 70% P=0.07) compared to patients with only PIK3CA-MUT (2/5; 40%, P=1.0) or only MAP3K1-MUT tumors (1/2; 50%, P=1.0). Only 2/19 (11%) patients with PIK3CA-WT/MAP3K1-WT cancers benefitted from treatment. Both MAP3K1 and PIK3CA alterations were each also associated with increased PFS (p=0.03 and p=0.009, respectively). Three of 5 (60%) patients with tumors with FGFR1 amplification achieved clinical benefit (including a MAP3K1-MUT tumor and a PIK3CA-MUT/MAP3K1-MUT tumor), suggesting that FGFR1 may preferentially signal via PI3K and/or FGFR1 amplifications are not associated with resistance to the combination of aromatase inhibitors and PI3K inhibitors.

Conclusions: Both MAP3K1 and PIK3CA are mutated at higher frequencies in luminal A breast cancer, suggesting that this alteration pattern (MAP3K1-MUT + PIK3CA-MUT) is a surrogate for low grade ER+ breast cancers with PI3K dependence. It is also possible that MAP3K1 mutations may predispose tumor cells to sensitivity to PI3K inhibition, but this speculation requires further investigation. Finally, patients with ER+/FGFR1-amplified cancers appeared to derive clinical benefit from combined therapy with letrozole and buparlisib.
Title: Predicting outcome and benefit to first-line bevacizumab in advanced/metastatic hormone receptor (HR)+/HER2-negative breast cancer (BC) treated with endocrine therapy: A correlative science study from the LEA phase III clinical trial (GEICAM/2006-11_GBG 051)

Prat A, de la Haba-Rodriguez J, Guerrero Á, García-Sáenz JA A, Morales S, Antón A, Muñoz M, Ramos M, Martínez-Jáñez N, Margelí M, Servitja S, Rojo F, Galván P, González S, Cruz J, Sánchez-Rovira P, Perelló A, Rodríguez-Martin C, Casas M, Carrasco E, Caballero R and Martín M. Translational Genomics Group, Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain; Hospital Clínico de Barcelona, Barcelona, Spain; Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC)–H. Universitario Reina Sofía, Universidad de Córdoba, Córdoba, Spain; Instituto Valenciano de Oncología, Valencia, Spain; Hospital Clínico San Carlos, Madrid, Spain; Hospital Universitari Arnau de Vilanova de Lleida, Lleida, Spain; Hospital Universitario Miguel Servet, Zaragoza, Spain; Centro Oncológico de Galicia, A Coruña, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain; Hospital del Mar, Barcelona, Spain; Fundación Jiménez Díaz, Madrid, Spain; Hospital Universitari Mutua de Terrassa, Terrassa, Barcelona, Spain; Hospital Universitario de Canarias, La Laguna, Tenerife, Spain; Complejo Hospitalario de Jaén, Jaén, Spain; Hospital Universitari Son Espases, Palma de Mallorca, Spain; GEICAM, Spanish Breast Cancer Group, Madrid, Spain and Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense, Madrid, Spain.

Body: Introduction: The role of bevacizumab in combination with chemotherapy in metastatic BC is controversial, and no biomarker exists as of today that predicts benefit to this agent. In the LEA clinical trial, a numerical, statistically non-significant benefit from the addition of bevacizumab to endocrine therapy (ET) was observed in the first-line metastatic setting (18.4 vs. 13.8 months of Progression-Free Survival (PFS), p=0.14). Here, we explored various gene expression-based predictors of outcome and benefit to bevacizumab.

Methods and materials: LEA trial randomized 380 patients with HR+/HER2- advanced disease to bevacizumab in combination with ET (ET+B) vs. ET alone. Primary endpoint was PFS. Expression of BC selected genes was evaluated in formalin-fixed paraffin-embedded (FFPE) primary tumors using the nCounter platform from patients randomized in Spain that consent for biomarker analyses. The following variables were evaluated: 1) research-based PAM50 intrinsic subtypes (categorical variable; Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like); 2) research-based PAM50 signatures (continuous variable; scores showing the distant of the gene expression values of an individual sample compared to the centroid gene values for each PAM50 intrinsic subtype); 3) risk of recurrence (ROR) groups (low, medium and high); 4) the 13-gene hypoxia/VEGF signature (continuous); and 5) Ki67 by immunohistochemistry (continuous). Uni- and multivariable Cox models for PFS were used to test the prognostic significance of each variable. To determine whether each variable is predictive of bevacizumab benefit, we tested the interaction term of each variable by treatment arm in a Cox model.

Results: Tumor samples from 103 patients were analyzed: 55 (53%) in ET+B arm and 49 (47%) in ET arm. Subtype distribution was as follows: 57 (55.3%) Luminal A, 32 (31.1%) Luminal B, 5 (4.9%) HER2-enriched, 1 (1.0%) Basal-like, and 8 (7.8%) normal-like. In a univariate analysis, Luminal B tumors had a poorer outcome using Luminal A as reference (13.8 vs. 21.3 months, respectively; hazard ratio, HR=1.80, 95% CI 1.10-2.95, p=0.019). Concordant with this finding, Luminal A signature was associated with a better outcome. Similarly, ROR-P high group showed a poorer outcome than ROR-P low group (8.5 vs. 19.4 months; HR=2.88, 95% CI 1.30-6.35, p=0.009). Neither VEGF-13 signature nor Ki67 were found to be associated with PFS. Similar findings were obtained after adjustment for treatment, age, previous ET, ECOG, visceral disease and number of metastatic sites. In terms of treatment benefit, the HER2-enriched signature was the only variable found predictive of bevacizumab PFS benefit in univariate (p=0.010) and multivariate (p=0.015) analyses.

Conclusions: In advanced HR+/HER2- disease, intrinsic subtype (i.e. Luminal A vs. B) independently predicts PFS following first-line ET. In addition, HR+/HER2-negative tumors with high expression of the HER2-enriched signature, a biomarker of estrogen-independence, benefit the most from bevacizumab. Further validation of these prognostic and predictive biomarkers is warranted.
Title: DNA methylation patterns correlating with outcome in patients treated with first-line bevacizumab for metastatic breast cancer

Gampenrieder SP P, Rinnerthaler G, Weinhäusel A, Pulverer W, Hugnagl C, Hackl H, Romeder F, Monzo Fuentes C, Hauser-Kronberger C, Mlineritsch B and Greil R. Salzburg Cancer Research Institute with Laboratory of Immunological and Molecular Cancer Research and Center for Clinical Cancer and Immunology Trials, Paracelsus Medical University Salzburg, Salzburg, Austria; Business Unit for Molecular Diagnostics, AIT – Austrian Institute of Technology GmbH, Vienna, Austria; Division of Bioinformatics, Biocenter, Innsbruck Medical University, Innsbruck, Austria and Paracelsus Medical University Salzburg, Salzburg, Austria.

Body: Background: Biomarkers predicting response to bevacizumab containing therapy in breast cancer are of urgent need for a personalized treatment approach. DNA methylation is involved in regulation of angiogenesis and development of treatment resistance and could therefore provide predictive information for bevacizumab efficacy.

Patients and methods: A genome-wide methylation profiling using the Illumina Infinium HumanMethylation450 BeadChip was performed in FFPE specimen of 36 patients with HER2-negative metastatic breast cancer treated with chemotherapy in combination with bevacizumab as first-line therapy (discovery set). Twenty-eight (78%) samples came from primary tumor and 8 (22%) from metastasis (2 lung mets., 1 pleural met., 1 liver met., 2 soft tissue mets., 1 ovarial met., 1 bone marrow infiltration). Based on progression-free survival (PFS) and breast cancer subtype (ER+ vs. triple-negative) patients were divided in responder (R) and non-responder (NR). By biostatistical methods differences in the methylation pattern between R and NR were detected. The 48 most interesting genes (e.g. because of their involvement in angiogenesis or carcinogenesis) showing a differential methylation status between R and NR are currently validated in two further metastatic breast cancer cohorts treated with (main set) and without bevacizumab (validation set), respectively. These validated results will be presented at the meeting.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-44

Title: Abstract Withdrawn

Body:
Title: Bone turnover marker levels and clinical outcomes in patients with breast cancer and bone metastases treated with bone antiresorptive therapies

Lipton A, Stopeck A, Body J-J, Von Moos R, De Boer R, Paiva Gadelha Guimaraes A, Tonkin K, Fujiwara Y, Zhu L and Warner D. Penn State Hematology Oncology, Hershey, PA; Stony Brook Cancer Center, NY, NY; Université Libre de Bruxelles, Brussels, Belgium; Kantonsspital Graubünden, Chur, Switzerland; Royal Melbourne Hospital, Melbourne, Australia; Cancer Hospital AC, Carmargo, Brazil; Cross Cancer Institute, Edmonton, Canada; National Cancer Center Hospital, Tokyo, Japan and Amgen Inc., Thousand Oaks, CA.

Body: INTRODUCTION AND OBJECTIVES: Patients with advanced breast cancer (BC) and metastatic bone disease typically have elevated serum levels of bone turnover markers (BTMs). Potent antiresorptive agents, such as denosumab and zoledronic acid, can significantly reduce BTM levels (Stopeck et al. J Clin Oncol 2010). Prior studies have provided evidence that higher BTM baseline levels may be associated with worse clinical outcomes (Ali et al. Ann Oncol 2004). In this analysis, we assessed the association between BTM levels after treatment with antiresorptive agents and overall survival (OS), disease progression (DP) and disease progression in the bone (DPB) in patients with advanced BC and bone metastases.

METHODS: This post-hoc analysis included data from patients with BC and bone metastases enrolled in an international, blinded phase 3 trial who were randomized to receive either denosumab (120 mg SC) or zoledronic acid (4 mg IV, adjusted for creatinine clearance). The BTMs urinary N-telopeptide (uNTx) and bone-specific alkaline phosphatase (BSAP) were measured at study entry and at study month 3. The clinical outcomes of OS, DP, and DPB were compared in patients with BTMs above or below median levels at month 3 of antiresorptive therapy. These covariate analyses were based on Cox models stratified by treatment, prior SRE before month 3, prior bisphosphonate use, chemotherapy at randomization, and region (Japan or other countries).

RESULTS: A total of 1705 patients were measured for uNTx serum levels, with 895 patients ≥ and 810 < the median of 10.40 nmol/mmol at month 3. Similarly, BSAP levels were measured in a total of 1708 patients, with 855 patients ≥ and 853 < the median BSAP level of 10.89 ng/mL at month 3. Patients with uNTx levels ≥ the median at month 3 had a significantly reduced OS (by 54%) and a greater risk of both DP (by 21%) and DPB (by 23%) than patients with uNTx levels < median (see Table). Similarly, patients with BSAP levels ≥ the median level at month 3 had an increased risk for reduced OS (by 197%), DP (by 67%) and DPB (by 56%) compared with patients whose BSAP levels < median. After adjusting for risk factors suggestive of more advanced disease such as visceral metastases or > 2 bone metastases, the correlation between elevated BTMs and reduced OS and greater risk of DP and DPB was maintained.

CONCLUSIONS: Patients with BTM levels ≥ median at month 3 of antiresorptive therapy had generally worse clinical outcomes than patients whose BTM levels were < median. Assessment of uNTx and BSAP serum levels after treatment with antiresorptive therapy can add to our understanding of which patients with breast cancer and bone metastases are most at risk for decreased OS, or increased DP or DPB.

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Hazard Ratio (95% Confidence Interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decrease in OS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uNTX(^a)</td>
<td>1.539 (1.270, 1.866)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BSAP(^b)</td>
<td>2.966 (2.422, 3.633)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Increase in DP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uNTX(^a)</td>
<td>1.214 (1.071, 1.377)</td>
<td>≈0.0024</td>
</tr>
<tr>
<td>BSAP(^b)</td>
<td>1.666 (1.470, 1.888)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Increase of DPB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uNTX(^a)</td>
<td>1.231 (1.054, 1.437)</td>
<td>≈0.0087</td>
</tr>
<tr>
<td>BSAP\textsuperscript{b}</td>
<td>1.563 (1.342, 1.821)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Comparing $\geq$ median to $<$ median. \textsuperscript{a}Median uNTx levels=10.40 nmol/mmol. \textsuperscript{b}Median BSAP level=10.89 ng/mL.
Title: CYPTAM-BRUT 3: Endometrial thickness cannot be used as a marker for tamoxifen metabolization in postmenopausal breast cancer patients

Body: Background:
Tamoxifen is commonly used for the treatment of all stages of estrogen receptor (ER) positive breast cancer (BC), the largest group of BC. In postmenopausal women, known endometrial side-effects are cystic appearance, hyperplasia, polyps and endometrial cancer. Tamoxifen is converted through several CYP450 enzymes into the active metabolite endoxifen. Patients with particular single nucleotide polymorphisms (SNPs) in those CYP450 enzymes (e.g. CYP2D6) have lower endoxifen concentrations and therefore could experience less benefit from tamoxifen. However, the clinical significance stays controversial in the literature as results remain contradictory. Our primary hypothesis is that women with lower endoxifen levels do not have the typical tamoxifen-induced increase in endometrial thickness.

The aim of this study is to test the association between endoxifen concentration and the increase in double endometrial thickness (DET).

Patients and methods:
CYPTAM-BRUT 3 is a prospective, multicentric study including postmenopausal women with an ER positive BC receiving tamoxifen in the adjuvant setting. Primary objective is the association between serum endoxifen levels and change in DET between baseline and follow-up after 3 - 6 months. Secondary objectives are investigating the relation between serum endoxifen levels and other endometrial changes (i.e. the presence of cysts/polyps), menopausal symptoms assessed with a questionnaire and serum levels of follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG). Other objectives are the association between the 'CYP2D6 tamoxifen activity score', based on SNPs and co-medication, and DET, other endometrial changes, FSH and SHBG. The CYPTAM-BRUT 3 study is a sub-study of the CYPTAM study in Leiden, The Netherlands, with survival as primary outcome.

Results:
144 women were included in 19 hospitals. There was no significant association found between endoxifen and change in DET (p=0.888), as for the association between endoxifen and the development of cysts/polyps (p=0.208). For the questionnaire items no correlation was found (p≥0.051). Changes of FSH and SHBG were in the range of what would be expected during tamoxifen treatment in the general population and there was no association with endoxifen (p=0.726 and p=0.181). In addition, no association was found between the CYP2D6 TAS score and DET (p=0.613), other endometrial changes (p=0.196), FSH (p=0.976) and SHBG (p=0.900).

Conclusion:
Our study is one of the first studies that prospectively investigated the relation between tamoxifen metabolization, in correlation with endometrial thickness and SNPs.

We can conclude that the typical increase in endometrial thickness seen in a significant proportion of postmenopausal women under tamoxifen cannot be used to predict that those women have a high endoxifen concentration or that women without an increased endometrial thickness have a significantly lower endoxifen concentration. The same conclusions can be made for the
other tamoxifen-induced changes as cysts and polyps, menopausal symptoms, FSH and SHBG. It may also apply to tamoxifen efficacy but further prospective studies looking at survival are needed to answer that question.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-47

Title: Comprehensive profiling of metaplastic breast carcinoma reveals frequent over-expression of PD-L1


Body: Background: Metaplastic breast carcinoma (MBC) is a rare subtype of breast carcinoma less responsive to conventional chemotherapy relative to usual breast carcinomas (UBCs) such as ductal and lobular subtype. In molecular terms MBC usually clusters with triple negative breast cancer (TNBC), but MBCs portray a worse prognosis in comparison to TNBCs. Published studies investigating MBCs for specific biomarkers of therapy response are rare and limited by the methodological approaches.

Methods: This study included 132 patients with 38 histologically confirmed MBCs and 94 UBCs. Amongst the 94 UBCs, 44 were estrogen receptor positive, 33 were triple negative and 17 were HER2 positive. Direct sequencing analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tissue (FFPE) using the Illumina MiSeq Next Generation Sequencing platform (NGS). Immunohistochemistry for PD-L1 (SP142, Spring Bioscience), PD-1 (NAT105, Ventana) and EGFR (31G7, Life Technologies) was performed using automated procedures. Additionally, mutation analysis for *EGFRvIII* was performed on RNA extracted from FFPE tissue.

Results: At the genomic level, numerous cases of MBC had multiple genomic alterations with the most frequent genetic mutation in *TP53* gene (14/24), similar to the TNBC controls (17/33). *BRCA1* mutations were detected in 2/10 cases. Potentially actionable mutations were rare and included *PIK3CA* gene. Importantly, PD-L1 expression on cancer cells was detected in significantly higher proportion of MBCs (37%) than in the UBC cohort (6%) or TNBC control (14%) (p=3.7x10^-5 and p=0.03, respectively). PD-1 positive tumors infiltrating lymphocytes (TILS) varied greatly in MBC (0 to >50/mm²). Over-expression of *EGFR* was frequent in MBCs (62%); however no mutations in the gene including *EGFRvIII* were detected.

Conclusion: Comprehensive profiling of a large cohort of this rare carcinoma highlighted predominance of *TP53* mutations, wild type *EGFR* gene expression, a distinct increase in proportion of PD-L1 expression in carcinoma cells, and PD-1 expression in TILS. The latter properties can be exploited in clinical trials utilizing immune check point inhibitors.
Title: Prediction of complete pathologic response to veliparib/carboplatin plus standard neoadjuvant therapy in HER2 negative breast cancer: Exploratory protein pathway marker results from the I-SPY 2 trial


Body: Background: In the I-SPY 2 TRIAL, HER2- patients were randomized to receive standard chemotherapy or chemotherapy plus the oral PARP inhibitor veliparib in combination with carboplatin (V+C), which graduated in the HR-/HER2- arm. Exploratory analysis of protein signaling was performed to identify biomarker candidates that correlated with pCR in the HER2- population. We evaluated 110 key signaling proteins using reverse phase protein microarray (RPPA) data from pre-treatment LCM purified tumor epithelium.

Methods: Of 115 patients, 97 (V+C: 61 controls: 36) had RPPA and pCR data. RPPA data was correlated to pCR in both the treated and control patients using parametric (t-test) or non-parametric (Wilcoxon) statistical analysis, depending on data distribution. Only analytes whose pre-treatment levels were associated with response in the V+C but not in the control arm were identified (P<0.05). Markers are analyzed individually; p-values are descriptive and were not corrected for multiple comparisons. Results: 11 protein/phosphoprotein markers were significantly associated with pCR in the V+C arm but not in controls. Two were positive predictors of response: YAP S127 p= 0.03 and LC3B p=0.04. Negative predictors of response included Cyclin D1 p=0.001, and a number of phosphorylated RTKs: ROS Y2274 p=0.03, IGF1R Y1135/Y1136-IR Y1150/Y1151 p=0.03, ERBB4 Y1284 p=0.002, total HER2 p=0.04, and total IGF1R p=0.01. Moreover, a number of AKT-mTOR pathway proteins were found to be negative predictors of V+C response: ACC S79 p=0.005, p70S6K S371 p=0.01, and B-RAF S445 p=0.01.

Conclusion: Our sample size is too small to draw definitive conclusions and the results are exploratory. Coordinated RTK-mTOR pathway activation appears to be a hallmark signature of lack of response to veliparib in HER2- tumors. We also found that HER2 levels were correlated paradoxically with lack of response in this HER2- population, suggesting potential added clinical value of quantitative HER2 measurement techniques. Such exploratory results merit evaluation in larger trials with HER2- breast cancer patients.
Title: Residual cancer burden (RCB) with veliparib/carboplatin in the I-SPY2 trial

Liu MC, Symmans WF Fraser, Yau C, Chen Y-Y, Rugo HS S, Olopade OF F, Datnow B, Chen B, Feldman M, Kallakury B, Hasteh F, Tickman R, Ritter J, Troxel M, Mhawech-Fauceglia P, Duan X, Berry D, Esserman L and DeMichele A. Mayo Clinic, Rochester, MN; MD Anderson, Houston, TX; Buck Institute for Research on Aging, Novato, CA; University of California, San Francisco, CA; University of Chicago, Chicago, IL; University of San Diego, San Diego, CA; University of Pennsylvania, Philadelphia, PA; Georgetown University, Washington, DC; Swedish Medical Center, Seattle, WA; University of Minnesota, Minneapolis, MN; OHSU, Portland, OR; Keck Hospital of USC, Los Angeles, CA and Loyola University Health System, Maywood, IL.

Body: Background: I-SPY2 is a multicenter phase 2 trial in high risk stage II/III breast cancer (BC) using adaptive randomization within biomarker subtypes to evaluate novel agents added to standard neoadjuvant chemotherapy. The first regimen to graduate based on the predicted probability of a higher pCR rate within predefined subsets was veliparib/carboplatin + paclitaxel (VC+T→AC vs T→AC) in triple negative BC (TNBC). In TNBC the residual cancer burden (RCB) is prognostic, whether as a continuous index or grouped into classes, with pCR (RCB-0) and RCB-I classes having identical survival. Therefore, we evaluated the use of RCB to further discriminate between investigational and control arms. Methods: Site pathologists reported RCB for 99% of subjects in the primary efficacy analysis based on pCR (n=114/115). We compared the distribution of RCB reported as a continuous index in each treatment-subset combination to matched concurrently randomized controls using the Wilcoxon rank sum test for RCB index, and Fisher's Exact test for RCB classes (RCB-0/I vs RCB-II/III). The statistics are descriptive rather than inferential, and given the small sample size have no claim on generalizability. We modified the Bayesian model used to compute the estimated probability of success in a future, randomized, phase 3 trial of 300 subjects, if response were defined by either pCR or RCB-I (RCB0/I), or separately if it were defined by pCR alone. Results: VC+T→AC led to a significantly lower RCB index than T→AC in TNBC (p=0.0021), with a near-significant trend when those with pCR were excluded (p=0.06). There was no significant difference in RCB distributions in the other breast cancer subtypes treated. In TNBC, the odds ratio (OR) for achieving RCB-0/I in the VC+T→AC arm vs control was 8.2 (95% confidence interval (CI): 2.1–35), whereas the OR for achieving pCR was 4.56 (95% CI: 1.25–19.53). The simulations using response information from I-SPY2 to predict the probability of success for VC+T→AC for TNBC in a future phase 3 trial estimated this probability to be 0.99 if modeled using RCB-0/I as the response endpoint, and 0.90 if modeled using pCR as the response endpoint. Conclusions: Use of RCB index and classes provided additional insight into the effect of adding VC to T, appearing to magnify the improved treatment response that had been observed with pCR rates in TNBC. It will be important to test in randomized trials whether a decrease in the RCB index relative to controls, and/or increased rates of RCB-0/I class, are predictive of survival benefit in TNBC.
Title: Early and accurate prediction of pathological response by magnetic resonance imaging and ultrasonography in patients undergoing neoadjuvant chemotherapy for operable breast cancer

Kaise H, Ishikawa T, Miura D, Hasegawa Y, Horiguchi J, Hayashi M, Takao S, Kim SJ, Tanino H, Miyashita M, Konishi M, Shigeoka Y, Yamagami K, Suzuki M, Taguchi T, Akazawa K and Kohno N. Tokyo Medical University Hospital, Tokyo, Japan; Yokohama City University Medical Center; Toranomon Hospital; Hirosaki Municipal Hospital; Gunma University Hospital; Tokyo Medical University Hachioji Medical Center; Hyogo Cancer Center; Osaka University Hospital; Naga Municipal Hospital; Konan Hospital; Hyogo Prefectural Nishinomiya Hospital; Yodogawa Christian Hospital; Shinko Hospital; Niigata University Medical and Dental Hospital; Kobe Kaisei Hospital; National Hospital Organization Chiba Medical Center and University Hospital, Kyoto Prefectural University of Medicine.

Body: Background: Neoadjuvant chemotherapy (NAC) reduces tumor size, and increases the frequency of breast-conserving surgery in operable breast cancers. Response predictions to NAC are made based on diagnostic imaging. Although various studies have reported the optimal timing for diagnostic imaging, this still remains unclear.

Purpose: To identify the optimal timing of diagnostic imaging for the response prediction to NAC, and to evaluate the accuracy of response prediction.

Methods: We evaluated 146 cases enrolled in the JONIE-1 study (a randomized controlled trial comparing zoledronic acid plus chemotherapy with chemotherapy alone as a NAC in patients with HER2-negative primary breast cancer). The chemotherapy regimen was FEC100×4 courses followed by weekly paclitaxel 80×12 courses (± zoledronic acid). Statistical analysis of the association between the tumor reduction ratio and the histopathological response and the prediction of pathological complete response (pCR) was performed using JMP software. The maximum tumor diameter was evaluated using magnetic resonance imaging and ultrasound on each patient 3 times (before NAC, after FEC treatment, after NAC) and tumor reduction ratios were calculated.

Results: The average age of the patients was 49.8 years old. The menopause status was pre-menopause in 84 patients, and post-menopause in 58 patients. Regarding the subtype classification, 116 patients were of the luminal type (Lum) and 26 patients were triple negative (TN), and the Ki-67 labeling index had a median of 25% (1%-93%). Pathological examination demonstrated that 16 patients had pCR(11.3%, Lum; 9; TN: 7), and 126 patients had non-pCR (88.7%, Lum:107; TN:19). Seven patients had clinical-CR (4.8%, Lum: 4; TN: 3) at post-FEC, and 26 patients (17.8%, Lum: 20; TN: 6) at post-NAC. The prediction of pCR at post-FEC and post-NAC was evaluated by single variable analysis, resulting in an AUC (0.75645) p=0.0017 at post-FEC, and AUC (0.76563) p=0.0001 at post-NAC. The sensitivity / specificity / positive predictive value / negative predictive value were 0.625 / 0.873 / 0.385 / 0.948 at post-FEC, 0.250 / 0.976 / 0.571 / 0.911 at post-NAC, respectively. In TN cases, the values were 0.714 / 0.947 / 0.833 / 0.900 in post-FEC, and 0.429 / 1.000 / 1.000 / 0.826 in post-NAC.

Conclusions: Diagnostic imaging evaluation performed after FEC treatment was useful for the prediction of pCR. Furthermore, the reliability was high in Triple Negative Sub type, but is affected by the existence of residual tumors in Luminal type.
Title: Regulation of DNA methyltransferases via TRAF6 determines breast cancer response to decitabine


Body: Background: Tumorigenesis involves both genetic and epigenetic changes. Epigenetic alterations are reversible and are promising cancer therapeutic targets. Decitabine (5-aza-2'-deoxycytidine), a DNA methyltransferase inhibitor, is FDA approved for hematological malignancies. However, the effect of decitabine in breast cancer is not completely understood. Previous reports indicated that one decitabine mechanism involves regulation of protein levels for DNMT1, the major DNA methyltransferase that methylates hemimethylated CpG di-nucleotides in DNA. However, the E3 ligase involved in this process has not been identified. Whether decitabine also regulates DNMT3A and 3B in a similar fashion remains unclear. Therefore, our goals were to 1) understand mechanisms underlying decitabine action, 2) test the antitumor activity of decitabine in breast cancer models and 3) identify biomarkers associated with response to decitabine.

Methods and Results: Western blots of breast cancer cell lines showed that DNMT1, DNMT3A, and DNMT3B protein levels decreased following decitabine treatment without a reduction in mRNA levels. Bioinformatic analysis of DNA methyltransferase sequences revealed a potential TRAF6 binding motif, and the interaction with TRAF6 (TNF receptor-associated factor 6) was confirmed by IP. TRAF6 functions as an E3 ligase. To determine whether TRAF6 might be the E3 ligase responsible for the degradation of DNMTs after decitabine treatment, we knocked down TRAF6 by RNA interference or knocked out the TRAF6 gene by CRISPR/Cas9. Down regulation of TRAF6 attenuated DNMT ubiquitination and increased DNMT protein levels, suggesting that TRAF6 might mediate proteasome-dependent degradation of all three DNMTs. This was further confirmed by reconstituting the knockout cells with WT and a TRAF6-C70A mutant, followed by assessing DNMT protein levels. Global DNA methylation was also increased after TRAF6 depletion and was confirmed in TRAF6 knock out cells in which DNMT levels were unaffected by decitabine. Cell cytotoxicity and colony forming assays showed that TRAF6 knockout cells were resistant to decitabine, suggesting that a major decitabine mechanism of action is through the regulation of TRAF6 which, in turn, degrades DNMTs, leading to decreased global methylation. Finally, decitabine significantly induced TRAF6 at both mRNA and protein levels, a process that might create positive feedback leading to increased degradation of DNMT proteins upon decitabine treatment. Based on these results, we further hypothesized that levels of the three DNMTs might influence decitabine response. Using 18 breast cancer patient derived xenograft (PDX) models, we found a wide range of DNMT protein levels regardless of ER/HER2 status. DNMT levels in the PDX models were directly associated with sensitivity to decitabine treatment, confirming our hypothesis.

Conclusion: Our data showed that decitabine might be an effective agent for treating breast cancer and revealed a novel mechanism underlying decitabine treatment. Baseline DNMT protein levels may serve as a biomarker for predicting decitabine drug response.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-07-52

Title: Identification of serum biomarkers associated with Akt inhibitor MK-2206-induced toxicity in a pre-surgical breast cancer (BC) trial


Body: Background: The PI3K/Akt/mTOR pathway is an important oncogenic driver in BC. A major hurdle in clinical Akt inhibitor development has been dose-limiting toxicities, such as rash. To facilitate the risk assessment of Akt inhibitor associated toxicity, we hypothesize that circulating biomarkers can be identified in proteins secreted by the tumor or tumor microenvironment and systemic response after treatment. Exosomes are small membrane bound vesicles containing proteins, mRNA, miRNA, and lipids that are secreted from host cells and remain viable after long-term storage of blood. In this study, we focused on identifying biomarkers associated with drug rash from serum exosomes in BC patients treated with the Akt inhibitor MK-2206.

Methods: In an open-label pre-surgical trial, 2 doses of weekly MK2206 were administered to patients (pts) with stage I-III invasive BC: first at day -9 and second at day -2 from surgery. Sera were collected before and after MK2206. 200 µL of serum was used to isolate total exosomes by precipitation and centrifugation, followed by trypsin digestion and multiplexing labeling analysis. The Orbitrap mass spectrometer was used to acquire LC-MS/MS data. 1,053 unique proteins were identified from the uniProt database. Maximum false discovery rate level (FDR) for predictive biomarkers was controlled at 26% (q<0.26). Analysis was conducted on pre-MK-2206 and post-MK-2206 treated sera from pts to develop a protein signature associated with rash and identify candidate biomarkers of MK-2206-associated rash.

Results: The study was discontinued after 12 pts were enrolled due to toxicity. Notably, an acneiform/maculopapular rash was observed in 5 pts. Unsupervised principal component analysis on the pre-MK-2206 specimens and the entire set of 1,053 proteins demonstrated that 4 of the 5 pts with rash formed a distinct cluster. 30 proteins were differentially expressed in pre-MK-2206 samples from pts who developed rash vs. no rash (q<0.26), with ≥1.5 fold difference in expression level in those with rash after MK-2206. Ingenuity pathway analysis revealed statistically significant over-representation of pathways involved in lipid metabolism (including MALRD1, AWAT2), nucleic acid synthesis (PPAT, ADSLL1), and protein synthesis (PPIB). 45 proteins were significantly different in post-MK-2206 samples (q<0.285). Lipid metabolism was the most significantly over-represented pathway in post-MK-2206 samples.

Conclusions: We demonstrated that mass spectrometry-based proteomic analysis of patient-derived serum exosomes is a promising approach to study drug-induced toxicity. We found significant changes of circulating proteins before and after MK-2206. Increased expression of different proteins involved in lipid metabolism appears to predict skin toxicity, commonly seen with PI3K/Akt pathway inhibitors. Since the PI3K/Akt signaling pathway plays a role in physiological regulation of lipid metabolism, lipid metabolic profiles of BC patients might be important for predicting the risk and controlling toxicity induced by Akt inhibitors. These toxicity-associated biomarkers should be validated and then assessed prospectively in clinical trials.
Chromosomal instability as a predictor of sensitivity to paclitaxel

Cavalcante LL L, Denu R, Zasadil L, Weaver BA A and Burkard M. University of Wisconsin, Madison, WI.

Body: Background: Paclitaxel is one of the most effective therapies for breast cancer, although many patients do not benefit. Our goal is to identify those who will benefit, by understanding how this drug contributes to chromosomal instability (CIN). CIN is the gradual gain/loss of whole chromosomes that can occur with mitotic errors as tumors proliferate. Some breast cancers inherently have CIN whereas others lack CIN. Previous work suggests low rates of CIN can promote tumor growth by creating genetic diversity. By contrast, high rates of CIN are lethal, apparently due to a high incidence of deleterious karyotypes. We hypothesize that paclitaxel operates by increasing CIN, and that this has preferential anticancer effects in tumors with preexisting low CIN. Methods: To assess rates of underlying CIN in human breast cancer, we performed 6-chromosome FISH on 354 human breast cancers and correlated with outcomes on a cohort with median 8.4 year follow-up. We measured the physiologic levels of paclitaxel that occur in human breast tumors. To do this, we treated 5 women with neoadjuvant paclitaxel 175mg/m2, performed tumor biopsy at 20 hours, performed LC to quantify intratumoral levels and analyzed mitotic spindles by IHC. Additionally, we performed timelapse videomicroscopy to analyze mitosis in fluorescently-labeled breast cancer cells in the laboratory after exposure to these levels. To evaluate whether CIN controls paclitaxel sensitivity we artificially introduced low levels of CIN into breast cancer cell lines by doxycycline-inducible expression of GFP-Mad1, a protein involved in the mitotic checkpoint, and tested whether this enhanced sensitivity to physiologic doses of paclitaxel.

Results: A total of 77% (270/349) of breast cancer have detectable underlying CIN, (average percentage of non-modal chromosomes averaged for 6 chromosomes) greater than the normals (n=11). CIN is higher in HER2+ and TNBC subtypes compared to HR+. CIN does not correlate with the proliferation marker, Ki67 (r2 = 0.04), which does not support the idea of a growth advantage. CIN greater than median levels correlated with worse breast cancer-specific survival (p=0.022 log rank), but no difference in OS or RFS. Paclitaxel in human breast cancer reaches a level mimicked by 5-50nM exposure in laboratory experiments. In the laboratory, breast cancer cells exposed to these levels exhibit multipolar divisions, and similar abnormal mitoses can be found in patient tumors. In breast cancer cells lacking CIN, chromosome analysis demonstrates that it can be artificially induced by conditionally expressing GFP-Mad1. Inducing GFP-Mad1 expression increases sensitivity to paclitaxel, demonstrating that CIN enhances taxane sensitivity.

Conclusions: These data support the idea that excessively high levels of CIN can be lethal to cancer cells and that paclitaxel enhances CIN. We predict that the anticancer effects of paclitaxel are marked in tumors with intrinsic CIN, as the enhanced levels are lethal. Thus CIN may be an effective biomarker to predict which women will benefit from taxane therapy. Ultimately, this could be applied in the clinic to substantially improve patient care by decreasing primary resistance or by reducing side effects associated with paclitaxel use.
Title: Insulin-like growth factor 1 receptor expression and polymorphism are associated with response to neoadjuvant chemotherapy in breast cancer patients: Results from the NEOZOTAC trial (BOOG 2010-01)


Body: Background
The insulin-like growth factor 1 (IGF-1) pathway is involved in cell growth, proliferation and cell cycle progression and associated with tumor genesis and therapy resistance. This study aims to elucidate whether variation in the IGF-1 pathway is predictive for pathologic response in early breast cancer (BC) patients taking part in the phase III NEOZOTAC trial, randomizing between 6 cycles of neoadjuvant TAC chemotherapy with or without zoledronic acid.

Method
Formalin-fixed paraffin-embedded (FFPE) tissue samples of pre-chemotherapy biopsies and operation specimens were collected for analysis of IGF-1 receptor (IGF-1R) expression using IHC (n=216) and for analysis of 8 candidate SNPs in genes of the IGF-1 pathway (n=184) using OpenArray® RealTime PCR. Optionally, blood samples were collected immediately before chemotherapy for determination of glucose, insulin, IGF-1, IGF-2 and IGF-BP3. Associations with patient and tumor characteristics and chemotherapy response according to Miller and Payne (MP) pathologic response were performed using chi square and logistic regression analyses.

Results
High IGF-1R expression was associated with estrogen receptor expression (P=0.001). During chemotherapy, a significant number of the tumors (47.2%) showed a decrease in IGF-1R expression, while in a small number of the tumors an upregulation was seen (15.1%). IGF-1R expression before treatment was not associated with pathological response, however absence of IGF-1R expression after treatment was associated with a better response in multivariate analyses (P=0.012) and patients with a decrease in expression during treatment showed a better response to chemotherapy as well (P=0.008). Moreover, the variant T allele of 3129G>T in IGF-1R (rs2016347) was associated with a better pathological response in multivariate analyses (P=0.032). In addition, high glucose and insulin levels were associated with positive lymph node status before chemotherapy in multivariate analysis (P=0.019) and (P=0.031), respectively.

Conclusion
Neoadjuvant chemotherapy induced changes in the IGF-1R expression in most of the tumors. Absence or diminished expression of IGF-1R after treatment was associated with a better pathological response. Additionally, we found a SNP (rs2016347) in IGF-1R as a potential predictive marker for chemotherapy efficacy in BC patients treated with TAC. These findings may help to select patients who might benefit from (co-)treatment with an IGF-1 pathway inhibitor.
Title: Predictive value of O6-methylguanine-DNA methyltransferase (MGMT) promoter gene methylation in triple-negative breast cancer patients receiving carboplatin

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Body: Background: The epigenetic profile of triple-negative breast cancer (TNBC) showed a wide prevalence of MGMT promoter methylation. Aberrant methylation of MGMT seems to be an independent predictor of poor survival in patients with basal-like breast cancer. Moreover, patients with MGMT-negative basal-like tumors who received cyclophosphamide had significantly improved DFS and OS compared with MGMT-positive tumors. However, the impact of MGMT methylation in the context of modern therapy concepts is not clear.

Methods: We retrospectively evaluated 174 TNBC tumors of patients enrolled into the neoadjuvant GeparSixto trial from 08/2011 to 12/2012. Patients were randomized to receive 18 weeks of neoadjuvant treatment with paclitaxel (80mg/m²/week) and non-pegylated liposomal doxorubicin (20mg/m²/week) with or without addition of carboplatin (AUC 2.0-1.5/week). Hormone-receptor status, HER2 status, and Ki67 were centrally confirmed prior to randomization. We defined pathological complete response (pCR) as ypT0/is ypN0. MGMT promoter methylation status was determined by PCR using EZ DNA Methylation Kit™ (Zymo Research); TNBC tumors were considered to be methylated if they had an average methylation ≥ 10%, some tumors were considered borderline due to high heterogeneity among CpG islands. We investigated the effect of MGMT methylation on pCR and its correlation with baseline characteristics.

Results: A total of 210 tumors from the TNBC cohort of the GeparSixto trial (n=315) were available with a tumor content >20%. In 174 tumors the methylation assay was performed successfully. The number of tumors with methylated MGMT was similar in carboplatin vs. non-carboplatin treated cohorts. In the carboplatin group 19.3% (17/88) of TNBC were methylated, 65.5% (58/88) unmethylated, and 14.8% (13/88) borderline. In the non-carboplatin group 20.9% (18/86) of TNBC were methylated, 62.8% (54/86) unmethylated, and 16.3% (14/86) borderline. In the entire cohort, there was no association between MGMT methylation status and pCR (p=0.522). Non-carboplatin cohort: 33.3% (6/18) of patients with methylated MGMT achieved pCR vs. 51.9% (28/54) of unmethylated and 51.9% (28/54) of patients with methylated MGMT achieved pCR vs. 51.9% (28/54) of unmethylated and 51.9% (28/54) of patients with methylated MGMT achieved pCR vs. 51.9% (28/54) of unmethylated and 51.9% (28/54) of patients with methylated MGMT achieved pCR vs. 51.9% (28/54) of unmethylated (p=0.079). Carboplatin cohort: 52.9% (9/17) of patients with methylated MGMT achieved pCR vs. 55.2% (32/58) of unmethylated and 76.9% (10/13) of borderline (p=0.320). In TNBC patients with methylated MGMT, the addition of carboplatin resulted in a 20% increased pCR rate (p=0.241).

Conclusion: In this study no statistically significant association between MGMT methylation and pCR was found. Patients with MGMT methylation seemed to have a lower possibility to achieve a pCR and the addition of carboplatin seemed to reverse this effect. However, a clear classification of the borderline MGMT samples and further studies in larger series of TNBC are warranted.
**Title:** Predictive value of tertiary lymphoid structure assessed by high endothelial venule count in neoadjuvant setting of triple-negative breast cancer

Song IH, Lee HJ, Park IA, Yu JH, Ahn J-H and Gong G. University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

**Body:**

**Background**

Tertiary lymphoid structure (TLS) is an ectopic lymph node-like structure characterized by lymphoid aggregation with high endothelial venules (HEVs), and is an important source of tumor-infiltrating lymphocytes (TILs). TILs have a strong prognostic and predictive significance, particularly in triple-negative breast cancer (TNBC). We previously analyzed expression of immune-related genes in pre-neoadjuvant chemotherapy (NAC) biopsy samples using NanoString assay and showed that gene expression of follicular helper T cell marker CXCL13, which is closely associated with TLSs, was an independent predictive factor for pathologic complete remission (pCR) in TNBC. However, measuring gene expression of biopsy sample is not easy to perform in daily pathology practice. Therefore, we evaluated TLSs by assessing hematoxylin and eosin (H&E) stained slides and immunohistochemistry, and investigated their clinical importance.

**Methods**

A total of 108 patients diagnosed with primary TNBC and treated with NAC (anthracycline, cyclophosphamide, and taxane) were included. The amounts of TILs and TLSs were histopathologically measured in H&E slides. Immunohistochemical studies were done in 55 cases with available tissue samples. The numbers of CD3-, CD8-, and CD20- immunopositive cells in tumor areas were counted by the image analyzer. MECA79- immunopositive HEV densities were calculated. Their relationship to CXCL13 gene expression by NanoString assay was also analyzed.

**Results**

The overall rate of pCR was 30.6% (33 of 108 tumors). Lower pre-NAC clinical T stage and higher level of TIL and TLS assessed by H&E slides were predictors of pCR in all cases. The mean number of MECA 79-immunopositive HEV in pre-NAC biopsy samples was 12 (range, 0-72). The amounts of TILs and TLSs, numbers of CD3-, CD8-, and CD20- positive cells, HEV density, and expression of CXCL13 showed good correlation with one another. Higher HEV density, CD20- positive cell number, and CXCL13 expression were predictors of pCR. Higher CD8- positive cell numbers and CXCL13 expression were associated with better disease-free survival rate.

**Conclusion**

The amount of TLSs assessed by H&E slides and MECA 79-immunopositive HEV densities was well correlated with level of TILs, numbers of CD3-, CD8-, and CD20-positive cells, and gene expression of CXCL13, and was significantly associated with pCR in TNBCs. Therefore, assessing HEV density by MECA 79 immunohistochemistry in pre-NAC biopsy samples might be an objective and valuable tool for predicting pCR of TNBC in routine pathology practice. Further investigation of mechanism of TLS development might help to improve immunotherapeutic strategy.
Title: Development of a 6-gene qPCR RUO-validated assay as a predictive biomarker for response of vantictumab (OMP-18R5; anti-frizzled) in HER2- breast cancer patients


Body: Background: We have developed a monoclonal antibody, vantictumab that blocks canonical WNT/β-catenin signaling through binding of five FZD receptors (1, 2, 5, 7, 8). This antibody inhibits the growth of several tumor types, including breast. Vantictumab reduces tumor-initiating cell frequency and exhibits synergistic activity with standard-of-care (SOC) agents (Gurney et al, 2012). To target breast cancer patients most likely to respond to vantictumab, we undertook a predictive biomarker study.

Methods: We have identified a 6-gene Wnt pathway-related signature, FBXW2, CCND2, RHOU, CTBP2, WiFi1, and DKK1, based on microarray gene expression data from 8 breast cancer patient derived xenograft (PDX) models with established in vivo response to vantictumab plus SOC. This signature successfully predicted the response of 8 additional and independent PDX breast tumors. We further developed a qPCR Research Use Only (RUO) assay for the 6 genes to be used on FFPE human breast tumor samples. Multiple assays targeting different regions spanning each mRNA transcript were tested and selected based on PCR efficiency, specificity and sensitivity. We compared assay sensitivity under different cDNA synthesis and pre-amplification conditions: random vs. gene-specific priming, number of pre-amplification cycles, pre-amplification reaction volumes, and cDNA synthesis kits. A repeatability study was performed to test assay performance. The pre-amplification and PCR were repeated over three separate days and across two independent labs.

Results: Our results showed that cDNA synthesis by gene-specific priming followed by 18 cycles of pre-amplification performed the best and the assay is robust with minimal starting FFPE RNA input. The results of the repeatability study were consistent among the different days and the different labs (<5% CV). Using the 6-gene qPCR RUO assay, the signature score from the microarray data was further refined using 12 PDX HER2- breast tumors with known in vivo response to vantictumab with SOC. The prevalence of the 6-gene signature was established using ~100 HER2- breast cancer samples.

Conclusions: A robust 6-gene RUO-validated assay was developed as a predictive biomarker for vantictumab in HER2- breast cancer. The assay is currently being evaluated in a Phase 1b study of vantictumab with paclitaxel in HER2- breast cancer.
Title: CD44v as a potential predictive biomarker for pathologic complete response in primary HER2+ breast cancer: Utility of adaptive response biopsy in preoperative therapy

Yamauchi T, Imamura CK K, Yamauchi H, Jinno H, Takahashi M, Kitagawa Y, Nakamura S, Lim B, Krishnamurthy S, Reuben JM M, Liu D, Tripathy D, Zujewski JA, Chen H, Takebe N, Saya H and Ueno NT T. Division of Medical Oncology, St. Luke's International Hospital, Tokyo, Japan; Keio University, School of Medicine, Tokyo, Japan; St. Luke's International Hospital, Tokyo, Japan; Keio University, School of Medicine, Tokyo, Japan; Showa University School of Medicine, Tokyo, Japan; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; Cancer Therapy Evaluation Program, National Cancer Institute, Rockville, MD and Division of Gene Regulation, Institute for Advanced Medical Research, Keio University, School of Medicine, Tokyo, Japan.

Body: Background: Preoperative dual anti-HER2 therapy with lapatinib and trastuzumab in combination with conventional chemotherapy demonstrates a higher pathologic complete response rate (pCR) than trastuzumab with chemotherapy in patients with HER2+ breast cancer. Preoperative chemotherapy has been reported to increase the fraction of cancer stem-like cells (CSCs) in breast cancer, but this effect has not been well validated in clinical setting. Cancer cells with the epithelial-mesenchymal transition (EMT) phenotype also exhibit stem cell–like properties with drug resistance. Our goal was to determine the quantitative values of various biomarkers in baseline and adaptive response biopsy specimens and in subsequent surgical specimens to predict pCR in patients treated with dual anti-HER2 therapy as demonstrated by reduction of CSCs, phosphorylated receptors and signaling kinases, and circulating tumor cells (CTC) with the EMT phenotype. Methods: Eighteen patients with operable primary HER2+ invasive breast cancer (≥T2 excluding inflammatory breast cancer, any N) were eligible. Patients received lapatinib (1000 mg PO daily) + trastuzumab (4 mg/kg at loading, then 2 mg/kg IV weekly) for the first 6 weeks, then lapatinib (750 mg daily) + trastuzumab (2 mg/kg IV weekly) + paclitaxel (80 mg/m2 IV weekly) for 12 weeks, followed by surgery (ClinicalTrials.gov Identifier: NCT01688609). Tumor and blood specimens were collected before (baseline), after the 6 weeks of dual anti-HER2 therapy (adaptive response biopsy), and at 18 weeks, after 12 weeks of dual anti-HER2 therapy + paclitaxel (surgical specimens). We measured CSC biomarkers CD44 variant (CD44v) and aldehyde dehydrogenase-1 in tumor tissues, EMT markers in CTCs (TWIST1, SNAIL1, SLUG, ZEB1, and FOXC2) in blood samples by quantitative RT-PCR, and the ratios of phosphorylated EGFR (pEGFR)/EGFR, pHER2/HER2, pERK/ERK, and pAkt/Akt in tumor tissues. All tissue and CTC biomarker levels at all three time points were evaluated for their association with response via Fisher's exact test, McNemar's test, and Wilcoxon rank sum test according to the variables. Results: Eight of 18 patients (44.4%) achieved pCR after dual anti-HER2 therapy + concurrent paclitaxel. All patients who achieved pCR showed reduction or disappearance of CD44v+ cells over the treatment course. Five of the 10 non-pCR patients showed consistent CD44v expression or enrichment after dual anti-HER2 therapy in both the adaptive response biopsy and the surgical specimens. None of the eight pCR patients had detectable CD44v in the 7-week adaptive response biopsy specimen (Fisher exact test, two-tailed, P = 0.0359). None of the other markers significantly predicted pCR. Conclusion: Persistent expression or enrichment of CD44v was suggested to be predictive for non-pCR in breast cancer patients treated with preoperative dual anti-HER2 therapy plus concurrent cytotoxic chemotherapy. A single evaluation of biomarkers before therapy is insufficient for prediction of clinical response. Application of the adaptive response biopsy during the course of preoperative therapy might play a significant role in the success of therapeutic strategies that target CSCs.
Title: Stem cell markers are overexpressed in tumorspheres cultured from circulating epithelial tumor cells (CETCs) in patients with breast cancer

Zimon D, Pizon M, Mayer V and Pachmann K. Transfusion Center, Bayreuth, Germany.

Body: Background:
Breast malignancies continuously shed tumor cells which may enter the circulation, spread to other tissue and initiate metastases, but only a small subpopulation among CETCs is capable of metastasis formation. The subpopulation of CETC capable of forming tumorspheres in vitro and carrying stem cells properties is assumed to have metastatic potential. The aim of this study was to characterize the pattern of expression of the tumorspheres as compared to CETCs.

Methods:
Blood samples were obtained from patients diagnosed with breast tumors. CETCs were enumerated with maintrac method and subsequently cultivated in stem cell-selective medium. The expression of stem cell marker genes EpCAM, Nanog, Okt4 Sox2, ALDH and CD133 were detected by RT-PCR in single CETCs and tumorspheres.

Results:
0.1- 10% of CETCs could form non-adherent tumorspheres under stem cell-selective conditions after a period of 14 days. Array qRT-PCR analysis revealed that putative stem cell markers, such as Oct4, Sox2, Nanog, EpCAM, ALDH1 and CD133 are overexpressed in relation to house-keeping genes RPL13a and GAPDH in tumor spheres in contrast to the significantly lower expression level of these stem cell markers in individually isolated CETCs. High expression level of pluripotency genes in tumorspheres was associated with aggressive tumor behaviour in terms of tumor progression and type of cancer.

Conclusion:
Thus, differential expression of stem cell markers in CETCs and tumorspheres substantiates the hypothesis that only a small population of circulating tumor cells possess the stem cell properties and are responsible for metastasis formation.
Title: Impact of high SPARC expression of a primary tumor as a strong risk factor for disease recurrence and overall survival in patients with triple-negative breast cancer

Zhu A, Yuan P, Du F, Ding X and Xu B. Cancer Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China.

Body: Background: SPARC (secreted protein acidic and rich in cystein) is a secreted glycoprotein that interacts with extracellular matrix protein and acts as a regulator of critical cellular functions such as proliferation and cell migration. An increasing number of studies have shown altered expression of SPARC in several malignancies. However, the role of this potential biomarker in breast cancer development and progression is controversial. Triple-negative breast cancer (TNBC) is known for its poor prognosis and high recurrence probability. There is a need for prognostic biomarkers to guide treatment decisions for this subtype.

Objective: To explore the association between SPARC and the prognosis of triple-negative breast cancer.

Methods: In this study, a total of 211 samples of triple-negative breast lesions from 2004 to 2008 were collected in Cancer Hospital, Chinese Academy of Medical Sciences. SPARC expression was evaluated by immunohistochemistry using an immunoreactive score (IRS) from patients with up to 10 years clinical follow-up data. The current study set out to examine both the expression of SPARC in primary tumor tissue and to demonstrate if a link existed between the levels of SPARC and the clinical outcome. Cox proportional hazards model was used to estimate HRs and 95%CI, stratified on tumor grade, TNM stage, lymph node status and vascular invasion.

Results: High SPARC expression (IRS≥3) was observed in 52.1% of all primary tumors. Patients expressing low levels of SPARC had better disease-free survival (DFS) (HR=0.632, 95%CI:0.405-0.987, P = 0.044) as well as overall survival(OS)(HR=0.576, 95%CI:0.351-0.946, P = 0.029) compared to those with high SPARC expression levels.Furthermore, the presence of high SPARC expression was an independent prognostic factor for both DFS (HR=1.67, 95%CI:1.04-2.69, P=0.034) and OS(HR=1.77, 95%CI:1.04-3.01, P=0.037) of triple-negative breast cancer patients, stratified on tumor grade, TNM stage, lymph node metastasis and vascular invasion, with adjustment for age, hormonal status, tumor size, adjuvant radiotherapy and adjuvant chemotherapy.

Conclusion: Our results suggest that the presence of higher SPARC expression could be an indicator of high aggressiveness and may be a strong prognostic factor for triple-negative breast cancer.
Title: Predictors of response and follow up biomarkers for metronomic chemotherapy with cyclophosphamide and celecoxib in advanced breast cancer patients

Perroud HA A, Alasino CM M, Rico MJ J, Menacho-Marquez MA A, Mainetti LE E, Queralt F, Pezzotto SM M, Rozados VR R and Scharovsky G. Institute of Experimental Genetics, School of Medical Sciences, National University of Rosario, Rosario, Santa Fe, Argentina; Rosario Institute of Oncology, Rosario, Santa Fe, Argentina; Argentinean Council of Science and Technology - CONICET, Buenos Aires, Argentina and Institute of Immunology, School of Medical Sciences, National University of Rosario, Rosario, Santa Fe, Argentina.

Body: Low-dose metronomic chemotherapy (MC) with Cyclophosphamide (Cy) and Celecoxib (Cel) has demonstrated to be effective and well-tolerated in advanced breast cancer patients (ABCP) but predictive markers of response or follow-up are lacking. Given the antiangiogenic properties of MC we analyzed several angiogenesis-related biomarkers and evaluated their potential role as predictors of response or treatment follow-up of ABCP treated with MC. Treatment plan: Patients received Cy 50 mg p.o./day + Cel 200 mg p.o./bid. Cellular parameters: Circulating endothelial cells (CEC) and Circulating progenitor endothelial Cells (CEP) were determined by Flow Cytometry. Serologic parameters: Serum levels of vascular endothelial growth factor (VEGF), VEGF-C, soluble VEGF Receptors 2 and 3 (sVEGFR-2, sVEGFR-3) and Thrombospondin-1 (TSP-1) were determined by ELISA. Blood samples were collected before and during treatment. Twenty patients were enrolled. Response Rate was 5% and Clinical Benefit (CB) 55%. Most of the patients showed prolonged stable disease (SD ≥ 24 weeks). Biomarkers were determined in all patients. Levels of CEC and CEP showed no clear trend variations during treatment. However, levels of CEC significantly increased at the time of disease progression in those patients who showed CB (P=0.014). Also baseline values of CEC and CEP showed marginally significant associations with Time To Progression. Serum VEGF concentration decreased during treatment (P=0.050) while sVEGFR-2 increased (P=0.005). VEGF-C, sVEGFR-3 and TSP-1 showed non-significant variations. VEGF/sVEGFR-2 ratio decreased during treatment (P=0.041), whereas VEGF/TSP-1, and VEGF-C/sVEGFR-2 ratios showed non-significant variations. Baseline values of VEGF, and VEGF/sVEGFR-2 showed negative and significant associations with TTP (P=0.0354 and P=0.0300, respectively) while sVEGFR-2 did not. When considering the two variables together, the goodness of prediction was not improved. To confirm the value of baseline VEGF and VEGF/sVEGFR-2 as predictors of response, we used the 50th percentile as a cutoff value to analyze the % of progression free survival. Patients with values lower than the 50th percentile for both biomarkers showed longer TTP (P=0.0001 and P=0.0008, respectively). The treatment had anti-angiogenic effect (VEGF decrease and sVEGFR-2 increase). The absence of variation in VEGF-C and sVEGFR-3 would indicate the lack of effect on lymphangiogenesis. Increased levels of CEC could be useful for detecting progression. If confirmed with a higher number of patients, baseline VEGF and VEGF/sVEGFR-2 values could be useful as early predictors of response.
**Title:** Association of overexpression of hypoxia inducible factor 1α with response to neoadjuvant chemotherapy in early stage breast cancer

Zhi WI Iris, Huang C-Y, Gabrielson E, Tully E, Cimino-Mathews A, Santa-Maria C, Jeter S, Kai C, Semenza G and Stearns V. Hematology/Oncology Fellowship Training Program, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; Breast and Ovarian Cancer Program, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; Division of Biostatistics and Bioinformatics, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD and Sun Yat-Sen Memorial Hospital., Yanjiang West Rd. #2, GuangDong, China.

**Body:**

**Background:** Hypoxia inducible factor 1 alpha (HIF-1α) is a master transcription factor involved in multiple oncogenic processes. In breast cancer, HIF-1α overexpression is associated with increased resistance to radiation therapy, chemotherapy, and inferior disease-free and overall survival. Patients who achieve a pathologic complete response (pCR) following neoadjuvant therapy have improved survival outcomes. Limited data are available regarding the association between HIF-1α expression and rates of pCR. We investigated the relationship between HIF-1α overexpression and pCR rates following neoadjuvant chemotherapy for early breast cancer.

**Methods:** Eligible women were those with HER2-negative, stage II-III breast cancer, who received anthracycline- and taxane-based neoadjuvant chemotherapy from 2002 to 2012, and were included in an institutional review board-approved Integrated Breast Cancer Research Database at Johns Hopkins. The database includes patient age, sex, menopausal status, breast cancer diagnosis, tumor histopathology, treatment history, laboratory data, and outcomes. Both diagnostic and surgical tissue blocks were retrieved from pathology archives. Whole section slides were prepared and analyzed by immunohistochemical staining with appropriate negative and positive controls. The intensity of cells positive for HIF-1α was estimated visually by a pathologist blinded to clinical data. A semi-quantitative scoring of nucleus expression was used to score HIF-1α expression: score 0 is defined as less than 1%, 1 is defined as 1-5%, 2 as 5-20% and 3 as >20% tumor cells positive for HIF-1α. Overall tumor HIF-1α negativity was defined as (0, 1) and positivity as (2, 3). We compared baseline HIF-1α status among responders (pCR defined as no invasive tumor in the breast or lymph nodes) and non-responders (no pCR) using Fisher's exact test, and evaluate the association between baseline and surgical specimens in those who did not achieve pCR using McNemar's test.

**Results:** A total of 122 women meeting the eligibility criteria underwent a definitive surgical procedure following neoadjuvant chemotherapy. Of those, 50 patients had no tissue blocks available at baseline and additional 16 patients' blocks did not contain sufficient tissue for analysis. Thus, tumors from 56 women were available for analysis. Median age was 50 (range 33-78), 54% were White and 35% Black; 41% and 59% of women had triple negative and hormone receptor-positive tumors, respectively; 71% women had Ki67>30%, and 80% were node-positive. Overall pCR was observed in 12 women (21%). We did not detect a significant association between HIF-1α score on the diagnostic specimen with pCR status ($p=0.627$). However, a positive HIF-1α score was significantly associated with positive lymph nodes ($p=0.01$). We observed a significant decrease in HIF-1α score following chemotherapy ($p<0.001$).

**Conclusions:** We did not observe a clear association between HIF-1α expression and response to neoadjuvant chemotherapy. However, HIF-1α expression on a diagnostic specimen was statistically higher than following chemotherapy and was associated with lymph node positivity. Our study is limited by its retrospective nature and a small sample size.
Title: Ki67 cut-off point to predict the benefit of adjuvant chemotherapy in ER+ HER2- breast cancer patients


Body: BACKGROUND: The benefit of chemotherapy for patients with estrogen receptor(ER)+ HER2- breast cancers is an ongoing question. The evaluation of the tumor's proliferation by Ki67 may guide the indication but no consensual predictive cut-off has been set yet.

MATERIALS AND METHODS: This study included women with a first ER+HER2- invasive breast cancer treated by primary surgery between 2003 and 2008. Data was collected prospectively. Ki67 cut-off was sequentially set each 1% from 5% to 30%. For each threshold, the interaction between Ki67 and adjuvant chemotherapy was integrated in a multivariate Cox model to determine when it became statistically significant in predicting distant-disease free survival (DDFS). Using different Ki67 cut-offs, we also compared DDFS of patients who had or not an indication of chemotherapy, depending on whether they actually received it or not.

RESULTS: Among the 3221 breast cancers, median Ki67 was 10%, with a mean of 15% (S.D = 14). Ki67 was an independent prognosis factor whether the cut-off was set to 14% or to 20% (p<0.001). The interaction between Ki67 and chemotherapy became significant only for a threshold of Ki67 above 20% (p<0.05). When adjuvant chemotherapy was not indicated according to St Gallen guidelines 2013 with a Ki67 cut-off set to 20%, DDFS was not significantly improved if patients did receive chemotherapy.

CONCLUSION: In ER+ HER2- breast cancers, a Ki67 level of expression >= 20% was predictive of benefit from adjuvant chemotherapy. A threshold at 14% was not as discriminant. Based on this large cohort study, we recommend the use of a cut-off at 20% to decide whether patientes with ER positive tumors should receive chemotherapy or not.
Title: Association between gene variants in SULT1A1 and UGT1A4 and disease outcomes in patients enrolled in SWOG S0226 and treated with anastrozole alone or in combination with fulvestrant for metastatic breast cancer


Body: Background: Anastrozole (A) blocks estrogen production by inhibiting the activity of CYP19 aromatase. Fulvestrant (F) blocks estrogen receptor (ER) signaling by competitive binding, leading to ER degradation by ubiquitination. SWOG S0226 ("Phase III Randomized Trial of Anastrozole versus Anastrozole and Fulvestrant (250mg LD) as First Line Therapy for Post Menopausal Women with Metastatic Breast Cancer," ClinicalTrials.gov Identifier:NCT00075764) demonstrated that combination of A+F is superior to A alone as first-line therapy for patients with ER positive metastatic breast cancer (Mehta et al, NEJM, 2012). Our functional preclinical studies have shown that single nucleotide polymorphisms (SNPs) in SULT1A1 and UGT1A4, drug conjugation enzymes that inactivate A and F, result in decreased enzyme activity toward these drugs (Edavana et al, DMD, 2013; Edavana et al Pharmgenomics Pers Med 2013). We therefore hypothesized that these SNPs will be associated with disease outcomes in S0226 patients due to altered drug levels.

Methods: Germline DNA was available for 295 (43.5%) patients enrolled in S0226 overall (157 on A and 138 on A+F). SNPs in SULT1A1 and UGT1A4 were determined either by direct sequencing or allele-specific PCR (TaqMan) assays.

Results: There was no difference in progression-free survival (PFS) or overall survival (OS) comparing patients with or without available germline DNA (p = 0.86 and 0.36, respectively). The SULT1A1 G902A allele (rs6839), which confers decreased mRNA and enzymatic activity, was associated with improved PFS (GG/GA vs. AA; HR 0.74, 95% CI 0.56-0.98, p=0.033) and OS (HR 0.70, 95% 0.50-0.98, p=0.039). In exploratory subset analyses of PFS, the SULT1A1 G902A association was similar across both treatment arms (A HR=0.75; 95% CI 0.51-1.10; A+F HR=0.73; 95% CI 0.48-1.11). For OS there was some evidence of a difference by treatment (A HR=0.60; 95% CI 0.38-0.96; A+F HR=0.82; 95% CI 0.50-1.32), though no significant interaction was evident (p=0.30).

The UGT1A4 G-163A promoter variant, which leads to decreased protein expression, was not associated with PFS (AA/AG vs. GG HR 0.88, 95% CI 0.68-1.14, p=0.33); however, this variant was associated with OS (HR 0.71, 95% CI 0.52-0.96, p=0.027). In subset analyses with OS, the difference was marginally stronger in the A arm (HR 0.63, 95% CI 0.42-0.97, p=0.035) compared to the A+F arm (HR 0.77, 95% CI 0.49-1.21, p=0.25), though the interaction was not significant (p=0.40).

Conclusion: SULT1A1 and UGT1A4 gene variants resulting in decreased enzyme activity were associated with better PFS, OS or both in patients enrolled in SWOG S0226. Planned validation studies correlating these SNPs with drug levels and disease outcomes in additional patient cohorts will establish their clinical utility in identifying patients who benefit from A and F alone or in combination.

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Title: ER pathway activity as a predictive biomarker for neoadjuvant endocrine therapy


Body: Approximately 75% of all breast cancer patients is treated with endocrine therapy, based on stratification using estrogen receptor (ER) and progesterone receptor. To date, these classical biomarkers are the only ones available to predict the response to (neo)adjuvant endocrine therapy. Response to endocrine therapy depends on the presence of an active tumor-driving ER signalling pathway and, in the case of treatment with aromatase inhibitors (AI), also on aromatase-induced estradiol as the pathway activating ligand. Conventional nuclear staining for ER is not necessarily indicative of an estradiol-activated ER signalling pathway. We evaluated a recently described diagnostic computational model (Verhaegh, Cancer Research 2014) which identifies ER pathway activity based on tissue-derived target gene mRNA levels, for its clinical utility to predict neoadjuvant AI response in ER positive breast cancer patients.

Tumour tissue from pre-treatment biopsies and post-treatment resection material was collected from patients with early breast cancer (>2 cm and >50% ER expression) participating in the TEAM-IIA trial, which were treated with neoadjuvant exemestane for 3 to 6 months. Using Laser Capture Microdissection (LCM), tumour cells were isolated and the probability of ER pathway activity was assessed with RT-qPCR. In total, 77 FFPE samples were analysed (51 biopsies + 26 paired resection cases). In a preliminary analysis, results were correlated with clinical response based on palpation and mammography.

ER pathway activity significantly decreased during therapy (0.45 vs 0.27, two sided t-test p=0.001). Based on mammography, baseline ER activity in biopsy predicted therapy outcome after 3 months, with a higher probability of ER activity in responders, n=7, compared to non-responders, n=12, (0.73 vs 0.44, one sided t-test p=0.003). When therapy was continued up to 6 months, no correlation was found suggesting that other factors influence overall outcome of neo-adjuvant therapy. Baseline ER-pathway activity significantly predicted progressive disease by palpation at the end of therapy (mean treatment duration of 174 days, range 86-288 days) with a mean ER-pathway activity at biopsy of 0.53 vs 0.16 (p=0.01).

The significant difference in baseline activity with progressive disease indicates that low ER pathway activity could be used to predict low response rates. This is supported by the observation that all progressive disease cases at end of therapy had low baseline ER activity. Furthermore, baseline activity particularly predicted early radiological response based on mammography.

These preliminary results indicate that our ER pathway activity model could be able to predict response to endocrine neoadjuvant therapy. Further evaluation will be performed in order to assess the influence of post-treatment activity and other markers for response (Ki-67).

*First and second author contributed equally to this work.
Title: Efficacy and gene expression results from eribulin SOLT1007 neoadjuvant study

Prat A, Ortega V, Paré L, Galván P, Oliveira M, Nuciforo P, Lluch A, Morales S, Amillano K, Lopez R, González R, Manso L, Martínez J, Llombart A, de la Peña L, di Cosimo S, Rubio IT, Harbeck N, Baselga J and Cortés J. Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; Translational Genomics Group, Vall d’Hebron Institute of Oncology (VHIO), Bracelona, Spain; Hospital Universitari Vall d’ Hebron, Barcelona, Spain; Translational Genomics and Targeted Therapeutics in Solid Tumors. Institut de Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; Molecular Oncology Laboratory, Vall d’Hebron Institute of Oncology, Valencia, Spain; Hospital Clínico Universitario de Valencia, Lleida, Spain; Hospital Universitari Arnau de Vilanova, Lleida, Spain; Hospital Sant Juan de Reus, Tarragona, Spain; Complejo Universitario de Santiago de Compostela, Santiago de Compostela, A Coruña, Spain; Hospital Universitario Virgen del Rocio, Sevilla, Spain; Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Virgen de la Arrixaca, Murcia, Spain; Hospital Universitari Arnau de Vilanova de Valencia, Valencia, Spain; SOLTI Breast Cancer Research Group, Barcelona, Spain; Istituto Nazionale dei Tumori, Milan, Italy; Brustzentrum am Klinikum der Universität München, Munich, Germany and Memorial Sloan Kettering, NY, NY.

Body: Background: Eribulin is a non-taxane, microtubule dynamics inhibitor approved for the treatment of patients with locally advanced or metastatic breast cancer. To date, no biomarker exists to prospectively select patients who will derive the maximum benefit from this chemotherapeutic. In this study, we explored, in a prospective clinical trial, the efficacy and the association of pre-treatment expression of mRNA with response in patients with HER2-negative breast cancer treated with neoadjuvant eribulin. Results of the HR+ cohort are presented here.

Methods: SOLT1007 is a phase II, open-label, single-arm, exploratory pharmacogenomic study of single agent eribulin as neoadjuvant treatment for stage I-II HER2-negative breast cancer (n=100 HR+ and n=100 HR-negative). Patients received 1.4 mg/m² of eribulin mesilate intravenously on Days 1 and 8 every 21-day cycle, for 4 cycles. Baseline, C2D1, and post-treatment (surgical) formalin-fixed, paraffin-embedded tissue samples were collected and gene expression profiled. The PAM50 genes and 492 additional breast cancer-related genes were evaluated using the nCounter platform. Research-based intrinsic subtype and the Risk of Relapse based on subtype and proliferation (ROR-P) were evaluated using the Parker et al. JCO 2009 algorithm. The association of each signature and pathological complete response in the breast (pCRb) was evaluated using univariate logistic regression models. Genes found differentially expressed between screening and surgical specimens were identified using a paired two-class Significance Analysis of Microarrays.

Results: 92 evaluable patients were included. Mean age: 55.7. Mean tumor size: 3.7 cm. Mean Ki-67 %: 30.3. The overall pCRb rate was 5.4%. Distribution of the intrinsic subtypes was as follows: Luminal A (n=36, 39.1%), Luminal B (n=34, 37.0%), Basal-like (n=10, 10.9%), Normal-like (n=10, 10.9%) and HER2-enriched (n=2, 2.2%). pCRb rates differed significantly by subtype (p=0.009): Luminal B (11.7%, 4/34), HER2-enriched (50%, 1/2), Luminal A (0%, 0/36), Basal-like (0%, 0/10), Normal-like (0%, 0/10). pCRb rate differed significantly by ROR-P (p=0.018): ROR-P high (16.7%, 4/24), ROR-P med (1.9%, 1/52), ROR-P low (0%, 0/16). Ki67 % did not predict pCRb (p=0.406). Subtype change at surgery occurred in 48.3% (14/29) of Luminal B disease. 78.6% (11/14) of subtype changes in Luminal B disease were to Luminal A. Compared to screening biopsies, 136- and 141- genes were found up- and down-regulated in surgical specimens (False Discovery Rate <5%). The up-regulated gene list was enriched for response to hormone stimulus (e.g. ESR1, BCL2 and ERBB4), negative regulation of apoptosis (e.g. IL6, EGFR and PTEN) and angiogenesis (e.g. ANGPTL4, HIF1A and TGFBR2). The down-regulated gene list was enriched for cell cycle (e.g. CCNB1, RAD17 and MKI67), DNA-repair (e.g. BRCA1, BRCA2 and ATR) and microtubule cytoskeleton organization (e.g. AURKA, CENPA and KIF23).

Conclusions: From a response and biological perspective (i.e. induction of a Luminal A phenotype), patients with Luminal B disease might benefit the most from eribulin therapy. Strategies combining eribulin with endocrine therapy seem warranted in Luminal B breast cancer.
Title: Chemosensitivity and endocrine sensitivity predicted by MammaPrint and BluePrint in clinical luminal patients in the prospective NBRST study

Pellicane JV V, Whitworth P, Beitsch P, Baron P, Beatty J, Murray MK K, Dul CL L, Mislowsky AM M, Nash CH H, Richards PD D, Lee LL L, Stork-Sloots L, de Snoo F, Untch S, Gittleman M, Akbari S and Rotkis MC C. Virginia Breast Center, Bon Secours Cancer Institute, Richmond, VA; Nashville Breast Center, Nashville, Nashville, TN; Dallas Surgical Group, Dallas, TX; Breast & Melanoma Specialists of Charleston, Charleston, SC; The Breast Place, Charleston, SC; Akron General Hospital, Akron, OH; St. John Hospital & Medical Center, Detroit, MI; Coastal Carolina Breast Center, Murrells Inlet, SC; Northeast Georgia Medical Center, Gainesville, GA; Blue Ridge Cancer Care, Roanoke, VA; Comprehensive Cancer Center, Palm Springs, CA; Agenda Inc, Irvine, CA; Breast Care Specialists, Allentown, PA; Virginia Hospital Center, Arlington, VA and Northern Indiana Cancer Research Consortium, South Bend, IN.

Body: Background
Ideally classification by subtype predicts treatment response and overall outcome. BluePrint 80-gene functional molecular subtype is based on mRNA expression (as is intrinsic subtype) associated with intact translation to protein (unlike intrinsic subtype). BluePrint (BP) classifies patients into Luminal, HER2 or Basal-type. Presently subtype is approximated using conventional immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) (“conventional subtype”) or assigned by gene expression profiling. The aim of the prospective NBRST study was to compare chemosensitivity as defined by pathological Complete Response (pCR), or endocrine sensitivity as defined by partial response (PR) using 80-gene functional subtype vs. conventional IHC/FISH subtyping. In this analyses we present the results of all IHC/FISH Hormone Receptor (HR) positive/Her2 negative patients.

Methods
The neo-adjuvant NBRST study enrolled over 1000 US patients between June 2011 and December 2014. Women aged 18–90 with histologically proven breast cancer, who were scheduled to start neo-adjuvant chemotherapy (NCT) or neo-adjuvant endocrine therapy (NET), and who provided written informed consent were included. Additional inclusion criteria were no excision biopsy or axillary dissection, no confirmed distant metastatic disease, and no prior therapy for breast cancer. Treatment was at the discretion of the physician adhering to NCCN approved or other peer-reviewed, established regimens. BluePrint in combination with MammaPrint classifies patients into four molecular subgroups: Luminal A, Luminal B, HER2 and Basal. pCR is defined as T0/isN0 and PR is according to RECIST and defined as at least a 30% decrease in the tumor bed.

Results
418 IHC/FISH HR+/Her2- T1-4, N0-3, patients were enrolled (median age 54, range 22-87). MammaPrint and BluePrint (MP/BP) classified 28% as Luminal A, 54% as Luminal B, and 18% as Basal-type. The distribution of locally assessed ER% in BP Luminal tumors was 2% ER 0-10%; 3% ER 11-50%; and 95% ER>50% compared to 45% ER 0-10%; 28% ER 11-50%; and 27% ER >50% in BP Basal tumors. Tumors classified as BP Luminal 370 (89%) received NCT and the overall pCR rate was 9%. There was a significantly higher (p<0.0001) pCR rate of 26% in BP Basal tumors compared to the pCR rate in BP/MP Luminal A and B tumors (3% and 6%). 43 (10%) patients received neo-adjuvant ET and 72% had a PR.

Conclusions
Molecular subtyping using MammaPrint and BluePrint classified 1 out of 5 pathological luminal patients as BP Basal-type with a significant higher response rate to NCT compared to BP Luminal patients. BP Luminal patients have an excellent partial response rate to NET.
Title: Population-based validation study of Adjuvant! for primary breast cancer patients in the Netherlands

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Body: Background Adjuvant! is an online risk prediction tool that can support breast cancer patients and physicians in clinical decision-making regarding adjuvant systemic treatment. The aim of this study was to validate Adjuvant! on a Dutch population-based cohort of women diagnosed in 2003 and assess its calibration and discriminatory accuracy.

Methods All Dutch patients diagnosed with invasive primary breast cancer in 2003 in the Netherlands meeting the criteria of Adjuvant!; unilateral, unicentric invasive breast cancer, no evidence of metastatic or residual disease, pathologically staged I-III and no pT4 features, were included. Local treatment consisted of breast conserving therapy or ablative surgery and axillary staging. Adjuvant systemic treatment was given according to national guidelines. Patient, tumor and treatment characteristics were retrieved from the Netherlands Cancer Registry. Endpoints were ten-year overall survival, breast cancer specific survival and event free survival.

Findings A total of 8,195 patients were included. Out of all patients, 4,389 (53.6%) received some form of adjuvant systemic therapy. Throughout a ten-year follow-up, 2,156 (26.3%) patients died and 1,953 (23.8%) patients developed a recurrent disease (locoregional, distant or contralateral invasive cancer or DCIS). Ten-year observed overall survival was underestimated by -1.4% (95% CI -2.3 to -0.4, p=0.006), breast cancer specific survival was underestimated by -3.3% (95% CI -4.1 to -2.6, p<0.0001) and event free survival was underestimated by -3.5% (95% CI -4.5 to -2.6, p<0.0001). Discrimination was moderate for overall survival and breast cancer specific survival but poor for event free survival.

Interpretation Adjuvant! does not accurately predict breast cancer specific survival and event free survival in this Dutch population-based study. Dutch patients showed better ten-year survival rates than predicted by Adjuvant!. For specific patient groups, the model could be used to estimate overall survival. We conclude that Adjuvant! should be used with caution in clinical practice in the Netherlands but probably also in other countries and the US.
Purpose: Some studies suggest that telomere shortening due to repeated cell divisions may be associated with increased breast cancer risk and mortality. Obesity is also associated with increased breast cancer risk and mortality. Few studies have examined telomere length as a potential mechanism/biomarker mediating the obesity-breast cancer association. One study published of a diet, physical activity and support intervention in prostate cancer patients found a positive association between lifestyle changes and relative lengthening of telomeres, and another study of weight loss in healthy postmenopausal women observed no effect of weight loss on leukocyte telomere length. The purpose of our study was to examine the effect of a 6-month diet- and exercise-induced weight loss intervention vs. usual care on telomere length in 100 breast cancer survivors.

Methods: 100 breast cancer survivors with BMI ≥ 25 kg/m2 were randomly assigned to a weight loss counseling intervention with either telephone or in-person counseling (n = 67) or usual care group (n=33). Weight loss counseling included eleven 30-minute counseling sessions over 6 months, focusing on reducing caloric intake, increasing physical activity and behavioral therapy. Body composition (height, weight, and DEXA scans), physical activity and diet were measured at baseline and 6-months. Fasting blood samples were also collected at baseline and 6 months. Relative telomere length (T/S: telomere length/single copy of gene albumin) was measured by quantitative-polymerase chain reaction (qPCR) done on buffy coat extracted genomic DNA. Mean baseline to 6-month changes were compared between groups (intent-to-treat) using generalized estimating equations and Pearson correlation coefficients.

Results: Baseline characteristics were similar for women randomized to each group. Women were 59±7 years, with BMI 33.1±6.6 kg/m2 and were 2.9±2.1 years from diagnosis; 91% were non-Hispanic white, and 51% were diagnosed with Stage I breast cancer. Average 6-month weight loss was 6.2% and 2.0% for weight loss and usual care groups, respectively (p=0.0004). At baseline, higher % body fat was associated with shorter T/S (r = -0.31, P=0.012). After 6 months, women randomized to weight loss experienced a 4% T/S lengthening compared to a 5% T/S shortening in the usual care group (P=0.10) (Table 1).

CONCLUSION: Our results indicate that higher % body fat is associated with shorter leukocyte telomere length, and weight loss was associated with an increase in leukocyte telomere length, suggesting that telomere length may be a mechanism mediating the relationship between obesity and breast cancer risk and mortality.
Title: Comparison of strategies for weight loss maintenance among rural breast cancer survivors: The rural women connecting for better health randomized controlled trial


Body: Background: Breast cancer survivors who reside in rural areas represent one of the largest medically underserved populations of breast cancer survivors in the nation and have higher obesity prevalence compared to their urban counterparts. Given the evidence linking obesity with poor breast cancer prognosis, trials are needed to demonstrate ability to produce long-term weight loss maintenance in this hard-to-reach group. Group phone-based counseling via conference calls is a low-technology approach with excellent reach to rural areas. This treatment delivery approach capitalizes on the support benefits of in-person groups by allowing participants to interact in real time while also diminishing costs.

Methods: In this 2 phase trial, overweight and obese (BMI 27 to 45 kg/m2) rural breast cancer survivors (with initial stage 0-III disease) were randomized to one of two extended care interventions for weight loss maintenance (Phase 2) subsequent to an initial 6-month weekly group phone-based behavioral weight loss intervention (Phase 1). To be eligible for randomization for maintenance, participants must have lost ≥ 5% of their baseline weight during Phase 1. In Phase 2, participants were randomized to continued group phone-based counseling reduced in frequency to every other week during maintenance vs every other week mailed newsletters that followed the same content.

Results: 210 breast cancer survivors with a mean time since treatment of 3.5 years ± 2.4 years, mean age of 58.1 ± 9.9 years, and mean BMI of 33.9 ± 4.4 kg/m2 residing in a three state region of the rural Midwest were entered in the 6-month weight loss phase. Retention from baseline to 6 months (Phase 1) was 91%. Mean percent weight loss at 6 months for the total sample was 12.9% with 82% of enrolled participants ≥ 5% below baseline weight. 172 participants with a mean initial loss of 14.0% of baseline weight (12.8 ± 4.9 kg) were randomized to a maintenance intervention. Retention from 6 to 18 months (Phase 2) was 92%. Intent-to-treat analyses with imputation of missing data revealed participants in the group phone condition regained less weight (3.3 ± 4.8 kg) compared to participants in the newsletter condition (4.9 ± 4.8 kg; p = 0.03). Mean percent weight loss from baseline to 18 months did not significantly differ between the group phone condition (10.2 ± 7.5%) and the newsletter condition (9.2 ± 7.9%). However, at 18 months 75.3% of participants in the group phone condition remained ≥ 5% below baseline weight compared to 57.8% in the newsletter condition (p = .02).

Discussion: The initial group phone-based weight loss intervention exceeded typical weight losses reported in the literature with over 80% of enrolled participants achieving clinically meaningful weight loss. Continued group phone counseling was modestly better in sustaining weight loss at 18 months than a mailed newsletter. However, for both maintenance approaches, the majority of participants maintained a weight at 18 months that was 5% or more below baseline.
Title: Mobile phone multimedia messaging intervention for breast cancer screening

Lee HY Y, Le C, Ghebre R and Yee D. University of Minnesota, Twin Cities, Minneapolis, MN.

Body: Background. Korean American women have one of the highest breast cancer mortality rates and lowest breast cancer screening rates among American women. In response to the need to enhance breast cancer screening, this study aims to develop and test a 7-day mobile phone application (app)-based Mammogram (mMammogram) intervention designed to promote breast cancer screening among Korean American women. To date, mobile app technology has not been used for mammogram promotion.

Methods. Using FBM Model, we developed a mammogram intervention designed to increase knowledge of breast cancer screening, intent to receive mammogram, and the receipt of a mammogram. A series of focus groups were conducted to inform the development of the intervention. A randomized controlled trial was conducted with baseline, one week post-intervention, and 6-month follow-up testing among 120 Korean American women who were aged 40 and older and had not had mammograms within the last 2 years. The intervention group (60) received an individually and culturally tailored text messages via mobile app with health navigation services. The control group (60) received a brochure including information on breast cancer, screening guidelines, and a list of clinics that offer low-cost or free mammography without health navigation services.

Results. At one week post-test, statistically significant between-group differences were found; intervention subjects reported higher scores of knowledge in breast cancer and screening guideline than subjects in control group (mean differences: 1.70, p < 0.05). No statistical between group differences identified in intention to receive screening. However, significant between-group difference was found in the receipt of mammogram at 6-month follow-up test; 40.0% (24/60) of the intervention group received mammograms whereas 25.0% (15/60) of the brochure group received mammograms after intervention (p < 0.05). 100% of the participants expressed satisfaction with the intervention and 98.3% reported that they would recommend the program to their friends.

Conclusions. This study provides evidence of the effectiveness and feasibility of the mammogram intervention with health navigation services in promoting breast cancer screening. Mobile application-based intervention is a promising tool to increase both knowledge and receipt of mammograms. Given the widespread usage of mobile phone among minority populations, a mobile phone-based health intervention could be an effective method of reaching hard-to-recruit populations with high breast cancer burden, using individually tailored messages that cover broad content areas and overcome restrictions to place and time of delivery.
Title: Improving sleep to reduce breast cancer risk in shift workers

Gotay C, Aronson K, Campbell K, Demers P, Fleming J, Gelmon K, Goodfellow E, Munoz C, Neil-Sztramko S, Pollak M, Shen H and Spinelli J. The University of British Columbia, BC, Canada; Queen’s University, ON, Canada; University of Toronto, ON, Canada; BC Cancer Agency, BC, Canada; McGill University and BC Cancer Research Centre, BC, Canada.

Body: BACKGROUND: Shiftwork that involves circadian disruption has been designated as a 2A (probable) breast cancer carcinogen by the International Agency for Research on Cancer. Night shift workers experience many lifestyle disturbances including disrupted sleep. While the specific biological mechanisms that confer increased breast cancer risk are not yet clear, sleep disruption is hypothesized to have both direct (lowering melatonin levels) and indirect (obesity status) impacts on risk status. However, few interventions of any type have been reported for shift workers to potentially reduce their risk for breast cancer. An effective intervention to improve sleep quality would be one way to potentially reduce breast cancer risks in these women.

METHODS: 47 female shift workers aged 40-65 who had experienced high circadian disruption (rotating or permanent night shifts) at least 3 times per month for at least 2 years participated in a single arm study examining the impact of a sleep intervention on health behaviours and breast cancer risk. Over the course of 10 months, women received with a 10-session (plus 2 booster sessions), telephone-delivered sleep hygiene intervention. The program was adapted from a hospital-based sleep clinic protocol based on cognitive behaviour therapy (CBT) principles and aimed to improve sleep quality and quantity. Sleep quality was assessed by the Pittsburgh Sleep Quality Index (PSQI). Data were assessed at baseline and 6 and 12 months.

RESULTS: Mean age was 47 years, 68% were partnered, and 78% had diploma level education or higher. Participants were nurses (49%), emergency communications personnel (17%) and paramedics (13%), and others. At baseline, 79% had “poor” sleep quality (score above 5 on the PSQI), decreasing to 54% at 6 months and 49% at 12 months (p<0.001 for both changes between baseline and 6 months, and between baseline and 12 months, based on McNemar’s test). Significant correlates of better sleep included younger age, being married, and having more education, but not obesity (measured by body mass index). We also investigated chronotype, which characterizes sleep time preferences that reflect underlying circadian rhythms; individuals may be “morning types” or larks (preferring early awakenings and bedtimes), “evening types” or owls (preferring late nights and mornings), or intermediate. We found that our intervention was more significantly effective for larks (where 11% reported good sleep at baseline and 67% at 12 months) and intermediates (where 28% reported good sleep at baseline and 62% at 12 months) than for owls (where 15% reported good sleep at baseline and 18% at 12 months).

DISCUSSION: Most female shift workers in this study report impaired sleep quality. Our CBT-based sleep intervention led to significant improvements in sleep quality in 6 months, and these improvements were maintained at one year. This approach was more effective for some chronotypes than others, and “night owls” may require a different intervention. Sleep is a modifiable risk factor that is increasingly linked with cancer-related outcomes, including cancer incidence. Interventions to improve sleep quality such as the program used here offer a novel approach with the potential to reduce breast cancer risk.
The use of chemoprevention increases significantly with oncology trained providers and with application of a risk assessment model

Oseni T, Perkins S, Deutsch E and Soballe P. Naval Medical Center San Diego, San Diego, CA and Uniformed Services University, Bethesda, MD.

**BACKGROUND**
Large clinical trials have proven the efficacy of selective estrogen receptor modulators in reducing the risk of breast cancer in high risk women. However, despite these studies the use of chemoprevention in high risk women remains low. The goal of this study was to determine in a clearly identified high risk population, the utilization rate of chemoprevention and factors that affect its use, notably, surgeon training and the Gail model.

**METHODS**
This was a retrospective chart review of all women diagnosed with Atypical Ductal Hyperplasia (ADH) at our institution from 2008 to 2013. We examined the use of chemoprevention and screening recommendations after diagnosis. Other factors evaluated included family history, Gail model, surgeon specialty and menopausal status.

**RESULTS**
Ninety-four women with ADH were treated at our facility in the study time frame. The overall use of chemoprevention in our study population was 41.5%. Of those who were not on chemoprevention, 56.4 % were offered chemoprevention and declined. In addition, after the diagnosis of ADH, annual mammography was recommended for 75% of women. However, this was preferentially seen in women on chemoprevention (95% vs. 65%). Menopausal status and use of the Gail model were statistically significant in predicting annual screening after a diagnosis of ADH. The Gail score was calculated preferentially in women who chose chemoprevention. Prior breast biopsy, family history of cancer, first degree relative with cancer, age and menopausal status were not found to be statistically significant in the use of chemoprevention. Oncology training and use of the Gail model were found to be statistically significant in chemoprevention use.

**CONCLUSION**
The use of chemoprevention in high risk women is significantly improved with oncology training and the use of the Gail model. Concern regarding side effects continues to result in low utilization of selective estrogen receptor modulators. Education regarding newer agents for chemoprevention with minimal side effects may result in increased utilization of chemoprevention.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Chemoprevention n (%)</th>
<th>No Chemoprevention n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>58 (37-78)</td>
<td>52 (30-77)</td>
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</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>0.790</td>
</tr>
<tr>
<td>Caucasian</td>
<td>18 (46.1 %)</td>
<td>33 (60.0 %)</td>
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</tr>
<tr>
<td>Asian/PCI</td>
<td>6 (15.4 %)</td>
<td>5 (9.1 %)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>2 (5.1 %)</td>
<td>2 (3.6 %)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (5.1 %)</td>
<td>1 (1.8 %)</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>10 (25.6 %)</td>
<td>18 (32.7 %)</td>
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</tr>
<tr>
<td>First degree</td>
<td>26 (66.7 %)</td>
<td>32 (58.1 %)</td>
<td>1.000</td>
</tr>
<tr>
<td>Gail score calculated</td>
<td>29 (74.3 %)</td>
<td>20 (36.3 %)</td>
<td>&lt;0.001</td>
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<tr>
<td>Gail score median (range)</td>
<td>3.4 % (0.8-17.7)</td>
<td>2.7% (0.2-8.6)</td>
<td>0.169</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>17 (43.6 %)</td>
<td>19 (34.5 %)</td>
<td>0.796</td>
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<tr>
<td>Specialty provider</td>
<td>37 (94.9 %)</td>
<td>38 (69.1 %)</td>
<td>0.002</td>
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<tr>
<td>Prior breast biopsy</td>
<td>13 (33.3 %)</td>
<td>16 (29.1 %)</td>
<td>0.438</td>
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</table>

The views expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.
Intermittent fasting (IF) regimens have gained widespread attention in recent years for their possible role in human health and disease risk. In mice, IF schedules that are aligned with sleep-wake cycles appear to positively influence metabolic processes related to cancer risk and may have direct effects on carcinogenesis; however the impact of these regimens on cancer risk in humans remain unclear. We examined associations between nighttime fasting duration (a form of IF aligned with sleep-wake cycles) and (1) biomarkers demonstrated to be associated with breast cancer prognosis; and (2) long-term clinical outcomes in a sample of breast cancer survivors from the Women's Healthy Eating and Living (WHEL) Study. Dietary data were available for 3,061 non-diabetic women enrolled in the WHEL Study. Nighttime fasting duration was calculated using time-stamped 24-hour dietary recalls collected at the baseline, Year 1, and Year 4 study assessment periods. Approximately 3-4 dietary records were collected per subject at each assessment period, and these records were averaged to yield a single estimate of nighttime fasting duration per time point. Glycosylated hemoglobin (HbA1c) and C-reactive protein (CRP) levels were ascertained from blood specimen collected at baseline. Clinical outcomes recorded during the study follow-up include breast cancer events (recurrence or new primary) and mortality. Linear regression models examined the associations of nighttime fasting with baseline concentrations of HbA1c and CRP. Delayed-entry Cox proportional hazard models were used to assess the association between nighttime fasting duration, recorded at the baseline, Year 1, and Year 4 assessments, with clinical outcomes. These models used a counting process method to account for repeated measures. All models controlled for basic demographic factors, participant characteristics (BMI, comorbidity status, sleep duration), breast cancer characteristics (stage, grade, anti-estrogen use), and dietary variables (total calories, evening calories, eating frequency). Women fasted an average of 12.5 hours per night (SD=1.6 hours). There were 520 new breast cancer events, and 569 deaths during study follow up. HbA1c level was significantly and inversely related to nighttime fasting duration. Each 2-hour increase in the nighttime fasting duration was associated with a 0.2-unit decrease in HbA1c ($\beta=-0.21$; $p=0.03$ with HbA1c expressed as mmol/mol), and there was no evidence of mediation or effect modification by participant characteristics, e.g., BMI. No associations were observed between nighttime fasting duration and CRP. In longitudinal models, women who fasted less than 13.1 hours per night (bottom two tertiles of nightly fasting distribution) had roughly a 50% higher hazard for experiencing a breast cancer event, compared to women who fasted at least 13.1 hours per night (HR: 1.46; 95%CI: 1.11-1.93; $p<0.01$). Nighttime fasting duration was not associated with mortality. Findings suggest that increasing the length of the nighttime fasting interval could be a simple, feasible, and novel strategy to improve glucose control and reduce breast cancer risk. Randomized trials confirming the link between nighttime fasting duration and breast cancer risk are warranted.
2015 San Antonio Breast Cancer Symposium

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Title: Early life residence, fish consumption and risk of breast cancer

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Body: Introduction: Few studies exist on the effect of diet during different periods of life, on breast cancer risk later in life. Great differences existed in food consumption between the capital and rural areas in Iceland in the middle of the 20th century, with very high fish consumption in coastal areas.

Objectives: Our aim was to explore the effect of diet and residence during early life and midlife on breast cancer risk later in life.

Methods and data: We used data from the Reykjavik Study, a population-based Icelandic cohort of 10049 women born between 1907 and 1935, and examined the association of residence in early life, used as a proxy for dietary habits, and risk of breast cancer. To further explore this association, we also used food frequency data at different periods of life, including adolescence, from the AGES-Reykjavik cohort, a subgroup of the Reykjavik Study, established in 2002. Participants provided information on residence in early life. By linkage with the Icelandic Cancer Registry, information on breast cancer diagnoses was available throughout 2013. Adjustments were made for a series of potential confounders, including residence for dietary analysis.

Results: During a mean follow-up of 27.3 years, 744 women were diagnosed with breast cancer. We found a significant inverse association for breast cancer diagnosis among women who lived through the puberty period (20 years or more) in coastal villages compared with women residing in the capital area (HR = 0.74, 95% CI: 0.58, 0.94). In the subgroup analysis, we found that women with high fish consumption in midlife had lower risk of breast cancer in older age, compared with women with lower consumption, (OR = 0.60, 95% CI 0.38, 0.94). However, we did not observe a statistically significant association between high fish intake in adolescence and breast cancer (OR = 0.84, 95% CI 0.62 - 1.13).

Conclusions: Our results suggest that high fish consumption in early- to midlife may be associated with reduced risk of breast cancer.
Title: Combined effects of soy isoflavones and a high fat diet on the mammary gland in an animal model of diet-induced obesity

Kurrat A, Diel P, Blei T, Kluxen F, Mueller D, Pichotta M, Soukup S, Kulling S and Oden C. Institute of Cardiovascular Research and Sports Medicine, German Sports University Cologne, Cologne, Germany; Max Rubner-Institut, Karlsruhe, Germany, Karlsruhe, Germany and Clinic for Cattle, Endocrinology, University of Veterinary Medicine, Hannover, Germany.

Body: Scope: Obesity is a major risk factor for the development of breast cancer whereas isoflavone (ISO) exposure is discussed to reduce this risk. Aim of this study was to investigate effects of dietary soy ISO intake on proliferation and estrogenicity of ISO in the mammary gland of obese female Wistar rats.

Methods: Female Wistar rats (5 – 7 rats / group) grew up on low fat ISO-depleted diet (LF IDD) or ISO-rich diet enriched with a soy based commercial extract (LF IRD; ISO: 467 mg / kg diet). Starting postnatal day 83, ovariectomized (OVX) and intact animals received high fat diet for 12 weeks to induce obesity in the absence (HF IDD) or presence of ISO (HF IRD, ISO: 431 mg / kg diet). A special diet switch group (HF IRD switch OVX), grew up on LF IDD but switched to HF IRD after ovariectomy. This mimics the short term exposure to ISO in postmenopausal Western women who take ISO supplements. Two groups receiving LF diet either with or without ISO (LF IRD, LF IDD) lifelong served as control. From ablactation until the end of the experiment body weight and food consumption were monitored twice a week. After 12 weeks of HF diet animals were sacrificed. Body weight, visceral fat mass, and serum leptin were measured, and breast tissue was excised. Protein expression of proliferating cell nuclear antigen (PCNA) and progesterone receptor (PR) in breast tissue was analyzed by both immunohistochemistry (IHC) and Western Blot as markers for proliferation and estrogenicity of ISO, respectively.

Results: Analysis of ISO plasma levels revealed 1400 nM in LF IRD group and 300 – 700 nM in HF IRD groups. HF diet increased body weight, visceral fat mass and serum leptin levels compared to LF diet. In the mammary gland HF increased expression of proliferation marker PCNA and PR as compared to LF groups. Lifelong but not short term (HF IRD switch OVX) ISO exposure reduced body weight, visceral fat mass and leptin levels in HF OVX rats. In the mammary gland lifelong ISO exposure reduced PCNA expression in both LF and HF intact animals whereas in HF OVX animals lifelong ISO exposure increased PCNA expression compared to short term ISO exposure. PR expression increased in HF IRD OVX compared to HF IDD OVX.

Conclusion: The ISO plasma levels of the rats are comparable to the average ISO plasma levels as found in Asian population. Our results show that lifelong ISO intake reduces the risk to develop obesity in female rats. In the mammary gland lifelong ISO exposure decreases cell proliferation and shows estrogenicity by increasing PR expression. Effects of short term ISO exposure are less strong compared to lifelong ISO exposure. This supports the hypothesis that only lifelong but not short term ISO exposure can reduce the risk to develop breast cancer.
Title: Growth inhibitory effects of Cornus officinalis on a model for triple negative breast cancer

Telang NT T, Nair HB B and Wong GYC YC. Palindrome Liaisons Consultants, Montvale, NJ; Texas Biomadical Research Institute, San Antonio, TX and American Foundation for Chinese Medicine, NY, NY.

Body: Background: The triple negative breast cancer (TNBC) molecular subtype is characterized by the absence of estrogen receptor-α (ER-α), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) expressions. TNBC is refractory to endocrine and to HER-2 selective therapy, while options for the treatment of choice involving high dose Anthracyclin/Taxol based conventional chemotherapy and/or small molecule inhibitor based targeted therapy are associated with systemic toxicity and acquired tumor resistance, leading to compromised efficacy. These limitations emphasize a need to identify efficacious non-toxic agents for secondary prevention/therapy of TNBC. Cornus officinalis (CO) is a Chinese herb of a nutritional nature in the form of a fruit. It is a major ingredient herb in some well-known Chinese herbal formulations for health management purposes. Growth inhibitory efficacy of CO in a model for ER positive Luminal A molecular subtype of clinical breast cancer is associated with abrogation of estradiol promoted growth via generation of anti-proliferative estradiol metabolites [Telang et al: Mol. Med. Rep. 5: 20-28, 2012]. The present study examines the growth inhibitory effects of CO, and identifies possible molecular targets for its efficacy in a pre-clinical cell culture model for TNBC.

Experimental model, Herbal Extract and Biomarkers: The human mammary carcinoma-derived ER-α negative, PR negative and HER-2 negative MDA-MB-231 cell line represents the model for TNBC. Non-fractionated aqueous extract from CO represents the test agent. Anchorage dependent growth, anchorage independent (AI) colony formation, cell cycle progression, cellular apoptosis and relevant pathway specific mechanistic assays represent the biomarkers for efficacy.

Results: Treatment of MDA-MB-231 cells with CO extract induces substantial dose dependent cytostatic growth arrest (IC50:0.1%; IC90:0.5%), and strongly inhibits AI colony formation. Within the cytostatic range CO inhibits G1 to S phase transition leading to G1 arrest that is accompanied by decreased expressions of Cyclin D1 and p-RB. Cellular apoptosis induced by CO is associated with modulation of the BAX-BCL-2 pathway, and up-regulation of the pro-apoptotic Caspase 3/7 activity.

Conclusions: These data provide a mechanistic evidence for efficacy of CO as a naturally occurring nutritional substance in a cell culture model for TNBC. The present study validates a mechanism based approach to prioritize efficacious non-toxic herbal extracts for secondary prevention/therapy of the TNBC molecular subtype of clinical breast cancer.
Title: A pilot feasibility study of the WISDOM study, a preference-tolerant randomized controlled trial evaluating a risk-based breast cancer screening strategy


Body: Background: For almost 30 years, annual mammograms for women over 40 have been a cornerstone of the US strategy to reduce breast cancer mortality. Introduction of the 2009 USPSTF screening guidelines, though based on a thorough review of the scientific literature, has triggered scientific debate and a stalemate. The solution is not to prolong the controversy with repetitious reviews of past studies, but rather to test and implement a personalized model that leverages advances in breast cancer biology, risk assessment, and imaging to provide screening recommendations based upon well-characterized measures of risk. Our WISDOM (Women Informed to Screen Depending On Measures of risk) study, a preference-tolerant randomized controlled trial funded through PCORI, will evaluate whether such a risk-based screening strategy, compared to annual screening, is as safe, is less morbid, enables prevention and is preferred by women. This upcoming pilot study will test the feasibility and technical implementation of the WISDOM trial, focusing on recruitment, enrollment, and randomization processes, a coverage with evidence development approach to enable rapid adoption, and patient experience and satisfaction. Findings will directly inform implementation of the full trial, slated to begin in fall 2015 throughout the Athena Breast Health Network, a research and care collaboration across the five UC Medical Centers and Sanford Health.

Trial Design: 225 participants will be recruited from Athena patients receiving care at UCSF. Participants must be female; between age 40 and 75; have had a normal mammogram at UCSF in the past 6 months; and in the Athena research cohort. Exclusion criteria are a breast cancer or DCIS diagnosis; inability to provide consent; or inability to speak English. After education about the trial, patients will be asked if they are willing to be randomized to either the risk-based or annual screening schedule; if not, they can self-assign to their preferred schedule. The randomized and self-assigned cohorts will receive the same interventions. The risk-based screening strategy will incorporate risk assessment based on the latest Breast Cancer Surveillance Consortium model along with established and recently validated genetic risk factors, co-morbidities, and breast density, and will be used to tailor individual recommendations for starting and stopping age, frequency, and screening modality. A saliva assay will be administered to participants in the risk-based arm to screen for genetic breast cancer risk factors (BRCA1 & BRCA2 + 9 additional genes + 81 single nucleotide polymorphisms).

Statistical Methods: Descriptive statistics for the proportion of women who are willing to be randomized, choose the risk-based arm in the self-assigned cohort, and are willing to accept their assigned or chosen screening schedule, as well as the distribution of participant anxiety scores, will be analyzed. This will inform statistical design for the full trial, including the number of women who should be approached to enroll 65,000 randomized participants and the sample size needed to measure anxiety and decision regret in the randomized cohort. Results from the pilot will be available September 30, 2015.
Differential response of inflammatory cytokines to omega-3 fatty acid supplementation based on menopausal status

Kimler BF F and Fabian CJ J. University of Kansas Medical Center, Kansas City, KS.

Background: High dose marine omega-3 fatty acid supplementation is currently undergoing assessment in early phase primary prevention trials. A potential mechanism of action is thought to be inflammation resolution. Since post-menopausal women may have higher systemic levels of pro-inflammatory cytokines, and hormones may affect response to fatty acids, we conducted two parallel pilot trials of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as Lovaza® supplementation in women at high risk for development of breast cancer. This post-hoc analysis examines whether five cytokines involved in inflammatory processes (HGF, MCP-1, NGF, PAI-1, TNF-alpha) are impacted by menopausal status.

Methods: Separate trials were conducted in pre-menopausal (N=36) or post-menopausal (N=35) women. Blood (fasting) and benign breast tissue (non-fasting, sampled by random periareolar fine needle aspiration) was acquired before and after intervention with 4 g Lovaza® daily for 6 months. In addition to standard risk and response biomarkers, panels of cytokines associated with inflammatory processes were assayed by Luminex® technology: HGF, insulin, MCP-1, NGF, PAI-1, resistin, TNF-alpha. For breast tissue, values were normalized to protein content.

Results: Thirty-four women in each trial completed the intervention and provided pre-study and post-study specimens. At baseline, higher values for post-menopausal compared to pre-menopausal women were observed for serum levels of MCP-1 (p=0.002, non-parametric Mann-Whitney test), PAI-1 (p=0.001), and TNF-alpha (p=0.019). The same was observed for off-study specimens. There was no statistically significant (non-parametric Wilcoxon signed rank test) effect of the intervention in either cohort for MCP-1 or PAI-1. However, for TNF-alpha, while there was no modulation observed in pre-menopausal women, there was a modest decrease (medians of 3.7 to 3.4 pg/ml; p=0.016) for post-menopausal women. An effect on TNF-alpha would not have been detected if the two cohorts had been combined.

For breast tissue, higher values for post-menopausal women at baseline were observed for HGF (p=0.029), MCP-1 (p=0.013), NGF (p=0.001), and TNF-alpha (p=0.001), but not PAI-1 (p=0.79). Off-study values were also typically higher in post-menopausal women. As with serum levels, there was a significant decrease in TNF-alpha values over the course of the study (p=0.042) in post-menopausal women but not pre-menopausal women. A decrease was also observed for HGF (p=0.002) and MCP-1 (p=0.001) for post-menopausal women but not pre-menopausal women.

Conclusion: This post-analysis suggests caution when admixing pre-menopausal and post-menopausal women in small clinical trials of omega-3 fatty acid supplementation, especially when assessing biomarkers of inflammatory processes. For trials with both, sufficient subjects should be enrolled that stratification prior to randomization is possible.
Title: Receipt of breast cancer risk assessment and personalized prevention information among women diagnosed with a benign breast lesion (BBL) in a one stop breast unit: A prospective assessment


Body: Background: Women's awareness about their personal breast cancer (BC) risk in the general population is generally low. Mass screening and mass prevention interventions have as yet been moderately efficient in breast oncology. "Personalized prevention" including risk communication, personalized screening and primary prevention recommendations is a promising. A personal history of BBL slightly increases subsequent BC risk.

Objectives: the main objective was to evaluate the acceptability of a mathematical tool- based breast cancer risk assessment and subsequent proposal of a personalized BC prevention program in a BBL population. Secondary objectives were to evaluate information receipt, awareness, satisfaction, and anxiety.

Methods: Women were eligible for the study if aged 40-74, were recently diagnosed with a benign breast lesion at the one stop breast Unit of the center, had no personal history of cancer or atypical lesions and were not BRCA carriers. Women were proposed a personalized risk assessment using a mathematical tool (BCSC score adapted to the French population-Ragusa et al) together with personalized information on risk, BC screening and prevention, release of a personalized program and evaluation of their receipt. The main end point was the proportion of women willing to have a risk assessment and personalized counseling. A cut-off point of 70% was considered critical to consider acceptability. Secondary end points were perceived BC risk, satisfaction, anxiety and distress levels at day 2 using standardized questionnaires, as well as adherence with the proposed programs.

Results: Of 150 women proposed BC risk assessment and personalized prevention information between 02/2014 and 03/2015, 129 (86%) accepted. Median age: 53.6 years. 33% had a low BC risk (< 1.1% at 5 yrs [mean risk of 50 yrs-old women in France]), 53% a moderate risk (1.1-1.66% at 5 yrs), while 14% were high risk (> 1.66% at 5 yrs). 87% had never had any previous information on BC risk. 3 pts required a genetic assessment.

Participants were globally very satisfied with physicians' and nurses' interpersonal skills, availability and provision of information (mean score > 4; range 2-5). The mean scores of clarity of the BC risk information (4.14±1;range 2-5) and screening program information (4.21±0.93; range 2-5) were high.

The mean score of perceived risk level was estimated to 33.5% (SD=21.9).

Mean scores of state anxiety (36.7±12.2; range 20-71), trait anxiety (39.5±8.9; range 23-59), depressive symptoms (3.4±3.3; range 0-12) and psychological distress indicated low levels of all. Higher level of state-anxiety was associated with lower scores of satisfaction with doctors and nurses human qualities (r = 0.26, p<.05) and with lower scores of clarity of information about screening program (r = 0.25, p<.05).

Conclusion: The receipt of breast cancer risk assessment and personalized prevention information among women diagnosed with BBL was high (86%). Information need is high given the low level of real risk awareness. Such population may benefit from personalized prevention. Anxiety and distress scores were low and satisfaction rates high.
Title: Telapristone acetate suppresses the expression of genes involved in cell proliferation, stem cell self-renewal, and anti-apoptosis in MNU-induced rat mammary tumors

Lee O, Clare SE E, Ivancic D, Scholtens DM M and Khan SA A. Northwestern University, Chicago, IL.

Body: Background: We previously reported that telapristone acetate (TPA), an anti-progestin, significantly suppressed mammary tumor formation and growth accelerated by progesterone (P4) and medroxyprogesterone acetate (MPA) in N-methyl-N-nitrosourea (MNU)-induced carcinogenesis model[1]. This suggests TPA holds promise for the prevention and treatment of human breast cancer. The purpose of this study is to identify the genes modulated by TPA in rat mammary ER/PR+ tumors, which may be translated into a therapeutic biomarker of TPA therapy in ER/PR+ breast cancer.

Methods: Rat mammary tumors from MNU carcinogenesis were used for study [1]. Treatment groups were: control, MPA, P4, MPA+TPA, and P4+TPA. Doses were 30mg of TPA and 25mg of P4 or MPA (90 day release pellets). Tumor epithelium was isolated by laser capture microdissection and RNA extracted. 100 ng of RNA per sample was used for targeted -gene expression profiling by nCounter Gene Expression analysis (Nanostring Inc.). 112 genes related to PR, GR, AR signaling were selected by MetaCore pathway analysis (Thomson Reuters, Inc.). The data was normalized by positive control and RNA content using nSolver. The genes significantly modulated by hormones and TPA were reported as means ± standard deviations (SD) for each group as well as a p-value for pairwise t-test comparison.

Results:

mRNA expression, log mean (log SD).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control P4</th>
<th>P4 vs. P4+TPA</th>
<th>p</th>
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<tbody>
<tr>
<td>Survivin</td>
<td>0.7 (0.5)</td>
<td>3.9 (0.2)</td>
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<tr>
<td>Ki67</td>
<td>7.6 (0.3)</td>
<td>7.1 (0.3)</td>
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<tr>
<td>FANCA</td>
<td>4.3 (0.2)</td>
<td>3.8 (0.3)</td>
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<tr>
<td>EZH2</td>
<td>5.4 (0.2)</td>
<td>5.1 (0.2)</td>
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<tr>
<td>RFC3</td>
<td>5.6 (0.2)</td>
<td>5.3 (0.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>C.MYC</td>
<td>5.9 (0.4)</td>
<td>5.5 (0.3)</td>
<td>0.04</td>
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<tr>
<td>TK1</td>
<td>5.2 (0.3)</td>
<td>4.8 (0.2)</td>
<td>0.05</td>
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</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control MPA</th>
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<th>p</th>
</tr>
</thead>
<tbody>
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<td>FKBP5</td>
<td>4.7 (0.4)</td>
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<tr>
<td>TNFSF11</td>
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<td>2.8 (0.9)</td>
<td>0.02</td>
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<tr>
<td>TEK</td>
<td>3.7 (0.5)</td>
<td>3.1 (0.5)</td>
<td>0.03</td>
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<tr>
<td>PLZF</td>
<td>2.8 (0.9)</td>
<td>4.0 (1.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>EPAS1</td>
<td>5.5 (0.5)</td>
<td>4.9 (0.4)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Compared to the control, P4 significantly reduced CHD5 expression, a tumor suppressor in a mouse model, and its down-regulation contributes to the development and progression of human breast cancer. Comparing the P4 and P4+TPA groups, the genes involved in cell proliferation and DNA repair (C-MYC, FANCA, Ki67, RFC3, and TK1), EZH2 (stem cell self-renewal), and anti-apoptosis (survivin) were significantly down-regulated by TPA. On the other hand, MPA induced the up-regulation of FKBP5 (androgen-regulated protein), PLZF (GR responsive gene), TEK (angiogenesis-regulating gene) and TNFSF11 (RANKL) compared to the control. Comparing the MPA and MPA+TPA groups, TPA significantly reduced the expression of EGFR, SNAI2 and endothelin-1 (EMT initiation or maintenance) and increased AR expression.

Conclusions: TPA inhibits P4/PR mediated cell proliferation, and increases apoptosis. The tumorigenic effect of MPA is likely through AR and GR rather than a PR specific manner. In addition to the PR, MPA binds with high affinity to AR and GR, and therefore has the potential to elicit actions distinct to those of P4, which does not bind AR.

2015 San Antonio Breast Cancer Symposium

Publication Number: P3-11-02

Title: Weight maintenance initiated at midlife reduces mammary tumor incidence but metformin treatment does not mimic the effect

Cleary MP P, Mizuno NK K, Yang D-Q, Liao J and Grossmann ME E. University of Minnesota- Hormel Institute, Austin, MN.

Body: Weight gain and/or obesity are now considered to be risk factors for the development of postmenopausal breast cancer. Thus, weight loss and/or maintenance resulting from calorie restriction (CR) is recommended however success rates are infrequent. Metformin (MET) is a safe and effective treatment for type 2 diabetes mellitus and its use has been linked to reduced breast cancer incidence and mortality in comparison to other forms of diabetes treatments. Interestingly, MET's mechanism of action is considered to be similar to CR. The goal of the present investigation was to directly compare the effect of CR implemented at midlife with the administration of metformin on the development of mammary tumors (MT) in a relevant mouse model. Further, since these interventions would likely be used by obese women we studied both lean and obese mice. Female MMTV-TGF-α mice which develop MTs in the second year of life were fed ad libitum (AL) either a low fat (LF) (10.2% by calories) diet or a moderately high fat (HF) (33.5% by calories) diet from 10 until 30 weeks of age. At 30 weeks the mice on each diet were divided into AL, MET (250 mg/kg/bw/day) or CR (25% reduction in calories) subgroups and then followed until 90 weeks of age. HF-AL and HF-Met mice were significantly heavier than the other 4 groups whose weights were similar. The survival curves had an overall P value of P<0.0001 with LF-CR having the best survival of 100% while the HF-AL and HF-Met had the poorest survival with rates of 68% and 63% respectively. HF-CR mice had a significantly better survival rate compared to HF-AL and HF-Met (P<0.0001 for both). MT incidence (histologically confirmed) was significantly reduced in LF-CR (6%) compared to all other groups (45%-69%) (Chi-squared analysis). Tumor incidence in the HF-CR group (51%) was reduced compared to HF-AL (69%) and HF-Met (60%) mice but not significantly different due to adjustment for multiple comparisons, although the HF-CR vs HF-AL comparison was P<0.02. However, when the incidence rate for tumors detected by palpation prior to euthanasia was examined LF-CR mice did not have any MTs although the palpable incidence rates were not significantly different due to the need for multiple comparisons (LF-CR vs LF-AL was P<0.0097). The HF-CR group had significantly reduced palpable MT prior to euthanasia as compared to both HF-AL and the HF-Met (P<0.0002 and P<0.0001 respectively). Total tumor weight, grade and multiplicity were also examined for the effects of weight maintenance and MET. Presently the role of the AMPK pathway in these effects and serum measurements are being determined. In conclusion, the results to date indicate that weight maintenance during midlife can have a significant impact in delaying MT formation regardless of body weight status although the effects of MET are less dramatic.

Supported by NIH-NCI CA157012, The Hormel Foundation and Paint the Town Pink.
Title: Preventive effect of metformin in combination with atorvastatin on 7,12-dimethylbenzanthracene induced breast cancer in high fat diet fed C57BL/6 mice

Ramdhave AS Satish and Nandave M. SPP School of Pharmacy and Technology Management, SVKM's NMIMS University, Mumbai, Maharashtra, India.

Body: Metabolic syndrome is a cluster of common clinical and biochemical abnormalities that includes insulin resistance, impaired glucose tolerance, central obesity and dyslipidemia. While the underlying mechanisms are not exactly understood, increasing evidence supports that insulin resistance causes perturbation of Insulin-like growth factor-1 (IGF1) axis which forms the core reason of carcinogenesis. IGF1 receptor (IGF-1R) overexpression has also been reported to stimulate transcription of the fatty acid synthase (FASN) gene, a key enzyme involved in neoplastic lipogenesis. Thus, we hypothesize that by modulating these metabolic syndrome linked abnormalities, we may attenuate cancer development. We investigated the effects of High Fat Diet (HFD: D12492) induced metabolic abnormalities on growth of 7,12-Dimethylbenzanthracene (DMBA)-induced breast cancer model. Preventive effect of Metformin (Met) in combination with Atorvastatin (Ato) on DMBA induced breast cancer in HFD fed mice was designed and assessed using the Chou-Talalay method. Female C57BL/6, aged 4-5 weeks were fed with HFD for 16 weeks to induce metabolic abnormalities and it was confirmed by various physiological, biochemical and histological parameters. From 16th week onwards, these female C57BL/6 mice were orally administered 0.2 ml olive oil containing 1 mg DMBA for 6 weeks. The preventive treatment of Met and Ato was initiated on the same day before first carcinogen dose. Met was administered at a dose of 50, 100 and 200mg/kg. Similarly Ato was administered at a dose of 6.25, 12.5 and 25mg/kg. The combinations were administered at a dose of Met 50mg/kg + Ato 6.25mg/kg, Met 100mg/kg + Ato 12.5mg/kg and Met 200mg/kg + Ato 25mg/kg. These test drugs were administered in drinking water for another 18 weeks. During the experiment, the animals were weighed weekly and palpated for the presence of mammary tumors. Tumor incidence, latency, frequency and tumor volume were also recorded. It was observed that the tumors from mice fed with only HFD were almost twofold the volume of mice fed with normal fat diet (NFD). These findings were correlated with the observation that mice fed with HFD had significantly elevated plasma glucose, insulin, IGF-1, triglyceride, cholesterol, LDL and FASN levels. Furthermore, Met and Ato individually at higher dose reduced the tumor volume by one fourth as compared to HFD group. Likewise, Met and Ato in combination at higher dose significantly attenuated the tumor volume to one tenth as compared to HFD group. Combination index (CI) was calculated with the computerized Chou–Talalay method and was found to be less than 1, demonstrating a synergistic effect of two drugs. Thus, our study suggests a potential role for this combination in the management of a metabolically defined subset of breast cancer.
Metabolic profiles in female Sprague-Dawley rats receiving either a standard (4% fat) or Western (20% fat) diet and changes in rats bearing mammary cancers

Thompson MD D, Grubbs CJ J, Moeinpour F, Steele VE E, Miller MS S and Lubet RA A. National Institute of Health/NCI, Rockville, MD; University of Alabama at Birmingham, Birmingham, AL; University of Alabama at Birmingham, Birmingham, AL; National Institute of Health/NCI, Rockville, MD; National Institute of Health/NCI, Rockville, MD and National Institute of Health/NCI, Rockville, MD.

Body: The methylnitrosourea(MNU)-induced model of ER+ mammary cancers in female Sprague-Dawley rats has been routinely used in our laboratories for screening chemopreventive agents. We recently reported that metformin was ineffective in preventing mammary cancers in this model when given to rats on a standard diet (Thompson, et al, Cancer Prev Res. 2015;8:231-9). In this study, we evaluated metformin in rats placed on either standard diet (4% fat) or a Western diet (20% fat, low calcium). As fat calories, these diets were 8% and 42%, respectively. The rats were placed on standard diet or Western diet at 43 days of age, given MNU (via the jugular vein) once at 50 days of age, and administered metformin or vehicle at 57 days of age for the remainder of the study. Serum of the rats in the various groups was obtained at 78 days of age, and at the end of the study (when mammary tumors were present). The levels of approximately 500 metabolites were compared in the serum based on data obtained by Metabolon (Research Triangle Park, NC). These studies showed that each of the four groups [(standard diet (early and late) or Western diet (early and late))] yielded four clearly distinct patterns based on an unsupervised principal component analysis. Certain of the metabolites which were differentially expressed in serum from standard vs Western diets at the early time point were alpha-10-undecanoate, 13-methylmyristic acid, 4-hydroxy-benzoate, 2-amino-heptanpate, tocopherol, and nicotinamide. Certain of these were fatty acids and lipid soluble vitamins that one would expect to be altered. Comparing serum from standard vs Western diets at the late time point confirmed many of these metabolic differences. We also compared serum from the early and late time points since the latter sera were typically from animals with a significant number of mammary cancers. We observed a number of metabolite changes including 4-hydroxy-butyrate, acetyl-carnitine, oxalate, and threonate. These cancer-related profiles will be discussed at greater lengths. Altered profiles caused by the administration of metformin and other chemopreventive agents will also be discussed. Of interest, metformin was consistently ineffective in preventing mammary cancers in rats given either standard or Western diet. Supported by NCI contract HHSN261201200021I.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-11-05

Title: RANK ligand is a target for breast cancer prevention in BRCA1 mutation carriers

Lindeman GJ J, Nolan E, Pal B, Vaillant F, Giner G, Whitehead L, Mann GB B, Lok SW W, Shackleton K, Kathleen Cuningham Foundation Consortium (kConFab), Smyth GK K and Visvader JE E. The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; The Royal Melbourne Hospital, Melbourne, Victoria, Australia; The Royal Women's Hospital, Melbourne, Victoria, Australia and The Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia.

Body: Background: BRCA1 mutation carriers often undergo prophylactic mastectomy to minimize their risk of breast cancer. The value of targeting ovarian hormones to prevent breast tumorigenesis remains contentious and the identification of an effective and acceptable chemoprevention strategy remains a 'holy grail' for the field. Recently, luminal progenitor cells have been identified as the likely cell-of-origin for BRCA1-associated breast tumors. In addition, deregulated progesterone signaling has been implicated as a potential mechanism underlying tumor development in Brca1-deficient mammary glands, although its role in luminal progenitor activation in BRCA1 mutation carriers is unknown. RANKL (Receptor Activator of Nuclear Factor-kappa B Ligand) has been identified as a key paracrine effector of progesterone-induced mammary epithelial proliferation in both mouse and human tissue. Notably, RANKL and its receptor RANK play a critical role in the development of breast cancer, with inhibition of RANKL resulting in attenuation of tumorigenesis in mouse models of hormone-driven mammary carcinogenesis.

Methods: We explored a role for the RANK/RANKL pathway during the preneoplastic phase in freshly isolated, histologically normal specimens from BRCA1 mutation carriers using a combination of strategies. RANK and RANKL expression in breast cancer was also evaluated in formalin fixed paraffin embedded (FFPE) archival sections by IHC. All samples were obtained with relevant IRB approval. A role for RANKL inhibition in attenuating tumor onset was studied using models that recapitulate human basal-like cancer.

Results: A RANK+ subset of luminal progenitor cells was identified in histologically normal breast tissue from BRCA1-mutation carriers. The RANK+ luminal progenitors exhibited higher proliferative activity compared to RANK- progenitors. RNA profiling revealed a distinctive molecular signature, consistent with the RANK+ subset being a possible target for neoplastic transformation. In established BRCA1-associated breast tumors, a four-fold higher incidence of RANK expression was observed, compared to tumors from non-carriers. In ongoing work, histologically normal pre-neoplastic BRCA1mut/+ tissue is being studied using ex vivo breast organoid assays to determine whether RANKL inhibition can attenuate breast epithelial proliferation.

Conclusions: Our data raise the possibility that RANK signaling is implicated in the initiation of tumorigenesis in BRCA1 mutation carriers (and possibly other high risk women) and that RANKL is a promising chemoprevention target. The findings are of sufficient interest to have led to a clinical trial, BRCA-D (Registered as ACTRN12614000694617). A finalized abstract will be submitted in early September, during the Late-Breaking Abstract submission period.

References:
Title: Locoregional failure rates do not vary by breast cancer subtype after mastectomy in a modern cohort of patients with T1-2 tumors with 1-3 pathologically involved lymph nodes

Bazan JG G, Majithia L, Quick AM M, Terando AM M, Agnese D, Mrozek E, Farrar W and White JR R. The Ohio State University, Columbus, OH.

Body: Purpose/Objective(s): A recent meta-analysis of 22 randomized trials accrued between 1964-86 demonstrated significantly higher rates of locoregional failure (LRF), total failure (TF) and breast-cancer mortality in women with 1-3 positive (+) axillary lymph nodes (ALN) who did not receive radiotherapy after mastectomy (mast.). Given the improvements in diagnostic and therapeutic approaches, the challenge today is whether breast cancer patients with T1-T2 tumors with 1-3+ ALN have similar substantial risk that routinely warrants the delivery of post mastectomy radiotherapy (PMRT). We further set out to explore whether the risk of failure varies by breast cancer subtype.

Materials/Methods: We reviewed patients with pathologic T1-2N1 breast cancer treated with initial mast. and adjuvant systemic therapy (ST) from 2000-2013. The primary endpoint was LRF, defined as a recurrence in either the ipsilateral chestwall or regional lymphatics (axillary, internal mammary, or supraclavicular nodes). Secondary endpoints include rates of TF (LRF or distant metastases), disease-free survival (DFS, failure or death), and overall survival (OS). Patients were classified into 3 basic subtypes: hormone receptor positive/HER2 negative (HR+), HER2 positive (HER2+), and triple negative (TN). Survival analysis was performed using the Kaplan-Meier method. The log-rank test was used to compare survival between groups.

Results: We identified 550 eligible patients from our prospectively maintained cancer registry. Median follow-up was 5 years. Baseline characteristics included median age 53 yrs, 61% pathologic T2, 39% grade 3, 48% with lymphovascular invasion. Subtypes included 72% HR+ (n=393), 16% HER2+ (n=89), 12% TN (n=66) and 0.4% unknown (n=2). Treatment included chemotherapy in 78% (n=428), PMRT in 15% (n=82), and anti-endocrine therapy in 70% (n=385). A median of 18 ALN (range, 1-68) were removed, 10% (N=55) had sentinel-lymph node biopsy only, and 17%(N=95) had micrometastases (N1mic) only. A total of 296 pts had 1+ node, 165 pts 2+ nodes and 89 pts 3+ nodes. The 5 yr LRF rate for the entire cohort was 3.9% and patients with 1+, 2+, and 3+ nodes had 5 yr LRF of 2.6%, 4.7% and 6.4%, respectively (p=0.79). The 5 yr LRF for HR+, HER2+ and TN was 3.9%, 1.5%, and 6.6%, respectively (p=0.39). When stratified by 1+, 2+ or 3+ nodes, the 5 yr LRF for HR+ vs. HER2+ vs. TN were 2.4%, 6.8%, and 0% vs. 5.8%, 15.4%, and 0% vs. 5.7%, 0%, and 4.8%, p=0.43. The 5 yr TF, DFS, and OS rates for HR+, HER2+ and TN were 90.5% vs. 88.5%. vs. 83.6% (p=0.76); 84.9% vs. 82.6% vs. 79.2% (p=0.85); and 91.4% vs. 86.2% vs. 81.3% (p=0.83).

Conclusions: In a cohort of patients with T1-2N1 breast cancer treated with modern therapy, we found low rates of LRF which did not vary amongst HR+, HER2+ and TN patients. In particular, HR+ patients with 1+ LN had extremely low rates of LRF. Given these low recurrence rates, caution should be given in routinely recommending PMRT for every woman with 1-3+ ALN after mast. and adjuvant ST.
Patient Prognostic Score and Hazard Ratio (HR) Comparing Mortality between Radiotherapy Group and non-Radiotherapy Group

<table>
<thead>
<tr>
<th>Patient Prognostic Score</th>
<th>Number of patients in -RT group</th>
<th>Number of patients in +RT group</th>
<th>Weighted HR of BCM 95% CI</th>
<th>Weighted HR of OM 95% CI</th>
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<tr>
<td>0</td>
<td>782</td>
<td>1388</td>
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| 5                        | 223                           | 248                           | 0.29                     | 0.09 - 0.43             | 0.21 - -
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</table>

Abbreviation: RT, Radiotherapy; BCM, Breast Cancer Mortality; OM, Overall Mortality; N.A., not available
Title: Utilizing the genomically adjusted dose (GAD) to personalize radiotherapy in adjuvant breast cancer management


Body: Background: We have previously validated a multi-gene model of tumor radiosensitivity (RSI) with validation in multiple independent cohorts including breast, rectal, esophageal, head and neck, glioblastoma, and prostate malignancies. Utilizing the linear quadratic model and RSI, we derived an expression for the genomically adjusted dose (GAD) to model radiation dose effect for individual patients.

Methods: As RSI models the surviving fraction of cells at 2 Gy (SF2), we are able to derive a patient specific alpha. These terms were used in the linear quadratic model to calculate a GAD to model radiation effect and its association with local control. A higher GAD implies a higher predicted radiation therapy effect. Clinical and array-based gene expression were obtained from 75 ER negative patients from the Netherlands Cancer Institute (NKI) and the Institut Curie treated with breast conservation therapy.

Results: Median follow-up for all patients was 10 years with a median age of 42 years (range: 23-50 years). Adjuvant radiation dose to the whole breast was 50 Gy (range: 45-55 Gy). When assessing local recurrence on multivariate analysis, we found GAD to be a significant predictor of local recurrence when dichotomized at the median (GAD-low vs GAD-high Hazard Ratio (HR) 4.5; 95% CI 1.7-13.5; p=0.0031). GAD was also significant per unit change (HR 0.91; 95% CI 0.83-0.99; p=0.021). We then modeled GAD for an escalated dose up to 60 Gy and found an additional 48% of GAD-low patients could achieve a GAD-high with dose escalation.

Conclusions: We found GAD to be significantly correlated with local control following breast conservation therapy. Modeling dose escalation with GAD, we identified a select population of patients whom we hypothesize may benefit from genomically guided increased dose in the adjuvant setting. This population may represent a cohort for future clinical trial enrollment.
Title: The genomically adjusted radiation dose (GAD) and its association with distant metastases in breast cancer: A feasible approach to precision medicine in radiation oncology

Ahmed KA A, Scott JG G, Diaz RJ J, Fulp WJ J and Torres-Roca JF F. H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

Body: Background: Clinical validation studies in over 2,200 patients across 8 different disease sites, including breast cancer, have shown the radiosensitivity index (RSI), a gene expression signature, predicts outcomes in patients treated with radiation. We hypothesize that an approach to personalize radiation dose could be developed by integrating RSI into the linear quadratic model of dose and fractionation.

Methods: Utilizing the linear quadratic model and RSI, we derived an expression for the genomically adjusted dose (GAD) to model radiation dose effect for individual patients. A higher GAD implies a higher predicted radiation therapy effect. GAD was evaluated as a predictor of clinical outcome in two independent datasets of breast cancer patients treated with surgery and radiation. The association between GAD and distant-metastasis free survival (DMFS) and relapse-free survival (RFS) using univariate (UVA) and multivariate (MVA) Cox proportional hazard models was assessed. Clinical and array-based gene expression were obtained from two independent, previously described cohorts from the Karolinska Institutet and Erasmus University Medical Center.

Results: Full radiation treatment details were available for 263 patients in the Erasmus dataset, median follow-up 60 months. GAD-low patients (<75% GAD distribution) were found to have decreased DMFS when compared to GAD-high patients (≥25% GAD distribution) (Hazard Ratio (HR) = 2.31 (95% CI 1.25, 4.25), p=0.006). On MVA, GAD was an independent predictor of DMFS for the whole cohort (HR= 2.11 (1.13, 3.94), p=0.02). When the analysis was restricted to the ER positive cohort, GAD was an independent predictor of outcome both as a continuous (HR=0.977, (0.955, 1.0), p = 0.049) and as a dichotomous variable (HR = 3.42, (1.53, 7.67), p=0.003). These results were independently confirmed in the second Karolinska dataset. The 5 year RFS was 95% for GAD-high patients and 76% in GAD-low patients (p=0.027) and GAD was a significant predictor on MVA for RFS (HR =7.42, (1.41, 137.6), p=0.014). In the Karolinska cohort, we estimate a significant proportion of GAD-low patients (59%) would achieve GAD-high with dose escalation up to 70 Gy.

Conclusions: In this study, we develop and validate GAD, a novel and patient-specific measure of radiation dose effect. Importantly, GAD is a clinically actionable metric by adjusting radiation dose. We propose that GAD based radiation dosing is a feasible approach to precision medicine in breast radiation oncology.
Title: Technique and outcome of post-mastectomy adjuvant chest wall radiotherapy – The role of tissue equivalent bolus in reducing risk of local recurrence

Turner JY Y, Zeniou A, Williams A and Jyothirmayi R. Kent Oncology Centre, Maidstone, Kent, United Kingdom and St. James Institute of Oncology, Leeds, Yorkshire, United Kingdom.

Body: Introduction
Adjuvant chest wall radiotherapy is used in post-mastectomy patients with high risk histological features, to reduce the risk of loco-regional recurrence. This treatment can be given with or without a tissue equivalent bolus to increase skin surface dose. The additional benefit of using a bolus remains unclear; however it is known to be associated with higher incidence of skin toxicity. This retrospective cohort study looks at patients treated between 2005-2010 at the Kent oncology centre (KOC), and compares rates of local chest wall recurrence in patients treated with chest wall radiotherapy with and without a bolus.

Methods
This was a retrospective cohort study of 319 consecutive patients who had received chest wall radiotherapy at the KOC between 2005-2010, identified from radiotherapy planning records. Data was collected on key histological, demographic and treatment parameters, as well as incidence and grade of acute skin reactions. Data was also collected on treatment outcomes, including chest wall recurrence, disease-free and overall survival.

Results
One hundred and one patients received treatment with a bolus compared to 213 patients without a bolus. At median follow-up time of 60 months, there was 1 chest wall recurrence in the bolus treatment group and 4 in the no bolus treatment group. Using Fisher's Exact test no statistically significant difference could be shown between the two groups. However a significantly higher incidence of acute skin toxicity was seen in the bolus treatment group (p<0.001). The radiotherapy plans of the 5 patients with chest wall recurrence were evaluated in further detail regarding dose distribution and skin surface dose coverage. Data was also collected on incidence of metastatic relapse and overall survival. Sixty-six patients (21%) had metastatic relapse, 24 (23.8%) in the bolus treatment group and 42 (19.7%) in the no bolus treatment group, (p=0.41). The median time to relapse 29.5 months. At last follow-up 70.4% of patients had no evidence of disease. A further 6.1% were alive with disease, 6.1% had died with no evidence of disease and 17.5% had died of disease. Overall survival in both bolus and no bolus treatment groups 76%.

Conclusion
No statistically significant difference can be demonstrated in the rate of chest wall recurrence between those treated with radiotherapy with and without a bolus to the chest wall. This is consistent with limited previous literature on the topic. This study invites further evaluation of the role of a bolus in post-mastectomy chest wall radiotherapy, considering the increased toxicity that use of a bolus generates.
Title: Active breathing control in the management of left-sided breast cancer with irradiation: A dosimetric analysis of cardiac and lung dose

Gogineni ES S, Kehrer JD D, Jones GC C, Premo C and Stinson S. New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY; Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD and Suburban Hospital, Johns Hopkins Medicine, Bethesda, MD.

Body: Purpose: Deep inspiratory breath hold (DIBH) is a technique that has been increasingly utilized over the past several years for the purpose of reducing the radiation dose received by the heart and ipsilateral lung during radiotherapy (RT) for breast cancer. The purpose of this study was to provide a comparative analysis of cardiac and lung dose in patients treated with and without moderate DIBH with an active breathing control (ABC) device during RT for left-sided breast cancer at a community radiation oncology center.

Methods: From June 2013 to December 2014, 83 patients with stages 0-III left-sided breast cancer were treated with RT. Of these, 45 patients underwent CT simulation with an ABC device and the remaining 38 patients underwent standard free-breathing CT simulation for RT planning. Twenty-eight patients in the ABC cohort were treated with standard tangent fields and 18 were treated with locoregional RT. The non-ABC cohort included 26 patients treated with standard tangents and 12 treated with locoregional RT. A 4- or 5-field technique was utilized in all locally advanced patients to allow for coverage of the supraclavicular fossa and internal mammary lymph nodes with inclusion of low axillary coverage based on individual risk factors. All patients were treated with 3D-conformal techniques utilizing multi-leaf collimators to block the heart. Comparisons were made between mean heart dose, heart V5, mean lung dose, and lung V5 with and without the use of ABC. Kruskal-Wallis tests were used to determine significance between groups with \( \alpha \) for significance set at \( p \leq 0.05 \).

Results: On combined analysis, the use of DIBH with ABC resulted in a reduction in heart V5 (1.5 versus 5.4%; \( p=0.018 \)). Mean heart dose, mean lung dose, and lung V5 were not significantly improved with the use of ABC. On subgroup analysis, patients treated with locoregional RT had reduced mean heart dose (161 cGy versus 309 cGy; \( p=0.014 \)) and heart V5 (2.3% versus 14.8%; \( p<0.001 \)) compared to patients treated with a standard free-breathing technique.

Conclusions: The use of ABC provided a significant reduction in heart V5 in all patients with left-sided breast cancer and in mean heart dose and heart V5 in patients treated with locoregional RT. This retrospective, single-institution analysis demonstrates the feasibility of implementing moderate DIBH with ABC technology in the treatment of left-sided breast cancer in a community radiation oncology center.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-12-07

Title: Prechemotherapy disease involvement in inflammatory breast cancer is missed with current modern radiation planning

Nowak KA A, Lockamy V, Cristofanilli M and Simone NL L. Sidney Kimmel Medical College at Thomas Jefferson University, Sidney Kimmel Cancer Center, Philadelphia, PA.

Body: Background: Radiation is a crucial for the treatment of inflammatory breast cancer since trimodality therapy has been shown to improve patient survival. At this time, radiation therapy for inflammatory breast cancer is not standardized with institutional preferences dominating decisions of the treatment targets, amount of bolus, total dose and fractionation schedule. For the first time, we are evaluating the pre-chemotherapy skin involvement to determine if standard radiation fields would potentially underdose the initial extent of disease.

Methods: Eight consecutive patients with inflammatory breast cancer were seen in the radiation oncology clinic prior to beginning any therapy for their disease. At the time of initial visit, patients underwent a pre-chemotherapy radiation planning session during which extent of skin involvement as well as standard field borders were wired out and photographed. The pre-chemotherapy radiation planning scan was done to delineate both the initial skin involvement as well as standard borders. This was contoured based on wire marks and photographs. Plans were generated using standard tangent borders using Eclipse planning treatment system (Varian). If skin disease extended outside of the traditional borders, a second plan was generated to account for the extent of skin involvement. Plans were subsequently compared with respect of target coverage.

Results: Of the eight patient scans examined, six had skin involvement that extended outside of the standard chest wall borders. The involved skin coverage was less than ninety percent in the plan using standard borders for half of the patients. Average coverage was 92.6% in the standard plan and 99.8% in the plan accounting for skin involvement (p=0.008).

Conclusions: The role of pre-chemotherapy radiation planning should be evaluated in a larger scale trial since we have shown that a majority of patients have initial disease that is underdosed using standard radiation borders. While it is unclear how this may alter local recurrence rates, this study highlights the importance of a multidisciplinary approach and evaluation at initial presentation.
Title: Are different therapeutic approaches required after skin and nipple sparing mastectomies for locoregional control? A single institution's experience

Hopkins ZH H, Frandsen J, Poruk KE E, Agarwal J and Poppe MM M. Huntsman Cancer Hospital, Salt Lake City, UT; Johns Hopkins Hospital, Baltimore, MD and University of Utah Hospital, Salt Lake City, UT.

Body: Introduction
Nipple sparing (NSM) and skin sparing (SSM) mastectomies are gaining popularity. These procedures leave breast tissue at the skin/breast interface with the intent to better cosmesis. However, the impact of NSM versus SSM on risk of local recurrence in the remaining breast tissue is not well characterized, nor is the effect of post mastectomy radiotherapy (PMRT) in these patients.

Methods
A single institution retrospective study was conducted on women treated with NSM or SSM from 2005 to 2011 with follow up through 2015. Chest wall and chest wall or axillary recurrence were assessed. Factors associated with recurrence were examined. Kaplan Meier estimates and Cox proportional hazards models were used to analyze chest wall recurrence (CWR) and chest wall or axillary recurrence (CWAR), with CWAR as the primary outcome variable.

Results
This analysis identified 181 women who underwent a SSM (n=103, 58 (56%) with PMRT) or NSM (n=78, 35 (45%) with PMRT). Women undergoing SSM were older (56.0 ± 13.6 years, mean ± SD) than NSM (44.6 ± 11.3, p <0.0001) while follow-up times were similar (4.91 ± 0.43 and 5.43 ± 0.27 respectively, p = 0.15). Women undergoing PMRT were younger (49.2 ± 13.6 vs 53.1 ± 13.9 years, p = 0.008) but more likely to present with lymphovascular space invasion (LVSI)(42% vs 16%, p = 0.0003 by Chi-square), and were more likely to receive chemotherapy (83% vs 47%, p <0.0001). The majority of women (62%) in the group not receiving PMRT had stage I disease, and 79% were node negative. For those undergoing PMRT, 83% were stage II or III, and 69% were node positive (p <0.0001 for both differences). Despite the higher apparent risk of the PMRT group, the total number of chest wall or axillary recurrences was similar (8 in PMRT, 6 in no PMRT). Event-free survival for CWAR at 5 years was 92% for PMRT and 96% for no PMRT (p=0.42) and at 7.5 years, 85% and 84% respectively (p=0.42). In univariate Cox regression among all patients, age was the strongest predictor of CWAR (HR = 1.103 per year of age, 95% CI 1.053-1.154, p<0.0001). CWAR occurred in 2.6 % of NSM patients as compared with 11.8% of SSM patients (p=0.025 by Fisher’s exact test). SSM versus NSM was associated with increased hazzard for CWAR with HR = 4.6 (95% CI 1.03-21, p=0.046) on univariate analysis. However, this apparent risk became non-significant (HR = 2.24, 95% CI 0.48 – 10.5) with adjustment for age. Other variables associated with CWAR on univariate analysis included receipt of chemotherapy (HR = 0.28, 0.09-0.86, p=0.027) and estrogen receptor positive status (HR = 0.34, 0.12-0.98, p=0.046) but these also became non-significant with adjustment for age.

In multivariate Cox regression analysis, use of PMRT was associated with a non-significant higher risk of CWAR (HR = 1.45, 0.33-6.4, p=0.63 ) adjusting for age, LVSI, mastectomy type, stage, and ER status.

Conclusions
The risk of a chest wall or axillary recurrence for early stage breast cancer after a SSM or NSM appears to be low at five years. Radiation can likely be omitted in this group. Furthermore, despite presenting with more advanced disease, women who underwent PMRT experienced excellent locoregional control. Further research is needed on this topic.
Title: Neoadjuvant radiochemotherapy in breast cancer- A safe and effect method for patients

Matuschek C, Boelke E, Budach W, Audretsch W, Wollandt S, Speer V and Nestle-Krämling C. Heinrich Heine University, Dusseldorf, NRW, Germany; Sana Hospital Dusseldorf, Dusseldorf, NRW, Germany and Marienhospital, Dusseldorf, NRW, Germany.

Body: BACKGROUND:
Neoadvant radiochemotherapy (NRT-CHT) is the standard of care for many solid tumors. It could be also an alternative option for treating patients with locally advanced non inflammatory breast cancer (LABC). Surgeons are afraid of wound healing problems and fear bad cosmetic results. The purpose of this investigation was to find out if there are any acute or late side effects in breast conserving and mastectomy patients after NRT-CHX.

PATIENTS AND METHODS:
From 1991 to 1998 a total of 315 LABC patients (cT1-cT4/cN0-N1) were treated with NRT-CHX. Preoperative radiotherapy (RT) consisted of external beam radiation therapy (EBRT) of 50 Gy (5 × 2 Gy/week) to the breast and the supra-/infraclavicular lymph nodes combined with a consecutive electron boost in 214 cases or - in case of breast conservation - a 10-Gy interstitial boost with (192)Ir afterloading before EBRT. Chemotherapy was given prior to RT in 192 patients, and concomitantly in 113; 10 patients received no chemotherapy. Also we investigated the acute side effects in 10 patients with NRT-CHX who were treated with this method from 2012-2015.

The cosmetic outcome was assessed by patient questionnaire, panel evaluation, and breast retraction assessment (BRA). Quality-of-life was investigated by EORTC QLQ-C30 and BR23 and acute and late radiation side effects by LENT/SOMA scale.

RESULTS:
The long term results of 64 patients after breast conserving surgery and 152 patients after mastectomy were available. Most patients rated their overall cosmetics as excellent or good (94% breast conserving, 55.8% mastectomy). Patient and panel ratings on all cosmetic outcomes were similar between the two groups. After a follow up of 14-23 years we did not detect any grade III or IV fibrosis in any of our groups. The median BRA score after breast conserving surgery was 2.9 and the over-all quality of life (QLQ-C30) was rated “excellent” or good in 82%. Furthermore we did not detect any grade 3 or 4 acute side effects in our 10 patients who were recently treated with NRT-CHX.

CONCLUSION:
NRT-CHX is safe method and it is not associated with severe grade 3 or 4 acute or late side effects.
Title: Overall survival in triple-negative breast cancers - Prognostic influence of type of surgery and adjuvant radiotherapy: A systematic review and meta-analysis

O'Rorke MA A, Murray LJ J and Bhoo Pathy N. Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom and Julius Centre University Of Malaya, Kuala Lumpur, Lembah Pantai, Malaysia.

Body: BACKGROUND: Triple negative breast cancers (TNBC) are an aggressive, poorer prognostic tumour subtype which are negative for oestrogen, progesterone and human epidermal growth factor receptor 2 (HER-2) expression. Whilst the role of radiotherapy (RT) following breast conserving therapy (BCT) is widely accepted, there is no agreed consensus on the use of post-mastectomy RT, particularly in early-stage TNBC patients.

METHODS: Four electronic databases were searched from their inception to April 2015 including PubMed, MEDLINE, EMBASE and web of science. Observational studies investigating overall survival and locoregional/distant recurrence outcomes in women with TNBC according to type of surgery and radiotherapy receipt were included. Authors were contacted directly for further data. A random effects model was used to pool study effect estimates using mastectomy (MT) only patients as the reference group.

RESULTS: 8 studies including data from 3,456 patients were included. The median age and duration of follow-up was 54 years and 3.6 years respectively. The adjusted pooled hazard ratio (HR) and 95% confidence interval (CI) for overall survival comparing BCT and MT+RT to MT only was 0.52 (95% CI 0.31, 0.87; I²= 70.9%) and HR 0.60 (95% CI 0.38, 0.96; I²=41.5%) respectively. In subgroup analysis of MT+RT vs. MT alone, there was no significant interaction with early (T1-2, N0-1) vs. late stage (T3-4, N2-3) disease p=0.773 or age at diagnosis (<40, 40-64, ≥65 years) p=0.320. 5 studies evaluated local regional recurrence. Comparing BCT and MT+RT to MT only, the pooled HR was 0.61 (95% CI 0.41, 0.92) and HR 0.83 (95% CI 0.55, 1.26) respectively, with low heterogeneity detected (I² 0.0%). 4 studies assessed distant metastases. The pooled HR comparing BCT and MT+RT to MT only was 0.88 (95% CI 0.62, 1.23; I²= 0.0%) and HR 1.95 (95% CI 0.85, 4.49; I²= 75.1%) respectively.

CONCLUSIONS: The addition of adjuvant radiotherapy appears to favourably impact overall survival, irrespective of the type of surgery received. BCT may reduce the risk of locoregional recurrence in comparison to MT only. The small number of contributing studies and often high study heterogeneity suggest cautious interpretation of the findings. Further epidemiological studies or pooled analyses of individual patient data are warranted.
**Title:** One-year follow-up results of a multi-center trial of intra-operative radiation therapy using electronic brachytherapy at the time of breast conservation surgery for early stage breast cancer

Syed AMN, Chang H, Schwartzberg BS S, Bremner AK K, Lopez-Penalver C, Coomer C, Boylan S, Chakravarthy A, Vito CA A, Bhatnagar A, Proulx GM M, Dooley WC C, Davis M, Golder SL L, Ivanov O, Fernandez K and Rahman S. Todd Cancer Institute, Long Beach Memorial Medical Center, Long Beach, CA; David Geffen School of Medicine at UCLA, Los Angeles, CA; Sarah Cancer Research Institute at Rose Medical Center, Denver, CO; Breastlink, Murietta, CA; Doctors Hospital, Miami, FL; Staten Island University Hospital, Staten Island, NY; Sentara Northern Virginia, Woodbridge, VA; Vanderbilt University, Nashville, TN; City of Hope National Medical Center, Duarte, CA; Cancer Treatment Services, Casa Grande, AZ; Exeter Hospital, Exeter, NH; Oklahoma University, Oklahoma City, OK; Swedish Medical Center, Englewood, CO; Shannon Cannon Cancer Center at Parkridge Medical Center, Chattanooga, TN; Florida Hospital, Orlando, FL; MedStar Health, Baltimore, MD and Diablo Valley Oncology Hematology Medical Group, Pleasant Hill, CA.

**Body:** Objectives: To describe observations of one-year follow-up of subjects treated on a multi-center, non-randomized study with a single fraction of intra-operative radiation therapy (IORT) using the Xoft® Axxent® Electronic Brachytherapy System® (eBx®) immediately following surgical resection of early stage breast cancer.

Methods: Two-hundred forty three (243) subjects were treated at seventeen (17) US hospitals. Upon meeting the inclusion/exclusion criteria, patients underwent partial mastectomy, placement of a balloon applicator suitable to the surgical bed in the lumpectomy cavity and inflated with saline (30 – 75 cc). The skin was temporarily closed over the balloon and ultrasound examination performed to confirm that the balloon surface-to-skin distance was > 1.0 cm. A single fraction of intra-operative radiation therapy was delivered to the lumpectomy cavity using the Xoft System. The prescribed dose was 20 Gy at the balloon applicator surface, and the mean treatment time was 10.2 minutes. After treatment, the balloon was deflated and removed, and skin sutured.

Results: Two-hundred forty two (242) subjects received the prescribed dose of 20 Gy; one subject received 21 Gy. Eighteen (18) subjects were removed from the primary analysis post-IORT due to positive surgical margins (N=2), positive sentinel lymph nodes (N=13), or balloon surface-to-skin distance < 1 cm (N=3). However, these eighteen subjects will continue to be followed for the duration of this 10-year study. The mean follow-up for the two-hundred twenty five evaluable subjects is 494 days (range 300-465 days). The mean patient age was 65 years (41-89). Forty-nine subjects (21.8%) had ductal carcinoma in situ, one-hundred seventy one (76%) had invasive ductal carcinoma, and five (2.2%) had unknown histology. The DCIS nuclear grade was evenly distributed between high (N=18) and low/intermediate (N=23); 5 were unknown. Invasive cancer was Grade 1-2 in 142/171 cases. Two-hundred twelve subjects (94.2%) had T1 lesions, eight (3.6%) had T2 lesions, and five (2.2%) were unknown. The mean tumor size was 10.6 mm ± 6.4 mm. At the time of the last subject visit, 49/318 reported adverse events were Grade 2 or higher, and only 1/100 had serious side effects, i.e. infection. One patient died of aortic aneurism and two developed secondary malignancies, i.e. ovarian cancer and chronic lymphocytic leukemia. The most frequent side effects were seroma (12.5%), erythema (9.1%), and induration (7.5%). Cosmesis was excellent to good in 95% of cases.

Conclusions: IORT using the Xoft System as part of the conservative treatment of breast cancer is safe, with low morbidity. Early results from this multi-center trial demonstrate this short, convenient course of radiation therapy for select patients with early stage breast cancer has excellent-to-good cosmetic results and a low rate of low-grade adverse events.
Title: Incidence of internal mammary node, sternum, and manubrium failure as detected by FDG-18 PET/CT


Body: Introduction:
Elective radiotherapy to the internal mammary (IM) lymph nodes remains an ongoing subject of debate. While the incidence of occult IM involvement on extended mastectomy ranges from 15-65%, reported rates of IM failure are substantially lower (approximately 1%). Interest in this subject has resurfaced recently as randomized trials have shown a survival benefit to adjuvant regional node irradiation including the IM chain. The mechanism by which extended field radiotherapy leads to improved systemic outcomes has not been clearly demonstrated and the degree of benefit directly attributable to IM irradiation remains to be seen. We hypothesized that the IM lymphatic chain may provide a direct route for tumor cell dissemination into the the sternum or manubrium. As such, sternal metastases may be a manifestation of IM involvement rather than true hematogenous metastases. We sought to better elucidate patterns of failure by evaluating the incidence and timing of IM, sternal, or manubrial involvement identified by PET/CT imaging following diagnosis of metastatic breast cancer.

Patients and Methods:
Between 2007 and 2014, 96 patients with invasive breast cancer were found to have metastatic disease as diagnosed on FDG-18 PET/CT. Site of recurrence was scored as breast/chest wall, axilla/supraclav, IM chain, sternum/manubrium, or distant. IM or sternum/manubrium failure was scored as isolated (occurring without distant metastatic disease), synchronous (involved at initial diagnosis of distant metastatic disease), or metachronous (involved at any time after diagnosis of metastatic disease).

Results:
Isolated IM failures were observed in 3.1% of patients while isolated sternum/manubrium failures were recorded in 7.3% of patients. Isolated involvement of the sternum/manubrium or IM nodes occurred in 11.4%. The rate of synchronous IM failure was 11.4% with the rate of metachronous failure being 13.5%. The rate of synchronous sternum/manubrium failure was 17.7% with the rate of metachronous failure being 23.9%. The incidence of sternum/manubrium or IM involvement at the initial diagnosis of distant metastatic disease was 29.2% with the rate of involvement at any point increasing to 36.5%.

Conclusion:
The rate of internal mammary node failure by PET/CT at the time of metastatic diagnosis is higher than the incidence reported in previous trials. This discrepancy is likely due increased sensitivity of PET/CT and the difficulty of accurately assessing this region once patients have been found to have metastatic disease. Interestingly, sternum and manubrium were more often the first site of recurrence than the IM nodes. This could be due to a tropism of hematogenous metastases for these bones or could represent direct tumor cell dissemination from the internal mammary chain. This is of special interest as this region is incidentally included in the radiotherapy fields when targeting the IM nodes. Overall, our findings suggest that historic patterns of failure studies may underestimate the local benefit of internal mammary node radiotherapy.
Body: Background: Radiation is the current choice treatment for non-operable metastatic breast-brain cancer. When cancer lesions are located in sensitive areas like the brain or have excessive amounts of metastatic sites, radiation usually proves to be a more viable option than excision. Ionizing (X-ray and gamma) radiation is non-selective and affects all the tissue it penetrates. In order to concentrate the dose on tumors, high energy radiation from multiple directions is typically used, reaching the highest dose where the radiation crosses. This type of multiple angle treatment minimizes the dose to normal tissue by increasing overall normal tissue irradiation. The objective is to achieve sufficient radiation in the tumor tissue to cause the DNA strands to break and to disrupt the reproduction and maintenance of cancer cells while keeping the damage to normal tissue in a reasonable range for tissue preservation.

Metal nanoparticles have shown promising results for reinforcing the radiation dose effect. High atomic number (Z) elements absorb a greater amount of radiation because the higher density raises the probability of interaction. The metal nanoparticles interact with the energy of the ionizing radiation by either scattering or absorbing, or accumulating the energy, thus increasing the number of DNA strand breaks in the nucleus of cells.

Methods: Four breast cancer cell lines (BT-474, MDA-231, BT-549 and MCF-7) were incubated with 1-2 nm platinum nanoparticles (0-1000 µg/mL) produced with a cysteine coating. 24 hours later cells were exposed to 2 Gy radiation with a C-arm (Toshiba Infinix VF-i/SP) using 125 KV to deliver a spectrum of KeV low energy X-rays. After 24 hours the cells were washed and analyzed using a bioluminescence assay to assess cell proliferation based on ATP production.

Results: Of the four cell lines tested the BT-474 and BT-549 demonstrated limited reduction in cell proliferation at up to the highest treatment concentration 1000 µg/mL with no radiation exposure. As a result of the limited toxicity of the platinum nanoparticles the effect from increased radiation can be more readily observe when 2 Gy radiation is added resulting a in platinum nanoparticle dose dependent decrease in proliferation in the BT-474 cell line.

<table>
<thead>
<tr>
<th>Concentration of Platinum Nanoparticles (µg/mL)</th>
<th>0</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-231</td>
<td>0.995±0.012</td>
<td>0.974±0.013</td>
<td>0.979±0.014</td>
<td>0.777±0.014</td>
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<tr>
<td>BT-549</td>
<td>1.000±0.013</td>
<td>1.003±0.017</td>
<td>0.969±0.017</td>
<td>0.894±0.009</td>
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<tr>
<td>MCF-7</td>
<td>0.960±0.015</td>
<td>0.927±0.022</td>
<td>0.851±0.022</td>
<td>0.769±0.032</td>
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</tr>
<tr>
<td>BT-474</td>
<td>0.961±0.029</td>
<td>0.957±0.033</td>
<td>0.965±0.063</td>
<td>0.985±0.065</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Indexed values for cell proliferation for the BT-474 cell

<table>
<thead>
<tr>
<th>Concentration of Platinum Nanoparticles (µg/mL)</th>
<th>0</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>1.000±0.024</td>
<td>0.957±0.033</td>
<td>0.965±0.063</td>
<td>0.985±0.065</td>
<td></td>
</tr>
<tr>
<td>2 Gy</td>
<td>1.027±0.038</td>
<td>0.908±0.034</td>
<td>0.870±0.031</td>
<td>0.799±0.037</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Indexed values for cell proliferation for the BT-474 cell line 0 and 2 Gy radiation doses, 6 averages. * Student T-TEST P<0.05
Conclusions: At moderate doses of low energy radiation, a reduction in cell proliferation can be detected. This data supports follow-up experiments to add a targeting protein to facilitate uptake by cancer cells based on cell receptor expression. Experiments are current being done to utilize the HER2⁺ cell receptor upregulation to increase internalization of the particles to achieve a greater effect.
Title: Lung dose analysis in loco-regional hypofractionated breast radiotherapy

Bahadur YA A, Constantinescu C, Eltaher MM M and Attar MA A. King Abdulaziz University Hospital, Jeddah, Saudi Arabia; King Faisal Specialist Hospital & Research Center, Jeddah, Saudi Arabia and National Cancer Institute, Cairo, Egypt.

Body: Objective

Hypofractionated radiotherapy (HFRT) and minimizing toxicity to organs at risks are recent trends in breast carcinomas (BC) therapy.

To date, there are no clear recommended dose-volume constraints for the ipsilateral lung. However, a recent meta-analysis showed that the strongest Dose-Volume-Histogram (DVH) parameters associated with ipsilateral lung radiation-induced pneumonitis are percentage of lung volume receiving 20 Gy or more (V_{20Gy}) and mean lung dose (MLD), and recommended to keep the V_{20Gy} < 24% and MLD < 15 Gy.

We are reporting the ipsilateral lung dosimetry data of our patients treated with breast loco-regional HFRT, seeking for possible correlations between lung dose, patient characteristics, and treatment delivery parameters.

Methods

One-hundred-fifty patients treated for BC after breast conservative surgery or mastectomy by HFRT were identified and their CT-based 3D-CRT treatment plans retrospectively reviewed. All patients received 42.4 Gy in 16 fractions by tangential and supra-clavicular fields. Lymph nodes were contoured according to RTOG consensus guidelines. Lungs were delineated using threshold auto-contouring. All treatment plans used field-in-field forward planning optimization for 6 MV, 18 MV or mixed beam energies.

Ipsilateral lung dosimetric data, such as V_{20Gy} and mean lung dose were recorded. Correlations between lung dose, patient characteristics (lung volume, chest wall separation, depth and coverage of ALN and SCLN), and treatment delivery parameters (beam energy, breast board angle, posterior supra-clavicular field)) were assessed by a logistic regression test and a p value of < 0.05 was considered significant.

Results

We identified 65 (43%) left-sided and 85 (57%) right-sided BC patients; 46 (30%) had conservative surgery and 104 (70%) had mastectomy.

A weak, but statistically significant correlation was found between lung dose and lung volume (R^2 = 0.027, p = 0.043).

Table 1. Ipsilateral lung dosimetric data (n=150).

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>V20Gy (%)</th>
<th>MLD (Gy)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean ± SD</td>
<td>range</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>Ipsilateral lung dose</td>
<td></td>
<td>24.6±4.1</td>
<td>11.-33.</td>
<td>11.9±1.6</td>
</tr>
<tr>
<td>Energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 MV</td>
<td>24 (16%)</td>
<td>27.8±2.3</td>
<td>25.5-32.5</td>
<td>13.±1.1</td>
</tr>
<tr>
<td>6&amp;18 MV</td>
<td>81 (54%)</td>
<td>25.4±3.1</td>
<td>18.5-33.</td>
<td>12.2±1.2</td>
</tr>
<tr>
<td>18 MV</td>
<td>45 (30%)</td>
<td>21.2±4.</td>
<td>11.-27.</td>
<td>10.8±1.9</td>
</tr>
<tr>
<td>Breast board angle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 deg</td>
<td>9 (6%)</td>
<td>22.9±3.9</td>
<td>11.-28.</td>
<td>11.2±1.5</td>
</tr>
<tr>
<td>15 deg</td>
<td>132 (88%)</td>
<td>24.5±3.7</td>
<td>11.5-33.</td>
<td>11.9±1.5</td>
</tr>
<tr>
<td>20 deg</td>
<td>9 (6%)</td>
<td>25.2±7.5</td>
<td>17.-32.5</td>
<td>12.1±3.1</td>
</tr>
<tr>
<td>Posterior supra-clavicular field</td>
<td>yes 116 (77%)</td>
<td>24.3±4.1</td>
<td>11.-33.</td>
<td>11.9±1.6</td>
</tr>
</tbody>
</table>
The lung dose assessed by $V_{200\text{Gy}}$ was decreasing with patient separation and depth of ALN and SCLN ($p < 0.0001$). Lung $V_{200\text{Gy}}$ was significantly increasing with ALN ($p = 0.001$) and SCLN ($p = 0.003$) coverage measured by $V_{95\%}$. The addition of a posterior supra-clavicular field had no effect on the lung dose. Due to the small number of patients treated on breast board with 10 and 20 deg angles, analysis of the correlation between ipsilateral lung dose and board angle was not performed.

**Conclusion**
Acceptable ipsilateral lung doses can be achieved in hypofractionated breast 3D-CRT with tangential and supra-clavicular fields. Our data suggest that the use of high energy photon beams and low breast board angulation can significantly reduce the lung dose.
Title: Effect of hypofractionated radiotherapy for the treatment of early stage breast cancer: Meta-analysis on efficacy and safety

Nazário ACP Celso Pinto, Andrade TRdM Raquel de Moraes, Segreto HRC Regina Comodoro, Segreto RA Araújo and Fonseca MCM Cunio Machado. UNIFESP, São Paulo, Brazil.

Body: Objectives: To evaluate the short and long term effect of hypofractionated radiotherapy on efficacy and safety in women with early stage breast cancer that underwent breast-conserving surgery. Methods: We searched in Embase, Medline, Cochrane Library, and Lilacs for randomized controlled trials comparing conventional unconventional fractioning. Two reviewers obtained data independently and disagreements were solved by consensus. We measured the effect of fractioning within 5 years and after 5 years of treatment by means of the relative risk (RR) obtained from a meta-analytic random effects model comparing unconventional and conventional fractioning in relation to local, loco-regional and distant recurrence, mortality, disease-free survival (number of patients with disease-related events), ischemic heart disease, rib fractures and lung fibrosis. Results: We included five medium/ high quality studies totalizing 7,802 women. Unconventional fractioning does not change within 5 years and after 5 years, respectively: (1) local recurrence RR 0.90 (95% CI 0.68 to 1.18; P = 0.44) and RR 0.98 (95% CI 0.83 to 1.17; P = 0.86); (2) locoregional recurrence RR 0.99 (95% CI 0.71 to 1.36; P = 0.84) and RR 0.97 (95% CI 0.77 to 1.23; P = 0.79); (3) distant recurrence (RR) 1.04 (95% CI 0.73 to 1.46; P = 0.84) and RR 1.02 (95% CI 0.79 to 1.32; P = 0.88); (4) mortality RR 0.89 (95% CI 0.77 to 1.05; P = 0.16) and RR 0.96 (IC 95% from 0.89 to 1.08; P = 0.48); (5) disease-free survival RR 0.96 (95% CI 0.78 to 1.18; P = 0.69) and RR 0.96 (95% CI 0.84 to 1.09, P = 0, 49); (6) cardiac ischemia (radiotherapy in both breasts) RR 0.73 (95% CI 0.34 to 1.57; P = 0.42) and RR 0.61 (95% CI 0.33 to 1.15; P = 0.13); (7) cardiac ischemia (radiotherapy only in the left breast) RR de 0.84 (95% CI; 0.21 to 3,.7; P= 0.80) and RR de 0.72 (95% CI, 0.28 to 1.86; P= 0.49); (8) rib fractures RR 1.02 (95% CI 0.25 to 4.20; P = 0.98) and RR 1.08 (95% CI 0.26 to 4.53; P = 0, 91); (9) pulmonary fibrosis RR 2.42 (95% CI 0.50 to 11.71, P = 0.27) and RR 3.16 (95% CI 0.89 to 11.21, P = 0.07). In a subanalysis, removing the fractioning not recommended by ASTRO, unconventional fractioning increases the occurrence of pulmonary fibrosis after 5 years RR 4.17 (95% CI, 1.05 to 16.56; P = 0.04). In another sub-analysis to verify the possible influence of tumor-bed radiation boost we observed that unconventional fractioning increases disease-free survival within 5 years RR 0.82 (95% CI, 0.69 to 0.97; P = 0.02), decreases distant recurrence after 5 years of the treatment RR 0.80 (95% CI, 0.66 to .96; P = 0.02) but increases the occurrence of pulmonary fibrosis after 5 years RR 4.17 (95% CI, 1.05 to 16.56; P = 0.04). Conclusion: Hypofractioning does not affect, neither in the short nor in the long term, the occurrence of local, loco-regional and distant recurrence, disease-free survival and mortality, of ischemic heart disease, pulmonary fibrosis and ribs fracture in women with early stage breast cancer that underwent breast-conserving surgery.
Title: Hydrosorb® versus control (water based spray) in the management of radio-induced skin toxicity: Results of multicentre controlled randomized trial


Purpose: To report the results of a randomised study comparing the efficacy of Hydrosorb® versus control (water based spray) in the topical treatment of grade 1 and 2 radiation dermatitis in population of patients treated for early stage breast cancer (BC) with normo fractionated radiotherapy (RT).

Patients and Methods: Breast cancer patients with grade 1-2 radio-induced dermatitis during normo fractionated postoperative radiotherapy were eligible (according to the CTCAE v3 scale). They were randomised to receive either Hydrosorb® (A) or water based spray (B). The primary endpoint was local treatment failure defined as interruption of radiotherapy because of skin radiotoxicity or and/or change of local cares for skin alteration. Secondary endpoints were the evaluation of skin colorimetry, pain, and quality of life. Pain was assessed according to two classes with a VAS cut-off of 2.

Results: Two-hundred seventy eight patients were enrolled (A = 142, B = 136). There were 186 successfully treated patients (82 in Hydrosorb® arm, and 74 in the control arm). There were 60 “failures” in the Hydrosorb® arm, and 62 in the control arm (p = 0.72), but mostly without interruption of the radiotherapy. Twenty-four patients stopped the radiotherapy treatment for local cares (16 in Hydrosorb®, arm and 8 in control arm). No risk factors were associated with failure to local treatment. The average absolute reduction of colorimetric levels between day 28 and day 0 was 4 in the Hydrosorb®, and 4.2 in the water spray groups, respectively (p = 0.36). Forty-eight patients in the Hydrosorb® arm had a VAS > 2 versus 51 patients in the placebo arm, i.e. 34% and 38%, respectively (p = 0.45). A significant reduction of pain was observed on D7 (p = 0.04) and D21 (p = 0.01) in the Hydrosorb® arm. Sixty patients in the Hydrosorb® arm and 55 patients in the placebo arm had moderately to severely altered quality of life (p = 0.76).

Conclusions: The present study showed no significant difference between Hydrosorb® and simple water spray in the treatment of acute radio-induced dermatitis even if there was a trend to an improvement in pain at the first weeks after the treatment. Systematic prevention measures and modern breast cancer radiotherapy techniques now allow excellent tolerability, but the place of topical treatment to optimize this tolerability has yet to be defined.
**Title:** Can radiation dosimetric parameters explain severe skin reaction during adjuvant whole breast irradiation applying field-in-field technique?

Yoon WS, Lee NK, Lee JA, Yang DS, Kim CY, Son GS and Chang YW. Ansan Hospital, Korea University, Ansan, Gyeonggi-do, Korea and Ansan Hospital, Korea University, Ansan, Gyeonggi-do, Korea.

**Body:**

**Aim:** Although modern radiotherapy such as field-in-field technique decreased the radiation toxicity, skin reaction is still frequent and main problem during adjuvant whole breast irradiation. Our study investigated various radiation dosimetric and clinical parameters as the risk factors of severe skin reaction.

**Methods:** From January 2012 to December 2014, total 219 patients with breast conserving surgery and adjuvant whole breast irradiation were retrospectively reviewed. All patients took both whole breast irradiation (50 Gy/25 fractions) and boost to the tumor bed (10 - 15 Gy). Skin reaction was measured by comparing the photography of radiation field between the first day of whole breast irradiation and boost therapy. For each axilla and inferior fold, the intensity (score 1 to 5) and extent (score 0 to 1) of erythema were recorded and summed. The severe skin reaction was defined as score 5 or 6. The relations of various radiation dosimetric parameters for radiotherapy planning, personal breast characteristics and clinical factors to severe skin reaction were evaluated using the Logistic regression tests.

**Results:** Total 75 (34%) and 57 (26%) patients showed the severe skin reaction to axilla and inferior fold, respectively. The variables of P < 0.2 in univariate analyses including age, the body mass index, the breast height, the $V_{100}$, the calculated point dose in radiation planning system, the breast separation, the field size, and the gradient of inferior fold entered the multivariate analyses. Age ($P=0.013$ (OR = 0.950, 95% CI 0.913 - 0.989)), the body mass index ($P = 0.015$ (OR = 1.123, 95% CI 1.023 - 1.233)), the calculated axilla point dose ($P = 0.091$ (OR = 1.064, 95% CI 0.990 - 1.142)), and the gradient of inferior fold ($P = 0.073$ (OR = 1.029, 95% CI 0.997 - 1.063)) were risk factors for severe axilla skin reaction, whereas age ($P = 0.018$ (OR = 0.948, 95% CI 0.907 - 0.991)) and the $V_{100}$ ($P < 0.001$ (OR = 1.005, 95% CI 1.003 - 1.007)) were for severe inferior fold skin reaction.

**Conclusion:** In addition to clinical factor and personal breast characteristics, the radiation dosimetric parameters such as calculated point dose and $V_{100}$ could be another predictive factors of severe skin reaction.
Title: Radiation-induced CD8 T-lymphocyte apoptosis as a predictor of late toxicity after radiotherapy: Results of the prospective multicenter French trial

Azria D, Riou O, Castan F, Coelho M, Nguyen TD, Peignaux K, Lemanski C, Lagrange J-L, Kirova Y, Lartigau E, Belkacemi Y, Bourgier C, Noel G, Clippe S, Mornex F, Hennequin C, Kramar A, Pêlegrin A and Ozsahin M. Institut Cancer Montpellier, Montpellier, France; Institut Jean Godinot, Reims, France; Centre GF Leclerc, Dijon, France; AP-HP Henri Mondor, Créteil, France; Institut Curie, Paris, France; Centre Oscar Lambret, Lille, France; Institut Gustave Roussy, Villejuif, France; Centre Paul Strauss, Strasbourg, France; Centre Marie Curie, Valence, France; Centre Hospitalier Lyon Sud, Pierre-Bénite, France; Hopital Saint-Louis, Paris, France; Institut de Recherche en Cancérologie de Montpellier, Montpellier, France and Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Body: Purpose/Objective(s): We and others showed in retrospective and monocentric studies that radiation-induced CD8-lymphocyte apoptosis (RILA) can significantly predict differences in late toxicity between individuals and can be used as a rapid screening for potential hyper-reactive patients to radiation therapy (RT). We present here the clinical results of the prospective multicenter French trial (NCT00893035) evaluating the predictive role of RILA as a predictor of late effects after RT.

Materials/Methods: A total of 502 consenting breast-cancer patients (pts) treated by conservative surgery and adjuvant RT were included by 10 French centers. Lymphocytes apoptosis was assessed before RT by associated condensation of DNA. The incidence of late toxicities was obtained using CTCAEv3.0 grading scale. Complication-free survival (CFS) and complication-relapse-free survival (CRFS) curves were estimated by the Kaplan-Meier method. The log-rank test was used to identify significant categorical variables for each of the survival curves. Cox model was used for multivariate analysis.

Results: Four hundred and fifty-four pts (90.4%) were included in the final analysis (clinical, biological and dosimetric data available). One hundred and eight pts (24%) received both whole breast (WB) and nodal irradiation (NI). A boost dose of 10-16 Gy was given in 448 pts (99%). Adjuvant hormonotherapy (tamoxifen or aromatase inhibitor) was delivered to 346 pts (76%).

Three categories of absolute change in the percent CD8 cells in apoptosis before and after exposure to 8-Gy in vitro RT were constructed around the 33 percent quantiles, <12%, 12-20%, and >20%. In a median follow-up period of 38.5 months, grade 2 and 3 late fibrosis was observed in 54 (12%) and 3 (0.7%) pts, respectively. A decreased percentage of grade 2 or more late toxicity was observed for increasing values of CD8 apoptosis (p=0.001). No grade 3 late toxicity was observed for patients with RILA ≥12%. The 3-year CFS rates were significantly lower for patients with low levels of CD8 radiation-induced apoptosis, 79% (95% confidence interval [CI]: 72–85%), 90% (95% CI: 84–94%), and 93% (95% CI: 87–96%) for CD8 <12%, 12–20%, and >20%, respectively (p=0.001). Similar results were observed for the CRFS rates (p<0.001). In multivariate analyses, prognostic factors for CFS were RILA<12% (p=0.001), smoking history (p<0.001), and adjuvant hormonal treatment (p=0.008). Negative predictive value for grade 2 or more toxicity was equal to 83% for CD8 >20% and positive predictive value was equal to 22% for CD8 <12% where the overall prevalence of grade 2 or more late side effects was estimated at 14%.

Conclusion: RILA significantly predicts differences in radiation-induced late toxicity between individuals. This study validates the use of RILA as a rapid screening for potential hyper-reactive pts to radiotherapy.
Impact of young age on local control after partial-breast irradiation in early-stage breast cancer


Background: Although the rate of breast-conserving surgery (BCS) increased, the receipt of adjuvant radiotherapy after BCS decreased especially for young patients. The long-term daily visit to radiation facilities must be the most relevant barriers to receiving radiation therapy. The use of partial-breast irradiation (PBI) is considered an alternative option. However, there are limited data to be seen how safe PBI is as an option of adjuvant radiation therapy in young patients compared with whole-breast irradiation (WBI). In this report, we reviewed our single-institution experience with PBI compared with WBI in young breast cancer patients.

Methods: We evaluated 443 consecutive patients with T≤3-cm N0–1 breast cancer who underwent breast-conserving therapy (BCT) between November 2007 and May 2015. 268 patients received PBI using interstitial multicatheter brachytherapy. The interstitial brachytherapy was performed in an accelerated fashion with a dose of 32 Gy in eight fractions over 5-6 days. 185 patients received WBI with a dose of 50 Gy in fractions of 2 Gy. Patients with risk factors such as positive margins and young age received a subsequent 10 Gy boost to the tumor bed, and the regional nodal irradiation was added in patients with ≥ 4 positive nodes. Patients who underwent neoadjuvant chemotherapy were excluded from the analysis. Our primary objective was to assess outcome rates of ipsilateral breast tumor recurrence (IBTR), disease-free survival (DFS), and overall survival (OS), and compare the patterns of treatment failures between the cohorts.

Results: Patients aged <50 years with a minimum follow-up period of 6 months were selected for the analysis. Of those patients who could be completely followed, there were 95 women receiving PBI and 81 women receiving WBI. In PBI cohort, 4 patients also received WBI because of adverse histological features with positive nodes or positive margins by final pathology. Median follow-up was 4.0 years for PBI patients and 3.9 years for WBI patients. Median age was 43.9 years old for PBI and 42.1 years old for WBI cohort. Mean tumor size was equivalent for the cohorts (12 mm). Positive lymph nodes were seen more frequently in WBI cohort (9.5% and 29.6%, p < 0.05). There was no significant difference in the 3-year probability of disease-free survival (97.4% and 98.1% for PBI and WBI, respectively; p = 0.95). No breast cancer related death was observed. With our follow-up period, there were 5 IBTR (2.8%). Of these IBTRs, 4 were true recurrences (2 were in PBI and 2 were in WBI). There was 1 elsewhere recurrence in PBI cohort. The actual rate of IBTR was 3.2% and 2.5% in PBI and WBI, respectively (p = 0.64).

Conclusions: We observed equivalent IBTR rates between PBI and WBI cohorts in young breast cancer patients. If there are no differences in survival between the two radiotherapy regimens, PBI may be a better option than WBI after BCS in such a population. To our knowledge, this is the first report describing that the efficacy of PBI after BCS is comparable with WBI in young breast cancer patients in Asia. However, our data are limited by our short median follow-up with small number of patients. The application of PBI should still be carefully considered until mature Phase III trial data are available.
Title: The impact of everolimus on radiation-induced pulmonary fibrosis in wistar albino rats: Results of an experimental study

Eren MF Fuat, Ay Eren A, Yücel B, Elagöz S, Özugüen Y, Altun A, Kiliçkap S, Matsuno R and Bese N. Ministry of Health-Marmara University Pendik Education and Research Hospital Radiation Oncology Clinic, Istanbul, Turkey; Cumhuriyet University School of Medicine, Sivas, Turkey; Cumhuriyet University School of Medicine, Sivas, Turkey; Trakya University School of Medicine, Edirne, Turkey; Cumhuriyet University School of Medicine, Sivas, Turkey; Hacettepe University School of Medicine, Ankara, Turkey; University of California School of Medicine, San Diego, CA and Acibadem Maslak Hospital Breast Health Unit, Istanbul, Turkey.

Body: Objectives:
This study was performed to evaluate the effects of everolimus on pulmonary fibrosis, given concurrently or sequential with irradiation in rats.

Materials and Methods:
Forty female Wistar albino rats were randomized into five groups. The first group (Group 1: sham) was observed with identical conditions of the animals in other groups. The second group (Group 2) had only everolimus application. The third group (Group 3) had thoracic irradiation first and they had everolimus injection 22 hours after radiotherapy. The fourth group (Group 4) had irradiation to whole thoracic region in 2 hours of following everolimus administration. The last group (Group 5) had irradiation to whole thoracic region. As an end point the percentage of lung with fibrosis for each rat was quantified with image analysis of histological sections of the lung. Groups were compared using the linear regression method, Fisher's exact and chi-square tests were used to assess the association between treatment groups.

Results:
There was a statistically significant difference in the distribution of fibrosis scores among all groups (p=0.0022). Linear regression analyses revealed that all treatment groups showed an increased risk of lung fibrosis compared to control group. Group 2 (p=0.0020), group 3 (p<0.0001), group 4 (p<0.0001), and group 5 (p=0.0002). Chi-square test demonstrated that there was no statistically significant difference between the sequentially and concurrent treatment groups.

Conclusion:
This experimental study showed that of everolimus by itself may lead pulmonary fibrosis and addition to radiotherapy either sequentially or concomitantly increased the severity of the lung damage in rats.

Keywords: Radiotherapy, everolimus, rats, pulmonary fibrosis.
Title: Post-mastectomy radiotherapy for T3N0 breast cancers: A retrospective, multi-institution review

Frandsen JE E, Cannon G and Poppe M. University of Utah Huntsman Cancer Hospital, Salt Lake City, UT and Intermountain Medical Center, Murray, UT.

Body: Purpose
The role of post-mastectomy radiotherapy (PMRT) for node-negative breast cancers that are larger than five centimeters remains undefined. The purpose of this study was to report outcomes for women with T3N0 breast cancers treated with and without PMRT.

Methods
T3N0 breast cancers diagnosed between 1985 and 2014 were collected from the tumor registries of two large healthcare systems. For the purpose of our analysis, patients were divided into two groups: (1) women who received PMRT and (2) those who did not receive PMRT. Chi-square and students t-tests were used to compare baseline characteristics. Kaplan Meier estimates were used to analyze freedom from locoregional recurrence (FFLR), relapse free survival (RFS), and overall survival (OS).

Results
This analysis identified 93 women with T3N0 breast cancers. Of these, 53 received PMRT and 40 did not. Median follow up was 6.2 years and 5.3 years in the non-PMRT and PMRT cohorts, respectively. Women not undergoing PMRT were more likely to be diagnosed in the 1980s and 1990s than women receiving PMRT (p< 0.05). Adjuvant chemotherapy and endocrine therapy were less frequently given in patients not receiving PMRT (p<0.05), again associated with an earlier treatment era. There was a trend toward increased FFLR in the women receiving PMRT (p=0.15). FFLR in the PMRT cohort was 97.9% and 97.9% at five and ten years, respectively. For women not receiving PMRT, FFLR was 88.4% and 88.4% at five and ten years, respectively. RFS (p=0.66) and OS (p=0.48) were similar.

Conclusions
Women not receiving PMRT in our study had a locoregional failure rate of only 11.6% at ten years despite receiving inferior systemic treatment by current standards. Locoregional control after PMRT for T3N0 breast cancers was excellent with a 2.1% failure rate at ten years. Further research is needed to define PMRT indications for this patient population when receiving chemotherapy and endocrine therapy in line with current guidelines.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-12-22

**Title:** Reproducibility of automated coronary artery calcification scoring on radiotherapy treatment planning computed tomography scans of breast cancer patients


**Body:**

**Introduction:** Presence of coronary artery calcifications (CAC) is a major independent risk factor for cardiovascular (CV) disease. CAC can be visualized on CT scans. Most breast cancer patients planned for radiotherapy (RT) receive planning CT scans. These scans may provide a reliable estimate of a patients' CV risk. This study evaluated the feasibility and reproducibility of an automated algorithm for CAC scoring on RT planning CT scans of breast cancer patients.

**Methods:** This study was conducted within the Utrecht cohort for Multiple BReast cancer intErvention studies and Long-term evaLuAtion (UMBRELLA), and includes 562 breast cancer patients undergoing RT at University Medical Center Utrecht. Planning CT scans were performed using a 16-slice scanner (16 x 0.75 mm collimation, 3 mm thickness, 120 kVp, with or without breath hold (BH), without ECG synchronization). CAC were automatically scored using an algorithm developed with chest CT scans that considers lesions >130 Hounsfield units as CAC. CAC were identified using a supervised pattern recognition based on texture, size, and spatial features. To test validity of automated CAC scoring, manually scoring by an expert was performed in all scans with CAC (n = 80) and a random sample of scans without CAC (n = 83). Interscan reproducibility of automated CAC scoring was assessed in patients having two scans (n = 295). All scans with CAC score ≥1000 were manually checked and corrected if appropriate. Agatston calcification scores were analyzed continuously and categorically (0, 1-10, 11-100, 101-400, >400).

Agreement and reliability for categories were determined with proportional agreement (%) and linearly weighted kappa. Reliability of Agatston scores were assessed with Intraclass correlation coefficients (ICC).

**Results:** Of 562 patients, 129 (23%) patients had CAC scores > 0 with a mean of 93 (standard deviation: 166). Four patients had CAC scores ≥1000, which were erroneous and corrected. Of the 163 CT scans scored manually and automatically, 58 (36%) were performed with BH. Proportion of agreement was 79% (95% Confidence Interval (CI): 0.72-0.85) for all 163 scans: 88% (0.76-0.95) for 58 scans with BH and 74% (0.65-0.82) for 105 scans without. Proportion of agreement beyond chance was 0.80 (95% CI: 0.74-0.87) for all scans: 0.86 (0.77-0.96) with BH and 0.77 (0.684-0.853) without. Agatston score ICC was 0.86 (95% CI: 0.82-0.90) for all scans: 0.95 (0.91-0.97) with BH and 0.67 (0.55-0.76) without. For the interscan reproducibility (n = 295), the majority of patients (81%) had one scan with BH and one scan without. Proportion of agreement was 84% (95% CI: 0.79-0.88) and reliability was 0.61 (95% CI: 0.50-0.72). Agatston score ICC was 0.75 (95% CI: 0.69-0.80).

**Conclusion:** Automated CAC scoring on RT planning CT scans of breast cancer patients is feasible. Agreement with manually scored scans is high and higher in CT scans performed with BH. Interscan reproducibility is fair. Automated CAC scores ≥1000 should to be manually checked and corrected if necessary. Automated CAC scoring on RT planning CT scans of breast cancer patients is available for all patients undergoing RT, and can provide information on CV risk at no additional cost.
Title: Breast and chest wall edema during and following radiotherapy in breast cancer patients: Prevalence, risk factors and quality of life


Body: PURPOSE/OBJECTIVE
Innovations in loco-regional breast cancer treatment, such as oncoplastic surgery and neoadjuvant chemotherapy, have been suggested to increase the risk of breast and chest wall edema, which may impair quality of life (QoL) during and after treatment. The objective of this study is to evaluate prevalence and risk factors of breast and chest wall edema and its effect on quality of life.

METHODS
We conducted this study within a prospective observational cohort of breast cancer patients indicated to undergo radiation treatment after being treated with surgery (Utrecht cohort for Multiple BREast cancer intervention studies and Long-term evaluation, UMBRELLA). At the time of inclusion all participants consented to the collection of clinical data and ‘patient reported outcomes’ (PROMs) at regular intervals during and after treatment. Presence of breast and chest wall edema was registered by radiation oncologists according to CTCAE V4.0 scoring system, at weekly follow-up visits during radiation treatment, and at standard follow-up intervals after radiation treatment. When present, edema was defined as ‘acute’ (i.e. breast and chest wall edema within 0-90 days after the start of radiation treatment), ‘late’ (i.e. >90 days) or both. Information on potential risk factors, such as patient and tumor characteristics, and treatment (e.g. surgical procedure, RT target volumes, (neo)adjuvant chemotherapy) was collected from electronic patient files and questionnaires. We performed univariate and multivariable logistic regression analysis to identify determinants that were (independently) associated with breast and chest wall edema. PROMs on quality of life and pain (i.e. EORTC QLQ-C30/BR23) were collected regularly (i.e. baseline, 3, 6 and 12 months) and compared between patients with and without edema.

RESULTS
We included 427 patients with at least 3 months follow-up (median follow-up 48 weeks). Sixteen percent (70/427) had acute edema, 23% (73/314) had late edema and 8% (25/314) had both acute and late edema. The proportion of women with acute edema was significantly higher in patients treated with oncoplastic surgery (31% vs. 15%, p=0.03) or mastectomy (31% vs. 14% p<0.01). Risk factors for late edema were oncoplastic surgery (p=0.04), mastectomy (p=<0.001), axillary lymph node dissection (ALND) (p=0.01), loco-regional radiotherapy (p=0.02) and acute edema (p=<0.001). Mean QoL scores were lower, and mean pain scores were higher, in patients with edema compared to those without edema at all intervals in time (i.e baseline, 3, 6 and 12 months; figures will be presented at the symposium including stratified analyses).

CONCLUSION
Breast and chest wall edema is associated with reduced quality of life during the first year of treatment. Oncoplastic surgery and mastectomy increase the risk for acute edema, while oncoplastic surgery, mastectomy, axillary treatment (i.e. ALND, radiation therapy) and the presence of acute edema are associated with late edema. Early treatment of acute edema may reduce the risk for late edema, prolonged pain and impaired quality of life.
**Title:** Accelerated partial breast irradiation versus whole breast irradiation: short term quality of life results from a phase 3 trial


**Body:**

**Introduction.** Accelerated partial breast irradiation (APBI) represents a valid option for selected early breast cancer (BC); potential advantages of APBI include shorter treatment time, improved safety profile, and a cost reduction compared with standard fractionation. We recently published the 5-years results of a phase 3 randomized trial (NCT02104895), showing a very low rate of disease failure. A significant impact on patients compliance in terms of acute and late toxicity was shown. The purpose of the present analysis is to compare the quality of life (QoL) of women with BC treated with either APBI or whole breast irradiation (WBI).

**Methods.** Eligible patients were women aged more than 40 years with early BC suitable for breast conserving surgery. At the end of radiotherapy, patients were asked to compile the EORTC QLQ-C30 as a reliable and valid measure for cancer patients in multicultural clinical research settings, and the BR23 module as a supplementary questionnaire for issues relevant to patients with BC. Overall 205 patients (105 APBI and 100 WBI) fully completed the given questionnaires.

**Results.** The scores of the functional and symptom scales of QLQ-C30 are reported in Table 1 and Table 2, respectively. Significant differences between the two arms emerged by global health status (p=0.0001) and most functional and symptom scales, with better outcomes in the APBI arm. Women treated with APBI reported a significantly better QoL in terms of physical, role, emotional and social functioning. We also found in the APBI arm better outcomes in term of symptoms (fatigue, pain, dyspnoea, insomnia, appetite loss).

Concerning the functional and symptom scales of BR23 module, the body image perception and the future perspective were significantly better in the APBI group; no significant difference emerged for sexual functioning. Significant differences emerged also for symptom scales (breast and arm symptoms), with better scores for APBI group.

**Conclusion.** Overall, women treated with APBI reported a significantly better short-term QoL outcome as compared with women treated using WBI.

**QLQ-C30 functional scale scores in the series: difference between arms and p-value from Mann Whitney U test**

<table>
<thead>
<tr>
<th>Domain</th>
<th>APBI</th>
<th>SD</th>
<th>WBI</th>
<th>SD</th>
<th>Score Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>QL2, mean</td>
<td>66.4</td>
<td>18.8</td>
<td>49.7</td>
<td>18.1</td>
<td>+16.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>PF2, mean</td>
<td>86.2</td>
<td>15.2</td>
<td>77.7</td>
<td>17.3</td>
<td>+8.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>RF2, mean</td>
<td>88.5</td>
<td>19.2</td>
<td>76.8</td>
<td>21.6</td>
<td>+11.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>EF, mean</td>
<td>78.6</td>
<td>17.6</td>
<td>66.3</td>
<td>24.3</td>
<td>+12.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>CF, mean</td>
<td>86.8</td>
<td>18.2</td>
<td>81.7</td>
<td>17.8</td>
<td>+5.1</td>
<td>0.014</td>
</tr>
<tr>
<td>SF, mean</td>
<td>88.9</td>
<td>19.4</td>
<td>82.3</td>
<td>19.5</td>
<td>+6.6</td>
<td>0.003</td>
</tr>
</tbody>
</table>

QL2, global health status; PF2, physical functioning; RF2, role functioning; EF, emotional functioning; CF, cognitive functioning; SF, social functioning

**QLQ-C30 symptom scale scores in the series: difference between arms and p-value from Mann Whitney U test**

<table>
<thead>
<tr>
<th>Domain</th>
<th>APBI</th>
<th>SD</th>
<th>WBI</th>
<th>SD</th>
<th>Score Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA, mean</td>
<td>21.6</td>
<td>18.7</td>
<td>35.8</td>
<td>21.2</td>
<td>-14.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Symptom</td>
<td>Mean 1</td>
<td>Mean 2</td>
<td>Mean 3</td>
<td>Mean 4</td>
<td>Mean 5</td>
<td>p-value</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>NV</td>
<td>5.2</td>
<td>9.6</td>
<td>8.0</td>
<td>11.2</td>
<td>-2.8</td>
<td>0.047</td>
</tr>
<tr>
<td>PA</td>
<td>13.2</td>
<td>19.3</td>
<td>23.7</td>
<td>19.6</td>
<td>-10.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>DY</td>
<td>12.4</td>
<td>21.3</td>
<td>20.3</td>
<td>23.6</td>
<td>-7.9</td>
<td>0.006</td>
</tr>
<tr>
<td>SL</td>
<td>19.7</td>
<td>24.8</td>
<td>28.7</td>
<td>25.1</td>
<td>-9.0</td>
<td>0.004</td>
</tr>
<tr>
<td>AP</td>
<td>5.7</td>
<td>13.4</td>
<td>20.0</td>
<td>24.2</td>
<td>-14.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>CO</td>
<td>13.0</td>
<td>20.4</td>
<td>20.7</td>
<td>23.6</td>
<td>-7.7</td>
<td>0.01</td>
</tr>
<tr>
<td>DI</td>
<td>4.8</td>
<td>14.9</td>
<td>5.0</td>
<td>14.5</td>
<td>-0.2</td>
<td>0.88</td>
</tr>
<tr>
<td>FL</td>
<td>14.9</td>
<td>31.0</td>
<td>8.0</td>
<td>14.3</td>
<td>+6.9</td>
<td>0.67</td>
</tr>
</tbody>
</table>

FA, fatigue; NV, nausea-vomiting; PA, pain; DY, dyspnoea; SL, insomnia; AP, appetite loss; CO, constipation; DI, diarrhoea; FI, financial difficulties
**Title:** Impact of routine cavity shave margins on time and money: Results from the SHAVE trial

Chagpar AB B, Longley PB B, Horowitz NR R, Killelea BK K, Tsangaris TN N, Li F, Butler M, Stavris K, Yao X, Harigopal M, Bossuyt V, Lannin DR R, Pusztai L, Loftus M, Davidoff AJ J and Gross CP P. Yale University School of Medicine, New Haven, CT; Yale-New Haven Hospital, New Haven, CT; Thomas Jefferson University, Philadelphia, PA; Yale Center for Analytical Sciences, New Haven, CT and Yale Cancer Outcomes, Public Policy, and Effectiveness Research Center, New Haven, CT.

**Body:** INTRODUCTION: Taking routine cavity shave margins (CSM) reduces positive margin and re-excision rates by 50%, but the impact of this technique on operative time and overall costs have not been well-elucidated.

METHODS: The SHAVE trial randomized 235 Stage 0-3 breast cancer patients undergoing partial mastectomy 1:1 to either have further cavity shave margins resected ("shave") or not ("no shave"). Randomization occurred intraoperatively after surgeons had completed standard partial mastectomy. Intraoperative time as well as actual direct costs incurred by the hospital were measured, for both the index case as well as any surgeries over the subsequent 90 days.

RESULTS: Median patient age was 61 (range; 33-94). 54 patients (23%) had invasive cancer, 45 (19%) had DCIS, and 125 (53%) had both. Median invasive tumor size was 1.1 cm (range; 0-6.5), and median DCIS size was 1.0 cm (range; 0-9.3). The "shave" and "no shave" groups were well-matched in terms of baseline characteristics, including the proportion having a sentinel node biopsy (75.6% vs. 69.8%, p=0.32) and/or axillary node dissection (9.2% vs. 7.8%, p=0.68) at the time of the initial surgery. The median number of additional CSM in the "shave" group was 4 (range; 3-6). At the initial surgery, those in the "shave" group had a longer operative time (median 76 vs. 66 minutes, p=0.005), and higher OR, pathology and total costs (see table). 48 patients required a subsequent surgery; 45 (93.8%) for margin clearance, 3 for sentinel lymph node biopsy alone (2 in the "shave" and 1 in the "no shave" group, p=1.00). There was a significantly lower re-excision rate for margins in the "shave" group (10.9% vs. 27.6%, p=0.001). Median time to re-excision was 22 days (range; 10-62). The mean cost of additional surgeries for those who required them was no different between the "shave" and "no shave" groups ($2636 vs. $3453, p=0.12); however, given the overall lower reoperation rate in the "shave" group (12.6% vs. 28.4%, p=0.003), the mean cost per patient for additional surgeries was significantly lower in the "shave" vs. "no shave" group. Taking into account all surgeries (including the index case and any additional surgeries within 90 days), there was no significant difference in cost (from a hospital perspective) between the two groups.

<table>
<thead>
<tr>
<th>Mean (± SE) Costs per patient</th>
<th>&quot;Shave&quot; (n=119)</th>
<th>&quot;No Shave&quot; (n=116)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index surgery:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR costs</td>
<td>$1315 (± $69)</td>
<td>$1138 (± $52)</td>
<td>0.042</td>
</tr>
<tr>
<td>Pathology costs</td>
<td>$1195 (± $43)</td>
<td>$795 (± $48)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total costs</td>
<td>$4758 (± $123)</td>
<td>$4133 (± $119)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Additional surgery:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR costs</td>
<td>$94 (± $24)</td>
<td>$247 (± $44)</td>
<td>0.003</td>
</tr>
<tr>
<td>Pathology costs</td>
<td>$51 (± $18)</td>
<td>$112 (± $21)</td>
<td>0.031</td>
</tr>
<tr>
<td>Total costs</td>
<td>$332 (± $88)</td>
<td>$983 (± $189)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Total 90 day surgery costs:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR costs</td>
<td>$1409 (± $76)</td>
<td>$1385 (± $64)</td>
<td>0.808</td>
</tr>
<tr>
<td>Pathology costs</td>
<td>$1247 (± $49)</td>
<td>$909 (± $52)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total costs</td>
<td>$5090 (± $166)</td>
<td>$5116 (± $214)</td>
<td>0.925</td>
</tr>
</tbody>
</table>
CONCLUSIONS: Taking routine CSM is associated with increased time and cost for the index surgery, but this is offset by the cost savings of reduced re-excision rates. While the strategies of "shave" and "no shave" are similar in terms of 90 day hospital-related costs, taking CSM is associated with a lower need for reoperative surgery, thereby reducing patient angst and improving utilization of surgeon and OR time.
Body: PURPOSE
Approximately 10-15% of breast cancer patients treated by breast conserving surgery (BCS) and adjuvant radiotherapy (RT) will develop ipsilateral breast tumor recurrence (IBTR). International guidelines suggest total mastectomy as treatment of choice for IBTR following lumpectomy and RT. Nevertheless, there is evidence that second BCS might be equally sufficient.

PATIENTS AND METHODS
Patients with IBTR diagnosed between 1990 and 2014 after BCS and RT were included (n=170). 34.1% women underwent secondary BCS, whereas 65.9% were treated by mastectomy. We determined predictive factors for time to local progression (TTP), disease free survival (DFS), and overall survival (OS) comparing these two groups.

RESULTS
Median follow-up after primary IBTR was 49 months (59 months for patients still alive at time of analysis). Five-year IBTR-free rate after secondary BCS was 77.6% (SD±6.1%) and 75.0% (SD±4.5%) for patients after mastectomy. Five-year DFS was 57.3% (SD±8.2%), and 61.9% (SD±5.5%), five-year OS was 84.7% (SD±5.8%), and 72.6% (SD±5.1%), respectively. Prior adjuvant systemic therapy, muscular invasion, and skin infiltration were independent significant risk factors for a shorter TTP. Additionally, lymphovascular infiltration (LVI) in the IBTR increased the risk for a shorter DFS. LVI, muscular invasion, and skin infiltration were identified as independent significant risk factors for a shorter OS.

CONCLUSION
No significant difference in local control, DFS, and OS was seen between IBTR patients treated either by secondary BCS or mastectomy. Our data suggest that secondary BCS for IBTR patients after initial BCS and RT is feasible in selected patients.
Title: Multicenter clinical trial of percutaneous laser ablation for early stage primary breast cancer. Results of 49 cases with radiographic and pathological correlation

Schwartzberg BS S, Abdelatif OMA MA, Lewin JM M, Bernard JM M, Brehm JL L, Bu-Ali HM M, Cawthorn SJ J, Chen-Seeto M, Feldman SM M, Govindarajulu S, Jones Li I, Juette A, Kavia S, Maganini RO O, Pain SJ J, Shere MH H, Shriver CD D, Smith SG G, Valencia A, Whitacre EB B and Whitney R. Sarah Cannon at Rose Medical Center, Denver, CO; Carondelet St. Joseph's Hospital, Tucson, AZ; Walter Reed National Military Medical Center, Bethesda, MD; Wheaton Franciscan Health System, Wauwatosa, WI; North Bristol NHS Trust - Southmead Hospital, Bristol, United Kingdom; Columbia University Medical Center, NY, NY; Norfolk and Norwich University NHS Trust - Norfolk and Norwich University Hospital, Norwich, United Kingdom; Mid-Essex NHS Trust - Broomfield Hospital, Chelmsford, United Kingdom; Alexian Brothers Health System, Barlett, IL and Breast Center of Southern Arizona, Tuscon, AZ.

Body: Introduction: Percutaneous laser ablation of early stage primary breast cancer remains investigational. A multicenter, international clinical trial (NCT01478438) was completed to determine feasibility of this technique. Methods: Patients with a single focus of biopsy proven infiltrating ductal carcinoma measuring 20 mm or less by pre-ablation MRI were treated by image-guided percutaneous laser ablation. A laser diode source (805 nominal nanometer wavelength) was used to perform the thermal ablation. Thermal sensors placed at the periphery of the tumor measured achievement of predefined temperature levels, indicating successful ablation. The patients were evaluated by post-ablation mammogram, ultrasound and MRI at 4 weeks post-ablation, after which they underwent surgical excision. Pathology specimens were evaluated by hematoxylin & eosin, CK 8/18, Ki-67 and estrogen receptor staining.

Results: Forty-nine of the 61 enrolled patients (ages 42-77, mean age 64 years) undergoing percutaneous laser ablation have finished protocol analysis and are reported in this series. Ablation was considered complete by the treating physician in all cases. The mean tumor size was 11.3 mm. The mean laser time was 15.7 minutes. There were no serious adverse events. Seven patients (14%) reported mild adverse events (pain, blisters, lump). Post-ablation cell viability was determined by MRI and by changes in CK 8/18, Ki67 and estrogen receptor staining. A post-ablation discordance between MRI and pathology was found in evaluation of 4 patients (8%). Three patients (6%) were considered "false negative" with a post-ablation residual tumor burden of less than 2mm which was not detected by MRI. One patient (2%) had a complete pathologic ablation but positive MRI ("false positive"). One patient (2%) had adjacent residual DCIS, visible in retrospect on the pre-ablation MRI and was considered a screening failure. Eight patients (16%) were found to have residual invasive cancer by both post-ablation MRI and pathologic analysis. Complete ablation was confirmed in 36 patients (73%) when evaluated by both post-ablation MRI and pathologic analysis.

Conclusion: Percutaneous laser ablation holds promise as an alternative to lumpectomy in the treatment of early stage breast cancer. There is a strong correlation (92%) between findings on post-ablation MRI and changes in CK 8/18, Ki67 and estrogen receptor staining in this series. Additional trials are necessary to determine the long-term curative potential of this technique.
Title: Radioactive seed localization in non-palpable breast cancer compared to wire-guided localization and radio-guided occult lesion localization: Results of a comparative study

Theunissen CIJM, Noorda EM, Rust EAZ, Bandel C, Ooster- van den Berg JGv van ‘t, Edens MA, Jager PL and Francken AB. Isala, Zwolle, Netherlands.

Body: Introduction Almost 25% of women diagnosed with breast cancer have a non-palpable tumor. In breast-conserving therapy, it is important to have oncologic clear margins while excising minimal healthy tissue with good cosmetic outcome. The three most used techniques for non-palpable tumor localization pre-operative are radioactive seed localization (RSL), wire-guided localization (WGL), and radio-guided occult lesion localization (ROLL). Besides the advantage of tumor localization, each technique has its own disadvantages. When RSL is used, the major concern is safe handling of the seeds because of the used radioactive material. In case of WGL, the ideal skin incision and accurate positioning of the wire do not always match. The tip of the wire is non-palpable during surgery, which increases the risk of positive margins and increases the risk of enlarging resection volume resulting in poor cosmetic outcome. Moreover, the wire can get displaced or can be transsected during surgery. Disadvantages of ROLL include diffuse spread of the radioactive tracer in the breast tissue, which can result in higher excision volumes and problems with the concurrent sentinel node procedure. Thus far, no study has been performed comparing these three techniques. Therefore, this study analyzed the outcomes of RSL, WGL, and ROLL in non-palpable breast cancer in a single institution.

Methods Women diagnosed with ductal carcinoma in situ or non-palpable invasive breast carcinoma (stadium I or II) and were operated from January 2011 to December 2013 were retrospectively included in this study (N=278). In all included women in this study, RSL (n=71), WGL (n=78) or ROLL (n=132) was performed for intra-operative tumour localization. Outcome measures were weight of the resected specimen, oncological margins, re-excision and recurrence of disease.

Results In total 278 lumpectomies were performed in 272 patients. Mean resection volumes were not significant higher in RSL (mean 97.8 cm3), compared to WGL (mean 80.9 cm3) and ROLL (mean 84.8 cm3). RSL was associated with significant less tumor-positive margins (n=55, 96.5%) compared to the WGL (n=55, 73.3%) and ROLL (n=111, 84.1%). Also, RSL was associated with significant lower re-excision rates (n=1, 1.4%) as compared to WGL (n=12, 15.4%) and ROLL (n=14, 10.6%). In only one patient with ROLL, recurrence of disease was seen. The median follow-up of all patients was 27 months (9 – 44).

Conclusion This study revealed that RSL is an effective technique for excision of non-palpable breast cancer and is associated with fewer tumor-positive margins and therefore lower re-excision rates.
Title: Cancer recurrence in long-term early stage breast cancer survivors after 25 years

Smart DK K, Kesarwala AH H, Purec G, Liu R and Camphausen K. Radiation Oncology Branch, National Cancer Institute, Bethesda, MD.

Body: Between 1979 and 1987, NCI protocol 79-C-0111 randomized 237 women with pathologically confirmed invasive breast tumors 5 cm or less to receive either breast conservation therapy (BCT) or modified radical mastectomy (MRM), with overall survival as the primary endpoint. Both arms of the trial received axillary dissection. Patients with node-positive disease were treated with doxorubicin and cyclophosphamide. BCT patients had radiation to the whole breast followed by a cavity boost. Most women on this study have been followed yearly since treatment to document outcomes. After median follow-up of 25 years, 95 patients remained on the study for evaluation. We performed additional analysis at a median follow-up of 30.9 years to determine patterns which could suggest risk factors for delayed cancer recurrence of all types in long-term survivors of early stage breast cancer. A chart review was conducted on all patients with a new cancer diagnosis (including recurrent breast) 25-30 years after their original breast cancer diagnosis. 7.4% of the cohort were documented to have a cancer recurrence between years 25 and 30. 71.4% of recurrent patients had received prior BCT. However, 33.3% of recurrences were non-breast cancer in origin. 42.8% of the women had a BMI greater than or equal to 27. All of patients who initially received BCT are currently living despite recurrences. These data suggest that there may be a significant proportion of women with history of early stage breast cancer who are at risk for late cancer recurrences. Therefore, yearly monitoring with mammograms and physical exams are suggested for long-term breast cancer survivors. Germline and tumor genetic testing are pending on the recurrent patient cohort, and may suggest molecular predictors of late cancer recurrence.
Title: The efficacy and long term results of intraoperative frozen section analysis to access resection margin in ductal carcinoma in situ

Choi JE, Yeu KJ, Park JY, Kang SH, Lee SJ and Bae YK. Yeungnam University College of Medicine, Daegu, Korea and Yeungnam University College of Medicine, Daegu, Korea.

Body: Introduction
Breast conserving surgery (BCS) is a standard procedure for early breast cancer and resection margin state is the most important risk factor of local recurrence. Re-operation is generally conducted in 20–40% after initial BCS to achieve negative margins, especially in breast cancer with carcinoma in situ components. In this study, we analyzed the long-term follow up results and efficacy of BCS using intraoperative frozen section analysis to access resection margin in ductal carcinoma in situ (DCIS) patients.

Methods
Between 2004 and 2006, 1016 patients were diagnosed with primary breast cancer and received breast cancer surgery. Among them, BCS was attempted as an initial operation for 523 patients. Superior, inferior, medial and lateral margin of resected specimen were evaluated according to the intraoperative frozen section analysis. If tumor cells existed less than 2mm from resected specimen margin, intraoperative further resection was done and if the further resection was impossible, initial BCS was converted to mastectomy. All medical records and pathologic reports were reviewed retrospectively.

Results
Of the 523 patients who had to undergo BCS, 13.3% (70/523) were converted to mastectomy during initial BCS. The number of the patients who had either only DCIS or invasive carcinoma carcinoma in situ component was 372 (71.1%, 372/523) and 17.2% (64/372) were converted to mastectomy. One hundred fifty one (28.9%, 151/523) patients had only invasive carcinoma and 3.97% (6/151) were converted to mastectomy. In this study, we analyzed 94 patients who had to undergo BCS with DCIS. The rate of intraoperative conversion to mastectomy was 13.8% (13/94) and 81 patients had successful BCS with 0–3 times of intraoperative frozen section analysis. There were no differences between patients who had BCS and final mastectomy in clinicopathologic characteristics such as physical examination of tumor, age of patients, DCIS subtypes, nuclear polymorphism, presence of necrosis, ER, PR, HER2 and Ki67. After permanent biopsy was reported, in 5 patients, resected specimen had tumor cells within less than 2mm from resected margin, not inked margin. They had no reoperations and no recurrences. Mean follow up period was 76.6 months. One locoregional and 3 local recurrences in BCS patients and 1 local recurrence in mastectomy patients were found. There was no difference in disease free survival between two groups (95.1% vs 92.3%, p=0.659). In these DCIS patients, reoperation rate was 0%.

Conclusion
Intraoperative frozen section analysis during BCS to access resection margin helps to avoid reoperations and increase intraoperative success rate of BCS in DCIS. It also shows oncological safe long term results. Further studies are needed to resolve the problem with cost-effectiveness of intraoperative frozen section analysis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-13-07

Title: Radiofrequency ablation (RFA) is a promising treatment option for primary breast cancer: Experience in 386 Japanese breast cancer patients

Takahashi M, Ito T, Oura S, Nagamine S, Yamamoto N, Yamamichi N, Earashi M, Doihara H, Imoto S, Mitsuyama S and Akazawa K.  NHO Hokkaido Cancer Center, Sapporo, Japan;  Division of Surgery, Rinku General Medical Center;  Division of Breast Surgical Oncology, Wakayama Medical University;  Division of Surgery, Okinawa Red Cross Hospital;  Division of Breast Surgery, Chiba Cancer Center;  Fukui Kousei Hospital;  Division of Breast Surgery, Yao General Hospital;  Breast and Endocrine Surgery, Okayama University Hospital;  Kyorin University School of Medicine;  Kitakyushu Municipal Medical Center and  Niigata University Medical and Dental Hospital.

Body: Background. Radiofrequency ablation (RFA) is used for the treatment of various solid tumors. Several small experiences have reported as a primary treatment for breast cancer using RFA. However, the clinical benefits remain uncertain. We retrospectively studied 386 patients and analyzed their RFA-related complications and outcomes.

Methods. A clinical database was constructed from 10 institutions. RFA was performed using an electrical generator connected to a single cooled-tip electrode or multiple electrodes. Adjuvant systemic therapy and whole breast radiation were administered according to the clinicopathological background of each patient. Follow-up periods ranged from 1 to 90 months (mean 45.4 months). RFA-related complications and risk factors for in-breast recurrence after RFA were evaluated. Variables evaluated included patient characteristics, pre- and post-operative imaging modalities, tissue sampling modalities, and RFA-related factors.

Results. Skin burns were observed in 7 patients (1.8 %) and RFA-induced damage to the nipple-areolar complex in 7 patients (1.8 %). Persistent induration of the breast after RFA was observed in 137 patients (35.5 %). Eleven patients (2.8 %) developed in-breast recurrence. In-breast recurrence was more frequent in patients with tumor size >2.0 cm, ER-negative tumor, HER2-positive tumor, positive nodes, no breast irradiation and adjuvant chemotherapy.

Conclusions. Skin burns were a major problem after RFA, but the frequency of burns was relatively low. Breast induration was also developed, but it did not bring harmful effect to the patients with this complication. We conclude that RFA is a promising treatment option for solid T1 breast cancer without malignant potential.
Title: A prospective, single-arm, multi-site, clinical evaluation of the SAVI SCOUT® surgical guidance system for the location of non-palpable breast lesions during excision

Cox CE, Prati R, Blumencranz P, Allen K, Banull C, Cline M, Howard T, Portillo M, Whitworth P, Funk K, Police A, Lin E, Combs F, Anglin B, King J and Shivers SC. University of South Florida Breast Health Program, Tampa, FL; Morton Plant Mease Hospital, Clearwater, FL; Nashville Breast Center, Nashville, TN; Pink Lotus Breast Center, Beverly Hills, CA; UC Irvine Health Pacific Breast Care Center, Irvine, CA and Medical Center of Plano Complete Breast Care, Plano, TX.

Body: Background and Objectives: The standard preoperative technique for localizing non-palpable breast lesions is wire localization (WL). Radioactive seed localization (RSL) is an alternative approach that addresses a number of clear disadvantages associated with WL but, the adoption of RSL has been impacted by considerable regulatory requirements for the handling of radioactive materials. To advance the progress made with RSL and eliminate issues associated with radioactive components, the SAVI SCOUT® surgical guidance system was developed. SAVI SCOUT is an FDA-cleared medical device that utilizes non-radioactive electromagnetic wave technology to provide real-time guidance during excisional breast procedures. The purpose of this study is to evaluate the performance of SAVI SCOUT in guiding the removal of non-palpable breast lesions.

Methods: Following a 50 patient pilot study that showed SAVI SCOUT to be safe and effective, IRB approval was granted for this prospective, single-arm, multi-site study for women with a non-palpable breast lesion. Pts underwent localization and excision with the SAVI SCOUT system, which consists of an electromagnetic wave reflective device (reflector), handpiece and console. Using mammographic or ultrasound guidance, the reflector was implanted into the target tissue. Before making an incision, the surgeon used the handpiece, which emits electromagnetic waves and infrared light, to detect the location of the reflector and subsequently plan the surgical incision. During the procedure, the surgeon used the handpiece to guide the localization and removal of the reflector along with the surrounding breast tissue. The console provides audible feedback of reflector proximity to the handpiece. Successful reflector placement, localization and retrieval were the primary endpoints.

Results: A total of 61 pts have participated in the study to date, along with 7 surgeons and 9 radiologists across 6 institutions. The reflectors were successfully placed in all pts, including 27 under mammographic guidance and 34 under ultrasound guidance. In 28 cases, the reflectors were placed on the same day as surgery. Otherwise, the reflectors were placed up to 7 days (average 2.9 days) before surgery. Thirteen pts underwent excisional biopsy and 48 pts had a lumpectomy. The intended lesion and reflector were successfully removed in all pts. Reflector migration did not occur and no adverse events occurred. Final pathology is currently available for 52 pts: 8/10 excisional biopsy pts had no invasive or in situ carcinoma identified. For pts with cancer and complete data, 39/39 had clear margins, but one patient was recommended for re-excision due to a close margin (1 mm) for DCIS.

Conclusions: The preliminary data from this prospective, multi-site study show that real-time surgical guidance with SAVI SCOUT is an accurate technique for directing the removal of non-palpable breast lesions and is reproducible at multiple clinical sites. At present, the study has yielded 100% surgical success with a re-excision rate of 3.0%. Ongoing accrual to this clinical evaluation study will validate these findings with planned enrollment of 150 pts at up to 15 total sites.
2015 San Antonio Breast Cancer Symposium

Publication Number: P3-13-09

Title: Improved frozen section examination of the retroareolar margin for prediction of nipple involvement in breast cancer

Piato JR M, Aguiar FN N, Mota BS S, Dória MT T, Alves-Jales RD D, Messias AP, Goncalves R, Mano MS S, Soares JM M, Ricci MD D, Filassi JR and Baracat EC C. Discipline of Gynecology, Hospital das Clínicas, Faculty of Medicine - University of São Paulo-FMUSP, São Paulo, SP, Brazil; Discipline of Pathology, Hospital das Clínicas, Faculty of Medicine - University of São Paulo-FMUSP, São Paulo, SP, Brazil and Division of Medical Oncology, Instituto do Câncer do Estado de São Paulo, Faculty of Medicine, Universidade de São Paulo-FMUSP, São Paulo, SP, Brazil.

Body: Introduction: Development of the nipple-sparing mastectomy (NSM) technique has constituted a significant advance in the surgical treatment of selected cases of breast cancer. The most important aspect of areolar complex preservation is the exclusion of carcinoma involving the nipple. The retroareolar surgical margin is usually sampled and subjected to an intraoperative evaluation by frozen section examination in order to avoid a second procedure. However, this method is not standardized resulting in variable rates of false-negative results. Here, a new technique is proposed for the intraoperative study of the retroareolar margin. This ex vivo study was conducted by performing a simulated NSM procedure for patients undergoing total mastectomy to assess the impact of these measures on the accuracy of retroareolar frozen section examination. Materials and Methods: Between September 2012 and April 2014, we studied 158 mastectomy specimens from patients undergoing total mastectomy for breast cancer at the Cancer Institute of the State of São Paulo. Inclusion criteria were stage Tis-T3 tumors, multifocal and multicentric breast carcinoma, unicentric carcinoma not suitable to quadrantectomy. Patients submitted to neoadjuvant chemotherapy were also included. To obtain the entire sample area, the terminal retroareolar milk duct bunch was isolated. Fragments approximately 1.5 cm in length were excised and sectioned in parallel at the base of the nipple using a cold bistoury. Three transverse histological sections (4 µm each) at 200 µm intervals that included the entire isolated fragments were subjected to frozen section examination. The sections were stained with hematoxylin-eosin (H&E) and were evaluated. The remainder of each fragment was embedded in paraffin and 4 µm sections were subsequently stained with H&E and examined. Results: A total of 158 mastectomy specimens involving mammary carcinoma of no special type were examined. These included 15 (9.5%) in situ stage tumors, 36 (22.8%) stage I tumors, 71 (44.9%) stage II tumors, and 36 (22.8%) stage IIIA tumors. Paraffin examinations identified 25 retroareolar fragments compromised by carcinoma, resulting in 16.1% prevalence. Of the frozen sections examined, 2/158 (1.3%) had false-negative results and 5/158 (3.1%) had false-positive results. For the former two cases, the corresponding paraffin examinations detected low-grade carcinoma in situ and a residual cell cluster with a diameter less than 1 mm. The latter was found in a mastectomy specimen from a patient that underwent neoadjuvant chemotherapy. For the three cases involving false-positive results, the corresponding paraffin examinations revealed no atypical ductal hyperplasia present, one sclerosing intraductal papilloma and one nipple syringomatous adenoma. Statistical analysis revealed that the frozen section examinations performed had a sensitivity rate of 92.0% and a specificity rate of 96.2%. In addition, the positive predictive value (PPV) was 82.1%, the negative predictive value (NPV) was 98.4%, and the accuracy was 95.4%. Conclusion: The frozen section examination technique described here detected nipple involvement in breast cancer with greater accuracy than the frozen section usually performed by most surgeons.
Minimally-invasive (percutaneous) stereotactic and ultrasound-guided lumpectomy for DCIS and small breast cancers

Whitworth PW W, Graham C, Schonholz S, Manahan E, Phillips R, Robertson Y and Hardwick MK. Nashville Breast Center, Nashville, TN; Southwest General, Middleburg Heights, OH; Baystate Noble Hospital, Westfield, MA; Dalton Surgical Group, Dalton, GA; Metro Surgical Associates, Atlanta, GA and TME Research, Allentown, PA.

Background
In spite of advances characterizing the biology of individual breast cancers, current analytics cannot adequately identify truly indolent lesions. As a result many indolent breast cancers and DCIS are over-treated in order to avoid under-treatment of truly life-threatening cancers. Reducing over-treatment for DCIS and small cancers is a current priority for breast cancer management, to optimize both patient care and resource utilization. Intact minimally-invasive excision (percutaneous lumpectomy) with radiofrequency basket capture can eliminate the need for open excision of many atypical ductal hyperplasia (ADH) breast lesions (Whitworth, et al, Ann Surg Oncol, 2011). Percutaneous lumpectomy provides standard margin and lesion analysis of the specimen, accomplishing the same goals as open excision. Here we report results of the IPEX study, a prospective phase 4 trial evaluating the same minimally-invasive strategy when employed to manage small breast cancers and DCIS.

Methods
94 women age 31-86 had minimally-invasive (percutaneous) removal of DCIS or small breast cancers using a 20 mm radiofrequency basket capture technique with stereotactic or ultrasound guidance. Tissue elasticity permitted removal through a small incision (less than 10 mm). A second 20 mm basket capture was used to shave and further evaluate margins. Procedures were conducted with adequate local anesthesia and P.O. sedation in the imaging department. Patient tolerance scores were recorded. Standard radiologic evaluation (specimen and breast) and histologic criteria were applied in all cases. Standard open sentinel node biopsy was offered to patients with invasive breast cancers. All patients provided informed consent for the study.

Results
Final histologic lesion size was 1-20 mm. Of 40 DCIS and 54 invasive lesions, 18 (19%) had positive margins and/or open re-excision, including one patient who had re-excision in spite of negative margins because of inadequate shaved-margin quality (no residual disease on pathology). 58 patients completed APBI (balloon or strut-based brachytherapy) and 16 had whole breast radiation. Of 54 patients with invasive cancers, 34 (age 66-86) had no sentinel node biopsy based on patient preference and low risk features. Patient pain scores averaged 1.9 out of 10 (range 0-7).

Conclusions
Minimally-invasive (percutaneous) lumpectomy provides satisfactory management of DCIS and small breast cancers, accomplishing the same goals as open surgery. The specimen is intact so the pathologist can conduct standard histologic analysis of the lesion, distinguishing DCIS, ADH and invasive cancer. Margin analysis is standard. Compared to open surgery, less tissue can be removed because of small lesion size with more precise targeting and resection. Minimally-invasive (percutaneous) lumpectomy reduces morbidity, distress, discomfort and expense associated with overtreatment of small breast cancers.
Title: Phase Ib/II study of ribociclib and alpelisib and letrozole in ER+, HER2– breast cancer: Safety, preliminary efficacy and molecular analysis

Juric D, Ismail-Khan R, Campone M, García-Estévez L, Becerra C, De Boer R, Hamilton E, Mayer IA, A, Hui R, Lathrop KI, Pagani O, Asano S, Bhansali SG, Zhang V, Hewes B and Munster P. Massachusetts General Hospital Cancer Center, Boston, MA; H. Lee Moffitt Cancer Center, Tampa, FL; Institut de Cancérologie de l'Ouest/René Gauducheau, Saint-Herblain, France; Hospital de San Chinarro, Madrid, Spain; Texas Oncology-Baylor Charles A. Sammons Cancer Center, Dallas, TX; Royal Memorial Hospital, Victoria, Australia; Sarah Cannon Research Institute and Tennessee Oncology, Nashville, TN; Vanderbilt-Ingram Cancer Center, Nashville, TN; Westmead Hospital, University of Sydney, Sydney, Australia; University of Texas Health Science Center at San Antonio, San Antonio, TX; Breast Unit and Oncology Institute of Southern Switzerland, Ospedale San Giovanni, Bellinzona, Switzerland; Novartis Institutes for BioMedical Research, Cambridge, MA; Novartis Pharmaceuticals Corporation, East Hanover, NJ and University of California San Francisco, San Francisco, CA.

Body: Background: The interaction between the PI3K/AKT/mTOR and cyclin D–CDK4/6–INK4–Rb pathways has been reported to play a critical role in ER-driven breast cancer (BC). Furthermore, in preclinical ER+ BC models, the combination of ribociclib (LEE011; LEE), alpelisib (BYL719; BYL), and letrozole (LET) has recently shown enhanced activity vs each agent alone. A Phase Ib/II, 3-arm study is investigating the combination of LEE, BYL, and LET in pts with ER+ advanced (a)BC (CLEE011X2107; NCT01872260). Here, we report on safety, preliminary efficacy, and molecular analysis focusing on Arm (A)3 (LEE + BYL + LET) of Phase Ib.

Methods: Postmenopausal women with ER+, HER2– aBC received a fixed dose of 2.5 mg LET QD (continuous) with escalating doses of either oral LEE QD (3-wks-on/1-wk-off) or BYL QD (continuous), or both, in 28-day cycles. The primary objective is to determine the MTD and/or RP2D of each combination by assessing DLTs in Cycle 1 using an adaptive Bayesian Logistic Regression Model with overdose control principle. Secondary objectives include safety, PK, and preliminary efficacy assessments. Potential biomarkers predictive of response were assessed by next-generation sequencing in all patients, and immunohistochemistry in available tumor samples.

Results: As of Mar 2, 2015, 41 pts received LEE + LET (A1), 21 pts received BYL + LET (A2), and 36 pts received LEE (300–500 mg) + BYL (200–250 mg) + LET (A3). Here, we present results from A3. The number of prior endocrine regimens for advanced disease was: 0 (14 pts); 1–2 (14 pts); 3–4 (7 pts); ≥5 (1 pt); 33% of pts had previously received PI3K/AKT/mTOR inhibitors for aBC. Fifteen pts discontinued treatment: 7 (19%) due to PD and 8 (22%) due to AEs. The most frequent study drug-related AEs (all grade >35%) were: nausea (all grade, 44%; G3/4, 6%), hyperglycemia (44%; 17%), neutropenia (42%; 22%), and fatigue (36%; 11%). The median duration of exposure was 8 weeks; 9 (25%) pts received treatment for ≥4 cycles. Of 27 evaluable pts, 2 (7%) pts had PR, 4 (15%) pts had unconfirmed PR, 6 (22%) pts had SD, 6 (22%) pts had non-CR non-PD (NCRNPD), and 5 (19%) pts had PD as best overall response. One pt with PR was treated for 9 cycles, had 5 lines of prior therapy including a PI3K/AKT/mTOR inhibitor, and had alterations in \( \text{PIK3R1} \) and \( \text{CDK4} \). The other pt with PR was treated for >9 cycles, had 3 lines of prior therapy, and had alterations in \( \text{PIK3CA} \) and \( \text{CCND1} \). In A1, an increase from baseline to C1D15 in pAKT and pS6(235) was observed in 5 and 3 pts, respectively; importantly, there was a more consistent reduction in Ki67 in A3 vs A1 or A2.

Conclusions: LET + LEE + BYL (A3) has an acceptable safety profile and demonstrates preliminary clinical activity in heavily pretreated pts with ER+, HER2– aBC. Preliminary data suggest CDK4/6 pathway activation is associated with improved response to triplet therapy. Importantly, the pathway analysis reported is supportive of a mechanistic combination effect whereby inhibition of the three pathways provides a sustained downregulation of Ki67, potentially preventing a feedback mechanism and hence delaying progression through therapy. Future randomized studies will compare LET + LEE or BYL with LET + LEE + BYL.
Title: Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer (TNBC): Evidence of efficacy and proof of concept from a phase I trial with dose expansion of mTOR inhibition in combination with liposomal doxorubicin and bevacizumab


Body: Background: Approximately 30% of TNBCs are characterized by microarray as claudin-low, mesenchymal or mesenchymal stem cell-like and, unlike basal TNBCs, these tumors frequently harbor aberrations in the PI3K/AKT/mTOR axis, raising the possibility of targeting this axis to enhance chemotherapy response. Assays to clinically identify mesenchymal TNBCs are under development, but published results confirm that up to 30% are metaplastic breast cancers (MpBCs), a chemo-refractory group of tumors that contain a mixture of epithelial and mesenchymal components, making them identifiable by microscopy. As such, MpBCs serve as surrogates of response for potential regimens to treat mesenchymal TNBC.

Methods: Patients (pts) with advanced TNBC (N=64) were treated with liposomal doxorubicin (D), bevacizumab (A) and the mTOR inhibitors temsirolimus (T) or everolimus (E). D and A were administered IV on day 1 with T (IV on days 1, 8 and 15) or E (continuous daily oral administration) using 21 day cycles. Response was assessed every 6 weeks using RECIST. When available, archived tissue was evaluated for aberrations in the PI3K pathway using standard assays.

Results: Fifty-two MpBC pts were treated with DAT (N=39) or DAE (N=13). Median age was 58 (range 37-79); median # of prior regimens for metastatic disease was 1 (range 0-5). The objective response rate (ORR) was 21% [complete response (CR)=4 (8%); partial response (PR)=7 (13%)] and 10 (19%) pts had stable disease (SD)≥6 months for a clinical benefit rate (CBR) of 40%. Tissue was available for testing in 43 pts and 32 (74%) had a PI3K pathway activating aberration (Table 1).

Response According to PI3K Pathway Aberration

<table>
<thead>
<tr>
<th>PI3K Pathway Aberration</th>
<th>N (%)</th>
<th>CR</th>
<th>PR</th>
<th>SD≥6months</th>
<th>CBR</th>
<th>ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any PI3K Pathway Aberration*</td>
<td>32 (74)</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>44%</td>
<td>31%</td>
</tr>
<tr>
<td>PIK3CA Mutation</td>
<td>19 (59)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>47%</td>
<td>26%</td>
</tr>
<tr>
<td>p.H1047R</td>
<td>12 (38)</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>50%</td>
<td>25%</td>
</tr>
<tr>
<td>p.E545K</td>
<td>6 (19)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>50%</td>
<td>33%</td>
</tr>
<tr>
<td>p.G1007R</td>
<td>1 (3)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>p.E545A</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>p.E1047Y</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>p.K111E</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>p.E542K</td>
<td>1 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PIK3CA Amplification</td>
<td>1 (3)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>PTEN Mutation</td>
<td>5 (16)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PTEN Loss</td>
<td>5 (16)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>40%</td>
<td>40%</td>
</tr>
<tr>
<td>AKT1 p.E17K Mutation</td>
<td>2 (6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>AKT2 Amplification</td>
<td>1 (3)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>PIK3R1 Mutation</td>
<td>2 (6)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>NF2 Mutation</td>
<td>1 (3)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>No PI3K Pathway Aberration</td>
<td>11 (26)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>45%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Some tumors had >1 aberration detected

*PI3K pathway activation was associated with a significant improvement in ORR (31 vs 0%; P=0.043) but not CBR (44 vs 45%; P=1.000) or progression-free survival (median 5.1 vs 2.9 months; P=0.352). A pt with 5 year+ durable CR (on maintenance everolimus) had a mutation in NF2. To emphasize the importance of pt selection, it is notable that 12 pts with non-metaplastic TNBC were also treated with DAT, and only 1 pt had a response (CR/PR=1; SD≥6 months=0), for a CBR that was significantly worse than pts with MpBC (8 vs 40%; P=0.045).

**Conclusions:** Using MpBC as a surrogate of response, DAT/DAE has significantly better activity in mesenchymal compared to non-selected TNBC. Response is enhanced in pts with PI3K pathway activation. DAT/DAE should be tested in non-metaplastic, mesenchymal TNBC once a diagnostic assay is available.
Title: Abstract Withdrawn
Effects of the dual selective CYP17 lyase inhibitor and androgen receptor (AR) antagonist, VT-464, on AR+ and ER+ tumor models in vitro and in vivo


VT-464 is a lyase-selective inhibitor of the dual-activity CYP17A1 enzyme that is required for the synthesis of androgens and estrogens in the gonads, adrenals, and tumors. In addition to its role as a CYP17A1 lyase inhibitor, we previously showed that VT-464 also functions as a direct and effective AR antagonist in prostate cancer models. In our current study, we evaluated the therapeutic potential of VT-464 in breast cancer by analyzing its effectiveness in several AR-positive breast cancer cell lines, including those that are ER-positive and ER-negative. Through in vitro assays and xenograft analysis, we compared the activity of VT-464 to enzalutamide, a second generation AR antagonist that is approved for the treatment of castration resistant prostate cancer and is in multiple Phase 2 breast cancer studies. Our results showed that VT-464 was highly effective in preventing proliferation of both ER-positive and ER-negative breast cancer cell lines in vitro. Importantly, significant inhibition was also observed in soft agar assays that assesses anchorage-independent growth. In mechanistic studies, VT-464 and enzalutamide both inhibited induction of AR target genes and recruitment of AR to target promoters, verifying direct AR antagonistic activity observed previously in prostate cancer cells. Furthermore, we evaluated the ability of VT-464 and enzalutamide to inhibit tumor formation in a tamoxifen-resistant model of breast cancer. Oral administration of either enzalutamide or VT-464 significantly decreased tumor growth in mice, with VT-464 achieving greater growth inhibition than enzalutamide. Together, these data provide further rationale for the future study of the AR as a viable therapeutic target in breast cancer, and importantly, suggest that AR inhibition can impact tamoxifen-resistant tumor growth. The dual effects of CYP17A1 lyase inhibition and AR antagonism that are achieved with VT-464 further supports its development as an effective oral therapy option for AR-positive breast cancer. A phase 1 / 2 clinical study of oral VT-464 in women with AR+ triple-negative breast cancer or ER+ cancer resistant to aromatase inhibitors will commence in 2015.
**Title:** Interim analysis of a phase 1 study of the antibody-drug conjugate SGN-LIV1A in patients with metastatic breast cancer

University of Alabama at Birmingham, Birmingham, AL; Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN; Cedars-Sinai Medical Center, Los Angeles, CA; Seattle Cancer Care Alliance, University of Washington, Seattle, WA; Karmanos Cancer Institute, Detroit, MI; Mayo Clinic, Rochester, MN; Memorial Sloan Kettering Cancer Center, NY, NY; Yale University School of Medicine, New Haven, CT; Seattle Genetics, Inc., Bothell, WA and Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN.

**Body:**

**Background**

LIV-1, a multispan transmembrane protein and downstream target of STAT3, is highly expressed in breast cancer cells (Sussman 2014). It has been associated with lymph node involvement and linked with malignant progression to metastasis (Manning 1994). 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 binds to tubulin and induces G2/M arrest and apoptosis.

**Methods**

A phase 1, open-label, dose-escalation study is ongoing to evaluate the safety, tolerability, antitumor activity, pharmacokinetics (PK), and the maximum tolerated dose (MTD) of SGN-LIV1A monotherapy (q3 wks IV) in women with LIV-1-positive, metastatic breast cancer (MBC) (NCT01969643). Patients (pts) with either hormone receptor-positive/HER2-negative (HR+/HER2–) or triple-negative (TN) disease were eligible if they had measurable disease and received ≥2 prior cytotoxic regimens in the metastatic setting. Pts with ≥ Grade 2 neuropathy were excluded. Response was assessed per RECIST v1.1, and pts with stable disease (SD) or better were eligible to continue treatment until disease progression or intolerable toxicity. Fresh tumor biopsies were obtained pre- and post-treatment to evaluate the LIV-1 expression-response relationship, mechanism of action, and tumor sensitivity to SGN-LIV1A.

**Results**

To date, 21 pts (17 HR+/HER2–, 4 TN) have received a median of 3 cycles (range, 1–7) of SGN-LIV1A at doses of 0.5–2.8 mg/kg. Median age was 58 yrs (range, 34–73), and pts had a median of 8 prior systemic metastatic therapies (range, 2–15). At baseline, 19 pts had visceral disease and 15 had bone involvement. No dose-limiting toxicities (DLTs) in Cycle 1 were observed in the 18 DLT-evaluable pts; MTD has not yet been identified. Treatment-emergent adverse events (AEs) reported in ≥30% of pts were primarily Grade 1/2 in severity and were: nausea (57%), fatigue and peripheral neuropathy (43% each), alopecia (38%), and vomiting (33%). Two pts discontinued treatment due to AEs (1 nausea, 1 tachycardia). Preliminary PK data suggest a linear increase in antibody-drug conjugate (ADC) exposure with increasing dose. In the 19 efficacy-evaluable pts, the overall response rate (ORR) was 11% (2 partial responses [PRs]) with clinical benefit of SD or better achieved in 63% (2 PR, 10 SD) of pts. Of note, all 4 pts with TN MBC achieved clinical benefit: 2 PR and 2 SD. Currently, the median duration of clinical benefit is 12.7 wks (range, 6.1–26.3). Four pts remain on treatment.

To date, of the 179 MBC tumor specimens evaluated for LIV-1, 156 (87%) were LIV-1-positive; moderate-to-high LIV-1 expression (H-score ≥100) was present in 91% (94 of 103) of HR+/HER2– and 81% (47 of 58) of TN samples.

**Conclusions**

LIV-1 is expressed in the majority of metastatic breast cancer tissue samples, with moderate-to-high expression in both HR+/HER2– and TN disease. To date, SGN-LIV1A monotherapy has been generally well tolerated and resulted in a clinical benefit of SD or better in 63% of these heavily pre-treated pts, including 2 PRs in the 4 TN MBC pts. Response duration data continue to evolve. Clinical subtype-specific expansion cohorts are planned.
Title: Evaluation of the therapeutic efficacy of a Rad6 small molecule inhibitor in triple negative breast cancer cells

Haynes B, Zhang Y, Li J, Petit S, Westwell A, Mao G and Shekhar M. Wayne State University, Detroit, MI and Cardiff University, Cardiff, Wales, United Kingdom.

Body: Triple negative breast cancers (TNBCs) lack estrogen and progesterone receptors and Her2/neu amplification, and are hence not treatable with therapies targeting these molecules. TNBCs have upregulated DNA damage response mechanisms, including the Rad6 postreplication repair (PRR) pathway, that potentially contribute to chemoresistance. Rad6 is a major component of the PRR pathway and its ubiquitin conjugating (UBC) activity is critical for its function. Rad6 expression is low in normal breast cells and tissues but the Rad6 homolog Rad6B is overexpressed in invasive, metastatic and chemoresistant BrCas. Constitutive overexpression of Rad6B in MCF10A cells induces resistance to cisplatin and doxorubicin. TCGA analysis of TNBC patient data showed an association between high Rad6B expression (but not Rad6A) and decreased overall survival. We recently reported the development of a novel Rad6-selective small molecule inhibitor (SMI#9) that inhibits Rad6 UBC activity, migration, and induces apoptosis in TNBC cells but has no effect on MCF10A cells. Since SMI#9 has limited aqueous solubility, in this study we synthesized a modified analog of SMI#9 to enable conjugation via a hydrolyzable ester bond to gold nanoparticle (GNP) and to improve delivery. GNP tethered SMI#9 (SMI#9-GNP) was characterized for purity, ligand conjugation and size by thermogravimetric analysis, atomic force microscopy, transmission electron microscopy, UV-Vis spectroscopy and zeta sizer, and for cellular uptake and drug release by FTIR and mass spectrometry. We compared the activities of SMI#9-GNP and free SMI#9 for cytotoxicity and intracellular localization in mesenchymal (MDA-MB-231 and SUM1315) and basal (MDA-MB-468 and HCC1937) subtypes of TNBC, and in MCF10A cells. Whereas free SMI#9 was cytotoxic to all TNBC cells, SMI#9-GNP demonstrated as good or better cytotoxicity than free SMI#9 only in mesenchymal TNBC cells. MCF10A cells were unaffected by both free and SMI#9-GNP. Consistent with cellular sensitivities, SMI#9-GNP is efficiently endocytosed and processed in lysosomes in mesenchymal TNBC cells, while uptake into basal TNBC cells is compromised by cell microenvironment induced SMI#9-GNP aggregation. SMI#9-GNP treatment induces mitochondrial dysfunction, and stabilization and hyperactivation of PARP-1 that was commensurate with autophagy (indicated by LC3-I to LC3-II conversion). Rad6 loss and PARP-1 hyperactivation are associated with mitochondrial dysfunction, and since inhibition of Rad6 induces both mitochondrial dysfunction and PARP-1 activation this implicates a potential novel role for Rad6 in linking these processes. In summary, our data show that SMI#9-GNP is a suitable delivery vehicle and that the SMI#9 released from GNP conjugate functions similarly as free SMI#9. Our data also illustrate how cell microenvironment induced changes in the physical properties of GNP-drug conjugates can have important implications in the application of nanoparticles in cancer therapy. Supported by NIH R21 CA178117.
**Title:** Intra-cellular dsRNA receptor RIG-I: A ubiquitous novel target for treatment of chemotherapy drug resistant breast cancer

Jasani B, Navabi N, Barrett-Lee P, Thompson A, Chester J and Mason M. Nazarbayev University School of Medicine, Astana, Kazakhstan; Velindre Cancer Centre, Velindre Hospital NHS Trust, Cardiff, Wales, United Kingdom; James Arrott Drive Ninewells Hospital & Medical School, University of Dundee, Dundee, Scotland, United Kingdom and Institute of Cancer & Genetics, Cardiff University, Cardiff, Wales, United Kingdom.

**Body:**

**INTRODUCTION:** Toll-like receptor 3 (TLR3), the cell surface receptor for double stranded RNA (dsRNA), expressed in ~40% of advanced primary breast cancers (T2/T3/N+) has been shown to be an effective therapeutic target for synthetic dsRNA poly A:U in combination with radiation and adjuvant chemotherapy, producing a significant decrease in risk of metastatic relapse (HR 1.85-2.0; 1.03-3.89; Cancer Res;71:1607).

**AIM:** The present pre-clinical study aimed to explore the therapeutic potential of a ubiquitously expressed intracellular dsRNA receptor, retinoic-acid-inducible gene-I (RIG-I) - a cytoplasmic pathogen recognition receptor directed at pathogen-associated molecular pattern (PAMP) motifs to differentiate viral from cellular dsRNAs. We have shown Ampligen, poly I:C12U (a synthetic dsRNA polymer designed to rapidly degrade in vivo to prevent the toxicity of long dsRNA polymers such as poly I:C) is capable of entering cells in its fragmented dsRNA oligomeric form (<1–2 kb) optimal for activation of intra-cellular RIG-I (J Exp Med 2008;205:1601–1610).

**METHODOLOGY & RESULTS:** Preliminary experiments with Ampligen on several human breast cancer cell lines (MCF-7 & MDA-MB 453) and normal human mammary epithelial & fibroblast cell lines (HMEC & HFC) unexpectedly showed it to consistently cause a significant loss of cell viability (CellTiter-Glo Luminescent Cell Viability Assay) in p53-deficient drug resistant (5-FU/doxorubicin) MDA-MB 453 cell line in contrast to cell growth arrest (Guava cell Cycle Assay) in p53 wild type MCF-7 cancer and the two non-neoplastic cells lines. This selective effect was confirmed using syngeneic clones of MCF-7 breast cancer cells stably transfected with a dominant negative p53 construct or vector alone: p53-function blocked (DD1) vs p53-function active (EV1) MCF-7 cell lines, respectively (J Nucl Med 2006; 47:1525–1530), and shown to be associated with RIG-I specific mRNA induction (RT-PCR) and Type I interferon pathway activation both inhibited by BX795 (selective inhibitor of IRF3 activation and IFN-b production). Decitabine (DNA demethylating drug capable of intra-cellular generation of dsRNA through transcriptional activation of Alu retrotransposons - PNAS 2012; December 10: E89–E98 ) was next tested as an alternative source of intra-cellular dsRNA, and found to produce results similar to Ampligen on DD1 and EV1. Work is in progress to examine dose response and time-course relationships of the effects Ampligen or decitabine added singly or in combination with chemotherapeutic drug (e.g. doxorubicin) on DD1 and triple negative breast cancer cell lines such as MDA-MB 453 to explore potential therapeutically most effective protocols.

**PROVISIONAL CONCLUSIONS:** Intra-cellular dsRNA receptor RIG-I constitutively expressed in all cells offers a more ubiquitous target compared to TLR3 for the treatment of breast cancer. Ampligen and decitabine with their selective growth inhibitory effect on drug resistant p53-deficient breast cancer cell lines, merit testing as novel drugs for treatment of p53-deficient drug resistant breast cancer e.g. triple negative breast cancer frequently (~70%) associated with drug resistance and altered p53 status.
**Body: Background:** Triple-negative breast cancer (TNBC), accounts for 15% of all invasive breast cancers (BCs) and has the poorest survival outcome of all BC subtypes. Due to its heterogeneity, TNBC lacks validated therapeutic targets compared with other BC subtypes (Sohn et al., 2014; Foulkes et al., 2010). Therefore, improved approaches to treatment of these cancers are unmet needed. Several molecular targets including: epidermal growth factor receptor (EGFR), poly ADP ribose polymerase (PARP), and hepatocyte growth factor receptor (c-MET) are under clinical investigation for the treatment of this disease (De et al., 2014; Cleator et al., 2007). The \textit{MET} oncogene encodes a membrane-bound tyrosine kinase implicated in the formation and/or progression of several cancer types including TNBC, and several studies have shown c-Met overexpression to be an independent predictor of poor outcome in BC (Ho-Yen et al., 2014), c-MET may play a critical role in the development of the most aggressive BCs and may be a rational therapeutic target (Graveel et al., 2009). Currently inhibitors targeting c-MET (including ARQ197) are undergoing clinical trials in a variety of cancers including TNBC (Gaule et al., 2014; ClinicalTrials.gov). Recently, PARP inhibitors in combination with chemotherapy, has shown promising results in TNBC in clinical and preclinical studies (Tutt et al., 2010; De et al., 2014). We argue that, blocking the PARP-mediated nuclear machinery for repairing DNA-damage in presence of cytotoxic DNA damaging agents in conjunction with co-targeting c-Met pathway dependent downstream effectors may have a robust anti-tumor activity in TNBC cell lines. **Methodology:** BT-20 (\textit{PIK3CA} mutated, H1047R), HCC70 (\textit{PTEN} null), HCC1937 (\textit{PTEN} null), MDA-MB-231 (\textit{KRAS/BRAF} mutated), MDA-MB-468 (\textit{PTEN} null) and SUM149PT (\textit{BRCA1} mutated) cells were used for this study. Growth inhibition, survival/proliferation, colony formation and apoptosis were examined using MTT assay, 2D proliferative/growth assay, 3D-ON-TOP assays, and annexinV staining respectively. **Results:** 1) For all TNBC cell lines, the IC\textsubscript{50} of single agent ARQ197 was from 0.5 µM to 1.5 µM (following 96 hours treatment) 2) ARQ197 as a single agent or in combination with ABT888 or in triple combination dose dependently decreased cell growth/proliferation 3) annexin V positive cells were increased following treatment with single agent ARQ197 or in combination with ABT888 or in triple combination 4) 70-99% anti-proliferative activities were observed on 3D-ON TOP colony formation assay with ARQ197 alone or in combination in all tested cell lines. **Conclusion:** Our preclinical \textit{in vitro} drug sensitivity data suggest that administration of c-MET inhibitor may enhance the antitumor activity of PARP inhibitor plus standard cytotoxic agent in TNBC models. Mechanism studies are ongoing, the results of which will be presented in the meeting.
Title: Targeting TMEPAI/ PMEPA1 inhibited triple negative breast cancer cell growth and metastasis through growth suppressive TGF-β signaling

Singha PK K, Pandeswara S, Venkatachalam MA A and Saikumar P. University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Background: Triple negative breast cancers (TNBC) that lack estrogen receptor (ER), progesterone receptor (PR) and hormone epidermal growth factor receptor 2 (HER-2/neu) are aggressive and cause high mortality among breast cancer patients. Transforming growth factor beta (TGF-β) dependency for their aggressive behavior (growth and metastasis) has been well established in many of these tumors. Although targeting TGF-β signaling has major potential to treat TNBCs, however it carries the risk of disturbing the tumor suppressive effects of TGF-β in early tumors and its homeostatic control of normal tissues. Hence there is a need to identify novel targets to impede tumor progression without compromising the beneficial effects of TGF-β signaling. To satisfy this unmet need, earlier we identified that transmembrane prostate androgen induced (TMEPAI/PMEPA1), a direct target gene of TGF-β could act as a "molecular switch" that converts TGF-β from a tumor suppressor to promoter in TNBC. Thus, we undertook the present study that identified a novel compound that blocked TMEPAI expression.

Materials and Methods: All cell lines were cultured according to the ATCC recommendations. Cell proliferation was measured by quantitation of total cellular DNA. Immunoblotting, migration, invasion, tumor xenografts and lung metastasis were performed using standard methods.

Results: We identified a terpenoid derivative (TD) which inhibited the expression of TMEPAI, enhanced TGF-β signaling and blocked proliferation, migration and invasion of several triple negative breast cancer cells in vitro. Our results showed that TD increased phosphorylation of Smad2/3 and increased PTEN, p21 and p27 proteins that cause growth suppression. Concomitantly, TD decreased Akt phosphorylation and reduced Snail and Slug that are required for cell growth and metastasis. Interestingly, TD did not affect the growth of normal human mammary epithelial cells and failed to cause associated molecular changes that can result in growth suppression. Notably, using mice models (nude mice and syngeneic BALB/c mice), we showed that TD reduced tumor burden significantly with little or no toxicity. Consistent with its ability to inhibit Akt phosphorylation and induction of Snail and Slug proteins, TD suppressed lung metastasis of TNBC in mice model. Importantly, reduced tumors derived from TD treated mice exhibited increased expression of pSmad2/3, PTEN, p21, p27 and reduced expression of pAkt compared to tumors obtained from vehicle treated mice.

Conclusions: Our results have two important dimensions: On one hand, TD inhibited TMEPAI induction to promote growth suppressive TGF-β dependent Smad signaling in TNBC but not in normal cells. On the other hand, TD also inhibited non-Smad signaling that promotes growth and metastasis of TNBC both in vitro and in vivo. Hence our findings suggest that drugs that target TMEPAI will not only inhibit growth and metastasis of TNBC but will also restore homeostatic functions of TGF-β to prevent new tumor development.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-14-10

**Title:** Phase Ia/Ib study of taselisib (GDC-0032), a potent and selective phosphoinositide 3-kinase inhibitor, in Japanese patients with advanced solid tumors or hormone receptor-positive locally advanced or metastatic breast cancer (JO29196 study)


**Body:**

**Background:**

Taselisib (GDC-0032) is an orally bioavailable, potent and selective phosphoinositide 3-kinase (PI3K) inhibitor. Preclinical data showed that taselisib had increased antitumor activity against PIK3CA (gene encoding the PI3Kα isoform) mutant tumors. This study aimed to investigate the safety, tolerability and pharmacokinetics (PK) of taselisib as monotherapy and in combination with fulvestrant in Japanese patients (pts).

**Materials and methods:**

A 3+3 design was used. In Phase Ia, pts with advanced solid tumors received taselisib tablet monotherapy (2, 4 or 6 mg once daily [QD]), and safety and PK were evaluated. In Phase Ib, pts with hormone receptor-positive locally advanced or metastatic breast cancer received taselisib (2 or 4 mg QD) in combination with fulvestrant (500 mg at a time), and safety and PK were evaluated. Maximal administered doses of 6 mg QD as a single agent and 4 mg QD in combination with fulvestrant were based upon prior clinical trial experience with taselisib (Juric D. et al. AACR 2013, Abstract LB-64; Juric D. et al. SABCS 2013, Abstract PD1-3).

**Results:**

As of 15 Mar 2015, 9 pts (PIK3CA mutant: 2 pts) were enrolled in Phase Ia and 3 pts in Phase Ib. Phase Ia dose-escalation study has been completed and Phase Ib is ongoing.

In Phase Ia, no dose-limiting toxicity (DLT) was observed at any dose level tested (maximum administered dose of 6 mg QD). Common (≥3 pts) adverse reactions (ARs) were stomatitis (4 pts), rash (3 pts) and diarrhea (3 pts); the only Grade ≥3 AR was neutropenia (1 pt). Partial response was observed in 1 pt who received taselisib 4 mg and had a PIK3CA mutant breast tumor. Stable disease was observed in 4 pts. Cmax and AUC indicated a dose-proportional PK profile of taselisib within the dose range tested. Moreover, taselisib PK in Japanese pts was consistent with the PK reported from North American and European pts (Juric D. et al. AACR 2013, Abstract LB-64).

In Phase Ib, 3 pts received taselisib 2 mg in combination with fulvestrant and no DLT was observed. Preliminary ARs were similar to those with monotherapy and no Grade ≥3 AR was reported. Confirmation of tolerability of taselisib 4 mg in combination with fulvestrant is under evaluation.

**Conclusion:**

Taselisib monotherapy was well tolerated in Japanese pts up to a dose of 6 mg, which is the recommended dose in non-Japanese pts. Promising preliminary activity of monotherapy was observed in advanced solid tumors, especially in a pt with PIK3CA mutant tumor. The combination of taselisib 2 mg with fulvestrant is well tolerated. Investigation of tolerability of taselisib 4 mg in combination with fulvestrant is ongoing. Final results of this study will be presented here at the Symposium this year.
Title: Gene-expression analyses identify altered transcription factors and supports the antitumor activity of novel bromodomain inhibitors in triple negative breast cancer

Ocana A, Perez-Peña J, Serrano-Heras G, Corrales-Sanchez V, Montero JC, Gascón-Escribano MJ, Picazo MG G., García-Olmo DC, Martin M and Pandiella A. Translational Oncology Unit, Albacete University Hospital, Albacete, Spain, Albacete, Spain; IBMCC-CSIC, Universidad de Salamanca, Spain, Salamanca, Spain and Medical Oncology Service Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Body: Identification of novel therapeutic targets in triple negative breast cancer (TNBC) is a main goal. In silico evaluation of human tumors by gene expression analyses can identify functions that could be pharmacologically inhibited. BET bromodomain inhibitors are a new family of compounds that reduce activity of oncogenic transcription factors through the inhibition of bromodomains.

Material and methods: We performed gene expression and functional analyses comparing triple negative tumors with normal breast samples. RT-PCR was used to evaluate genes down-regulated by treatment with the BET bromodomain inhibitors JQ1 and OTOX15 at different time points in MDA-MB231 cells. Cell proliferation and colony growth was measured by MTT uptake and 3D growth in matrigel in a panel of cell lines including HS578T, BT549, MDA-MB231 and HCC3153 treated with JQ1 and OTOX15. Evaluation of apoptosis and cell cycle was performed by flow cytometry using Annexin V and propidium iodide, respectively. Western-blot was used for evaluation of protein expression. Xenografted animals were used for the assessment of the in vivo activity of JQ1.

Results: Gene expression analyses identified upregulated genes including a number of transcription factors. These genes were more expressed in MDA-MB231 compared with MCF-10A. Treatment with JQ1 reduced the expression of some transcription factors at different time points. JQ1 and OTX015 reduced proliferation and cell growth in a panel of triple negative cell lines. They also led to arrest at the G0/G1 phase in HS578T and MDA-MB231 at 12 and 24 hours; that was confirmed by the biochemical evaluation of cell cycle mediators. An induction of apoptosis was observed at 48 hours in HS578T. JQ1 at 50 mg/kg daily reduced tumor growth in MDA-MB231 xenografted in nude mice.

Conclusion: Gene-expression profiling identified upregulated transcription factors in triple negative tumors compared with normal breast human samples. BET bromodomain inhibitors reduced the expression of some of these genes in cellular models. JQ1 shows clear antitumor activity in vitro and in vivo.
Title: Phase 0 study evaluating COX2 inhibition on circulating PGE2 levels from obese subjects

Lengfelder L, Brenner A, Bowers L, Apte S, Galván G, Kist K and deGraffenried L. The University of Texas at Austin, Austin, TX and UTHSCSA, San Antonio, TX.

Body: Introduction: Obesity is associated with poor breast cancer outcomes in postmenopausal women in response to aromatase inhibitor therapy. Our prior studies have shown an association between reduced recurrence rate and use of cyclooxygenase-2 (COX-2) inhibiting non-steroidal anti-inflammatory drugs (NSAIDs) in obese breast cancer patients. The mechanism proposed was a decrease in prostaglandin E2 (PGE2) and reduced activation of the aromatase promote locally in the breast.

Methods: Postmenopausal women of varying body habitus were recruited at the CTRC in San Antonio and underwent randomized assignment to 1 of 3 arms: ASA 81mg daily, 1500mg of docosahexaenoic acid (DHA) and 2500mg eicosapentaenoic acid (EPA) given daily, or combined ASA and DHA/EPA. Sera were collected prior to and following 28 days of exposure, and cytokines including prostaglandin E2 were assessed via enzyme-linked immunosorbant assay (ELISA). Conditioned media was generated by exposing macrophages to patient sera in order to see if the patient sera induced PGE2 concentration in vitro.

Results: Thirty of the planned 120 subjects have completed assessment. No toxicity has been noted. In 71% of the patients, serum PGE2 levels decreased, but only 60% demonstrated concurrent decrease in serum PGE2 levels as well as macrophage PGE2 production, while almost all (88%) of the patients whose serum did not demonstrate a decrease in PGE2 levels also demonstrated no decrease in induced levels.

Conclusion: NSAIDs appear to effectively decrease circulating levels of PGE2 in most obese women. However, one third of the subjects did not demonstrate concurrent suppression of induced PGE2 from macrophages. These data suggest that circulating levels of PGE2 may not be reflective of local tumor microenvironment levels, and other pro-inflammatory circulating factors may be responsible for regulating local inflammatory responses. Final analysis will be completed and presented at the SABCS meeting.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD4-01

Title: Longitudinal health related quality of life (HRQOL) and subsequent adherence to breast cancer chemotherapy among African American women

Liang Z and Rosenzweig MQ Q. University of Pittsburgh School of Nursing, Pittsburgh, PA.

Body: Background: Racial breast cancer survival disparity is attributed, in part, to disparity in cancer treatment. Changes in HRQOL influencing treatment adherence over the chemotherapy course may be under recognized as a potential etiology of racial disparity in breast cancer treatment.

Objective: To describe African American breast cancer women's HRQOL and the relationship of HRQOL with adherence (dose prescribed without delay) rates to prescribed chemotherapy over three time points of chemotherapy (baseline, midpoint and completion). Methods: Descriptive analysis of an ongoing randomized controlled trial of a psycho-educational intervention to encourage acceptance and adherence to chemotherapy for African American women with breast cancer. The present study used HRQOL data from the parent study and descriptively reported change of quality of life over three time points: baseline – pre chemotherapy (Time 1); at midpoint of chemotherapy (Time 2) and at completion of chemotherapy (Time 3). A further analysis of HRQOL and its role as a potential mediator with treatment adherence was assessed. Descriptive analysis of secondary aim of the parent study, The Attitudes, Communication, Treatment and Support (ACTS) intervention. The Functional Assessment of Cancer Therapy (FACT) was used to measure HRQOL including physical, social, functional and emotional subscales. Adherence data were extracted from medical records. Linear mixed modeling analysis method was used.

Results: One hundred and twenty six African American patients were included from the ongoing parent study for this analysis, 67 from the intervention group and 69 from the usual care group. Subjects were recruited from four cancer centers in western Pennsylvania and one from Western Ohio. Subjects were diagnosed with any stage invasive breast cancer and were undergoing chemotherapy. Overall HRQOL decreased significantly over three time points (p<0.01) primarily driven from the physical subscale. Adherence to prescribed chemotherapy was significantly correlated with HRQOL (r=0.32, p<0.01). Physical well-being subscales decreased significantly over time. Energy, treatment side effects, feeling ill and spending time in bed during chemotherapy were items that decreased in score (increased in severity) most over three time points (decreased score >0.5 from time1 to time3). Energy and pain were the items with the lowest scores for the physical well-being subscale at baseline and the two follow up assessments (mean score<3.0 out of 4).

Conclusions: Worsening in physical symptoms and functional status was demonstrated over time among African American women receiving breast cancer chemotherapy. Lack of adherence to prescribed chemotherapy was significantly correlated with declining HRQOL.

Implications for Practice: The influence of HRQOL on chemotherapy dose reduction and early treatment cessation was primarily from physical factors during chemotherapy for African American women. Clinically this emphasizes the importance of close QOL and symptom screening, encouraging symptom reporting and the need to exhaust symptom management options before early treatment termination.
Title: Health beliefs predict adherence to aromatase inhibitors


Body: Background: Post-menopausal breast cancer survivors are often prescribed aromatase inhibitors (AIs) to decrease the chance of cancer recurrence. Despite their efficacy, many survivors do not fully adhere to their AI regimen. To improve adherence rates, it is important to understand which patient factors are associated with adherence. Current research has mostly focused on demographic, cancer, and symptom variables, most of which cannot be modified. One relevant factor that may be modifiable is health beliefs, which include perceived susceptibility of cancer recurrence, perceived benefits of treatment, and perceived barriers to treatment. Among breast cancer patients, each of these has been found to be associated with adherence behaviors, such as mammography and tamoxifen adherence. In this study, we explored whether health beliefs also play a role in adherence to AIs.

Objective: The purpose of this longitudinal study was to determine whether patients with lower perceived susceptibility to cancer recurrence, higher perceived barriers to taking AIs, and lower perceived benefits of AIs were more likely to non-adhere to their AI regimen. Method: Four hundred and thirty-seven breast cancer survivors who were currently on an AI completed a survey that included the Health Beliefs and Medication Adherence in Breast Cancer (HBMABC) scales (a measure adapted from the Champion Health Belief Model Scales (CHBMS) for Mammography Screening), as well as questions about their demographics and symptoms. Exploratory and confirmatory factor analysis of the HBMABC yielded a 3-factor solution: perceived susceptibility, perceived benefits, and perceived barriers. Adherence data, including drug holidays (taking breaks from AI treatment) and premature discontinuation (stopping AI treatment early), were collected from physicians' notes in patients' medical charts dating from the day they completed the survey through the end-date of their prescribed AI treatment. Bivariate analyses were conducted to determine variables that were predictive of non-adherence. Variables found to be associated with non-adherence were entered into multiple logistic regression analyses. Results: Eighty-five patients (20.6%) exhibited some form of non-adherence (premature discontinuation, drug holiday, or both). Joint pain severity and the number of years a patient was on an AI at the time of the survey were both associated with non-adherence. After adjusting for these covariates, perceived barriers to AI treatment was significantly associated with non-adherence (OR 1.76, 95% CI: 1.03 – 3.00, p = 0.04). No relationship was found between perceived susceptibility or perceived benefits, and AI adherence. Conclusions: Breast cancer patients on AIs who perceive greater barriers to AI treatment are more likely to non-adhere to their AI regimen. This finding suggests that clinicians can intervene to help modify patients' negative beliefs and ultimately help improve patients' adherence levels.
Title: Adherence to diet, physical activity and body composition guidelines and breast cancer in the black women's health study

Nomura SJO JO, Yu J, Dash C, Rosenberg L, Palmer J and Adams-Campbell L. Georgetown Lombardi Comprehensive Cancer Center, Washington, DC and Boston University Sloane Epidemiology Center, Boston, MA.

Body: Background: While breast cancer incidence rates have declined in non-Hispanic Caucasian populations, rates have remained stable in African American women, who are often affected by more aggressive subtypes. Previous studies have found that adherence to the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) cancer prevention recommendations, and the similar American Cancer Society (ACS) guidelines, is associated with lower incidence of breast cancer. However, few African American women were included in these studies, and guidelines are based primarily on research among Caucasian women.

Objective: To evaluate the association between adherence to the WCRF/AICR cancer prevention recommendations and breast cancer incidence among African American women.

Design: The Black Women's Health Study is an ongoing prospective study of African American women from across the United States who were 21-69 years of age at baseline in 1995. They are followed biennially through health questionnaires. Among 49,103 women who were free of cancer at baseline and who provided relevant dietary and data on the baseline questionnaire, 1,827 incident cases of breast cancer were ascertained during follow-up through 2011. Questionnaire data on physical activity, body composition and diet were used to compute adherence scores for seven WCRF/AICR recommendations involving those factors. For each individual recommendation, participants were categorized as adherent (1 point), partially adherent (0.5 points) or non-adherent (0 points). Scores were summed to a total adherence score (maximum score: 7 points) and a diet only adherence score (maximum score: 5 points). Adherence scores (categorical and continuous) based on baseline data only and on time-varying data were assessed in relation to breast cancer incidence using Cox proportional hazards regression models, with control for potential confounding factors.

Results: In the analytic cohort, 8.5% of participants had an adherence score of 4.5-7.0, while 46% had a score less than 3.0. For individual recommendations, 15.2% were adherent to body weight recommendations, 24.7% were adherent to physical activity, and 5.4% were adherent to more than 4 diet recommendations. Participants were most likely to adhere to the alcohol recommendation (94.3%). In the time varying model, higher overall adherence (per 0.5 unit increase) was associated with lower breast cancer incidence (HR: 0.90, 95% CI: 0.84-0.96), with greater adherence to diet overall, physical activity, sugar beverage intake, and red and processed meat recommendations all significantly associated with reduced risk. The adherence score based on baseline variables was not associated with significantly reduced risk (HR: 0.96, 95% CI: 0.90-1.02), although meeting physical activity recommendations was associated.

Conclusions: Our findings suggest that adherence to the WCRF/AICR guidelines may lower risk of developing breast cancer in African American women. However, body weight and alcohol, factors that are widely considered important for breast cancer prevention appear to be less relevant in this population.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD4-04

Title: Psychosocial factors related to interruptions in adjuvant hormonal therapy among women with breast cancer: The breast cancer quality of care study (BQUAL)

Hershman DL, Kushi LH, Hillyer GC, Coromilis E, Buono D, Lamerato LE, Bovbjerg DH, Mandelblatt JS, Tsai W-Y, Jacobson JS, Wright JD and Neugut AI. Columbia University, NY, NY; Kaiser-Permanente of Northern California, Oakland, CA; Henry Ford Health System, Detroit, MI; University of Pittsburgh Cancer Institute, Pittsburgh, PA and Lombardi Comprehensive Cancer Center, Georgetown, DC.

Body: Background. Adjuvant hormonal therapy (HT) for hormone-sensitive breast cancer decreases risk of breast cancer recurrence and improves survival. However, some women are non-adherent to this life-saving treatment.

Methods. In a cohort of women recruited at diagnosis of breast cancer in an integrated healthcare system, we investigated factors related to HT interruption (≥90 day gap). Serial interviews were conducted at baseline and during treatment to examine psychological factors as well as sociodemographic factors, tumor characteristics, and treatment factors. A series of multivariate models assessed potential predictors of HT interruptions.

Results. Of the 569 women in our cohort who initiated HT, 137 (24%) interrupted it, including 18 (3%) who did so prior to the first follow-up interview. In a multivariate analysis of clinical and demographic factors, only household income remained associated with HT interruption (OR 0.42, 95%CI 0.24-0.76). At first follow-up, after controlling for income, race and age, lower scores on all quality of life subscales, lower scores on global treatment satisfaction, and poorer scores on the intrusive and avoidant thought subscales of the Impact of Events scale were associated with higher odds of HT interruptions (P<0.001 for all predictors). Scores on social support and on interpersonal processes of care measures were not associated with HT interruptions. However, a higher score on the single question "How often did your doctor speak too fast?" was associated with higher risk of HT interruptions (OR 1.32, p=0.02).

Conclusions: Patients under greater duress and those with lower physical, functional, emotional or social quality of life appeared to be at the highest risk of HT interruption and thus received poorer quality care. A better understanding of psychological factors that can result in poor quality care may pave the way to targeted interventions to improve adherence.
Body: PURPOSE: Adjuvant therapy is associated with improved survival for women with breast cancer, but not all women who could benefit initiate treatment. Women's belief systems are related to treatment initiation. It has been hypothesized that complementary and alternative (CAM) use is associated with decreased initiation of standard oncology treatments because patients may be exploring alternative treatment approaches. However, there are limited data on the association between CAM use and cancer treatment initiation. We examined the association between CAM use and initiation of adjuvant breast cancer chemotherapy in a prospective cohort of early stage breast cancer patients.

PATIENTS AND METHODS: Subjects participated in a multi-center prospective cohort study of women with early stage invasive breast cancer (n=1,156). National Comprehensive Cancer Network guidelines were used to define groups based on whether chemotherapy was indicated. Three subgroups were created: chemotherapy indicated for subjects <70 years, chemotherapy discretionary for subjects <70 years, and chemotherapy discretionary for subjects ≥70 years. CAM use was assessed based upon self-reported use of 5 CAM modalities, including vitamin/mineral supplements, herbal supplements, other over-the-counter natural products, mind-body based approaches, and body/energy-based treatments. Psychosocial factors potentially related to chemotherapy initiation were assessed. Multivariable logistic regression models evaluated the associations between CAM use and chemotherapy initiation, adjusted for demographic, clinical and psychosocial factors.

RESULTS: Current CAM use was reported by 87% of women and 38% reporting current use of ≥3 modalities. The most commonly used CAM modalities were mind body therapies (63%) and other natural products (41%). In bivariate analyses, among women <70 years where chemotherapy was indicated, women who reported current use of vitamins/minerals or current use of all 5 CAM modalities were less likely to initiate chemotherapy compared to non-users (P<.0001), but this was not observed among women for whom chemotherapy was discretionary. Psychosocial factors were also associated with high levels of current CAM use in this group, including higher expectations of adverse effects from chemotherapy, more concerns about the physical effects of chemotherapy, lower beliefs in the benefits of chemotherapy, and lower positive decision balance while making chemotherapy decisions (all P<.05). Among women age <70 years for whom chemotherapy was indicated, 89% initiated treatment, and current use of all 5 CAM modalities was inversely associated with initiation in multivariable analyses adjusted for demographic and clinical factors (OR=0.08, CI: 0.02-0.32). The association remained after separately adjusting for psychosocial factors (all P<.05), except for positive decision balance, which was no longer statistically significant.

CONCLUSIONS: High use of CAM was associated with decreased chemotherapy initiation among women with breast cancer for whom chemotherapy was indicated. It is important for oncologists to discuss CAM use with their patients, especially since high CAM use is associated with negative expectations and beliefs about chemotherapy.
Title: Acupressure for persistent fatigue in breast cancer survivors

Zick SM M, Wyatt GK K, Murphy SL L, Arnedt JT T, Sen A and Harris RE E. University of Michigan, Ann Arbor, MI and Michigan State University, East Lansing, MI.

Body: Background: Persistent fatigue is a common and debilitating symptom in breast cancer survivors (BCS), yet treatments remain limited. The purpose of this study was to examine the effect of two types of self-administered acupressure on fatigue versus usual care in BCS.

Methods: This was a 10-week randomized trial that enrolled adult female BCS (stage 0-III), who had completed cancer treatments at least 12 months previously and who reported persistent mild-moderate fatigue (≥ 4 on the Brief Fatigue Inventory [BFI]). BCS were randomized equally into relaxation acupressure (RA), stimulating acupressure (SA) or usual care (UC). The primary endpoint was change in BFI from baseline to week 6 when acupressure was stopped and at week 10 to assess carryover effects of acupressure. Secondary analyses were conducted on sleep quality using the Pittsburgh Sleep Quality Index (PSQI) and 14-day sleep diaries; quality of life was assessed with the Long-Term Quality of Life in BCS (LTQL). Intent-to-treat analyses were conducted using linear mixed models.

Results: 288 BCS were randomized (98 RA, 94 SA, and 96 UC). 228 BCS completed the 6-week visit and 223 the 10-week visit (71 RA, 69 SA, and 83 UC). There were no significant group differences on baseline sociodemographic, clinical characteristics, BFI, PSQI, sleep diary parameters, or LTQL. At week 6 the mean change in BFI from baseline was significantly lower in the RA and SA arms than UC (RA = -2.57 ± 1.5, SA = -1.98 ± 1.5, and UC = -1.07 ± 1.6; p < 0.001 for both RA and SA vs. UC), but there was no significant difference between acupressure arms (p = 0.29). At week 10 the mean change in BFI from baseline ± SD was maintained and continued to be lower in RA and SA arms than UC (RA = -2.27 ± 1.4, SA = -1.96 ± 1.5, and UC = -0.99 ± 1.5; p < 0.001 for both RA and SA vs. UC), but no significant difference between acupressure arms (p >0.99). At week 6 the mean change in PSQI from baseline mean ± SD for RA = -1.93 ± 3.3 was significantly different from UC = -0.46 ± 3.1 (p = 0.03) but not SA = -1.34 ± 3.2 (p = 0.96). At week 10 there was no significant difference on the PSQI between arms (RA vs. UC, p = 0.40; SA vs. UC, p <= .99; RA vs. SA, p = 0.79); however PSQI scores remained lower and stable in the RA arm. There were no significant differences between the three arms at any time point for the sleep diary parameters sleep efficiency, total sleep time, sleep onset latency, or wake after sleep onset. Women in the RA arm were significantly improved versus UC for three of four quality of life subscales at both 6- and 10-week visits (somatic, p=0.03week 6, p=0.04 week 10; physical fitness, p=0.04 week 6, p=0.01 week 10; and social support, p=0.03 week 6, p=0.04 week 10; spirituality, p=0.55 week 6, p=0.17 week 10). The SA group was not significantly different from UC for any subscale at any time point.

Conclusions: Both acupressure arms significantly reduced fatigue compared to UC, but only RA had a significant effect on improving sleep quality and quality of life in BCS. Improvements in fatigue, sleep, and quality of life continued to persist for 4 weeks after cessation of acupressure. Self-administered RA offers an inexpensive easy to learn method to manage fatigue, sleep quality and overall quality of life in BCS with persistent fatigue.
Impact of breast density legislation on Hispanic/Latinas in the Northeast, US

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Objective: Changes in health care delivery and policies resulting from translational research efforts are intended to benefit a broad segment of the affected population. Yet, uptake of new approaches may not occur at the same level and/or pace in all groups, inadvertently increasing disparities in cancer outcomes. Our objective is to explore the impact of recently enacted legislation associated with routine mammography screening on Hispanic/Latino women living in Connecticut. Background: Breast densities are the non-fat (epithelial and stromal) breast tissue observable on screening mammograms. They are associated with a 4 to 6 fold increase in breast cancer risk and complicate the reading of screening mammograms, resulting in lowered sensitivity. Connecticut (CT) and many other states have enacted legislation requiring supplemental testing to be offered to women with dense breasts. Per CT statute, insurance companies must cover the cost of ultrasound screening of an entire breast/breasts for women with heterogeneously or extremely dense breasts. Additionally, personal information on breast density must be included in the mailed result following a screening mammogram. The intent is to improve early detection in women with dense breasts and to increase awareness of the greater risk of associated with dense breast tissue. Methods: After this law was enacted in 2008, we undertook a large prospective study of mammography screening in community based Hispanic/Latinas. We enrolled women seeking care in primary health care settings in the 4 CT cities with the largest H/L populations. Eligible women were ages 40-75, self-identified as H/L, and had negative history for breast cancer or breast biopsy. With 75% participation for baseline interview and 98% consent for medical record review, we report baseline interview data and mammography results (medical records) over a 2.5 - 4 year follow-up on 668 H/L women, ages 40-79, living in CT at the time of enrollment (2009-2011). Results: The women in this study were mostly foreign or Puerto Rican born (84%), lower socioeconomic status (51% with household incomes less than $10,000 per year; 54% less than a high school education) than the general population; median age was 51. Nearly half (46.0%) reported no usual care provider. Only 14% reported speaking English "very well". Most women reported that they received a mammogram in the previous year (65.0%). 21.4% of women met the criteria for receiving additional bilateral ultrasound testing due to heterogeneously dense (19.2%) or extremely dense (2.3%) breast tissue on screening mammograms occurring during follow up. Of the 128 women eligible for follow-up ultrasound, 18 (14%) received this exam. Conclusion: Although state law requires patient notification of breast density and insurance coverage for supplementary bilateral ultrasound tests in women with moderate to extremely dense breasts, our results show low uptake in Hispanic/Latino women in CT. In this largely foreign born, English second language population, effective communication regarding breast cancer risk, breast density, and the availability of follow-up ultrasound or other testing may represent a significant cancer care challenge.
Title: Post-diagnosis physical activity and comorbidities, not BMI, explain mortality risk in the after breast cancer pooling project

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Body: Background: In its 2014 position statement, ASCO concluded that obesity is associated with worse prognosis after cancer diagnosis. However in the same year, a comprehensive review by the World Cancer Research Fund concluded that there was limited evidence that greater body fatness increases risk of overall or breast cancer mortality, indicating that further investigation into lifestyle factors are needed. The After Breast Cancer (ABC) Pooling Project has reported, separately, significant mortality effects of pre-diagnosis BMI and of post-diagnosis physical activity (PA). We investigate whether the effect of BMI can be limited to subgroups characterized by comorbidities and physical activity.

Methods: Data are from the three US cohorts that were harmonized in the ABCPP (n=9513) including: the Women's Healthy Eating and Living (WHEL), Life After Cancer Epidemiology (LACE), and Nurses’ Health (NHS) studies. Stepwise delayed entry Cox proportional hazards models examined each lifestyle predictor (BMI, PA, and comorbidities assessed after diagnosis) sequentially and together in multivariate models for breast cancer and all-cause mortality.

Results: In multivariate models without the other two target variables, PA was significantly associated with a 17% decrease in the risk of breast cancer mortality among women in the highest quartile of PA (MET hr/wk > 21.4), compared to the lowest quartile (MET hr/wk < 2.7) (HR=0.81, 95% CI= 0.67,0.97). In the model with major comorbidities, there was a significant 40% increase in the risk of breast cancer mortality among women diagnosed with both diabetes and hypertension (HR=1.40, 95% CI= 1.01,1.93). In the model with BMI, there was no significant association with risk of breast cancer mortality. These results were essentially unchanged with all variables in a single model.

For all-cause mortality, the PA-only model showed a significant PA effect with the hazard decreasing from 20% to 40% across quartiles (Q2 HR=0.80, 95% CI=0.71,0.90, Q4 HR=0.62, 95% CI=0.54,0.71). In the comorbidity-only model, both diabetes and hypertension significantly increased hazard of all-cause mortality 80% and 33%, respectively. Having both diagnoses was associated with a significant, 2.3 fold increase in all-cause mortality (HR=2.34, 95% CI= 1.95,2.81).

In the BMI-only model, being underweight was associated with a significant 2.4 fold increase in risk of all-cause mortality, and there was a 20 and 37% increase in risk associated with being categorized as obese I or II (Obese I HR=1.23, 95% CI=1.07,1.40, Obese II HR=1.37, 95% CI=1.16,1.61).

With all three variables in the model, the risk associated with being obese decreased and became non-significant (Obese I HR=1.06, Obese II HR=1.05), while the significance, strength, and direction of the association of comorbidities and PA with all-cause mortality remained constant.

Conclusion: These data suggest that post-diagnosis comorbidities and lack of physical activity, rather than high BMI, are the important risk factors for all-cause and breast cancer specific mortality. While needing further validation, these suggest that physical activity interventions and monitoring treatment for comorbidities should become standard of care for breast cancer survivors.
Title: HERA trial: 10 years follow up of trastuzumab after adjuvant chemotherapy in HER2 positive early breast cancer — Final analysis

Jackisch C, Piccart MJ J, Gelber RD D, Procter M, Goldhirsch A, DeAzambuja E, Castro Jr G, Untch M, Smith I, Gianni L, Baselga J, Al-Sakaff N, Lauer S, Mcfadden E, Leyland-Jones B, Bell R, Dowsett M and Cameron D. Sana Klinikum Offenbach, Offenbach, Germany; Institute Jules Bordet, Brussels, Belgium; Dana Farber Cancer Institute and Frontier Science and Technology Research Foundation, Boston, MA; Frontier Science, Kincaig, United Kingdom; European Institute of Oncology, Milan, Italy; University of San Paolo, San Paolo, Brazil; Helios Kliniken Berlin Buch, Berlin, Germany; Breast Unit Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom; Ospedale San Raffaele, Milan, Italy; Memorial Sloan-Kettering Cancer Center, NY, NY; Hoffmann-La Roche, Basel, Switzerland; Edith Sanford Breast Cancer Research, Sioux Falls, SD; Andrew Love Cancer Center Geelong Hospital, Geelong, Australia; Royal Marsden National Health Service Truzst, London, United Kingdom and University of Edinburgh, West General Hospital, Edinburgh, United Kingdom.

Body: Background: Trastuzumab (T), a recombinant monoclonal antibody against HER-2 receptor, significantly improves overall (OS) and disease-free survival (DFS) in women with HER2 positive (HER2+) early breast cancer (EBC) when administered concurrent with or sequentially after adjuvant chemotherapy.

Material and Methods: HERA (BIG 1-01) is an international, multicenter, phase III randomized trial involving 5102 women with HER2-positive (HER2+) EBC either nodal negative (tumor-size ≥ 1cm) or nodal positive. After completion of primary therapy, including surgery, chemotherapy and radiotherapy as indicated, patients (pts.) were randomized to T every 3 weeks for 1 yr, 2 years (yrs), or observation. Primary endpoint is DFS and secondary endpoints are OS and time to distant recurrence (TTDR). Here, we are presenting the HERA final analysis after 10 years of follow-up.

Results: The clinical data cut-off for this final analysis of the HERA trial is June 2015. Data are being cleaned and final safety and efficacy analyses will be available for presentation at the meeting. Cardiac toxicity remained low and occurred mostly during the treatment phase.
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Title: Moved to S1-05

Pituskin E, Mackey JR R, Koshman S, Jassal D, Pitz M, Haykowsky MJ J, Thompson R, Oudit G, Ezekowitz J and Paterson I. University of Alberta, Edmonton, AB, Canada; University of Manitoba, Winnipeg, MB, Canada; Mazankowski Alberta Heart Institute, Edmonton, AB, Canada; Cross Cancer Institute, Edmonton, AB, Canada; Bergen Cardiac Care Centre, Winnipeg, MB, Canada and Cancer Care Manitoba, Winnipeg, MB, Canada.

Body:
Title: NSABP FB-7: A phase II randomized trial evaluating neoadjuvant therapy with weekly paclitaxel (P) plus neratinib (N) or trastuzumab (T) or neratinib and trastuzumab (N+T) followed by doxorubicin and cyclophosphamide (AC) with postoperative T in women with locally advanced HER2-positive breast cancer

Jacobs SA A, Robidoux A, Garcia JMP M P, Abraham J, La Verde N, Orcutt JM M, Cazzaniga ME E, Calvo L, Aguirre E, Buyse M, Pogue-Geile KL L, Srinivasan A, Song N, Balousek AD D and Wolmark N. NSABP Foundation, Inc.; The University of Pittsburgh Cancer Institute; Centre Hospitalier de l'université de Montréal; Hospital Universitario Vall d'Hebrón; MedSIR, Barcelona; West Virginia University; Azienda Ospedaliera Fatebenefratelli e Oftalmico; Roper St. Francis Healthcare; Azienda Ospedaliera San Gerardo, Monza; Hospital Universitario; International Drug Development Institute (IDDI) and Allegheny Cancer Center, Allegheny General Hospital.

Body: Background:
Trastuzumab (T), the first anti-HER-2-directed therapy, dramatically changed the natural history of operable HER-2 positive breast cancer. A subsequent improvement occurred when pertuzumab was added to a T/docetaxel regimen in NeoSphere, a phase II randomized neoadjuvant trial, in which the pathologic complete response rate (pCR) in breast and nodes was increased from 21.5% to 39.3%, leading to accelerated approval of this combination by the FDA. Another dual anti-HER trial, NeoALTTO, which combined lapatinib with T, resulted in pCR (breast and nodes) increase from 27.6% to 46.8%. Neratinib (N) + paclitaxel (P) followed by AC was tested in the neoadjuvant I-SPY 2 trial resulting in pCR of 55% for hormone-negative, HER2-positive breast cancer. A 300-patient phase III trial of N or T with standard chemotherapy is planned. In the FB-7 phase II trial in HER2-positive patients (pts) with locally advanced disease, pts were randomly assigned to N or T or the combination (N+T) with weekly P followed by standard AC. Arm 3 of this trial, N+T+P, was based on the phase Ib results from NSABP FB-8, a study in heavily pretreated HER2-positive metastatic breast cancer pts, which demonstrated an overall response rate of 38% and clinical benefit rate of 52%.

Methods:
NSABP FB-7 opened in October 2010 as a two-arm trial (Arm 1, N+P→AC and Arm 2, T+P→AC). After accrual of 30 pts, accrual was suspended in December 2011 as NSABP FB-8, a phase Ib trial of neratinib, trastuzumab, and paclitaxel (N+T+P), was conducted and a recommended phase II dose for the combination of N+T+P was determined. NSABP FB-7 reopened in September 2012 adding Arm 3 (N+T+P→AC). 141 pts enrolled in this trial between October 2010 and November 2014. Three withdrew consent before treatment and were replaced. A total of 126 US, Canadian, and European pts were randomized to Arm 1 (N+P→AC), Arm 2 (T+P→AC) or Arm 3 (N+T+P→AC). 12 additional pts from the US were treated on Arm 3 as nonrandomized (NR) pts. These NR pts are included for toxicity but not for efficacy. Eligibility criteria included women >18 years of age, ECOG PS 0-1, stage IIB-IIIC invasive breast cancer, HER2-positivity by IHC 3+, FISH, or CISH as determined by local laboratories, hormone receptor positive or negative, LVEF >50%, and adequate laboratory parameters. The primary endpoint is pCR in breast and nodes.

Conclusions:
The last patient enrolled on this trial in November 2014. Datalock for the primary endpoint will occur on or before September 1, 2015. Pathologic complete response data and toxicity will be reported on the entire cohort of pts. Biomarkers will be analyzed on a subset of pts.

Support: Puma Biotechnology, Inc.
Title: Neratinib for ERBB2 mutant, HER2 non-amplified, metastatic breast cancer: Preliminary analysis from a multicenter, open-label, multi-histology phase II basket trial

Body: Background: Somatic ERBB2 (HER2) mutations occur in approximately 2% of patients with breast cancer and are found in a predominantly mutually exclusive manner with ERBB2 amplification. These mutations result in increased signaling and oncogenic transformation. Neratinib, a pan-ERBB irreversible tyrosine kinase inhibitor, potently inhibits growth of ERBB2 mutant tumor cell lines and xenografts. An ongoing signal-seeking phase II 'basket' study is evaluating neratinib in patients with multiple histologies harboring ERBB2 mutations (NCT01953926). Novel mutations identified in enrolled patients were characterized for biologic activity in a variety of in vitro model systems. A preliminary analysis of the HER2 non-amplified metastatic breast cancer cohort is presented.

Methods: Patients with ERBB2 mutant metastatic breast cancer documented by local testing methods received single-agent oral neratinib 240 mg once daily until progression or intolerable toxicity. High-dose loperamide prophylaxis was mandatory during cycle 1. The primary endpoint was the objective response rate at 8 weeks, defined using anatomic (RECIST 1.1) and/or metabolic (PET Response Criteria) assessments. Secondary endpoints were best overall response rate, clinical benefit rate, progression-free survival, duration of response, and safety.

Results: 17 patients with metastatic breast cancer were enrolled and received neratinib (13 patients are evaluable for efficacy to date). Patients had a median of 3 prior anticancer regimens. Other baseline characteristics were: median age 59 years; bone involvement 71%; visceral disease 82%. Tumor characteristics were: ductal/lobular 76%/24%; ERBB2 mutation single nucleotide variants/indels 82%/18%; HER2 amplified/non-amplified 0%/100%; hormone receptor positive/negative 82%/18%. Five patients (39%) had an objective response at 8 weeks (95% CI 14–68%). In the patients who responded, ERBB2 mutations were: 1 complete response (L755S); 4 partial responses (L755S, V777L, V777L, and L869R). The most common all-grade adverse events (in ≥15% of patients) across all cohorts (n=93) were: diarrhea (62%), fatigue (28%), nausea (36%), vomiting (30%), anemia (15%), and constipation (29%). The most common grade 3/4 adverse event was diarrhea (14%, all grade 3). Updated efficacy results, centralized genomic analyses on archival tumor samples, and in vitro characterization of novel ERBB2 mutants will be presented.

Conclusions: Single-agent neratinib shows encouraging signs of clinical activity in patients with heavily pretreated, ERBB2 mutant, HER2 non-amplified metastatic breast cancer. The breast cancer cohort demonstrated sufficient activity to meet the study's pre-specified efficacy requirements according to a Simon's two-stage design, and suggests that a confirmatory trial of neratinib for targeting ERBB2 driver mutations in metastatic breast cancer is warranted. Safety was acceptable and diarrhea was manageable with loperamide prophylaxis.
**Title:** Effects of perioperative lapatinib in early HER2+ breast cancer - The UK EPHOS-B trial (CRUK/08/002)

**Body:**

**Background:** Patients diagnosed with primary breast cancer (BC) often have a couple weeks interval between diagnosis and definitive surgery. This time window provides the opportunity for assessing biological drug effects in a treatment naive population. The EPHOS-B trial was designed to measure the effect of pre-operative anti-HER2 therapy on proliferation and apoptosis in HER2+ BC patients.

**Patients & methods:** EPHOS-B is a multicentre, 2-part randomized trial in patients with operable newly diagnosed HER2+ primary BC. In Part 1 patients were randomized (1:2:2) to no perioperative treatment (control), trastuzumab only or lapatinib only (11 days pre-operative therapy). Emerging evidence on the efficacy and safety of combination anti-HER2 therapy led to Part 2 in which patients were allocated to control, perioperative trastuzumab only or lapatinib and trastuzumab (1:1:2). The IDMC have agreed release of data from lapatinib Part 1 patients only.

Tissue samples were taken at the time of diagnostic core biopsy and surgery, and analysed centrally for Ki67, apoptosis (activated caspase 3), PgR, HER3 and Bcl2 by immunohistochemistry (IHC). Local ER and PgR status were also recorded.

**Results:** Between Nov-2010 and Jul-2013, 51 patients (pts) were allocated to perioperative lapatinib, with 49 (96.1%) receiving at least 1 dose. All pts were HER2+ (90% 3+ by IHC and 10% amplified by FISH, locally assessed) at entry. Median age was 51 years (IQR 48-60); 65% had tumours >2cm and 51% were grade 3 at surgery. According to local assessment, 61% were ER+ and 43% PgR+. Only 2 pts (4%) had a dose reduction and 1 pt (2%) discontinued lapatinib in the 3 days prior to surgery due to toxicity (rash, 3pts; nausea, 1pt). There were no delays in surgery.

Paired samples were valid for analysis in 43/51 (84%); invalid pairs were mostly due to inadequate samples for scoring. Overall, 67% (95% CI: 52 to 81) of pts demonstrated a ≥30% fall in Ki67 whilst a ≥30% rise in apoptosis was observed only in 30% of pts (95% CI: 17 to 46). When assessed as continuous variables, Ki67 fell significantly from pre-treatment but there was no significant change in apoptosis detected (results in table). No correlation was observed between Ki67 change and change in apoptosis (p=0.5). Neither HER-3 expression nor BCL2 predicted response.

<table>
<thead>
<tr>
<th></th>
<th>ER- HER2+, n=18</th>
<th>ER+ PR- HER2+, n=7</th>
<th>ER+PR+HER2+, n=17</th>
<th>All, n=43*</th>
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<tbody>
<tr>
<td>Ki67 % change from pre treatment</td>
<td>-51% (-69% to -21%), p&lt;0.001</td>
<td>-53% (-78% to -11%), p=0.02</td>
<td>-38% (-58% to -24%), p&lt;0.001</td>
<td>-45% (-57% to -32%), p&lt;0.001</td>
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<tr>
<td>Apoptosis % change from pre treatment</td>
<td>-24% (-37% to +30%), p=0.27</td>
<td>-13% (-48% to +125%), p=0.61</td>
<td>-13% (-65% to +43%), p=0.19</td>
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**Conclusion:** EPHOS-B demonstrates that ~11 days' lapatinib has a marked anti-proliferative effect in HER2+ve breast cancers. The trial is ongoing and when complete will provide a definitive analysis of the relative biological effects of perioperative treatment
with different anti-HER2 therapies (trastuzumab, lapatinib and their combination) in patients with HER2+ BC.
Title: The impact of early lapatinib-induced rash on disease-free and overall survival in patients treated within the ALTTO phase III randomized trial

Azim Jr HA A, Sonnenblick A, Agbor-Tarh D, Bradbury I, Daly F, Huang Y, Dueck AC C, Pritchard K, Wolff AC C, Jackisch C, Lang I, Untch M, Smith I, Boyle F, Xu B, Gomez H, Perez E, Piccart M and de Azambuja E. Institut Jules Bordet, Belgium; Frontier Science Scotland, United Kingdom; Novartis; Mayo Clinic Cancer Center; Sunnybrook Odette Cancer Centre, Canada; Johns Hopkins Sidney Kimmel Comprehensive Cancer Center; Sana Klinikum Offenbach, Germany; National Institute of Oncology, Hungary; Helios Klinikum Berlin-Buch, Germany; Royal Marsden Hospital, United Kingdom; Mater Hospital, Australia; Chinese Academy of Medical Sciences and Peking Union Medical College, China and Instituto Nacional de Enfermedades Neoplasicas Universidad Peruana Cayetano Heredia, Peru.

Body: Background: We have previously shown in a phase III neoadjuvant trial that early development of lapatinib-induced rash (i.e. within 6 weeks after lapatinib initiation) is independently associated with a higher chance of obtaining a pathological complete response (Azim et al; JCO 2013). In the current study, we aimed to investigate whether early lapatinib-induced rash is associated with improved survival in the context of a large phase III adjuvant trial.

Methods: This analysis is based on the ALTTO trial (BIG 2-06, Alliance N063D), in which patients with HER2-positive early breast cancer were randomized to adjuvant trastuzumab, lapatinib, their sequence or their combination for a total duration of 1 year. In this sub-study, we evaluated whether the development of rash (any grade) within 6 weeks of lapatinib initiation was associated with disease-free (DFS) and overall survival (OS). All analyses were tested in a multivariate model adjusted for treatment arm, treatment completion and trial stratification factors.

Results: A total of 6,098 lapatinib-treated patients were included in the current analysis; of whom 2,006 patients (32.9%) developed early lapatinib-induced rash, 1,025 (16.8%) developed rash after 6 weeks and 3,067 (50.3%) did not develop rash. No differences in patient characteristics were observed between the three groups apart from a higher frequency of younger patients (≤ 50) in the early rash group (54% vs. 47% and 44%, p<0.0001). At a median follow-up of 4.5 years, 876 (14.37%) and 377 (6.18%) patients in the lapatinib containing arms experienced a DFS and OS event, respectively. In a multivariate analysis confined to patients randomized to the lapatinib containing arms, the development of early rash was associated with improved DFS (HR: 0.80; 95%CI: 0.69-0.93, p=0.004) and OS (HR: 0.61; 95%CI: 0.48 - 0.78, p<0.001) compared to patients who did not develop early rash, with no interaction according to patient's age (p=0.9). No significant association was observed between the development of rash after 6 weeks of lapatinib initiation and survival. Compared to patients randomized to the trastuzumab alone arm (n=2,076), patients who developed early rash in the sequence (n=580) or combination (n=704) arms of trastuzumab/lapatinib had superior DFS (Sequence: HR 0.75 [95% CI: 0.58 – 0.98], p=0.034; Combination: HR 0.69 [95% CI: 0.54 – 0.89], p=0.005) and OS (Sequence: HR 0.57 [95%CI: 0.36 – 0.88], p=0.012; Combination: HR 0.59 [95% CI: 0.39 – 0.89], p=0.011). On the other hand, patients randomized to the lapatinib only arm who developed early rash (n=722) still had inferior DFS (HR 1.28 [95% CI: 1.04 – 1.59], p=0.02) with no difference in OS (HR: 0.95; 95%CI: 0.67 – 1.35, p=0.79) compared to patients randomized to the trastuzumab alone arm.

Conclusions: The results support our previous findings in the neoadjuvant setting that early development of skin rash within the first 6 weeks can identify patients who derive superior benefit of lapatinib treatment.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD5-08

Title: A biparatopic HER2-targeting antibody-drug conjugate demonstrates potent antitumor activity in primary tumor models that are refractory to or ineligible for HER2-targeted therapies


Body: Current HER2-targeted drugs are ineffective in killing cancer cells expressing relatively low levels of HER2. Therefore, more than 60% of breast cancer patients are ineligible for HER2-targeted therapies because of lack of HER2 overexpression and the vast majority of eligible patients who initially respond to the treatment will eventually relapse. MedImmune is developing a novel HER2-targeting antibody-drug conjugate (ADC) to address this unmet medical need. We show that a bivalent biparatopic antibody targeting two distinct non-overlapping epitopes on HER2 is able to induce receptor clustering on the tumor cell surface, which in turn facilitates internalization and promotes lysosomal trafficking and degradation. When conjugated with a tubulysin-based microtubule inhibitor, the biparatopic antibody can deliver a greater quantity of cytotoxin into the targeted cancer cells. As a result, it demonstrated superior antitumor activity over Kadcyla® (T-DM1) in HER2-overexpressing (HER2-positive) tumor models. It also induced complete tumor regression in a HER2-positive tumor model that had developed acquired resistance to T-DM1 through chronic exposure. Moreover, to explore the potential clinical applications in treating the HER2 non-overexpressing (HER2-negative) patients the biparatopic ADC was evaluated across 17 primary tumor models derived from HER2-negative breast cancer patients among which 13 were triple-negative. Other criteria were also considered in the selection of these 17 models, including the degree of heterogeneity in HER2 expression, ER/PR status and histopathologic subclass, to maximize the diversity of tumor subtypes in the study. The biparatopic ADC demonstrated potent antitumor activity regardless of the histopathologic subclass and ER/PR status of the tumor. At the dose of 1 mg/kg, 41% of the tumor models (7 out of 17) showed tumor regression and 6% (1 out of 17) showed tumor stasis. At the dose of 3 mg/kg, 71% of the models (12 out of 17) showed tumor regression and 12% (2 out of 17) showed tumor stasis. Overall, our findings underscore the potential use of this novel HER2-targeting ADC to treat a large patient population that is ineligible for or relapsed/refractory to current HER2-targeted therapies, and thus warrant investigation in the clinic.
Title: A case series of the Milky Way sign: A diagnostic finding of ductal carcinoma in situ (DCIS) and invasive breast carcinoma (IDC) on digital breast tomosynthesis (DBT)

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Body: Purpose: we have previously described the “Milky Way Sign”, which manifests as microcalcifications overlying noncalcified band-like density, best seen in digital breast tomosynthesis (DBT), in a patient with ductal carcinoma in situ (DCIS). Here, we describe a series of patients with DCIS, IDC, and rarely, Atypical Ductal Hyperplasia (ADH) with this sign.

Materials and Methods: we performed a systemic search of all stereotactic core biopsies for suspicious calcifications performed at our institution from April 15, 2014, when we first implemented DBT with synthesized 2D mammogram, to October 2, 2014, and identified all cases that were diagnosed as DCIS, IDC, or high-risk lesions (such as ADH, atypical lobular hyperplasia (ALH), and intraductal papilloma) on pathology. From 89 biopsied performed in 87 patients, a total of 13 cases of DCIS, IDC, or IDC with DCIS were identified in 12 patients. 17 cases of high-risk lesions were identified in 16 patients, including 10 ADH in 9 patients, 4 ALH, two intraductal papillomas, and one atypical columnar cell change.

Results: Among the 30 cases of suspicious calcifications with a diagnosis of DCIS, IDC, or high-risk lesions, 37% demonstrated the Milky Way Sign (11/30), including 71% of DCIS cases (5/7), 60% of cases of IDC (3/5), 100% of DCIS/IDC (1/1), and 12% of high-risk lesions (2/17). Both high-risk lesions with the Milky Way sign were ADH from the same patient. Among the 11 cases with the Milky Way sign, the breast density at the site of calcifications is either scattered (6/11) or heterogeneously dense (5/11), whereas the density at 1 cm surrounding the calcifications is either fatty (8/11) or scattered (3/11). The breast is less dense surrounding the calcifications in all but one case. The calcifications seen in the Milky Way cases are predominantly fine pleomorphic calcifications except one case of fine linear and linear branching calcifications. The calcifications are grouped (6/11), linear (3/11), or segmental (2/11) in distribution. Five patients also had contrast-enhanced breast MRIs. Among them, three (one each with the diagnosis of DCIS, IDC or DCIS/IDC) had non-mass enhancement on breast MRI that correlated to the Milky Way sign seen on mammogram. The other two patients (one with DCIS and one with IDC) had post biopsy change instead of Non-mass enhancement were seen in the corresponding areas.

Conclusion: The Milky Way sign, in the context of DBT, is a novel diagnostic sign for both DCIS and IDC. It may occasionally be seen in ADH cases as well. Features that often associate with the Milky Way sign include less dense tissue surrounding the calcifications, calcifications that are fine pleomorphic in morphology and grouped or linear in distribution. Preliminary results also suggest that the Milky Way sign may correspond to non-mass enhancement seen on contrast-enhanced breast MRI.

Clinical Relevance Statement: We hope that the Milky Way sign will facilitate detection of both DCIS and IDC in the context of DBT.
Title: Identification of histopathologic determinants of mammographic breast density as a cancer risk factor


Body: Background: Mammographic density and texture, the amount and appearance of fibroglandular tissue respectively, are known to be strong predictors of a woman's risk to develop breast cancer. However, how differences in underlying tissue biology are associated with the wide range of breast density patterns seen among women is currently unknown. Understanding the biological mechanisms driving breast density can be critical for developing targeted interventions aimed at reducing a woman’s risk for breast cancer.

Methods: Digital mammograms and formalin-fixed paraffin-embedded biopsy tissue sections from 73 women who donated breast tissue to the Susan G. Komen for the Cure Tissue Bank at the Indiana University Simon Cancer Center were included in this analysis. Hematoxylin and eosin (H&E) and AE1/AE3 cytokeratin stains were applied to the tissue sections, which were then visually characterized by an expert breast pathologist. H&E stains were used to assess the presence (in ≥5% of the tissue section) or absence of non-proliferative fibrocystic changes, proliferative fibrocystic changes without atypia, inflammatory and reactive conditions, or benign tumors. AE1/AE3 stains were used to quantify the extent of epithelial and stromal content, as well as the number of lobules and ductal structures seen in the section. Quantitative breast percent density (PD%) and whole-breast texture analysis was performed on the digital mammograms using validated software. Parenchymal texture measures recently demonstrated to be associated with breast cancer risk were assessed, including the gray-level co-occurrence features Cluster-shade, Correlation, Energy, Entropy, Inertia and Inverse Difference Moment. Texture was characterized within a 2.5cm² area of interest in the upper-outer quadrant of the mammograms corresponding to the approximate location of the biopsy. Partial-R² (pR²) analysis was applied in order to assess the proportion of the variability in the mammographic texture which can be directly attributed to underlying histological properties after adjusting for race, menopausal status, body mass index, PD% and mammography unit manufacturer, utilizing a false-discovery-rate (FDR) multiple comparisons correction.

Results: The presence of proliferative fibrocystic changes without atypia was found to have a significant association to Correlation (pR²=0.21), Energy (pR²=0.13), Entropy (pR²=0.16), and Inverse Difference Moment (pR²=0.14) at the FDR-corrected significance level of p<0.0042, even after adjusting for race, menopausal status, body mass index, PD% and the mammography unit manufacturer. These findings suggest that 13-21% of the variation in parenchymal texture patterns seen between women can be explained by the presence of proliferative fibrocystic changes without atypia, a common and well known risk factor for breast cancer.

Conclusion: In this study, we identified proliferative fibrocystic changes without atypia as one potential driver of image-based biomarkers of breast cancer risk. Ultimately, identification of radiographic biomarkers of tissue microenvironment could aid in the development of targeted chemopreventative interventions for women with dense breast tissue while simultaneously providing image-based biomarkers to monitor their efficacy.
Introduction: mammographic density is a strong risk factor for breast cancer and an essential determinant of screening sensitivity, but the breast density of Japanese women has yet to be objectively examined. Single mammographic examination fails to detect approximately half of breast cancers in women with dense breasts. We objectively evaluated mammographic density to clarify the relation between breast density and incidence of breast cancer in Japanese women.

Method: We enrolled 269 patients diagnosed with breast cancer and 397 healthy controls, who participated in screening mammography at Showa University Hospital. Breast density was measured using Volpara (a software application which analyses breast composition volumetrically).

Results:
The average age of both control and breast cancer groups was 55. Of the control group, 308 (78%) were characterized as having dense breasts, being defined as volumetric density grade (VDG) 3 or 4. More than 50% of the total population (all ages) had dense breasts (VDG 3 or 4) and more than 20% were extremely dense (VDG:4). Of the breast cancer patients, 232 (87%) were VDG 3 or 4. 52 (23%) of those with dense breasts showed negative results in single mammography examination. In primary breast cancer patients with dense breasts, 30% of those under age 70, but only 13% of those over age 70, showed no abnormal findings from mammographic examination.

Conclusion and Discussion: In comparison of our present results and other papers, In the Japanese control group, 72% of patients had dense breasts (VDG 3 or 4) at age 50 or greater, but in Holland, for example, only 36% of women had breasts classed as dense. 87% of Japanese women with breast cancer had dense breasts, approximately the same ratio (88%) as found in Korea. For Japanese and other Asians, women under 70 years old with dense breasts may warrant additional screening.
Title: Immunohistochemical and histological features of mammographic dense and non-dense tissue in breast cancer patients

dos Santos CC Cabello, Marshall P, Torresan R, Tinóis E, Duarte G and Teixeira S. State University of Campinas - UNICAMP; State University of Campinas - UNICAMP; State University of Campinas - UNICAMP; State University of Campinas - UNICAMP; State University of Campinas - UNICAMP and State University of Campinas - UNICAMP.

Body: Objective: We investigated immunohistochemical and histological composition of dense and non-dense breast tissue in 18 women undergoing mastectomy as the initial treatment for breast cancer. Materials and Methods: In each mammogram, we localized the dense and the non-dense areas. We used a localization technique based on a linear approximation method with interpolation of mammogram images and breast pictures. The selected areas were retrieved during mastectomy and analyzed. Results: Estrogen and progesterone receptors, Ki-67 and CD-34, were equally expressed in both tissues, as well as the percentage composition of fat. The percentage compositions of brownish spots among dense and non-dense tissues were significantly different (p = 0.0226). The number of terminal ductal lobular units was higher for dense than for non-dense breast tissues (p = 0.0019). In the non-dense breast tissue, there were no proliferative lesions with atypia, while we found flat epithelial atypia in 3 of the dense areas evaluated. Proliferative lesions without atypia and non-proliferative lesions were found in both tissues, but they were more frequent in dense than in non-dense breast tissues (23.5% vs 11.8%, p = 0.0455, and 17.6% vs 2.9%, p = 0.0253, respectively). Fibrosis was more frequently extensive or moderate in dense tissue, while it was predominantly mild in non-dense tissue (p = 0.03). Conclusion: There was no difference in the expression of the estrogen and progesterone receptors, Ki-67 and CD-34, in the dense and non-dense tissue areas in breast-cancer women. In addition, both stroma fibrosis and epithelial proliferation were responsible for higher mammographic density.
Title: Improving the quality of mammographic positioning


Body: Purpose:
Optimal breast positioning is a key component to high quality screening mammograms to allow the radiologist to make the best interpretation for the patient and referring physician. In addition, the success of newer imaging techniques also depends on breast positioning. The American College of Radiology (ACR) sets the standard of what images should include by outlining 13 criteria of breast positioning. An initial audit of over 100 mammograms at our institution in 2013 found that only a mean of 33% were achieving the ACR criteria. The goal of our project was to increase the percentage of screening mammograms achieving ACR criteria to 90% by June 2015.

Methods:
Our breast imaging center partnered with a quality improvement (QI) team driving a radiology department-wide program on quality improvement. Team members identified 5 key causes that barred achieving the ACR criteria: disagreement on what meets criteria, not having a standard work for acquiring and reading mammograms, lack of communication between the technologist and radiologist, not having a measurement system to track performance, and lack of coaching on technologist techniques for acquiring images. Developments to address these causes included: teaching modules on what meets ACR criteria, standard work for radiologists to recall mammograms that did not meet ACR criteria, system for the technologist to document why criteria were missed, auditing system to track performance, and feedback sessions between technologists and radiologists. Over 1,700 mammograms were audited from the time period of July 2014 to March 2015.

Results:
By October 2014, the percentage of mammograms achieving all 13 of the ACR criteria was 71%, with 4 criteria that prevented reaching the 90% goal. By March 2015, 10 of the 13 ACR criteria were being sustainably met by the target goal of 90% of mammograms, better in all criteria compared to our 2013 data, and better in all but one criterion compared to published 1993 data. Table 1 demonstrates that we have been able to sustain a composite percentage of 12 of the 13 ACR criteria greater than 90% for the last 2 consecutive months.

Table 1 shows the composite percentage of mammograms achieving 12 of the 13 ACR criteria over time.

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<tbody>
<tr>
<td>64%</td>
<td>67%</td>
<td>77%</td>
<td>82%</td>
<td>83%</td>
<td>81%</td>
<td>95%</td>
<td>96%</td>
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</table>

The excluded, most difficult criterion (visualization of the opposite breast cleavage) has been achieved at 32% per 1993 published data; we currently achieve it at 40%. 12/2014 audits were not performed due to holidays and changes in staffing.

Conclusion:
Few institutions have published positioning data, with the most recent QI publication on breast positioning dating to 1993. We have conducted a structured process to improve quality of mammographic positioning, including revision of processes that led to poor positioning outcomes and creation of an environment to sustain our improved outcomes. Three ACR criteria continue to be problematic in reaching the 90% goal, with future investigation into whether it is actually feasible to achieve the most difficult criterion at our goal of 90%. Future work also includes assessing how the recent hire of a mammography coach to spread best practices and real-time feedback is able to further improve results and maintain the infrastructure of ongoing QI.
Title: Microcalcifications as imaging biomarker in breast cancer: High-throughput radiogenomic analysis using microarray data

Body: Purpose: To investigate relationships between microcalcifications and gene expression pattern using microarray analysis in breast cancer.

Materials and methods: The institutional review board approved this retrospective study and waived the informed consent. Clinicopathologic finding, mammographic features, and gene expression data were evaluated in 133 women (mean age, 50.1 years; range, 21-79 years) with stage I-III breast cancer. Women with microcalcifications (n=33) and without microcalcifications (n=100) were compared. Immunohistochemical analysis of estrogen receptor, progesterone receptor, HER-2, and Ki-67 expression was performed. Differentially expressed genes (DEGs) using Affymetrix GeneChip® Human Gene 2.0 ST arrays (53,427 probes) were identified in tissue with microcalcifications versus without microcalcifications. In addition, genes included in the prediction analysis of PAM50, MammaPrint® and OncotypeDX® were also compared between two groups (microcalcifications versus no calcification). To further investigate the functions and underlying biology of DEGs, we performed enrichment analysis using the Gene Ontology database and pathway analysis using the Kyoto Encyclopedia of Genes and Genomes database and Ingenuity Pathway Analysis.

Results: Among clinicopathologic variables, HER2 positivity (p<0.001) and presence of comedo necrosis (p=0.024) are significantly higher in the calcification group. About 128 genes had differential expression (> 1.5 fold difference, adjusted p value<0.05). Among known gene signatures, GRB7 (fold change=2.26, p=0.006) and ERBB2 (fold change=2.13, p=0.001) which are known as associated with recurrence, cell invasion and poor survival were highly expressed. In contrast, ZNF385B which is associated with p53-mediated apoptosis and good prognosis was underexpressed (fold change=0.39, p=0.001) in calcification group. Significant gene ontology terms included response to wounding, coagulation, wound healing, and response to hypoxia. Network and canonical pathway analysis indicated that increased cellular movement, cellular growth and proliferation, cellular development, coagulation and atherosclerosis signaling in breast cancer with microcalcifications, suggesting biological aggressiveness.

Conclusion: Gene expression patterns are different according to microcalcifications status in breast cancer. Breast cancers with mammographic microcalcifications are associated with metabolic aggressiveness and poor prognosis.
Title: Recall for assessment after post-treatment surveillance mammograms in breast cancer

Wassermann M, Swinson C, Shrestha D, Kirkpatrick K and Ravichandran D. Luton and Dunstable University Hospital NHS Foundation Trust, Luton, Bedfordshire, United Kingdom.

**Body:** Background: Patients treated for breast carcinoma are usually followed up with regular mammograms. The aims are early diagnosis of locally recurrent disease in the ipsilateral breast or new cancers in the contralateral breast, and to provide reassurance. When the mammograms are considered abnormal patients are recalled for further assessment. The aim of this study is to review all mammographic recalls in a single institution over a 5-year period to study the patient population recalled, reasons for the recall and the outcome.

Materials and Methods: We identified all breast cancer patients who were recalled for assessment following a routine post-treatment surveillance mammogram from April 2010 to March 2015 from the breast unit database. We reviewed their original presentation with breast cancer, treatment received, reason for recall, the assessment process and the outcome. The mammographic follow-up policy of the unit during the study period was as follows; after breast conserving surgery (BCS), yearly bilateral mammograms for 5 years or until the age of 50 whichever the later; following mastectomy, mammogram of the opposite breast at years 1, 3 and 5 post-surgery, and if the patient is aged under 50 after 5 years, 2-yearly till 50 years of age. Patients were then discharged to UK National Breast Screening Programme where mammograms are performed 3-yearly.

Results: During the study period 1809 patients had 3685 surveillance mammograms. 149 patients were recalled (a recall rate of 8% of patients and 4% of mammograms over 5-years). 122 patients had BCS and 27 patients had mastectomy. The original diagnosis was invasive cancer in 131 and DCIS in 18. The reason for recall was a density or mass in 63, microcalcification in 61, distortion in 14 and a mixture of these or other reasons in 11. Eighty one patients were recalled for a problem on the ipsilateral side and 66 for contralateral side (2 patients had bilateral cancer).

Among 149 patients recalled, 50 had further imaging only (further mammographic views, ultrasound or both) after the recall and 79 underwent a needle biopsy under ultrasound or stereo guidance. 8 patients underwent US-guided aspiration of benign cysts. 21 biopsy results were malignant. The final diagnosis was ipsilateral recurrence in 10 patients and contralateral cancer in 11.

Conclusions: There is not much published data in the literature on the outcome of post-treatment surveillance mammograms despite the fact that such mammographic surveillance is commonly practiced. This study shows that over a 5-year period, 8% of the patients and 4% of the mammograms were recalled. A third of patients recalled only required further imaging. Although biopsies were performed in over half the patients, only 14% of patients recalled were proved to have ipsilateral or contralateral carcinoma after the assessment.
Only in lobular breast cancer MRI use is associated with a lower risk of positive surgical margins and a reduced number of mastectomies. A real-world analysis in The Netherlands

Tjan-Heijnen VC C, Lobbes MB B, Vriens IJ J, van Bommel AC C, Nieuwenhuijzen GA A, Smidt ML L, Boersma LJ J, van Dalen T, Smorenburg CH H, Siesling S and Voogd AC C. Maastricht University Medical Centre, Netherlands; Leiden University Medical Centre, Netherlands; Catharina Hospital, Netherlands; Maastro Clinic, Netherlands; Diakonessenhuis, Netherlands; Netherlands Cancer Institute, Netherlands and Netherlands Comprehensive Cancer Organisation, Netherlands.

Body: Background
The value of magnetic resonance imaging (MRI) for patients with breast cancer remains under debate. Breast MRI may contribute to the planning of local therapy, but also bears the risk of overtreatment. We analyzed the use of MRI and its impact on surgical treatment and risk of detecting contralateral breast cancer in the Netherlands.

Patients and methods
All patients who underwent primary surgery for stage I-III invasive breast cancer in the years 2011-2013 were identified through the Netherlands Cancer Registry. The following data were documented: year of diagnosis, hospital type and volume, age at diagnosis, clinical T and N stage, histological type and grade, presence of multifocality in resection specimen, hormone receptor status, HER2 status and use of MRI. We analyzed whether MRI use was related to type of surgery (primary or secondary mastectomy or breast conserving surgery), surgical margin involvement, and diagnosis of synchronous contralateral breast cancer.

Results
MRI was performed in 10,819 (29.8%) out of 36,333 patients newly diagnosed with invasive breast cancer and treated with primary surgery in the years 2011-2013 in the Netherlands. Use of MRI did not clearly increase in this period.

In the multivariate analysis, patients younger than 50 years of age compared to patients aged 70 years or older (OR 6.34, 95% CI 5.86-6.87), patients with lobular breast cancer compared to those with ductal carcinoma (OR 3.46; 95% CI 3.23-3.70) and patients with multifocal tumors compared to those without multifocality (OR 2.30, 95% CI 2.15-2.45) were more likely to undergo MRI. Hospital volume (<150 versus >150) was only marginally related to MRI use (OR 0.93; 95% CI 0.87-0.99).

Patients with invasive breast cancer undergoing MRI were more likely to undergo primary mastectomy than those without MRI (OR 1.21; 95% CI 1.15-1.28), but the subgroup with invasive lobular cancer undergoing MRI were less likely to undergo primary mastectomy (OR 0.85; 95% CI 0.75-0.98). A significantly lower risk of positive surgical margins was seen in patients with lobular breast cancer and breast conserving surgery who had undergone MRI as compared to those without MRI (OR 0.58, 95% CI 0.44-0.78) and, consequently, also a lower risk of secondary mastectomy (OR 0.60, 95% CI 0.41-0.87). Risk of positive surgical margins was not reduced by MRI use in patients with invasive ductal carcinoma (OR 0.91; 95% CI 0.77-1.07). Patients who underwent MRI were almost four times more frequently diagnosed with contralateral breast cancer, compared to those in whom MRI was not performed (OR 3.60, 95% CI 3.06-4.24).

Conclusion
Breast MRI was significantly more often used in younger patients, patients with lobular and/or multifocal breast cancer. Interestingly, MRI use was associated with less primary and secondary mastectomies in lobular invasive breast cancer, in contrast to an increased number of primary mastectomies in patients with invasive ductal cancer. MRI was further associated with an almost fourfold higher incidence of contralateral breast cancer.
**Title:** Did adding breast MRI decrease the surgical margin involved rate than conventional breast image? - A case controlled comparison analysis

Lai H-W, Chen S-T, Chen D-R, Wu H-K and Ku S-J. Changhua Christian Hospital, Changhua, Taiwan; Changhua Christian Hospital, Changhua, Taiwan; Changhua Christian Hospital, Changhua, Taiwan; Changhua Christian Hospital, Changhua, Taiwan and Changhua Christian Hospital, Changhua, Taiwan.

**Body:** Purpose: Resection of primary tumor with clear margin is the goal of surgical management for primary operable breast cancer. Surgical margin involvement was associated with increased local recurrence, and usually mandated further surgery. The objective of current study is to assess whether combining breast MRI would decrease the rate of margin involvement compared with conventional breast image.

Material and Methods: A retrospective, case controlled comparison study was conducted. Patients with primary operable breast cancer who received surgical management were searched from Changhua Christian Hospital (CCH) breast cancer database. The rate of surgical margin involvement was compared between two groups of patients with conventional breast image (Group A: mammogram and sonogram) or combined with breast MRI (Group B: mammogram, sonogram and MRI). Surgical margin involvement was defined as cancer cells present at surgical margin, or < 1mm. To further evaluate the effect of breast MRI on surgeon's margin involved rates, the index surgeons, defined as with more than 100 breast cancer operations in both Group A and Group B, were selected and analyzed.

Results: Group A, conventional breast image group, consisted of 741 breast cancer patients. Among them, 381 (51.4%) received partial mastectomy, and 360 (48.6%) received total mastectomy. Overall, 66 (8.9%) margin involved events were found in Group A. The margin involved rate in Group A patients received partial mastectomy or total mastectomy was 14.4% (55/381), and 3.1% (11/360), separately. The Group –B (combined with MRI) consisted of 736 breast cancer patients. Among them, 347 (47.1%) received partial mastectomy, and 389 (52.9%) received total mastectomy. The margin involved rate in Group B was overall 4.8% (35/736). The margin involved rate in Group B patients who received partial mastectomy or total mastectomy was 6.1% (21/347), and 3.6% (14/389), respectively. The surgical margin involved rate was decreased after combination of MRI to conventional breast image as showed in table 1.

<table>
<thead>
<tr>
<th>Margin involved rate with conventional breast image or combined with MRI</th>
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<tbody>
<tr>
<td><strong>Patient</strong></td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>Partial mastectomy</td>
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<tr>
<td>Total mastectomy</td>
</tr>
<tr>
<td>Rate of margin involvement</td>
</tr>
<tr>
<td>Partial mastectomy</td>
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<tr>
<td>Total mastectomy</td>
</tr>
</tbody>
</table>

Two index surgeons were selected for comparison of surgical margin involved rate before and after breast MRI. The "Surgeon A" 's margin involved rate in conventional breast image -> combined MRI were: overall: 7.6% -> 4.9%(0.202), partial mastectomy: 14.0% -> 8.1%(P=0.151), and total mastectomy: 1.3% -> 2.2%(P=0.8066). "Surgeon B" 's margin involved rate (from no MRI -> MRI ) was: overall: 9.0% -> 5.0%(P=0.121), partial mastectomy: 12.1% -> 3.9%(P=0.034), and total mastectomy: 6.2% -> 6.59%(P=0.722).

Conclusion: Adding breast MRI to conventional breast image decreased the surgical margin involved rate, with the cost of mild
increase the mastectomy rate. This decreasing surgical margin involved rate was not mainly due to increase mastectomy rate, but due to the selection of patients who were not suitable for partial mastectomy to receive total mastectomy.
The diagnostic accuracy of breast MRI in the prediction of malignant invasion of nipple areolar complex (NAC) of breast


Purpose: Nipple sparing mastectomy is increasingly used as a surgical treatment for breast cancer. To correctly predict the possibility of nipple invasion pre-operatively is critical important to prevent occult nipple invasion or early nipple recurrence. The objective of our study is to assess the diagnostic accuracy of breast MRI for the evaluation of malignant invasion of the nipple-areolar complex (NAC).

Material and Methods: From January 2011 to December 2013, patients with primary operable breast cancer diagnosed and treated at Changhua Christian Hospital (CCH), Taiwan were searched. The inclusion criteria were primary operable breast cancer patients, who received pre-operative breast MRI, and received breast cancer operation at CCH. The exclusion criteria were patients whose primary tumor was removed before definite cancer operation, those who received neoadjuvant chemotherapy, or patient's detailed data not available. Breast MRI examinations were retrospectively reviewed for nipple invasion or retraction, periareolar skin thickening, nipple areolar complex enhancement, relationship to the subareolar mass, malignant mass pattern, thickness of nipple-areolar complex enhancement, tumor-nipple distance and tumor size, and were correlated with pathologic findings. The accuracy of breast MRI to predict nipple invasion was compared with pre-operative image and post-operative pathologic reports.

Results: A total 704 primary operable breast cancers with pre-operative MRI and post operative pathologic reports were enrolled in our current study. In the total 704 patients, MRI showed signs of suspect NAC invasion in 160 (22.7%) patients. Total 41 (25.6%) patients were pathologic proven malignant invasion of NAC. In the final pathologic analysis, 57 pathologic confirmed NAC invasions were found in the 704 patients. The overall nipple invasion rate was 8.1% in this current study. The sensitivity of Breast MRI to predict NAC involvement was 71.9%. The specificity of breast MRI to NAC invasion is 81.6%. The positive predictive value of breast MRI is 25.6%. The negative predictive value of breast MRI is 97.1%. The accuracy of breast MRI to predict NAC involvement is 80.8%. In univariate logistic regression analysis, tumor size, lymph node metastasis, central location of tumor, unilateral nipple enhancement, relationship to tumor, and HER-2 overexpression were prognostic factors for NAC invasion. In multivariate analysis, unilateral nipple enhancement (hazard ratio=4.944, CI: 1.938-12.616, P=0.001) was the most significant independently risk factor associated with the increased risk of NAC involvement.

Multivariate analysis of factors related to nipple invasion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
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<tbody>
<tr>
<td>MRI tumor size</td>
<td>0.850</td>
<td>0.716-1.009</td>
<td>0.063</td>
</tr>
<tr>
<td>Distance to nipples (cm)</td>
<td>1.199</td>
<td>0.931-1.544</td>
<td>0.160</td>
</tr>
<tr>
<td>Location of tumor (Central)</td>
<td>1.683</td>
<td>0.724-3.912</td>
<td>0.226</td>
</tr>
<tr>
<td>Nipple enhancement (Unilateral)</td>
<td>4.944</td>
<td>1.938-12.616</td>
<td>0.001</td>
</tr>
<tr>
<td>Relationship (Yes)</td>
<td>1.616</td>
<td>0.625-4.177</td>
<td>0.322</td>
</tr>
<tr>
<td>MRI Lymph node metastasis (Yes)</td>
<td>1.674</td>
<td>0.870-3.224</td>
<td>0.123</td>
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Conclusion: MRI is an useful diagnostic image method for the evaluation of malignant invasion of the nipple-areolar complex. Through preoperative breast MRI evaluation of NAC status, more personalized oncoplastic breast surgery could be performed.
Title: Magnetic resonance imaging to predict nipple involvement in breast cancer patients

Body: Introduction. The implementation of Nipple-Sparing Mastectomy (NSM) as a treatment option for selected cases of breast cancer has risen great interest among breast surgeons. The preservation of the nipple-areola complex (NAC) can lead to extremely favorable psychological effects in breast cancer patients treated with this type of procedure. However, to ensure the oncologic safety of this technique it is of utmost importance to evaluate the likelihood of NAC involvement pre-operatively. In this study we evaluate the contribution of Breast Magnetic Resonance Imaging (MRI) in predicting the involvement in the NAC in breast cancer patients. Materials and Methods: We studied 170 mastectomy specimens from 165 breast cancer patients (five patients had bilateral disease) affected by Ductal Carcinoma in situ (DCIS)(n=19) or Invasive Ductal Carcinoma (n=151), stages I, II or IIIA. Every patient was pre-operatively studied using a 1.5 Tesla, 4-channel in vivo dedicated surface breast coil MRI. The parameters we investigated were: type of index lesion enhancement pattern (nodular or non-nodular), size of the index lesion, enhancement between the index lesion and the NAC, enhancement of the nipple, thickening of the areola, nipple retraction and size of the nipple in comparison with the contra-lateral nipple. The retro-areolar area and papilla were evaluated in histological sections of 4µm to identify DCIS and Invasive Ductal or Lobular carcinomas. One radiologist, blinded to the result of the histological evaluation of the papillae, performed the evaluation of the MRIs. Results. In univariate analysis, type of lesion enhancement in MRI, enhancement between index lesion and the papilla, distance between the index lesion and the papilla, enhancement of the papilla and nipple retraction had a statistically significant correlation with neoplastic involvement of the NAC (p<0.05). Using multivariate analysis, among the previously mentioned parameters, enhancement between the index lesion and the NAC, and nipple retraction remained as statistically significant predictors of nipple involvement in breast cancer patients (p < 0.001 e 0.010, respectively). The Negative Predictive Value of the combination of these two variables was 89.5%. According to this model that used the combination of those two variables, the probability of neoplastic involvement of the NAC was 73.9% in the presence of enhancement between the index lesion and the papilla combined with nipple retraction; 46% in the presence of enhancement between the index lesion and the papilla without nipple retraction in the MRI; 26.9% if there is only nipple retraction in the MRI; and 9.9% in the absence of these two characteristics. The sensibility of this model composed by those 2 variables to identify neoplastic involvement of the NAC was 29.7% (CI95%: 15.9% - 47%), specificity was 97.7% (CI95%: 93.5% - 99.5%), positive predictive value was 78.6% (CI95%: 49.2% - 95.3%) and negative predictive value was 83.3% (CI95%: 76.5% - 88.8%). Conclusion. We can conclude that the probability of the NAC being cancer-free is around 90% when there is no enhancement between the index lesion and the nipple, and there is no retraction of the nipple in the pre-operative MRI study of the breast.
Title: Predictors of MRI detection of occult lesions in newly diagnosed breast cancer

Wecsler JS S, Raghavendra A, Mack WJ J, Tripathy D, Yamashita M, Sheth P, Hovanessian-Larsen L, Sener SF F, Russell CA A, MacDonald H and Lang JE E. Division of Breast and Soft Tissue Surgery, USC Norris Comprehensive Cancer Center and Los Angeles County Medical Center, Los Angeles, CA; The University of Texas MD Anderson Cancer Center, Houston, TX; University of Southern California, Los Angeles, CA and Division of Breast Imaging, USC Norris Comprehensive Cancer Center and Los Angeles County Medical Center, Los Angeles, CA.

Body: BACKGROUND: The appropriate use of preoperative magnetic resonance imaging (MRI) in patients with newly diagnosed breast cancer remains a topic of debate. We aimed to determine the usefulness of MRI in the detection of occult multicentric, multifocal and contralateral lesions not seen by ultrasound or mammography.

METHODS: We performed a retrospective analysis of consecutive patients who underwent preoperative MRI for newly diagnosed biopsy-proven stage 0-III breast cancer who were treated surgically from January 2006-March 2013. All newly diagnosed breast cancer patients at our two affiliated institutions were evaluated with pre-operative MRI. Patients who received neoadjuvant systemic therapy or surgery at an outside institution were excluded from our study. Demographic, radiographic and pathologic data points including age, race, body mass index (BMI), lesion size, mammographic density, biopsy histology, and biomarkers were assessed for each patient with respect to the findings of multifocality, multicentricity, and the presence of contralateral lesions on all three imaging modalities. We performed univariate analysis associating factors separately in each of three models with multicentric, multifocal and contralateral disease on surgical pathology followed by multivariable analysis using logistic regression to calculate odds ratios.

RESULTS: Of 857 patients undergoing breast MRI within this time period, 770 patients were identified who met inclusion criteria. All patients underwent diagnostic mammogram and ultrasound followed by MRI. The patient population was 44.2% Hispanic, reflective of the population of our two institutions. Mean age was 54.7 years. MRI identified 86 patients with biopsy-proven multicentricity compared to 66 on conventional imaging. MRI identified 170 patients with biopsy-proven multifocality compared to 132 on conventional imaging. Finally, MRI identified 24 patients with biopsy-proven contralateral cancers compared to 7 on conventional imaging. Biopsy histology of invasive lobular carcinoma was predictive of the presence of multifocality on MRI (p=0.038, OR=1.95, 95% CI 1.09-3.48). Mammographic density was found to be a predictor of multicentricity (p=0.015, OR=2.22, 95% CI 1.13-3.33). Lesion size trended towards statistical significance on multivariate analysis of the multicentric lesions (p=0.057, OR=1.88, 95% CI 1.00-3.51). For contralateral cancers seen on MRI the presence of invasive lobular carcinoma on biopsy (p=0.027, OR=4.83, 95% CI 1.25-16.21) was predictive of finding a contralateral cancer.

CONCLUSIONS: Predictive factors including breast density, biopsy histology, and lesion size should be taken into account as clinical predictors of utility of pre-operative breast MRI. This data is being used to construct nomograms to predict multicentric, multifocal and contralateral disease to aid clinicians in evaluating the potential clinical utility of preoperative breast MRI.
Title: Improving efficacy of applying breast MRI to detect mammography-occult breast cancer

Zheng B, Hollingsworth AB B, Tan MY Y, Stough RG G and Liu H. University of Oklahoma, Norman, OK and Mercy Health Center, Oklahoma City, OK.

Body: Background: Mammography is the only clinically acceptable imaging modality in current population-based breast cancer screening, but it has a relatively low sensitivity in detecting early cancers. Although breast magnetic resonance imaging (MRI) is much more sensitive in detecting early or mammography-occult cancers, the low cancer detection yield in the general screening population (∼1%) prohibits breast MRI as a screening tool with wide applicability. In order to solve this clinical dilemma, in this preliminary study, we tested the potential of applying a new mammographic image feature analysis model that serves as a short-term breast cancer risk prediction model to identify women at high risk of harboring mammography-occult breast cancers, which can be detected by breast MRI.

Methods: An image dataset involving 30 women who had both mammography and breast MRI screening examinations was retrospectively assembled. All mammograms were interpreted as negative by the radiologists during the original image interpretation. When applying breast MRI examinations to these women immediately following the negative mammography, 5 women were positive with cancer detected and 25 remained negative. We developed a computer-aided detection scheme to process the bilateral CC view mammograms of the left and right breasts. Ten (10) texture-based mammographic image features were computed and compared from the bilateral mammograms. These features were then fused to build a new artificial neural network (ANN) based risk model. Based on the threshold of 0.5 applying to the ANN-generated risk prediction scores (ranging from 0 to 1), we stratified these 30 women into two groups with high and low risk of harboring mammography-occult cancer.

Results: Using the ANN-generated risk scores, 9 and 21 cases were assigned to high-risk and low-risk group, respectively. All 5 breast MRI-detected cancer cases were classified into the high-risk group (with 100% sensitivity), while 4 negative cases were also classified into the high-risk group resulting in a 16% false-positive rate (4/25). All 21 cases in the low-risk group were negative cases. Hence, our risk prediction model yielded an overall prediction accuracy of 86.7% in which 26 of 30 cases were correctly classified. The potential clinical impact is that based on the case stratification result, the maximum cancer detection yield of using breast MRI is 56% (5/9) in this dataset. Meanwhile, it can also eliminate 84% (21/25) unnecessary (negative) breast MRI screening examinations.

Conclusions: This is the first study, which demonstrated that by computing and analyzing the variation of mammographic image features, we are able to develop a new quantitative image feature analysis model that can be applied to predict the short-term risk of a woman having a mammography-occult cancer that can be detected by breast MRI. This new strategy has the potential to significantly increase cancer detection yield of breast MRI and thus make breast MRI a more cost-effective imaging modality in breast cancer screening.
Association between computer-derived features of the ipsilateral breast on DCE-MRI and the 70-gene signature in patients with invasive breast cancer

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Introduction
Molecular assays such as the 70-gene signature are increasingly used as prognostic indicators to select chemotherapy in individual patients. These assays are typically derived from postoperative excision specimens and require several weeks to complete. Earlier assessment of the results of such assays could open up new therapeutic options in subgroups of patients, potentially avoiding overtreatment of early breast cancer. Although molecular assays may be derived from biopsied tissue, tumor heterogeneity may cause uncertainty. Dynamic contrast-enhanced MRI (DCE-MRI) depicts some of the hallmarks of cancer that are tested by these molecular essays. The goal of this study was to investigate the association between the postoperatively derived 70-gene signature and computer-derived DCE-MRI features of the ipsilateral breast prior to surgery.

Material and Methods
Sixty-nine patients with node-negative invasive breast cancer were enrolled between 2003 and 2006. These patients received a preoperative MRI in study setting and a postoperative 70-gene signature assay. Association between preoperative features and the 70-gene signature was evaluated using a computer prediction model combining clinical features and automatically extracted MRI features. The clinical features were age at diagnosis and largest tumor diameter on MRI. The MRI features were rate of contrast uptake in the tumor, rate of wash-out, tumor volume, and two features from the intramammary blood vessel tree (total length and mean rate of contrast uptake). The features were transformed into an orthogonal feature set using principal component analysis. Association with the 70-gene signature (positive or negative indication for systemic therapy) was evaluated using binary logistic regression. Model performance was measured using the area under the receiver operating characteristics curve (AUC) after bootstrap validation using 200 iterations. Two operating points were examined: one to predict a positive 70-gene signature with high certainty (i.e., at high positive-predictive value (PPV)) and one to predict a negative signature with high certainty (i.e., at high negative-predictive value (PPV)).

Results
The average patient age at diagnosis was 48 years (range: 32-58). The median largest tumor diameter on MRI was 17 mm (range: 5-40). The 70-gene signature was positive in 29/69 (42%) patients. The computer prediction model achieved an AUC of 0.72 after bootstrap validation. At high PPV, 10/29 (34.5%) positive 70-gene signatures were identified preoperatively at the expense of 2/40 (5.0%) false-positive. The PPV was 10/12 (83.3%). At high NPV, 12/40 (30.0%) negative 70-gene signatures were identified at the expense of 1/29 (3.5%) false-negative signature. The NPV was 12/13 (92.3%).

Conclusion
Computer-derived DCE-MRI features from the ipsilateral breast in combination with clinical parameters show potential to preoperatively assess a negative outcome of the 70-gene signature in approximately one-third of the total patient group.
MRI changes in breast skin following preoperative therapy for inflammatory breast cancer (IBC)

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Purpose: Standard treatment for IBC includes preoperative systemic therapy (PST), followed by mastectomy (M) and adjuvant radiation (R). Determining the optimal sequencing of M and R after PST may be difficult when clinical changes suggest residual disease within breast skin. With the goal of selecting appropriate patients (pts) for M prior to R, we correlated pathologic disease response in breast skin with changes in skin thickness and enhancement determined by MRI.

Methods: An IRB approved database of IBC pts evaluated at Dana Farber Cancer Institute (DFCI) from 1997-2013 was used for retrospective analysis. 40 pts met criteria: confirmed diagnosis of IBC, completed PST followed by M without preoperative R. Baseline and post-PST breast MRI imaging was reviewed. Using the ACR BI-RADS lexicon, we recorded skin thickness, qualitative enhancement and kinetic analysis using computer-aided detection post-processing software. Findings were correlated with pathologic response in skin found at M.

Results: MRI showed baseline skin thickening in all 40 pts (median 6mm, range 3-13mm). Although 34 (85%) had persistent skin thickening post-PST (median 4 mm, range <3-13 mm), there was a significant overall reduction in skin thickness (p<0.001); median decrease =2mm, range 1-7mm.

MRI showed qualitative skin enhancement at baseline in 39/40 pts. 29 (73%) had medium/fast initial phase kinetics: 25 persistent delayed phase kinetics, 2 wash-out, 2 plateau. 20 pts had residual qualitative skin enhancement post-PST; 11 pts (28%) had medium/fast initial phase kinetics, all persistent delayed kinetics. The decrease in skin thickness was significantly greater among the 19 pts achieving resolution of skin enhancement post-PST compared with the decrease in skin thickness among the 20 pts with residual skin enhancement (p=0.02).

8 pts (20%) had residual tumor within the skin at M. All 8 pts had thicker skin on post-PST MRI (median 5 mm, range 3-13mm) compared with pts without residual disease in the skin (median 3.5mm, range <3-11 mm). Qualitative skin enhancement post-PST was seen in 63% of pts (5/8) with residual skin disease compared with 47% of pts (15/32) without disease.

Conclusion: Although IBC pts have skin thickening demonstrated by MRI at baseline, there is a statistically significant reduction in the skin thickness following successful PST. This correlates with a reduction in enhancement of the skin shown by MRI imaging. More substantial and persistent skin thickening with enhancement was seen in the setting of residual dermal lymphatic involvement following the completion of PST, though this study is too small to detect any significant correlation. Since an accurate assessment of residual disease in breast skin is vital in determining the optimal sequencing of M and R following PST in IBC pts, MRI evaluation of skin thickness and enhancement may be a useful tool to predict residual disease and guide surgical management of IBC.
Can MRI be used to determine pathological complete response following neo-adjuvant chemotherapy for breast cancer?

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Intro

Neo-adjuvant chemotherapy (NACT) is increasingly offered to patients with breast cancer, and it understood that patients with pathological complete response (PCR) have improved survival. It has been suggested that patients with radiological complete response may be spared surgery. We aimed to determine whether MRI findings can predict PCR following NACT.

Methods

This was a retrospective analysis of a prospectively maintained database. Most patients had NACT with 6 cycles of EC/DC+/-Traztuzumab and response was monitored using MRI. For univariate analysis $\chi^2$ and Fisher’s exact test was used for categorical data. Log rank test by Kaplan Meier method was used for survival analysis.

Results

310 patients had NACT from December 2007 to December 2014. Baseline MRI prior to embarking on NACT was achieved in 251 patients. MRI concorded with size on mammography/USS in the majority of cases (83%) and upgraded the overall size of the lesion in 16% (50% of unifocal lesions on USS upgraded to multifocal).

In the 247 patients who had baseline MRI, a radiological response seen on the 2nd MRI (early responders, n=138), was predictive of eventual PCR ($p=0.000$, sensitivity = 77%). Patients with Her2+ve non-luminal (65%), triple negative (TNBC) (64%) and Luminal B Her2-ve (65%) tumours were more likely to be early responders on MRI. The non-early responders with Her2+ve tumours all had late radiological response, however, the TNBC tumours had low rates of late radiological response. Complete radiological response on the final MRI correlated strongly with PCR; $p=0.000$, specificity=98%. Tumour vascularity correlated positively and significantly with PCR. There was no difference in survival between those with early and late radiological response (median survival 66.2 months and 66.9 months), but those with no radiological response had significantly poorer survival 53.2 months, $p=0.012$).

Conclusion

Response on MRI is predictive of PCR, particularly within the non-luminal Her2+ve and TNBC molecular subtypes. Early response has high sensitivity, and complete response on final MRI has high specificity. Further work on larger data-sets is required before we confidently spare patient from surgery in the event of complete response on MRI.
response to neoadjuvant chemotherapy assessed by magnetic resonance imaging (MRI) texture analysis compared with residual cancer burden (RCB) and tumor subtype in primary breast cancer

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Purpose
MRI has great potential in the imaging of response to neoadjuvant chemotherapy in primary breast cancer. The aim of this study was to ascertain whether changes in lesion heterogeneity, as measured using texture analysis (TA), are linked with pathological response to treatment in primary breast cancer, according to lesion immunophenotype.

Materials & Methods
Sixty-one consecutive patients with biopsy-proven primary breast cancer underwent anthracycline-based NAC with baseline (pre-NAC) and interim (post 3-cycles NAC) MRI examinations performed on a 32-channel 3.0T MRI scanner (Siemens Trio; Erlangen) using a 7-channel breast biopsy coil. T2-weighted images (voxel size: 1.1x1.1x1.1mm3) were used for texture analysis, which was executed using MaZda software (University of Lodz; Poland). Slices were matched between imaging time-points and regions of interest manually placed to encompass the lesion. Where multiple lesions were present, only the index lesion was used. Lesion heterogeneity was assessed using the entropy feature, as derived from the co-occurrence matrix, and percentage changes between baseline and interim examinations calculated.

Final pathologic response of the surgical resections was assessed using the Residual Cancer Burden (RCB) index, as calculated from tumor bed dimensions, residual cellularity and axillary node burden. Good responders (GR) were classified as those attaining a pCR or RCB-I score, while RCB-II and RCB-III lesions were considered poor responders (PR).

Cancers were subdivided into estrogen-receptor positive (ER+), HER2 amplified (HER2+) and triple negative (TN) groups. Changes in lesion heterogeneity in relation to RCB response category and tumor subtype were compared using Mann Whitney U tests with significance at p ≤0.05 level.

Results
Patients who ultimately achieved a good pathological response demonstrated significantly greater reduction in lesion heterogeneity at TA between baseline and interim (pCR: 20.0%, RCB-I: 10.6%) than those who had a poor response (RCB-II: 6.8%, RCB-III: 3.4%) (p<0.001; Mann Whitney U).

RCB response categories in each tumor subtype was as follows: ER+: GR- 4 patients, PR- 13; HER2+: GR- 7, PR- 13; TNBC: GR- 7, PR- 17. There were statistically significant differences in the degree of reduction of lesion heterogeneity between good responders and poor responders for the ER+ (p=0.010; Mann-Whitney U) and TNBC patients (p<0.001; Mann Whitney U), but not for the HER2+ cancers (p=0.097; Mann Whitney U).

Conclusion
Changes in lesion heterogeneity between baseline and interim MRI examinations, as measured using TA on T2-weighted images, correlate with pathologic response to NAC treatment in primary breast cancer using the RCB score. Larger reductions in lesion heterogeneity between baseline and interim examinations predict for a good response to treatment especially in ER+ and TNBC. Further evaluation of TA as a predictor of RCB score, with increased numbers in tumor subgroups, is required in a clinical trial setting.
Title: Value of magnetic resonance imaging in preoperative predicting tumor extent in each subtypes of breast cancer

Yang BS, Lee HW, Park JT, Lee HM, Ahn SG and Jeong J. Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea and Breast Center, MizMedi Hospital, Seoul, Korea.

Body: Background: It is well known that patients with triple-negative breast cancer (TNBC) have a high risk of local tumor recurrence in ipsilateral breast. To know the additional value of breast magnetic resonance imaging (MRI) in preoperative prediction of tumor extent for TNBC, we tested the accuracy of MRI in correlation with pathologic tumor size according to subtypes. In addition, we investigated the margin-positive rates by subtypes among the patients receiving breast-conservative surgery (BCS).

Methods: We retrospectively identified patients with invasive breast cancer who had preoperative breast MRI and ultrasound between 2011 and 2014. We excluded patients having large tumor more than 5cm or multiple tumors. Patients were classified into 4 subtypes (luminal A, luminal B/HER2, HER2, triple-negative breast cancer (TNBC)) based on the immunohistochemistry. Lin's concordance correlation coefficient was used to measure the agreement between the MRI or ultrasound and tumor extent. Also, patients were classified into three groups according to the accuracy of MRI in correlation with tumor extent (concordance, underestimation, and overestimation). Tumor extent was defined as pathologic tumor size including in situ carcinoma.

Results: In a total of 589 patients, 397(67.4%) women received BCS. Means of tumor size were 1.99 ± 0.91 cm by pathologic review, 1.91 ± 1.01 cm by MRI, and 1.76 ± 0.92 cm by ultrasonography, respectively. Correlation analysis revealed a similar concordance rate between MRI and ultrasound to pathologic tumor size (r= 0.622 and 0.573). Lin's concordance analysis showed that MRI showed more significant correlation with pathologic tumor size than ultrasound in patients with TNBC (r= 0.735 VS 0.593, p=0.0052). However, the proportion of concordant group did not differ significantly according to subtypes (p=0.279).

Conclusions: Our findings suggest that preoperative MRI does not increase the accuracy in predicting tumor extent or reduce the margin-positive rates in breast cancer.
Title: Breast MRI: Enhancing pre-operative planning in women with newly diagnosed breast cancer


Body: Hypothesis:
Preoperative breast MRIs in newly diagnosed breast cancer patients may lead to additional findings, including larger disease involvement, thus leading to a change in the operative plan. This information can lead to better surgical planning and decreased rates of surgical re-excisions.

Methods:
This was a single surgeon, single institution, retrospective analysis of women with newly-diagnosed breast cancer who had surgery between December 2013 and December 2014. A total of ninety-six patients underwent preoperative breast MRI. Exclusion criteria were patient's refusal of MRI or surgery, contraindication to MRI based on implants or body habitus, and male patients.

Results:
Overall, breast MRI most closely correlated to pathological tumor size (gold standard). MRI had a 64% correlation with pathology size, whereas mammography and ultrasound correlation were 44% and 43% respectively. As breast density increased, MRI correlation to pathologic tumor size improved. For breast density between 25-50%, tumor size by MRI correlated with pathology size in 27% of patients, mammogram correlation was 33% and ultrasound was 12%. When breast density increased to 51-75%, MRI correlation improved to 92%, whereas mammogram correlation was 56% and ultrasound was 68%. The majority of patients fell within these two density categories; correlation for breast density <25% and >75% were not statistically significant. Of the breast biopsies performed for MRI abnormalities not otherwise identified by mammography or ultrasound, 42% were positive for invasive cancer or DCIS. Thirty-eight percent of patients had a change in operative plan based on their MRI findings. Of the ninety-six patients, nine were changed from breast conserving surgery to mastectomy, and 29 underwent a larger area of resection than planned prior to MRI, due to findings of more extensive disease on MRI not otherwise appreciated by mammography or ultrasound.

Conclusion:
Pre-operative MRI played a significant role in the pre-surgical evaluation of patients with newly diagnosed breast cancer. In our series, breast MRI demonstrated better correlation with extent of disease compared to other imaging modalities. This is turn enhanced patient and surgeon decision making and led to more precise pre-operative surgical planning.
Title: The pattern of tumor shrinkage is associated with prognosis in low grade luminal early breast cancer during neoadjuvant chemotherapy

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Body: BACKGROUND: In neoadjuvant chemotherapy (NAC) for early breast cancer, the pathological response rate in estrogen receptor (ER)-positive tumors has been low in comparison with those of ER-negative tumors. Therefore, surrogate makers other than the pCR rate are needed during NAC for luminal breast cancer. Using MRI, we analyzed the patterns of tumor shrinkage after NAC as a surrogate prognostic factor in low grade luminal breast cancer. METHODS: Of 854 patients who had received NAC in a single institute from Jan. 2000 to Dec. 2009, 183 patients with low grade luminal breast cancer were retrospectively evaluated for this study. They were defined as ER and/or PgR positive in more than 10% of cancer cells and HER2 negative (IHC 0, 1+ or FISH <2.0) with nuclear grade 1 and 2. RESULTS: The median observation period was 67.9 months following surgery, and recurrence was observed in 31 patients (16.9%). The median age was 49 (22-76) years. One hundred eighty patients received anthracycline-containing chemotherapy, and 158 received taxane. There were 16 deaths (8.7%) related to breast cancer. We categorized the patterns of tumor shrinkage by MRI into 6 types: concentric shrinkage (CS), diffuse decrease (DD), reduction to small foci (RSF), decrease of intensity only (DIO), no change (NC), and enlargement (EL). According to our categorization, CS occurred in 97 (53.0%), RSF in 7 (3.8%), DD in 62 (33.9%), DIO in 7 (3.8%), NC in 5 (2.7%), and EL in 5 (2.7%). As expected, there were statistically significant differences in both the median DFS and OS in each pattern of tumor shrinkage (p <0.001 and p=0.001, respectively); in particular, the CS pattern had excellent prognosis. Multivariate analysis demonstrated that concentric shrinkage was the only significant good prognostic factor for OS (p=0.015). CONCLUSIONS: Tumor shrinkage patterns as revealed by MRI could be important surrogate prognostic factors for NAC in early low grade luminal breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-02-14

Title: Incidentally detected enhancing lesions found on preoperative breast MRI: Analysis of T2 signal intensity and apparent diffusion coefficient significantly improve classification

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Body: Purpose and Background– Reportedly, magnetic resonance imaging (MRI) detects mammographically and sonographically (US) occult incidental lesions in 10-29% of patients with a malignant primary breast lesion. We evaluated diagnostic performance of the BI-RADS reporting system and utility of T2- and diffusion weighted imaging (DWI) for MRI detected additional lesions.

Methods– This prospective study protocol included 3.0T structural breast MRI with T2-weighted imaging and DWI performed according to EUSOMA guidelines in 112 consecutive patients with primary breast lesions (mean age 57.0±12.7 years, range 38-80 years). Breast lesions with one or more suspective feature according to the BI-RADS lexigon were biopsied. T2 signal intensity (SI) and DWI findings were assessed.

Results– Altogether 33 (29.5%) patients had 36 primarily MRI detected incidental additional lesions. In addition, 36 sographically or mammographically detected lesions were histopathologically confirmed and a total of 8 lesions were followed up. Of incidental lesions, 16 (44.4%) proved to be malignant. Mean size was 0.82±0.29 cm (range 0.5 cm to 1.5 cm) and 1.5±1.61 cm (range 0.5 cm to 5.0 cm) for 29 mass lesions and 7 NMLE lesions, respectively.

In mass lesions, traditional morphological or kinetic features were not correlated to malignancy except for fast initial enhancement (P=0.003). The BI-RADS classification produced 100% sensitivity, 40% specificity, 53.3% PPV, 100% NPV and 51.7% overall accuracy. Both the low T2 SI (P=0.05) and low ADC values (P<0.001) significantly correlated with malignancy yielding improved 86.7%/90.5% specificity and 65.0%/90.5% negative predictive value, respectively (McNemar’s test, P<0.01). Combining low T2 SI, ADC values or morphologic or kinetic features did not improve the results.

Conclusion– A single suspective morphologic or kinetic MRI feature is the most sensitive parameter to discover an incidental breast malignancy, as some incidental lesions present with only a few suspicious MRI feature. Specificity of MRI is improved when T2 SI or DWI are addressed.
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Publication Number: P4-02-15

Title: Preoperative MRI of the breast and ipsilateral breast tumor recurrence: Long-term follow up

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Body: Introduction: Local recurrence after breast conserving surgery for invasive breast cancer is uncommon, reported in 5 to 10% of cases at 10 years after surgery. Prior studies with short term follow-up have shown that preoperative breast MRI does not reduce re-excision rates for positive margins or reduce local recurrence after lumpectomy and radiation therapy. This study aims to determine 1) if preoperative breast MRI is associated with reduced ipsilateral breast tumor recurrence (IBTR) rates in the longer term and 2) the IBTR rates of a high risk (triple negative (TN) and Her-2 positive) subgroup in those receiving or not receiving preoperative MRI.

Methods: Between 1999 and 2005, a cohort of patients with invasive breast cancer undergoing breast conservative surgery and radiation therapy were identified from a prospectively collected database and followed. The primary endpoint was IBTR rate. Secondary outcomes included the determination of factors associated with the use of preoperative breast MRI and prognostic factors related to IBTR. IBTR rate was calculated by Kaplan-Meier method. Univariate analysis was calculated using log-rank test and chi-squared test.

Results: The cohort consisted of 470 cases with invasive breast cancer undergoing lumpectomies with negative resection margins. All patients received adjuvant radiation therapy. 127 (27%) patients underwent preoperative breast MRI and 343 (73%) did not. Median follow-up was 97 months. The overall 10-year IBTR rate was 3.6%. Overall, there was no significant difference in IBTR rate at 10 years between those receiving preoperative MRI and those without (IBTR: 1.6% and 4.2%, respectively (p = 0.37). There were no differences in IBTR rate between MRI and no-MRI after adjusting for age, year of surgery, tumor size, and adjuvant treatments on univariate analysis. For patients who recurred, median time to recurrence was 26 months for MRI group vs. 25 months for no-MRI group. Factors associated with the receipt of preoperative MRI were age < 50 years, lesion > 2 cm and receipt of adjuvant chemotherapy. We also found that the TN and Her-2 positive combined subgroup had a higher IBTR rate than all others (9.8% vs. 3.1%, p = 0.03). In those that received preoperative MRI, there was no difference in IBTR between the high risk group (n= 33) and the remaining patients (3.3% vs 1.2%, p= 0.5), but in the group without an MRI, the IBTR rate of the high risk group (n= 75) was 11.8% compared to the remainder (vs. 4.0%, p= 0.0529). For the TN and Her-2 positive combined group, the difference in IBTR rate when this subgroup was subdivided if they had received preoperative MRI vs. no-MRI (3.3% vs. 11.8%, p= 0.3) was not significant.

Conclusion: With long term 10-year follow up, there is no overall significant difference in IBTR rate whether preoperative breast MRI is performed versus not. However, the high risk triple negative breast cancers and Her-2 positive populations combined have shown an increased IBTR rate, and this was more marked in those who did not receive preoperative MRI.
Title: Efficacy of contrast enhanced spectral mammography (CESM) in breast cancer detection – Retrospective review of 1000 CESM cases

Li L, Tinney E, Germaine P, Camacho JM M, Ren S and Liao L. Cooper University Health Care, MD Anderson Cancer Center at Cooper, Camden, NJ.

Body: Objective: Contrast enhanced spectral mammography (CESM) is a newly established study which utilizes contrast and dual energy digital mammographic technology to identify breast lesions. Limited studies have shown that CESM highlight contrast enhanced cancer that may be invisible on conventional mammogram or inconclusive on breast ultrasound. More studies are needed to assess the diagnostic accuracy of CESM as an adjunct to mammogram and/or ultrasound in breast cancer detection. Our research is to analyze sensitivity and specificity for each modality of CESM, Mammography, ultrasound, and CESM combined with ultrasound respectively.

Methods: We retrospectively reviewed 1000 CESM studies together with mammography and ultrasound completed in our institution from October 2012 to May 2015. GE Senographe Essential Full Field Digital System (SenoBright) was utilized for CESM studies. Both low and high energy images were obtained at 2 minutes and at 5 minutes after intravenous 75-100cc Isovue 370 administration at flow rate of 1.5-2cc per second. Images taken from mammography, ultrasound, and CESM were evaluated for each patient during two sessions 30 days apart. The cancer diagnoses were confirmed by tissue diagnoses. Sensitivity and specificity were calculated and statistical significance was analyzed using p value.

Table 1. The efficacy of CESM

<table>
<thead>
<tr>
<th>Study Modality</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
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<tbody>
<tr>
<td>CESM</td>
<td>98</td>
<td>86</td>
</tr>
<tr>
<td>Mammography</td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>92</td>
<td>78</td>
</tr>
<tr>
<td>CESM + Ultrasound</td>
<td>100</td>
<td>91</td>
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Results: The results from pathology identified 175 malignant breasts. The sensitivity and specificity for CESM were 98% and 86% respectively. The sensitivity and specificity for conventional mammography were 88% and 82% respectively. The sensitivity and specificity for breast ultrasound were 92% and 78% respectively. Sensitivity was significantly higher for CESM than it was for mammography or than it was for ultrasound (p<0.01). Sensitivity was 100% for CESM adjunct with ultrasound. Specificity for CESM was significantly higher than it was for mammography or than it was for ultrasound (p<0.01). Specificity was 91% for CESM adjunct with ultrasound, which was higher than single CESM, mammography or ultrasound. The smallest cancer can be detected by CESM is 0.5 cm.

Conclusions: Our study indicates that CESM had significantly higher sensitivity and specificity than mammography or ultrasound alone. Adding CESM to diagnostic workup adjunct with breast ultrasound increased sensitivity and specificity for breast cancer detection. With its easy accessibility and cost effectiveness, CESM may play an important role in breast cancer detection in conjunct with diagnostic mammography and breast ultrasound.
Title: Up-classification of suspicious breast masses using opto-acoustic imaging

Lavin PT T, Stavros AT T and Ulissey MJ J. Boston Biostatistics Research Foundation, Framingham, MA; Seno Medical Instruments, Inc., San Antonio, TX; Breast Diagnostic Center, Auburn, WA and Breast Diagnostic Center, Federal Way, WA.

Body: Purpose: Breast cancer diagnostic methodologies have been optimized to achieve increased sensitivity at the expense of relatively low specificity. Seno Medical’s opto-acoustic (OA) imaging fuses real time co-registered, temporally interleaved laser optic and ultrasound imaging showing dual functional (hemoglobin de-oxygenation) and morphology findings for breast masses using a hand-held probe. We present data from the PIONEER Pilot study (n=100). We have shown improved specificity for OA relative to the ultrasound component (IUS) and the site determination by conventional diagnostic ultrasound (CDU). We now examine the BI-RADS upgrades for 36 malignant masses achieved by OA versus IUS and the site determinations using CDU.

Materials and Methods: A total of 7 independent registration readers (IRRs) blindly assessed all 36 malignant masses using IUS first and OA second without any knowledge of clinical data or outcome. Among the cancers, there were 2 BI-RADS 4b, 12 BI-RADS 4c, and 22 BI-RADS 5 according to participating site radiologists’ CDU evaluations. IRRs trained to identify and score three OA internal features and two OA external features for all masses were immediately offered the results of two nomograms based on their OA feature scores to predict the Probability of Malignancy (POM), which was then used to assign a BI-RADS category.

Results: Combining data from all 7 readers, OA findings enabled upgrades of site CDU-determined BI-RADS categories 43% of the time for BI-RADS 4b and 29% for BI-RADS 4c; in contrast, the overall percentages of IUS upgrades versus site CDU were 21% for BI-RADS 4b and 10% for BI-RADS 4c. Overall, 12% of all OA reads resulted in upgrades in contrast to 4.4% for IUS compared to site CDU BI-RADS classifications. Relative to IUS, the overall percentages of OA upgrades were 58% for BI-RADS 4b and 34% for BI-RADS 4c. OA has comparable sensitivity to IUS.

Conclusions: OA was more likely than IUS to result in a BI-RADS upgrade of a malignant mass. If subsequently confirmed, OA findings may help identify more cancers prior to biopsy. The 2,095 PIONEER Pivotal Study will allow for confirmation.

Clinical Relevance: The ability to upgrade BI-RADS 4b and 4c for cancer masses is an unmet need. If verified, these findings could provide additional evidence to confirm a malignant mass earlier and spare subsequent diagnostic evaluations. This may help plan the efficient identification and excision of malignant masses.
**Title:** Detection of the tumor vasculature and the hypoxic status of breast lesions using second-generation photoacoustic mammography: An exploratory study

Takada M, Kawashima M, Kataoka M, Kanao S, Yamaga I, Torii M, Tokiwa M, Fakhrejahani E, Sakurai T, Asao Y, Haga H, Shiina T, Togashi K and Toi M. Graduate School of Medicine, Kyoto University, Kyoto, Japan; Division of Clinical Radiology Service, Kyoto University Hospital, Kyoto, Japan; Kyoto University Hospital, Kyoto, Japan; Canon Inc., Tokyo, Japan and Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

**Body:** Background: Tumor angiogenesis and hypoxia are associated with breast cancer growth and metastasis. Photoacoustic mammography (PAM) non-invasively visualizes hemoglobin distribution inside the breast by detecting thermoelastic waves from hemoglobin generated by the irradiation of a near-infrared laser pulse. Oxygen saturation (SO2) can be calculated using photoacoustic (PA) signals obtained by two laser pulses of different wavelengths. We further improved the spatial resolution of PAM by approximately 1 mm and enhanced detectability by using a high-sensitivity detector. This new PAM technique can obtain both PAM images and ultrasonography (US) images simultaneously. The aim of this study was to explore the clinical usefulness of this PAM technique.

Patients and methods: Women who had breast lesions were eligible for this study. The participants’ lesions were measured using the new PAM technique before they began treatment. The PAM images were evaluated by 5 physicians. First, the lesions were identified using only the PAM images. Second, we used US or contrast-enhanced magnetic resonance images (CE-MRI) to identify the locations of the lesions. Next, we evaluated the photoacoustic (PA) signals based on their locations. Peri-tumoral PA signals were defined as linear signals that congregated in the peri-tumoral area, boundary PA signals were defined as peri-tumoral signals that were disrupted at the lesion's boundaries, and intra-tumoral PA signals were defined as any significant PA signals inside the tumor. SO2 was illustrated using a color scale. The study protocol was approved by the institutional review board at Kyoto University Hospital, Japan (UMIN000007464).

Results: PAM was performed on 48 breast lesions in 45 patients, including 36 invasive carcinoma lesions, 8 ductal carcinoma in situ (DCIS) lesions, and 4 benign lesions. Evaluations of PA signals according to the locations of the lesion, with confirmation from US or CE-MRI, were successfully performed for 38 lesions. Peri-tumoral PA signals were detected in 33 lesions (87%), disrupted boundary PA signals were detected in 30 lesions (79%), and intra-tumoral PA signals were detected in 25 lesions (66%). The detection rates for peri-tumoral, boundary and intra-tumoral PA signals were 94%, 87%, and 65% for invasive carcinoma, and 60%, 40%, and 80% for DCIS, respectively. Intra-tumoral PA signals tended to be weaker than peri-tumoral PA signals in invasive carcinoma lesions, and they often displayed a spotty rather than a linear shape. Intra-tumoral PA signals were observed to have lower SO2 levels than peri-tumoral PA signals in 95% of invasive carcinoma lesions and in 75% of DCIS lesions. Although peri-tumoral and boundary PA signals were also detected in a 38-mm fibroadenoma, the intra-tumoral PA signals displayed a diffuse pattern.

Conclusions: We demonstrated that high spatial resolution and use in combination with US and CE-MRI facilitate the region-specific evaluation of PAM imaging. PAM could become a useful tool for the evaluation of the hypoxic status of tumors by enhancing its sensitivity.
Title: Dynamic tomographic optical breast imaging (TOBI) for neoadjuvant chemotherapy monitoring


Body: Background: Dynamic Diffuse optical imaging using near-infrared light was shown to be promising method for neoadjuvant therapy monitoring as an alternative functional imaging that is low-cost, non-invasive, portable, safe and simple to operate. While optical breast imaging methods rely on "static" assessments of tissue oxy- and deoxy- hemoglobin concentration without contrast agents, they are insufficient for clinical applications. Dynamic tomographic optical imaging induces tumor-sensitive hemodynamic variations, as a contrast mechanism, driven by fractional mammographic compression. These tumor contrast measurements are governed by interlay of tissue biomechanics and oxygen metabolism. In this study we seek to evaluate the predictive value of these biomarkers with respect to treatment outcome.

Methods: A group of 22 patients with locally advanced breast cancer were scanned using our dynamic TOBI system before and during neoadjuvant chemotherapy. In this analysis we focused on pre-treatment, day 7 and day 30 post-treatment dynamic TOBI scans. Both breasts are compressed in turn to 4-8 lbs of force (depending on size) and optical images are acquired every 2 seconds over 2 minutes. We calculate the time course of oxy (HbO), deoxy (HbR) and total (HbT) hemoglobin concentration as well as the hemoglobin oxygen saturation (SO2). Regions of interest are defined in the optical images to correspond to the radiology identified tumor location, and the healthy tissue in the same breast, respectively. We compare the time courses in the two regions at baseline, day 7 and 30 days after initiation of treatment.

Results: In this analysis we present results from 10 patients including 6 responders (defined as greater than 50% reduction in the largest tumor axis from baseline imaging and final pathology) and 4 non-responders. As the compression plates are held in place the tissue collagen matrix begins to stretch, effectively reducing the compression force. At baseline, all patients exhibit a decrease followed by delayed recovery in HbT, and SO2 in the tumor area, in contrast to immediate recovery in surrounding tissue. At day 7 and 30, this contrast is maintained in non-responders (<50% reduction in tumor maximum diameter); however, in responders, the contrast starts decreasing at day 7 and substantially disappears at day 30. Average changes in HbT and SO2, show that the contrast between normal and tumor increases somewhat at day 7 and more noticeably at day 30 in non-responders to NACT.

Comparing hemodynamic changes in responders and non-responders, it is clear that at three selected time points (30, 60 and 90 s) during the scan, the contrast between tumor and normal tissue in both ΔHbT and ΔSO2 is reduced for the responders at day 7 and day 30.

Conclusions: These initial results suggest that dynamic optical breast imaging can detect changes due to treatment and have predictive value for the treatment outcome. DTOBI can show the difference in hemodynamic response to compression between tumor and normal tissue and demonstrates the feasibility of using dynamic optical breast tomography for neoadjuvant chemotherapy monitoring. ongoing.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-03-05

**Title:** Wide-field optical coherence tomography (WF-OCT) for near real-time, point-of-care assessment of margin status in breast-conserving surgery specimens: Results of a feasibility study at a high-volume single-centre

Valic MS S, Leong WL L, Done SJ J, Wilson BC C, Kulkarni S, McCready DR R, Niu CJ J, Atachia Y, Munro EA A and Rempel D. Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada; The Princess Margaret Cancer Centre, University Health Network (UHN), Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Perimeter Medical Imaging, Inc., Toronto, ON, Canada and University Health Network, Toronto, ON, Canada.

**Body:** Wide-Field Optical Coherence Tomography (WF-OCT) is a non-destructive, non-contact light imaging modality capable of label-free visualization of the internal microscopic architecture of breast tissue specimens. Its unique combination of high-resolution imaging in near real-time with tissue penetration depths approaching 2-mm makes it a promising imaging modality for obtaining detailed surgical margin status in breast-conserving surgery (BCS) specimens. A prototype WF-OCT imaging platform developed by Perimeter Medical Imaging, Inc. (Toronto, Canada) has permitted fully-automated, dynamically-focused visualization of margin widths around the intact surfaces of freshly excised BCS specimens. Herein are reported the results of a feasibility study at a high-volume single-centre evaluating the routine use of WF-OCT for sampling of surgical margin status in BCS specimens at the point-of-care.

**Methods:** Women with biopsy confirmed breast cancer and scheduled for primary BCS were recruited at Princess Margaret Cancer Centre (Toronto, Canada). Standard medical care was not altered. Freshly excised BCS specimens including all lumpectomy samples were imaged by WF-OCT immediately prior to standard histological processing. The system acquired dynamically-focused, hemispherical coverage over two contra-lateral surfaces of the intact BCS specimen within the time constraints of the cold ischemic time window. High-resolution (10 µm) images of the tissue surface down to a 1 to 2-mm depth were obtained. Blinded assessments were performed on image data sets by two clinical readers (surgeon and radiologist) trained on a validated and unrelated data set correlating OCT images with histology slides. The readers were first asked to independently assess margin status using only blinded pre- and intra-operative knowledge (without OCT). Upon completion, the readers were provided OCT images of all scanned surface and similarly asked to assess the margin status with the additional OCT information. These assessments were subsequently evaluated by a breast pathologist comparing the OCT images and corresponding histopathology sections. The added utility of WF-OCT imaging information for margin prediction was studied.

**Results:** [Pending study completion in August 2015]. Through accurate correlation with the histopathologic gold standard, OCT demonstrated capability to differentiate tissue microstructures, including: distinctive patterns for adipose tissue, fibrous stroma, breast lobules and ducts, cysts and microcysts, as well as in-situ and invasive carcinomas.

**Implications:** The fully-automated WF-OCT imaging platform can integrate conveniently into standard pathological processing workflows to provide comprehensive sampling of surgical margin status in BCS specimens at the point-of-care. Clinical readers from surgical and radiological backgrounds can be trained to competently interpret WF-OCT images of BCS specimens for accurate prediction margin status. The implementation of WF-OCT at the point-of-care for routine surgical margin assessments will be further explored in future clinical trials.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-03-06

Title: Near-infrared diffuse optical imaging for early prediction to neoadjuvant chemotherapy in patients with primary breast cancer

Ogura H, Yoshizawa N, Ueda S, Hosokawa Y, Matsunuma R, Tochikubo J, Nasu H, Shigekawa T, Takeuchi H, Osaki A, Saeki T, Yoshimoto K, Ohmae E, Suzuki T, Ueda Y, Yamashita Y and Sakahara H. Breast Surgery, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; Radiology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; Breast Oncology, International Medical Center, Saitama Medical University, Hidaka, Saitama, Japan; Breast Oncology, Saitama Medical University, Iruma, Saitama, Japan and Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, Japan.

Body: Background: Diffuse optical spectroscopic imaging (DOSI) can be exploited as a marker of tumor blood volume quantified by tissue hemoglobin (tHb) concentration. In DOSI, frequent measurement is possible for breast cancer patients because of its non-invasiveness. The tHb concentration determined by DOSI is expected to be a new biomarker for prediction of breast cancer response to neoadjuvant chemotherapy (NAC).

Purpose: Our objective is to determine whether early change of tumor tHb concentration predicts pathological complete response (pCR) to NAC in patients with operable breast cancer.

Methods: In a prospective study, one hundred patients with primary breast cancer were enrolled for primary objective analysis. The regimens of NAC were according to the standard of care. Patients underwent sequential scans using DOSI at baseline, after 1st course and 2nd course of chemotherapy. The mean value of tHb (tHbmean) concentration of the targeted lesion was measured and the percentage change in tHbmean (ΔtHbmean) concentration was calculated. Receiver operating curve analysis demonstrated diagnostic performance of DOSI for predicting a pCR.

Results: In interim analysis, it was regarded as a good outcome that area under the curve (AUC) for ΔtHbmean after 1nd course was 0.797 (SE 0.104, 95%CI 0.633-0.911), and after 2nd course was 0.867 (SE 0.06, 95%CI 0.715-0.956).

Conclusion: DOSI could predict accurately a pCR to neoadjuvant chemotherapy in patients with primary breast cancer.
Title: Contribution of high-resolution ultrasonography in the diagnosis of non-palpable breast cancer with calcification

Arai T and Takebe K. Takebe Breast Surgery Clinic, Takamatsu, Kagawa, Japan.

Body: Background: In diagnosis of non-palpable calcificated breast cancer (NPCBC), stereo-guided mamnotome (ST-MMT) serves as the standard. However, ultrasound resolution has improved dramatically recently. We hypothesized that through ultrasonography, it will become possible to diagnose NPCBC by US guided examination such as fine needle aspiration cytology (FNAC) and core needle biopsy (CNB) with much less invasion than ST-MMT.

Method: (Study1) The results of ultrasonic detection ability and US guided examination were studied among the benign lesions of 35 cases and 90 NPCBC patients histologically diagnosed in our facilities from 2010 to 2012. Of the 90 histological types, 74 (82%) were DCIS, and 16 (18%) were invasive carcinoma. (Study 2) The results of 193 progress observation cases from 2008 to 2012 with Birads Category 3 and 4a calcification were examined.

Result: (Study1) Using ultrasonography (Toshiba Aprio XG), calcification was detected in 88 of the 90 NPCBC lesions as echogenic foci. Two non-detectable lesions were DCIS of Van Nuys 1 and Van Nuys 2. The results of the FNAC were 80% positive and 20% indeterminate (there were no negative cases). In the 88 cases of possible calcified substance extracted through FNAC, 99% were confirmed under microscopic observation. (Study 2) 163 of 193 cases were able to be followed-up every six months for two to four years. An increase in calcification was observed in nine cases. Five of these cases were diagnosed with DCIS: Four were Van Nuys 1; one was Van Nuys 2. All five cases were of low-grade-malignancy.

Conclusion: The diagnostic method using FNAC to visualize calcification using high-resolution ultrasonography is an effective method to reduce the non-efficient, invasive breast cancer detecting method of ST-MMT. The diagnostic method is particularly useful in women of small breast size in Asian countries. The few low-grade DCIS cases included were not detected with ultrasound. However, we believe it is not a diagnostic error. As a result, we stopped using the ST-MMT from 2010. In spite of this, in the MMG screening performed in our facilities from 2010 to 2012, the detection rate of NPCBC without mass on MMG was 0.17%. This detection rate is excellent in our country.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-03-08

Title: Abstract Withdrawn

Body:
Purpose: Challenge current axiom regarding hyperechogenic lesions in breast US categorized as mainly benign lesions. 2) Present frequency of malignant hyperechoic lesions, describe their sonographic and histological characteristics.

Method and Materials: IRB approved retrospective review of 2369 consecutive US-guided Core biopsies between 2006 and 2014. Lesions were considered hyperechogenic when more than 90% of its volume had higher echogenicity than surrounding subcutaneous tissue. Variables assessed were: age, size, histology, mass or non-mass, margins, echogenicity, acoustic effect, orientation, location, presence of a hypoechoic center, vascularization and BI-RADS category. Qualitative variables were described by percentage distribution, mean and SD.

Results: Thirty one (1.3%) lesions biopsied were hyperechoic: only 17 (54.8%) were benign. We found 14 patients (range, 39-81 years, mean 57.5 years) with hyperechoic cancers, accounting for 1.77% of all cancers. 12 (85.7%) were ductal and 2 (14.3%) lobular histology (mean 17.5 mm range 8-40mm). All of them exhibited a small central hypoechoic area and were partially or completely surrounded by fatty tissue. Shape, margins, orientation and vascular architecture were highly suggestive of malignancy at US, mammography and in MRI (BI-RADS 4B and 5). On histology, all lesions were surrounded by fatty tissue, with a low–cell count collagenous center with a higher peripheral cellularity, mixing and interweaving with adipose tissue.

Conclusion: Hyperechogenic breast cancers are rare (1.77%), and we challenge the current concept of high rate of benignity with a 45.2% of chances for malignancy. We propose that hyperechogenicity is given by interweaving bundles with adipose tissue, which provide higher amount of acoustic reflective surfaces, thus explaining its sonographic pattern.

Clinical Relevance/Application: Hyperechogenic breast lesions should not be considered as always benign. Malignancy thresholds should not be influenced by this sonographic characteristic. We propose histopathological explanation of this sonographic pattern.
Title: PDL-1 expression in primary breast cancers with germline mutations in BRCA 1 and 2

Audeh MW, Dadmanesh F and Yearley J. Samuel Oschin Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA and Merck and Co, Inc., Kenilworth, NJ.

Body: Background: The PD-1/PDL-1 pathway is a major inhibitory regulator of the immune response to tumors. Tumors responding to anti-PD-1 therapy appear to be characterized by a high "load" of somatic mutations, e.g. carcinogen-induced cancers (melanoma, lung) and tumors with a high intrinsic mutation rate (colorectal cancers with defects in DNA mismatch repair). High mutational load in these tumors may cause the expression of neoantigens which may induce an immune response unless the inhibitory PD-1/PDL-1 pathway is upregulated. Tumors in carriers of germline mutations in BRCA1/2 lack effective DNA repair and are genomically unstable, with a high mutational load and the possible expression of neoantigens. Breast cancers arising in carriers of germline mutations in BRCA1 and BRCA2 may rely on the PD-1/PDL-1 pathway to avoid immune destruction. Methods: Samples from thirty (30) treatment-naïve, primary breast cancers from 30 women with known germline mutations in BRCA 1 or BRCA2 were identified by records review in the Cedars-Sinai Department of Pathology, and selected for analysis of PD-1 and PDL-1 expression. Samples were analyzed for PD1 and PDL-1 expression utilizing immunohistochemical staining. Sections from FFPE tissue blocks were analysed with anti-PD-L1 clone 22C3(Merck); anti-PD-L1 clone SP142; or anti-PD-1 clone NAT105. IHC Scoring analyzed both tumor cell and non-tumor cell (inflammatory) infiltrate. A 0-5 scoring system (0 = neg, 1 = rare, 2 = low, 3 = mod, 4 = high, 5 = very high) was applied. Tumors with IHC scores of 2 or greater were considered "positive" for expression of PD1 or PDL-1. Clinical Data: Age range 39-90 years, median age 61. Twenty (20) tumors were from BRCA1 mutations carriers, and 10 were BRCA2. Sixteen (16) were basal type breast cancer (13 BRCA1, 3 BRCA2), 14 were estrogen receptor positive (ER+) (7 BRCA1, 7 BRCA2), with 7 Luminal A and 7 Luminal B. There were no HER2-amplified tumors in the cohort. Results: PD1 expression was observed in 11/30 (37%) of the cohort, and PDL-1 expression was detected in 21/30 (70%) of the cohort. PDL-1 expression was primarily seen in non-tumor, infiltrating immune cells. PD1-1 expression was seen in 15/20 (75%) of tumors from BRCA1 mutation carriers, and 6/10 (60%) of tumors from BRCA2 carriers. PDL-1 expression was present in 13/16 (81%) basal tumors and 8/14 (57%) ER+ tumors. Within the cohort of PDL-1 expressing cancers, 7/21 (33%) were scored as "high" or "very high", 5 basal breast cancers and 2 ER+ cancers. Conclusion: The current study has identified a high rate of PDL-1 expression in untreated primary breast cancers with germline BRCA1 and BRCA2 mutations, regardless of intrinsic subtype. Although the highest rate of expression was seen in basal breast cancers (81%), the majority of ER+ breast cancers, both Luminal A and Luminal B, also expressed PDL-1. Anti-PD-1 therapy has yielded a response rate of 19% in metastatic triple negative breast cancer, unselected for BRCA mutation. The current study suggests that tumors in carriers of germline mutations in BRCA1 or BRCA2, regardless of intrinsic subtype, may rely on immune checkpoint inhibition for their growth and survival, and that therapy directed against the PD-1/PDL-1 pathway may be of benefit in this cohort of patients.
Title: Amplification of chromosome 9p24 targeting PD-L1 and JAK2 correlates with altered tumor microenvironment expression profiles in triple negative breast cancer

Anderson KS S, Andreozzi M, Pockaj BA A, Hopper M, McCullough AE E and Barrett MT T. Biodesign Institute, Arizona State University, Tempe, AR; Mayo Clinic, Phoenix, AR; Mayo Clinic, Phoenix, AR and Mayo Clinic, Phoenix, AR.

Body: Introduction: In a pilot study, we recently described amplification of chromosome 9p24 encoding PD-L1, PD-L2, and JAK2 (the PDJ amplicon) in 29% of early stage triple negative breast cancers (TNBC). Amplification was associated with poor DFS and OS. The impact of this amplicon on immune function is not known.

Methods: Fresh frozen tumor samples from 36 subjects with newly-diagnosed TNBC (stages I-III) were evaluated for copy number aberrations using DNA-based flow cytometry to enrich nuclei based on ploidy status (tetraploidy or aneuploidy), followed by oligonucleotide CGH arrays. Targeted immune expression profiling was performed using the Nanostring PanCancer Immune Profiling Panel (n=770 genes).

Results: 23 samples had measurable abnormal ploidy (evidence of sufficient tumor content) for CGH analysis; the remaining samples were omitted from analysis. High level (log2ratio >1) amplification was detected in 6/23 (26.1%) of TNBCs, and low-level copy number gain (CN log2ratio >0 and<1) was detected in 3/23 (13.0%). The remaining samples had no copy number change (n=9) or low level copy number loss (CN log2ratio>-1 and <0, n=5). Increased RNA expression of STAT1, STAT2, STAT3, and STAT6 was associated with PDJ amplification, as well as upregulation of the immune regulatory genes CD47 and IDO1.

Conclusion: Amplification of chromosome 9p24.1 involving PD-L1, PD-L2, and JAK2 is present in a significant proportion of TNBCs. This amplicon is associated with altered gene expression of the tumor microenvironment.
Title: Targeting of phosphatidylserine by monoclonal antibodies augments the activity of immune checkpoint inhibitor PD-1/PD-L1 therapy in murine breast tumors


Body: Phosphatidylserine (PS) is a phospholipid that typically resides in the inner leaflet of the plasma membrane in many types of cells, including both tumor and tumor associated endothelial cells. Conditions that cause cellular stress, including those that occur from oxygen radicals, hypoxia, irradiation, and chemotherapy, cause a dramatic shift in PS localization. This change in localization results in PS shifting to the outer plasma membrane, allowing its recognition by components of the tumor microenvironment. Recognition of PS promotes an immunosuppressive environment that encourages tumor growth, in part by promoting the recruitment of myeloid derived suppressor cells, immature dendritic cells, and M2-like macrophages, in addition to inducing production of anti-inflammatory cytokines. Currently the chimeric PS-targeting antibody, bavituximab, is being used in combination with chemotherapies to treat patients with solid tumors in multiple late-stage clinical trials, where it is believe to help augment the efficacy of chemotherapeutics by blockade of PS-mediated immunosuppression and triggering an Fc-FcR mediated pro-inflammatory response in the tumor microenvironment. While the results with PS targeting therapies with chemotherapeutics are encouraging, the effectiveness of PS targeting therapies in conjunction with therapies towards immune checkpoint regulators remains largely unknown. To better understand the role of PS-targeting in breast cancers, and its effectiveness when used in combination with checkpoint inhibitors, immune competent mice harboring either EMT-6 or E0771 breast tumors were utilized. Treatments comprised PS targeting antibodies and an anti-PD1 antibody (to interrupt the PD-1/PD-L1 signaling axis) either alone or in combination with each other, and the effect on tumor growth and immune suppression determined. In both models, which showed differential sensitivity to therapy, the inclusion of PS targeting antibodies with the checkpoint blocker antibody had a significantly greater anti-tumor response than either single agent alone.
Title: Triple-negative (TN) and HER2+ breast cancers (BC) have different immune milieu in primary and metastatic tumors

Bianchini G, Riba M, Zambelli S, Safonov A, Oguya R, Jiang T, Hatzis C, Niikura N, Zambetti M, Iwamoto T, Pusztai L and Gianni L. Ospedale San Raffaele, Milan, Italy; Fondazione Centro San Raffaele, Milano, Italy; Yale Comprehensive Cancer Center, Yale School of Medicine, New Haven, CT; Tokai University School of Medicine, Isehara, Japan; Kyoto University School of Medicine, Kyoto, Japan and Okayama University Hospital, Okayama, Japan.

Body: Background: In TN and HER2+ early BCs, a high immune infiltration is linked to good prognosis and improved treatment benefit. Little is known about the characteristics of the immune milieu of BCs in metastatic disease. We aimed to investigate differences of the immune microenvironment between cohorts of primary early breast cancer (EBC) and metastatic (MBC) tumors according to molecular subtypes.

Methods: We identified publicly available gene expression profiles (GEPs) of MBCs profiled either on Agilent (n=12, cohort I) or AffymetrixU133A (n=36, cohort II). These included 21 ER-/HER2- (TN), 10 HER2+ and 17 ER+/HER2- (Luminal). From GEPs of EBC profiled on the same platforms, we randomly selected two cohorts with the same molecular subtype composition (n=65 and n=230) and compared them with MBCs. We assessed differential expression of 40 pre-selected immune genes belonging to six immune-related metagenes [CD8, IGG and MHC2, related to T cells, plasma cells and antigen presenting cells respectively; MHC1, STAT1 and IF.I related to HLA class I genes; and genes modulated by interferon (Gianni L SABCS 2012)]. We also evaluated β2-microglobulin (B2M), for its role in the MHC1 complex, and an immune signature associated with benefit from pembrolizumab in melanoma (Ribas A ASCO 2015).

Results: In cohort I (Agilent), only 33 genes were annotated. Overall, 16/33 (48.4%) genes had a significantly lower expression in MBC (p<0.05). In TN and HER2+ MBCs 18 and 11 genes were significantly lower than in EBC, respectively (p<0.05) (6 in both), while only one was lower in luminal MBCs (IGHM). In cohort II (Affymetrix), 26/40 genes (65%) had lower expression in MBC (p<0.05). Considering molecular subtypes, 25 and 19 genes were lower in TN and HER2+, respectively (17 in both), and only one in ER+/HER2- (IL7R). In ER+/HER2- one gene was higher in MBC (IFIT2). In TN and HER2+ the genes with lower expression in MBC belong to all immune functional categories, in particular MHC1 (HLA-A, B and C), STAT1 (STAT1, CXCL10, CXCL11, GBP1), MHC2 (HLA-DQB1 and DRB4) and T cells (CD52, IL7R and TRBC1). B2M was significantly lower in all MBC patients, and in HER2+ and TN groups both in cohort I (p=0.0002; p=0.006 and p=0.0005, respectively) and in cohort II (p<1E10-6; p=0.0008 and p=0.00004, respectively), while it was modestly lower in ER+/HER2- in cohort II only (p=0.027). The signature associated with benefit from pembrolizumab in melanoma was significantly lower in TN and HER2+ MBC in both cohort I (p=0.003) and cohort II (p=0.001), but not in luminal cases.

Conclusions: TN and HER2+ MBCs have a “colder” immune microenvironment than primary tumors, with significantly lower expression of genes related to immune response and to antigen presentation (B2M and MHC1). This is consistent with the lower TILs we have described in a small series of paired EBC-MBC (Oguya R ASCO 2015), suggesting the engagement of mechanisms of immune escape during the metastatic process. However, the “cold” immune milieu observed in MBC could also result from selection of low immunogenic tumors more likely to relapse. Our findings suggest that use of immune-checkpoints inhibitors in MBCs may require the combination with agents able to turn on an immunogenic response.
Title: Differential mRNA expression patterns in breast tumors with high vs. low quantity of stromal tumor–infiltrating lymphocytes


Body: Background: Tumor-infiltrating lymphocytes (TIL) have prognostic and potentially predictive significance in the (neo)adjuvant treatment of high-risk breast cancer. However, quantitative TIL measurement is not routinely performed. It is unclear why some tumors attract large quantities of TIL while others do not. We sought to confirm the association between TIL and pathologic complete response rate (pCR) and to further use next generation sequencing (NGS) to identify genes and gene pathways associated with the presence/absence of TIL.

Methods: We studied 140 women with high risk stage I-III breast cancer, enrolled in the Breast Cancer Genome Guided Therapy Study (BEAUTY), obtaining serial biopsies for DNA/RNA sequencing and MRI imaging to assess response to neoadjuvant chemotherapy (NAC) with taxane (+/- trastuzumab+/-pertuzumab for HER2+ disease) followed by AC or (F)EC. Diagnostic pre-NAC core needle biopsies and surgical resection specimens post-NAC were available from 110 patients. Stromal TIL were semi-quantitated on a scale of 1-4 (with 1: ≤10/hpf, 2: subtle infiltrate >10/hpf, 3: moderate infiltrate readily visible at low power magnification, 4: dense infiltrate with innumerable lymphocytes). For this analysis, low TIL was defined as scores of 1-2 vs. high defined as 3-4. Using pre-NAC biopsies, RNAseq was performed using the Illumina HiSeq2000 and the Mayo Analysis Pipeline for RNAseq (MAP-Rseq) for quality control, sequence alignment, and gene counts. The quantity of TIL was associated with transcripts across the transcriptome after conditional quantile normalization. Differentially expressed genes were obtained using EdgeR analysis, using a false discovery rate of 0.05, and pathways were evaluated using GAGE methods.

Results: The pCR and residual cancer burden (RCB)-0/I rates by stromal TIL status within each molecular subtype are presented in the table. A diverse spectrum of 1344 genes with differential expression between tumors with high vs. low stromal TIL was identified. The genes with >2.0-fold change (FC) and p<e-09 included S100A7 (4.49 FC), LCN2 (2.48 FC), and ART3 (2.82 FC) (genes known to be involved in immune regulation), as well as TDRD1 (2.71 FC) (a gene related to ERG [ETS-related gene] expression). In addition, the “regulation of actin cytoskeleton” pathway was upregulated in tumors with high TIL, while the “Hedgehog signaling” and “Wnt signaling” pathways were downregulated.

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>Stromal TILs</th>
<th>pCR rate n (%)</th>
<th>RCB-0/I rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>High</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Luminal A</td>
<td>Low</td>
<td>0/9 (0%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>High</td>
<td>1/9 (11.1%)</td>
<td>1/8 (12.5%)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>Low</td>
<td>3/24 (12.5%)</td>
<td>6/23 (26.1%)</td>
</tr>
<tr>
<td>ER+/HER2+</td>
<td>High</td>
<td>3/9 (33.3%)</td>
<td>4/9 (44.4%)</td>
</tr>
<tr>
<td>ER+/HER2+</td>
<td>Low</td>
<td>1/6 (16.7%)</td>
<td>1/6 (16.7%)</td>
</tr>
<tr>
<td>ER-/HER2+</td>
<td>High</td>
<td>8/9 (88.9%)</td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td>ER-/HER2+</td>
<td>Low</td>
<td>4/8 (50.0%)</td>
<td>6/8 (75.0%)</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>High</td>
<td>10/19 (52.6%)</td>
<td>13/19 (68.4%)</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>Low</td>
<td>7/14 (50.0%)</td>
<td>9/13 (69.2%)</td>
</tr>
</tbody>
</table>

Conclusions: We identified genes and gene pathways associated with high TIL expression in breast tumors prior to NAC that...
provide insight into the interactions between TIL and tumors. TIL can be easily semi-quantitated on H&E and along with these novel biomarkers, may contribute to the personalization of breast cancer therapy.
**Title:** Immune balance between tumor-infiltrating lymphocytes and tumor-associated macrophages impacts the outcome of triple negative breast cancer patients

Bottai G, Raschioni C, Losurdo A, Di Tommaso L, Roncalli M and Santarpia L. Oncology Experimental Therapeutics Unit, IRCCS Humanitas Clinical and Research Institute, Milan, Italy; IRCCS Humanitas Clinical and Research Institute, Milan, Italy and IRCCS Humanitas Clinical and Research Institute, Milan, Italy.

**Body:**

**Background:** Stromal tumor-infiltrating lymphocytes (TILs) are emerging as a robust prognostic factor in triple negative breast cancer (TNBC). However, the prognostic value of TILs may be also influenced by the complex landscape of the tumor immune microenvironment. In this study, we aimed to assess the correlation between TILs and tumor-associated macrophages with pro-tumoral functions (M2 TAMs), and to determine the prognostic role of TAM/TIL ratio in TNBC.

**Methods:** Formalin-fixed, paraffin-embedded tissues were retrospectively collected from 189 patients with histologically confirmed invasive ductal TNBC. Stromal TILs were evaluated on H&E slides. The content of CD163-positive M2 TAMs in tumor stroma was assessed by immunohistochemistry. Whole tumor slides were independently assessed by two pathologists blinded for patient characteristics and outcome. CD163 staining was dichotomized into absent/moderate or dense infiltration; 50% stromal TILs was used as threshold (Salgardo, 2015). Statistical analyses were performed using Spearman's correlation, Fisher's exact tests, Kaplan-Meier and Cox regression analyses.

**Results:** TNBC patients treated with adjuvant chemotherapy who experienced tumor recurrence showed a significant lower content of stromal TILs ($p < 0.0001$) and a higher infiltration of CD163-positive TAMs ($p = 0.020$) compared to patients without recurrence. Stromal TILs were also associated with lymph node positivity ($p = 0.036$). Univariate and multivariate analysis confirmed the importance of stromal TILs as a prognostic marker of recurrence-free survival (RFS), and overall survival (OS). Importantly, we found that stromal TILs inversely correlated with CD163-positive TAMs ($rs = -0.539; p < 0.0001$), indicating the significance of the balance between distinct immune components in the tumor microenvironment. Consistently, TNBC patients with a high TAM/TIL ratio had significantly shorter RFS (Log-rank $p < 0.0001$) and OS (Log-rank $p < 0.0001$) than patients with low TAM/TIL ratio. TAM/TIL ratio also retained its independent prognostic significance for RFS (HR = 7.02; 95%CI 2.09 - 23.59, $p = 0.002$) and OS (HR = 7.11; 95%CI 2.10 - 24.05, $p = 0.002$) in multivariate analysis.

**Conclusions:** Distinct immune cell subpopulation may have a specific role in modulating the immune response against breast tumor. A high content of TAMs with pro-tumoral functions may influence the recruitment of lymphocytes and suppress antitumor immunity. Indeed a high TAM/TIL ratio could help to identify a subset of TNBC patients at high risk for relapse and reduced OS. The understanding of the biological and clinical relevance of immune balance in tumor microenvironment warrants further investigations, and may be useful for the stratification of TNBC patients who may benefit from the addition of immunomodulatory therapies.
Title: Association between tumor infiltrating immune cells and disseminated tumor cells in the bone marrow of patients with primary breast cancer: A ten year follow-up

Kasimir-Bauer S, Spiekermann A, Friedrich CM M, Hoffmann O, Schmid KW W, Kimmig R and Bankfalvi A. University Hospital Essen, Essen, Germany; University of Applied Science and Arts, Dortmund, Germany and University Hospital Essen, Essen, Germany.

Body: Background: Disseminated tumor cells (DTCs) in the bone marrow (BM) of breast cancer patients (pts) were shown to be an independent prognostic factor with regard to reduced PFS and OS. Although this negative prognostic impact has been related to chemotherapy resistant and stem cell-like cells, the microenvironment of the primary tumor might also influence tumor cell spread and thus, outcome of the disease. After a ten year follow-up, we here analyzed the prognostic significance of infiltrating leukocytes in the tumor (TILs) and stroma (SILs) with regard to PFS and OS as well as their association with DTCs in 163 pts with primary breast cancer, diagnosed between 2001 and 2004.

Patients and Methods: Tissue microarrays (TMA) were stamped from paraffin embedded specimens and 5µm thick sections were subjected to immunohistochemistry using antibodies against CD3 (DCS, Hamburg, Germany), CD68, CD20, CD8 (all Dakocytomation, Glostrup, Denmark), CD4 (Zytomed Systems, Berlin, Germany) and CD45R0 (Labvision, Fremont, USA), respectively. Each TMA has been evaluated for TILs and SILs assessing intraepithelial and stromal tumor areas occupied by respective immune cells separately: score 0: no staining; score 1: ≤ 10%; score 2: 11-60%; score 3: ≥61%, respectively, using light microscopy at magnifications of x100-200. Statistical analysis was conducted with the R programming language (version 3.20) and Cox proportional hazard regression and the Kruskal-Wallis test. DTCs were studied at the time of primary diagnosis using immunocytochemistry, applying the pan cytokeratin (CK) antibody A45-B/B3.

Results: Univariate analyses showed significant associations between prolonged OS and high rates of CD8TIL (p=0.01) and CD4TIL (p=0.028). In the subgroup of triple-negative carcinomas, CD3 TIL and CD8SIL were positively correlated with OS (p=0.038 and p=0.046, respectively), whereas CD8TIL was significantly associated with longer OS (p=0.049) in the total group of 163 pts, whereas in the triple-negative subgroup, CD3TIL was significantly correlated with favorable outcome (p=0.044). In contrast, no significant associations were found between TILs/SILs and PFS. The results for DTCs were available for 92/163 pts resulting in a positivity rate of 53%. Intriguingly, despite failing overall associations between DTCs and TILs/SILs, an unfavorable CD4SIL/CD8SIL ratio in the tumors of DTC-positive pts predicted significantly shorter PFS and OS (p=0.009 and p=0.018, respectively). In the DTC-negative subgroup, high CD4SILs were a prognostic marker for shorter OS. Notably, no significant associations were found between CD20, CD45R0 and CD68 and any parameter tested.

Conclusion: Analyzing a variety of leukocyte subtypes, we here demonstrate that especially the distribution of CD4 and CD8 in the primary tumor and their ratio seems to be involved in the prognosis of DTC-positive as well as DTC-negative primary breast carcinomas. Since the results for DTCs were only available in a subgroup of pts, further studies will have to prove these findings.
2015 San Antonio Breast Cancer Symposium

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Title: Presence of tumor-specific cytolytic T cells in human primary breast carcinoma: Consequences for immunotherapy

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Body: Background: Immunotherapy through stimulatory antibodies targeting the CTLA-4 or PD-1 pathways has a clear clinical efficacy in a fraction of patients with various cancers. It is likely that the main immune effectors of these therapies are CD8+ cytolytic T lymphocytes (CTL) recognizing tumor-specific antigens. The antigenicity of human tumors has been demonstrated with studies conducted mostly on melanomas. However the genetic mechanisms leading to antigenicity, notably point mutations in the tumor cells, apply to all cancer types. Thus primary breast carcinoma cells do certainly bear tumor-specific antigens, even though the extent of this antigenicity is unknown. Most melanomas, which are highly antigenic tumors, are also immunogenic, i.e. they stimulate spontaneous anti-tumor CTL responses. This immunogenicity, of which the presence of tumor-infiltrating T cells (TILs) is probably a surrogate marker, might be a predictive marker for clinical benefit to immunostimulatory antibodies. Whether primary breast carcinomas are immunogenic is not known, mainly due the absence of autologous tumor cell lines to analyze patients’ T cells. However even in the absence of T-cell aimed immunotherapy the amounts of TILs have been positively correlated with patients’ survival. Here we wished to obtain evidence for the presence of tumor-specific CD8+ T cells in TILs from primary breast carcinomas.

Methods: From each tumor we isolated TILs and derived a random set of ±100 CD8+ clones maintained in culture by stimulation with anti-CD3 antibodies, thus irrespective of their antigenic specificity. We screened these clones for recognition of tumor-specific antigens present on the autologous tumor. In the absence of autologous tumor lines we restricted our analysis to mutated antigens selected on the basis of tumor exome sequencing and gene expression profiling. Indels and non-synonymous base substitutions were selected to synthesize candidate mutated peptides.

Results: Thus far we have analyzed two hormone receptor-positive HER2-negative primary carcinomas. For one patient we screened 144 T cell clones for recognition of 40 candidate mutated peptides, without any positive result. For the other patient, 6 out of 98 T cell clones recognized 4 out of 119 candidate mutated peptides. Two peptides were recognized by two different T cell clones, i.e. with different T cell receptor sequences. These 4 ‘antigenic’ mutations appear to be passenger, i.e. the four genes have a low published mutation frequency.

Conclusions: We conclude that some human primary breast carcinomas are immunogenic, as one tumor contained at least 6% of tumor-specific T cells among the CD8+ TILs. It suggests that the corresponding patient could benefit from the currently used immunostimulatory antibodies. More work is required to understand the reasons for the negative results in the first patient. We are pursuing the work on 2 HER2-positive and 2 triple-negative tumors, in which TILs are better correlated with prognosis. Our results warrant more investigations on the activation or inhibition of tumor-specific T cells at early stages of human breast cancer development.
Title: CD4 and CD8 T cell densities are increased in benign breast disease compared to normal breast tissue


Body: Introduction: CD4+ and CD8+ T cells are important effector cells in the adaptive immune response against cancers. In premalignant proliferative breast tissues, the role of the immune system remains relatively undefined. In this study, we sought to investigate and quantify the presence of CD4 and CD8 expressing immune cells in benign breast tissues and their association with breast cancer risk.

Method: Archived breast tissue samples from women with benign breast disease (BBD) or no clinical breast disease [Komen Tissue Bank (KTB)] were obtained for this age-matched case-control study. BBD samples included BBD cases (subsequently developed breast cancer) and BBD controls). Tissue sections were characterized for non-proliferative or proliferative disease and degree of lobular involution. CD4+ and CD8+ cell densities (cells/mm$^2$) were obtained by digital image quantitation for up to 10 individual lobules per sample and a per-sample estimate of cell density was calculated as the median cell density across lobules within a sample. Statistical analysis was performed using Wilcoxon signed-rank and Kruskal-Wallis tests.

Results: Among 267 women (median age 52, range 35-75) comprising 89 age-matched triplets, 2417 lobules were evaluated [783 KTB, 804 BBD case, 830 BBD control]. 1372 (57%) lobules were normal and 1045 (43%) were fibrocystic. CD4+ cells were present in 1903 (79%) lobules while CD8+ cells were present in 2214 (92%) lobules. CD4 and CD8 densities showed a moderate positive correlation ($r = 0.39$) with each other, but overall, lobules showed a lower density of CD4+ than CD8+ cells (mean difference -54 cells/mm$^2$, 95% CI: -68 to -39 cells/mm$^2$). At the per sample level (see Table), BBD controls showed significantly higher levels of both CD4+ (p=0.009) and CD8+ cells (p=0.0001) compared to KTB samples. In contrast, the BBD cases only showed elevated CD8 (p=0.007) and not CD4 (p=0.21) cell infiltrates compared to KTB samples. Comparing BBD cases to BBD controls, BBD cases showed significantly lower CD8 cell density as compared to BBD controls (p=0.002) and cases had lower CD4 cell density but the difference was not significant (p=0.07). Despite these differences in the cell densities, the ratio of CD4:CD8+ cells did not differ significantly across groups (p=0.83). The CD4:CD8+ ratio also did not differ significantly by histologic impression (p=0.74), degree of involution (p=0.27), or age (p=0.32).

<table>
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<th>BBD Controls</th>
<th>BBD Cases</th>
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<td>(n=89)</td>
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<td>CD4+ cells/mm$^2$</td>
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<td>CD8+ cells/mm$^2$</td>
<td>85 (49-131)</td>
<td>157 (84-225)</td>
<td>134 (80-168)</td>
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<td>Ratio (CD4:CD8)*</td>
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<td>0.41 (0.15-0.81)</td>
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*Ratio is missing for n=7 samples (3 KTB, 2 BBD control, 2 BBD case) with undefined ratio due to zero CD8+ cells for all lobules in the sample.

Conclusion: Our findings suggest that BBD is associated with increased infiltration of T cells into the breast tissue. Furthermore, these immune responses appear more robust in BBD patients at lower risk of developing breast cancer.
Title: Early-stage breast carcinomas are infiltrated by oligoclonal T cell populations highly enriched relative to the blood


Body: BACKGROUND: The immunogenicity of some human tumors towards T lymphocytes is well established. Recently, encouraging results have been obtained with immunotherapies inhibiting immune checkpoints in cancers such as melanoma, NSCLC and bladder cancer. Fewer studies explored these treatments in breast cancer (BC) as these tumors are often considered to be poorly immunogenic.

METHODS: We analysed the T cell receptor β-chains variable genes (TCRBV) repertoires of tumor-infiltrating T cells in 17 early BC. We looked for clonally amplified T cells as their presence is an expected consequence of tumor immunogenicity. RNA was extracted and reverse-transcribed from formalin-fixed, paraffin-embedded tumor tissues. A short random sequence was added to the cDNA and used as a unique molecular identifier (UMI) for each cDNA molecule. cDNA encoding TCRBV genes was then amplified and sequenced using high throughput sequencing. Usage of UMIs during this procedure strongly improved the accuracy of the analysis by avoiding amplification biases inherent to the construction of the TCRBV library and by allowing an absolute quantification of TCRBV mRNA molecules normalized with the RPP30 housekeeping gene. TCRBV sequences were aligned using IMGT/HighV-QUEST. The Simpson's index was used to evaluate TCRBV repertoires diversity (ranging from 0 = infinite diversity to 1 = no diversity). For 3 patients, the same procedure was applied on blood T cells collected a few days before tumor resection and the analysis was also carried out on 3 normal tissues obtained from breast reduction surgery.

RESULTS: T cell infiltration varied strongly from one tumor to another ranging from 5 to 2498 TCRBV/10^3 RPP30 mRNA molecules. TCRBV repertoires analysis indicated that infiltrated T cells corresponded to oligoclonal populations. We observed 3 clonotypes in the smaller repertoire and 74 in the largest one and the Simpson's index ranged from 0.01 to 0.65. Most tumors (16/17) contained at least one clonotype that made up ≥10% of the infiltrating T cells, with the highest observed proportions reaching 80%. Normal breast samples were infiltrated by a more diverse repertoire: 130 to 368 clonotypes were identified in those tissues and Simpson's index ranged from 0.002 to 0.008. Highest observed frequency among those clonotypes was 2%. For 3 BC patients, the frequencies of the most prevalent clonotypes in the tumor were compared to those of the same clonotypes in blood prior to surgery. These T cell clones were 250 to >34000 times more frequent in the tumor than in the blood.

CONCLUSIONS: Some early BC are infiltrated by oligoclonal T cell populations that are highly enriched relative to the blood. Quantitative T cell repertoire analysis allows to distinguish 3 types of BC: (1) tumors without T cell infiltration, (2) tumors with a high T cell infiltration and a small T cell repertoire, and (3) tumors with a high T cell infiltration and a large repertoire. Our observations suggest that anti-tumor T cell responses are ongoing in some early BC and this warrants boosting such responses with immune checkpoint inhibitors in selected patients. T cell repertoire evaluation could be used as a predictive biomarker to identify patients who will benefit from this treatment.
Preferential expression of the chemokine receptor 8 (CCR8) on regulatory T cells (Treg) infiltrating human breast cancers represents a novel immunotherapeutic target


Treg cells are identified by the expression of the transcription factor FoxP3 and preserve immune homeostasis by the establishment and maintenance of peripheral tolerance. This suppressive function however, limits anti-tumor immune responses and represents a critical obstacle to immunotherapy. Safely targeting Treg cells will require selective elimination of tumor infiltrating Treg cells, as systemic depletion will lead to immune related adverse events. We hypothesize that differential gene expression analysis of Treg cells isolated from human breast tumors and peripheral blood will identify a tumor specific means to target Treg cells for the immunotherapy of breast cancer.

Methods: Tumor infiltrating lymphocytes were isolated from fresh operative specimens of patients undergoing surgery for primary invasive breast carcinoma. Lymphocytes were also isolated from normal breast tissue and peripheral blood buffy coat. T cell subsets including Treg cells were isolated by fluorescent activated cell sorting and analyzed by RNAseq. Mixed bone marrow chimeric mice were generated by reconstituting irradiated immunodeficient mice with a mixture of CCR8^-/^- FoxP3^-/^- or CCR8^+/^ FoxP3^-/^- bone marrow, thus creating mice with Treg cells lacking CCR8 and controls.

Results: We found that Treg cells are more prevalent in breast tumors as opposed to normal breast tissue regardless of the biologic subtype of breast cancer (p<0.05). Gene expression profiling of Treg cells and CD4 T cells isolated from tumor or blood revealed a distinct tumor Treg cell gene signature. This signature was enriched for cytokine binding and chemokine receptor GO categories (FDR<0.005). Specifically, we identified CCR8 to be differentially and robustly expressed on tumor infiltrating Treg cells. This was validated by flow cytometry on over 50 primary breast cancers where the mean florescence intensity of CCR8 on Treg cells was at least twice that observed on conventional CD4 T cells (p<0.05). CCR8 expression on Treg cells also significantly correlated with higher-grade cancers (p<0.05). Using a data set generated by the Cancer Genome Atlas, we found that a high CCR8/FOXP3 gene expression ratio is strongly associated with worse disease free and overall survival of breast cancer (p<0.001) patients while FOXP3 gene expression level alone does not predict disease outcome. To investigate the role of CCR8 expression on Treg cells in a preclinical murine model of mammary carcinogenesis, we implanted syngeneic polyoma middle-T antigen-driven breast cancer cells in the mammary fat pads of mixed bone marrow chimeric mice in which Treg cells lack CCR8 expression. CCR8 deficiency in Treg cells significantly decreased primary tumor progression and distant metastases without any overt immunopathology (p<0.05).

Conclusions: Treg cells infiltrate human breast cancers and suppress anti-tumor immune responses. Our results demonstrate that CCR8 is selectively expressed by human breast cancer infiltrating Treg cells. Targeting CCR8 represents a promising immunotherapeutic approach for the treatment of patients with breast cancer. Depleting CCR8 antibodies are currently in development for additional preclinical and human studies.
Title: Enhancing the immunogenicity of breast cancer cells to stimulate innate immunity and augment the effects of cellular immunotherapy


Body: A promising strategy for treating metastatic breast cancer is engaging the immune system to destroy disseminated breast cancer cells. However, breast cancer is a challenge for immunotherapy because of its inherent genetic heterogeneity and decreased immunogenicity. There is an unmet need to develop new, targeted therapies for breast cancer that stimulate the immune system and can be used in combination with checkpoint blockade or adoptive cell transfer to fight metastatic disease. One potential approach is to generate dying breast cancer cells that operate like a vaccine to stimulate a tumor-specific immune response. This is termed immunogenic cell death (ICD) and is characterized by a unique molecular signature, involving the release of molecules that attract and stimulate phagocytes like dendritic cells (DCs) and that could sensitize tumor cells to killing by natural killer (NK) cells. With the discovery of new ICD-inducing agents, interest in this approach is increasing. However, the current doses of drugs used to induce ICD may be too high to translate into clinically relevant regimens. To address these problems, our group developed a novel cytotoxic peptide, CT20p, and a nanotechnology-based platform to deliver and concentrate CT20p in breast tumors. We found that treatment of a murine xenograft model with nanomolar amounts of CT20p, encapsulated in nanoparticles formed with a novel hyperbranched polyester polymer (HBPE-NPs), resulted in regression of breast cancer tumors, and that dying breast cancer cells expressed markers characteristic of ICD, such as the pre-mortem exposure of the "eat me" signal, calreticulin (Crt). The intracellular target of CT20p is a protein called chaperonin-containing T-complex (CCT), which is essential for the folding of actin and tubulin and other critical proteins into their native forms. Disruption of CCT could cause endoplasmic reticulum (ER) stress through the accumulation of misfolded proteins. ER stress in turn initiates intracellular pathways that generate the danger signals associate with ICD. To this end, we observed that cancer cells treated with CT20p displayed alterations in PERK, a key mediator of the unfolded protein response (UPR) and translocation of Crt. As a result, CT20p-treated cancer cells were more readily phagocytosed. More importantly, NK cells more effectively killed cancer cells pre-treated with CT20p. This suggests that CT20p may stimulate NK cell cytotoxicity by inducing activating signals on cancer cells, which would greatly augment the curative effects of adoptive transfer of NK cells. In our experiments normal breast epithelial cells, macrophages or NK cells, themselves, were unaffected by CT20p treatment due reduced CCT. These studies indicate that a single molecule, CT20p, can do both the killing of cancer cells and activation of immune cells and can be combined with cellular immunotherapy (e.g. expanded NK cells) to provide a more complete protection from breast cancer recurrence and metastasis.
Title: Comparison of tumor and stroma CD73 expression with TLR9 and survival in breast cancer

Selander K, Mella M, Kauppila J, Karihtala P, Jukkola-Vuorinen A, Auvinen P, Soini Y, Kauppila S, Haapasaari K-M, Harris K and Vuopala K. Oulu University Hospital, Oulu, Finland; Kuopio University Hospital, Kuopio, Finland; University of Alabama at Birmingham, Birmingham, AL and Lapland Central Hospital, Rovaniemi, Finland.

Body: CD73 is a 5' ectonuclease that catalyzes the conversion of cyclic AMP into the highly immunosuppressive adenosine in extracellular space. In addition to the cells of the immune system, CD73 is highly expressed in various cancer cell lines and clinical cancer tissues. Toll like receptor-9 (TLR9) is a cellular DNA-receptor that is highly expressed in breast cancer. Both CD73 and TLR9 expression have recently been associated with TNBC prognosis but the mechanisms how these proteins possibly contribute to TNBC pathophysiology remains poorly understood. TLR9 and CD73 expression has been shown to be mutually regulated in various cell types. Whether this is the case in cancer is unknown. The aim of this study was to investigate the mutual role of TLR9 and CD73 in breast cancer (BC). Specifically our hypothesis was that TLR9 and CD73 expression correlate in TNBC. We compared immunohistological tumor TLR9 and CD73 expression scores using a previously characterized breast cancer (BC) cohort (n=184) with follow-up time of > 10 years. We did not discover a connection between TLR9 and CD73 expression in tumor cells in BC. There was a trend for increased survival among patients that had high tumor cell CD73 expression, as compared with the lower tumor cell CD73 expression groups. There was a trend for a better survival among TNBC patients that had lower stromal CD73 expression, as compared with those TNBC patients that had higher stromal CD73 expression. No such difference was detected among patients with non-TNBC tumors. Our results suggest that stromal vs. tumor cell CD73 expression have opposite effects on survival in TNBC, but there is no connection between CD73 and TLR9 expression. Our conclusions are limited by low sample numbers.
Prediction of treatment responses to neoadjuvant chemotherapy in triple-negative breast cancer by analysis of immune checkpoint protein expression

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Purpose: "Avoiding immune destruction" has recently been established as one of the hallmarks of cancer. The programmed cell death (PD)-1/programmed cell death-ligand (PD-L) 1 pathway is also an important immunosuppression mechanism that allows cancer cells to escape host immunity. The effect of the tumor immune environment not only on immunotherapy effectiveness, but also on conventional anti-tumor therapy effectiveness and prognosis, has recently been demonstrated. Thus, improvement of the tumor immune environment is important. In other words, the tumor immune environment plays a role in the anti-tumor effects of conventional anti-tumor drugs. Moreover, immune checkpoint proteins such as PD-1, PD-L1, and PD-L2 may play an important role in improving the tumor immune environment. Given this background, the clinical significance of immune checkpoint protein expression was investigated in patients receiving neoadjuvant chemotherapy (NAC) for breast cancer using conventional anti-cancer drugs, and whether this would be useful as a marker to predict treatment response was evaluated.

Experimental Design: A total of 177 patients with resectable early-stage breast cancer were treated with NAC. All patients received a standardized protocol of NAC consisting of four courses of FEC100 (500 mg/m2 fluorouracil, 100 mg/m2 epirubicin, and 500 mg/m2 cyclophosphamide) every 3 weeks, followed by 12 courses of 80 mg/m2 paclitaxel administered weekly. Forty-five patients had HER2-positive breast cancer and were additionally administered weekly (2 mg/kg) or tri-weekly (6 mg/kg) trastuzumab during paclitaxel treatment. ER, PR, HER2, Ki67, PD-L1, PDL-2 and PD-1 status were assessed by immunohistochemistry on core needle biopsy specimens.

Results: There were 37 (20.9%) patients with high PD-1 expression, 42 (23.7%) patients had high PD-L1 expression, and 52 (29.4%) patients had high PD-L2 expression. The patients with high PD-1 and PD-L1 expressions had a significantly higher rate of triple-negative breast cancer (TNBC) (p=0.041) (p<0.001). Univariate analysis showed that PD-1 and PD-L1 expressions were associated with significantly shorter DFS (p=0.008, HR=2.752) (p=0.002, HR=3.194). However, although multivariate analysis showed that lymph node metastases were an independent poor prognostic factor (p=0.046, HR=4.330), PD-1 and PD-L1 expressions were not independent prognostic factors (p=0.492, HR=1.415) (p=0.084, HR=2.613). In TNBC, patients with high PD-1 and PD-L1 expressions had significantly higher rates of non-pCR (p=0.049, HR=1.415) (p=0.084, HR=2.613). In TNBC, patients with high PD-1 and PD-L1 expressions had significantly higher rates of non-pCR (p=0.003) (p<0.001). Univariate analysis showed that PD-1 and PD-L1 expressions also significantly shortened disease free survival in TNBC (p=0.048, HR=3.318) (p=0.007, HR=8.375). However, multivariate analysis found that only PD-L1 expression was an independent prognostic factor (p=0.041, HR=9.479).

Conclusions: PD-1 and PD-L1 expressions may be useful as biomarkers to predict treatment responses to NAC in breast cancer. Above all, PD-L1 expression may also be useful as biomarkers for more effective chemotherapy in TNBC.
Title: Porous silicon microparticle-potentiated dendritic cell vaccine for HER2 positive breast cancer

Shen H, Xia X and Ferrari M. Houston Methodist Research Institute, Houston, TX.

Body: Harnessing the patient's own immune system to eradicate malignant cells is a promising approach for cancer treatment, and therapeutic vaccine represents an important arm of cancer immunotherapy. However, there has not been significant progress in recent years on vaccine development for breast cancer. Besides the notion that breast cancers are generally considered as poorly immunogenic, lack of proper adjuvants to potently and sustainably stimulate dendritic cells in the suppressive tumor microenvironment has severely limited our ability to develop effective therapeutic vaccines for this deadly disease.

We have recently shown that porous silicon microparticles (PSMs) serve as a potent adjuvant for cancer vaccine (Cell Reports 2015). PSMs can stimulate type I interferon expression in dendritic cells (DCs). This action is mediated by the TRIF/MAVS pathways, and is independent of the cell surface or endosomal Toll-like receptors. In addition, the nanometer size pores in PSMs can be loaded with tumor antigen peptides, and package of antigen in PSM not only maintains sustained antigen release, but also alters the route(s) of antigen processing inside the DCs. Furthermore, treatment of mouse models of primary HER2 positive breast cancer with a DC vaccine comprising of bone marrow-derived DCs internalized with HER2 antigen peptide-loaded PSMs not only activates and expands antigen-specific CD8+ T cells, but also promotes a Th2-to-Th1 transition in the tumor microenvironment to favor anti-tumor activity. Thus, our result supports the development of PSM-potentiated DC vaccines for breast cancer.

Reference:
Title: Characterization of an autologous tumor cell vaccine against breast cancer in mice

Ravindranathan S, Kurtz SL L, Smith SG G, Koppolu B, Nguyen KG G and Zaharoff DA A. University of Arkansas, Fayetteville, AR and Tulane University, New Orleans, LA.

Body: The vast majority of breast cancer-related deaths are due to progressive recurrences of non-metastatic disease. Current adjuvant therapies aimed at preventing breast cancer recurrence are ineffective for tens of thousands of women each year. In addition, current adjuvant therapies carry significant co-morbidities. Active, specific immune-based strategies have the potential to train a patient’s immune system to recognize and eliminate occult metastases. Here, we explore autologous tumor cell vaccines (ATCVs) as a strategy to prevent tumor recurrence and improve survival. Because >90% of breast cancer patients undergo resection, autologous tumor cells are readily available for the production of personalized vaccine. Patient-specific vaccination is particularly attractive for breast cancer which is highly heterogeneous. The major disadvantage of ATCVs, is poor immunogenicity.

Thus, in order to develop an effective, immunogenic ATCV against breast cancer, we wanted to understand the features of immunogenicity. In this study, we evaluated the immunogenicities of two murine breast cancer cell lines, EMT6 and 4T1. For tumor protection studies, BALB/c female mice were given priming and booster vaccinations, ten days apart, with 1,000,000 irradiated (100Gy) cells. Ten days after the booster vaccination, mice were challenged with live tumor cells. Mice vaccinated with EMT6 cells were completely protected against a live EMT6 challenge in 80% of mice. However, mice vaccinated with irradiated 4T1 cells failed to provide any protection against a live 4T1 challenge. Most interestingly, when mice were vaccinated with a mixture of irradiated EMT6 and 4T1 cells, the protective response against EMT6 challenge was significantly diminished as 60% of mice developed tumors. This finding implied that non-immunogenic 4T1 cells released one or more immunosuppressive factors that inhibited anti-EMT6 immunity. Thus, we investigated the levels of different immunosuppressive cytokines, GM-CSF, IL-6, MCP-1, TGF-β and VEGF released by both 4T1 and EMT6 cells before and after irradiation.

Of the different cytokines measured, we found that only GM-CSF is produced at significantly higher levels by 4T1 cells than EMT6 cells. Since at higher levels (>200pg/ml) GM-CSF can induce the accumulation of large amounts of myeloid derived suppressor cells (MDSCs) in the tumor site and lymphoid organs, we believe it to be the reason for the failure of the hybrid vaccine. Future studies will determine if blocking GM-CSF production by 4T1 cells will decrease the accumulation of MDSCs and subsequently increase anti-tumor immunity.
**Title:** Evaluation of a prognostic immune gene expression signature in different breast cancer molecular subtypes

Sheen MA A, Connor CG G, Berglund AE E, Prabhakaran S, Rizk VT T and Soliman HH H. H. Lee Moffitt Cancer & Research Institute, Tampa, FL and USF Morsani College of Medicine, Tampa, FL.

**Body:**

**Background:** Cancer immunotherapy is increasingly important in many malignancies. Breast cancers with greater numbers of tumor-infiltrating lymphocytes (TILs) exhibit better responses to neoadjuvant therapy. Additionally, increased CD8+ TILs in the breast tumor microenvironment have better outcomes. The Total Cancer Care (TCC) biorepository at Moffitt Cancer Center allows for the collection of all clinical patient data and tissue for analysis of novel biomarkers with prognostic and predictive abilities. Using TCC consented patients, we sought to determine if an immune based signature score derived from gene expression profile data would have a significant prognostic ability, and determine if this signature is differentially expressed within different PAM50 molecular subtypes.

**Methods:** Tumors were arrayed on Affymetrix HuRSTA-2a520709 GeneChips (Affymetrix, Santa Clara, CA). The chips contain ~60,000 probe sets representing ~25,037 unique genes (Affymetrix HuRSTA-2a520709). Gene expression data was normalized using iterative rank-order normalization and de-batched using Principal Component Analysis (PCA). The immune score for each tumor was derived using the first component from a PCA model based on all immune related genes in breast primary tumor samples. Mean immune score between molecular subtypes was compared using one way ANOVA.

**Results:** A total sample of 310 non-metastatic patients with full clinical and gene expression data were available for the analysis. Mean age was 55. Staging breakdown was Stage I: 26%, stage II: 46%, and stage III: 17%. For tumor grades, 10% were well differentiated, 45% poorly or undifferentiated, 41% moderately differentiated, and 3% missing. Mean follow up for overall survival (OS) was 6.84 years and 6.10 years for recurrence free survival (RFS). For IHC subtypes 62% were hormone receptor positive, 18% triple negative, and 20% HER2 positive. The mean immune score across the whole population was 0.05 (range -2.22 to 2.27). Mean scores were higher in PAM50 basal (.379, 95% CI .143 to .615) and HER2 enriched (.316, 95% CI .143 to .6155) vs. luminal A (-.215, 95% CI -.34 to -.09)/B (-.168, 95% CI -.332 to -.004) subtypes. Immune score was not prognostic for OS hazard ratio (HR) 1.11 (95% CI: 0.91-1.36) p=.30, but was for RFS HR=0.71 (95% CI: 0.53-0.96) p=.02 in a multivariate analysis controlling for age, race, stage, grade, adjuvant treatment, and molecular subtype.

**Conclusions:** The mean immune score is higher in basal and HER2 enriched tumors, suggesting we can potentially identify immune infiltrate enriched patients for immunotherapy trials using existing gene expression profiling data routinely obtained on breast cancer primary tumors. Correlation between score and lymphocyte infiltrate on histology is ongoing and will be presented. A higher score also appears to be an independent predictor of RFS which will need to be confirmed in additional independent well annotated datasets.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-04-18

Title: High serum miR-19a levels correlated with favorable prognosis in patients with metastatic HER2+ breast cancer and might result from effective antibody-dependent cell-mediated cytotoxicity (ADCC) induced by trastuzumab and Th1-mediated antitumor immune response

Anfossi S, Huo L, Woodward WA A, Ueno NT T, Valero V, Calin GA A and Reuben JM M. The University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Introduction
HER2 amplification is found in up to 20% of breast cancer and is associated with poor survival. To date, no predictive biomarker has been validated for clinical practice in patients with HER2+ breast cancer. Type 1 T helper lymphocytes (Th1) are required to activate antitumor cytotoxic T lymphocytes (CTL) necessary for tumor clearance. Particularly, IFN-γ secreted by Th1 cells is necessary for the development of CTL-mediated antitumor response. Trastuzumab can induce antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells. In turn, trastuzumab-activated NK cells can trigger Th1 cells by driving their differentiation and maturation. Furthermore, NK-mediated ADCC can increase tumor cell membrane permeability through pore formation induced by perforin secreted by NK cells.

We found that: 1) high serum miR-19a levels in patients with metastatic HER2+ breast cancer were predictive of favorable prognosis; 2) Th1 cells expressed and secreted high miR-19a levels; 3) and breast cancer tissue expressed higher miR-19a levels than normal adjacent tissue. Therefore, the high serum miR-19a levels in patients with good prognosis may derive from tumor cells killed by NK-mediated ADCC and secretion from Th1 cells. MiR-19a may represent a marker of effective anti-tumor immune response.

Results
HER2+ metastatic breast cancer patients with high serum miR-19a levels (n=27) had longer PFS (7.9 vs. 4.1 months; p=0.003) and OS (median not reached vs. 13.1 months; p<0.0001) than patients with low serum miR-19a levels (n=24). Locally advanced breast cancer tissue (n=35) expressed higher levels of miR-19a than normal adjacent tissue (n=10) (p=0.048). KPL-4 cells (HER2-amplified) expressed higher miR-19a levels than SKBR3 cells (HER2-amplified) (p=0.010) and MCF-7 cells (non-HER2-amplified, used as control) (p<0.0001). In in vitro ADCC assay, trastuzumab induced an increased tumor cell killing by NK cells and consequent miR-19a release into the supernatants of MCF-7 (p=0.004; p=0.0005), SKBR3 (p=0.001; p<0.0001) and KPL-4 (p=0.0005; p<0.0001), respectively. Th1 cells expressed (p<0.0001) and secreted (p=0.0002) higher miR-19a levels than Th2 cells (Th1 antagonist). Patients with favorable prognosis had higher levels of IFN-γ-secreting Th1 cells (p=0.049) in the peripheral blood than patients with worse prognosis.

Conclusion
High serum miR-19a levels in patients with metastatic HER2+ breast cancer may result from an increased ability of trastuzumab to induce an effective NK-mediated ADCC and activation of Th1-mediated immune response. This may explain the different clinical outcome between patients with high and low serum miR-19a levels. Our results suggest that miR-19a may potentially represent a novel serum biomarker to evaluate trastuzumab response and Th1-mediated anti-tumor immunity in patients with metastatic HER2+ breast cancer.
Title: Primary breast cancer culture in a perfusion-based bioreactor suitable for in vitro testing of immune blockade therapy

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Body: Introduction: Interaction between cancer cells and immune system critically affects development, progression and treatment of human malignancies. Two-dimensional (2D) in vitro culture systems and in vivo animal models are the primary tools used to test cancer cell response to drugs but they are not suited for the development of immune-mediated therapies. Here we present an innovative method to culture breast cancer tissue in porous 3D scaffolds by using a perfusion-based bioreactor system that allows the maintenance and expansion of tumor microenvironment.

Experimental procedures: Freshly excised breast cancer specimens were fragmented and cultured in a 3D "sandwich-like format" between two layers of porous collagen scaffold under perfusion flow (U-CUP). DMEM/F12, supplemented with 10% autologous human serum, was used as a culture medium. We assessed the ability of tumor and non-malignant cells to survive and expand into the scaffold in perfusion culture, as well as their capacity to recapitulate features of the original breast cancer tissue. The maintenance of immune-infiltrating cells allowed testing of immune blockade therapy in vitro using anti-PD-L1 and anti-CTLA4 antibodies alone or in combination.

Results: The U-CUP culture system preserved tissue viability better compared to a static culture and promoted the expansion of breast cancer cells from surgical specimens together with accompanying stromal and immune cells into the porous scaffold. Tumor tissues were viable after 21 days and mostly recapitulating the initial histology with formation of glands. Administration of anti-PDL1 antibody, alone or in combination with anti-CTLA4, to the culture medium was associated with increased expression of markers of immune-activation (i.e. IFNg) and decreased expression of immunosuppressive cytokine IL10.

Conclusions: Our results show that culture of breast cancer tissue in a 3D perfusion-based bioreactor might represent a promising system for the pre-clinical evaluation of immune-mediated therapies. Preserving malignant, interstitial and immunocompetent cells comprised in surgically excised breast cancer samples might allow a direct evaluation of the effects of various treatments on the complex tumor microenvironment. This engineered in vitro model could be extended as a platform allowing the testing of innovative approaches for the treatment of human malignancies, possibly in the direction of personalized medicine.
Title: Subtype specific differential expression and immunogenicity of endogenous retrovirus elements in breast cancer

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Body: Background: Endogenous retroviruses (ERVs) are germline encoded DNA sequences that entered the human genome millions of years ago. While they are mostly inactivated due to accumulated termination codons and deletions, previous studies have demonstrated overexpression and antibody-targeted immunotherapeutic potential, of ERV-related env proteins in breast cancer. We sought to elucidate subtype specificity, immunogenicity, and correlation with innate immunity related gene signatures of ERVs in breast cancer.

Methods: We utilized publically available RNASeq gene expression data of breast cancer samples along and corresponding matched normals from TCGA. The dataset included 191 ER-/HER2- (TN), 197 HER2+, and 627 ER+/HER2- (Luminal) breast cancers. ERV expression was obtained by mapping bowtie2-aligned reads of recently annotated to be transcriptionally active to the RNAseq bam files (Rooney et al 2015, Mayer et al 2011). ERVs preferentially expressed in tumors compared normal tissue were identified as those for which the 5th percentile of ERV expression in the tumors exceeded the 95th percentile of ERV expression in the normal samples. A gene signature involving GZMB, PRF1, CXCL13, IRF1, IKZF1, and HLA-E was used as a measure of immune activity. To assess the immunogenic potential of the tumor-specific ERVs, we compared the expression level of the ERV within the lower and higher immune signature tertiles using the Wilcoxon signed-rank test. To elucidate mechanism of potential immune response, ERVs found to be significantly associated with immune response at a false discovery rate of < 10% were further analyzed for association with specific toll-like receptor (TLR) gene expression.

Results: Out of the 66 original annotated ERVs, 47 were found to be expressed at significantly higher levels in breast cancer compared to normal tissue and 22 were immunogenic. Examples include members of the ERV-K family, as have also been previously detected by flow cytometry and IHC. Subtype-specific immunogenic potential was demonstrated in 4 ERVs in TNBC (ERVK10, ERVK17, ERVFRD.1, ERVPABL.B.1) and in 7 ERVs in the luminal subtype (ERV3.1, ERVE.4, ERVFRD.2, ERVK.15, ERVK.19, ERVK.20, ERVK.25, ERVW.3). Twelve of the 22 immunogenic ERVs were significantly correlated with expression of all ten TLR evaluated, while four ERVs showed more specific correlation patterns with TLRs. High ERV3.1 expression was associated with high TLR3, TLR8, TLR9 that specifically target double stranded or single stranded RNA, suggesting a potential mechanism for mediation of ERV related immune response.

Conclusion: Our results suggest breast cancer subtype specific ERV dysregulation and immunogenicity. The potentially immunogenic ERVs were generally not self-correlated or located in the same amplicon as HLA genes, suggesting an independent immune response pathway. Furthermore, ERV expression correlates with specific endosomal nucleic-acid recognizing toll-like receptors, which may prompt further investigation into subtype-specific TLR-targeted therapy.
Two different ways of antitumor immune response in Russian and Dutch women with breast cancer stage I

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Objective: To study the differences in immune markers expression which participate in the anti-tumor immunity in two independent women populations (Russian and Dutch) with breast cancer stage I.

Materials and Methods: The clinical, morphological and survival data of 518 Russian and Dutch women with breast cancer stage I were analyzed. Russian women treated in RCRC and Clinic of RMAPE (n = 315); Dutch women treated in LUMC (n = 203) from 1985 to 2010. Paraffin blocks of tumor were investigated in the Research Lab, LUMC; immune markers expression (HC10, HCA2, HLA-E, HLA-G, Foxp3) was studied in TMA-slides by standard immunohistochemical analyzes. Was estimated population differences between immune markers expression; statistical analysis was made by SPSS 20.0.

Results: Was found significantly differences between Russian and Dutch women in immune characteristics of tumors (p<0.05). In Russian cohort were seen highly expression of the classical molecule HLA class I (HC10 in 91%, HCA2 in 48%; both expression-in 48% cases). But, was found high expression of non-classical markers HLA class I too (HLA-E in 99%, HLA-G in 26%) and strong tumor infiltration of Foxp3+ cells (in 73% tumors), which could suppress of anti-tumor response in Russian cohort. In contrast to it, tumors of the Dutch women presented of classical molecule HLA I class lower (HC10 in 74%, HCA2 in 41%, co-expression were seen in 38%), and express of non-classical markers HLA class I significantly lower too (HLAE expression was found in 48%); Foxp3+ cells were seen only in 43% Dutch tumors, p<0.05. So, in Russian cohort breast cancer tumor presented classical HLA I class molecules (HC10 and HCA2), which are necessary for activation of CD8+ killer cells, but high expression of non-classical molecule HLA-E and Foxp3+ cell tumor infiltration could cause a decrease in anti-tumor activity of NK-cells and CD8+ killer cells too. In contrast of it, in the Dutch cohort tumors presented lower expression of HLA class I markers (HC10 and HCA2), but had lower expression of non-classical molecules HLA-E and lower Foxp3+ cell tumor infiltration, which may indicate a high activity of NK-cells and CD8+ killer cells too. Those data may explain the high rates of cancer-specific survival in Dutch women with breast cancer stage I without adjuvant systemic therapy (5-years 97.7%; 10-years- 88.1%) and worse rate of cancer-specific survival in Russian women without adjuvant systemic treatment (5-years -90.5%; 10-years- 64.5%).

Conclusion: immune markers breast cancer expression has population and prognostic value in breast cancer stage I.
Title: Upregulation of the interleukin 17/granulocyte-colony stimulating factor/fibroblast growth factor axis in breast cancer and its negative association with treatment outcome

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Body: Background

Interleukin (IL) 17 is secreted by T helper 17 cells and tumor cells. It may induce the proliferation of mesenchymal stem cells (MSCs) in the tumor microenvironment directly and/or via fibroblast growth factor (FGF) and granulocyte-colony stimulating factor (G-CSF) production by tumor-associated macrophages. MSCs play various roles in tumor development and progression, by mediating tumor cell migration, promoting angiogenesis, regulating immune responses, and inducing anti-cancer drug resistance. Therefore, in this study, we examined the association of the IL17/FGF/G-CSF axis with both breast cancer development and response to chemotherapy.

Methods

Eighty-nine breast cancer patients (34% with luminal, 45% with HER2, and 21% with triple-negative breast cancer) and 55 healthy volunteers were analyzed. Serum IL17, basic FGF, and G-CSF levels were measured using a Luminex system. The primary objective was to evaluate the difference in IL17 serum levels between breast cancer patients and healthy volunteers. Secondary objectives were to determine the correlation of IL17 levels with the basic FGF and G-CSF levels, pathological complete response (pCR) (if neoadjuvant therapy was administered), and disease-free survival (DFS) in breast cancer patients. Statistical analyses were performed using the Mann-Whitney, chi-squared, and log-rank tests.

Results

Serum levels of IL17 (median, 91.9 vs. 40.3 pg/ml, p<0.0001), basic FGF (median, 63.9 vs. 3.15 pg/ml, p<0.0001), and G-CSF (median, 61.0 vs. 29.4 pg/ml, p<0.0001) were significantly higher in breast cancer patients than in healthy volunteers. IL17 levels were strongly correlated with basic FGF (r=0.86, p<0.0001) and G-CSF (r=0.73, p<0.0001) levels. Among 64 patients who received neoadjuvant therapy, those with low IL17 levels (<91.9 pg/ml) achieved a significantly higher pCR rate than those with high IL17 levels (≥91.9 pg/ml) (56.0% vs. 25.6%, p=0.014). In addition, the DFS at a median follow-up of 35.7 months was significantly higher in patients with low IL17 levels (p=0.0190).

Conclusion

The IL17/FGF/G-CSF axis was upregulated in breast cancer patients and the levels of these markers were inversely correlated with pCR and prognosis. These results may provide novel insight into the role of IL17 in tumor development and have important implications in the development of IL17 as a target in the treatment of breast tumors.
Title: Clinical importance of S100A9 and S100A2 in breast cancer

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Body: Background: The most frequent cause of cancer deaths throughout the world is breast cancer (BC). Therefore, preventing, diagnosing and treating BC has gained importance. Calcium-binding S100 group proteins are coded in the chromosome 1q21 and have many members. It is claimed that S100A12 and S100A9 play an important role in the development of cancer. S100A12 is normally expressed in neutrophil granulocytes, and from lymphocytes and monocytes in small amounts. S100A12 is closely related to inflammation and vascular invasion by tumor cells, and causes excessive inflammation and vascular invasion causes tumor recurrence and metastasis. S100A9 protein is found in human epithelial cells and cytosols in phagocytes and regulates the motility of phagocytes and activity of target proteins through interaction with danger signal molecules. Furthermore, it has also been stated that it is possible that it regulates the cellular growth through interaction with p53 like other S100 proteins and can modulate metastasis of malignant tumors. In this study, we aimed at diagnostic and clinic-pathological importance of serum levels of S100A9 and S100A12 with known cytokin-like pro-inflammatory effects in BC.

Material: A total number of 45 patients with breast cancer were first admitted in the study. Sixteen age-matched healthy women were enrolled in to this study. All the samples were analyzed with enzyme-linked immunosorbent assay for serum S100A9 and S100A12 levels before starting the systemic chemotherapy. The characteristics of the breast cancer patients with respect to age, weight, height, menopausal status, histopathological type, tumor size, tumor lymph node metastasis, tumor grade, and estrogen receptor (ER) and progesterone receptor (PR) and HER2, lymphovascular invasion (LVI), perineural invasion (PNI) status and stage were collected for data analyses. Clinicopathologic characteristics of BC and other blood parameters were compared in relation with serum S100A9 and S100A12 levels.

Results: While the serum S100A9 levels were found significantly higher as compared to healthy individuals (190.85±32.29 and 92.72±54, respectively) (p=0.001), it was observed that there were no differences in S100A12 (120.50±15.78 and 112.21±10.46, respectively) (p=0.056) levels. As regards the subgroup analysis in BC patients, no statistically significant results were found in body mass index, smoking, menopause status, histopathological type, grade, biological subtype of BC, tumor size, presence of lymph node metastases, LVI, PNI and stage. As regards the blood parameters and serum S100 A9, while only statistically significant results were found with anemia (209.05±33.12 and 181.75±28.21, respectively) (p=0.005), no statistically significant results were found with leukocytosis, thrombocytosis and tumor markers.

Conclusion: In this study, while we found the level of S100A9, which has a potential cytokine-like function in inflammation, significantly higher, we could not find any increase in S100A12 level. Therefore, it is possible that S100A9 can play a key role in inflammation-related BC. We therefore suggest that serum S100A9 can be the target marker in breast cancer both as a diagnostic tool and to suppress inflammation in treatment.
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Title: Network analysis of BRCA1-related functional associations in sporadic triple negative breast cancer

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Body: Triple negative breast cancers (TNBC)—so named since they do not express estrogen and progesterone receptors and lack HER-2/neu amplification—account for about 15% of all sporadic breast cancer cases. TNBC are mostly aggressive malignancies that present a clinical challenge. Their refractoriness to endocrine and HER-2 targeting therapy leaves chemotherapy as the mainstay of treatment. Deeper molecular understanding of TNBC would aid development of tailored therapeutic modalities and improve the poor outcome associated with this breast cancer subtype.

Most breast cancers arising in individuals who carry a germline BRCA1 mutation are also triple negative and share morphological, immunohistological, transcriptional, and clinical similarities and analogous genomic aberrations with sporadic TNBC. This phenotypic resemblance, along with the rare occurrence of BRCA1 mutations in sporadic breast cancer, including TNBC, sparked the speculation that derangement in BRCA1-related pathways may underlie sporadic TNBC (or at least a subset thereof) and provided the rationale for therapeutic exploitation of a presumed DNA repair defect, with so far inconclusive results.

In addition to its established role in the DNA damage response, BRCA1 has been implicated in many other cellular processes via interactions with diverse partner proteins. We therefore sought to devise a global analytical strategy to search for TNBC-specific perturbations, which would take into account the versatility of the BRCA1 protein and allow scrutiny of all known BRCA1-related processes. To this end, we combined database mining, literature curation, network-modeling, and transcriptome analysis. We first queried publicly available databases and the scientific literature to collect data on protein/protein interactions, co-complex memberships, and biochemical modifications involving BRCA1. We expanded the resulting list of known BRCA1 partners (interaction network) by adding high-confidence predicted functional links and generated a highly-connected BRCA1-centered network containing 1137 nodes (and 2941 edges) directly or indirectly associated with BRCA1. We next retrieved and curated 14 breast cancer datasets collecting, in total, 2022 sporadic primary breast cancer expression profiles. After uniform subtype discrimination, we computed meta-analytically the differential (TNBC vs. non-TNBC) expression of the network components across our compendium of transcriptional profiles. Finally, we identified TNBC-specific subnetworks of significantly hyperactive or suppressed BRCA1-functionally associated genes.

We present the workflow for this strategy and the identified presumptive TNBC signatures of BRCA1 pathway deregulation, which will serve as a framework for future analyses. Our next goal is to determine whether the TNBC-specific subnetworks can help to stratify patients based on 'BRCAness' and/or predict response to therapy. We also plan to use them as hypothesis-generating platforms to prioritize aberrant BRCA1-associated genes/proteins and related pathways for in-depth analyses and to pinpoint therapeutically exploitable vulnerabilities. These studies should help inform therapy selection, provide a rationale for innovative treatment, and, thereby, guide clinical trial design.
Inflammatory breast cancer cells demonstrate both canonical and non-canonical TGFβ signalling, affecting cancer cell motility


Body: Introduction. Inflammatory breast cancer (IBC) is an aggressive form of breast cancer with an elevated metastatic potential. Recent evidence suggest that TGFβ signalling may be an important driver of the disease. Here, we describe results from preclinical models and patient samples that corroborate this hypothesis.

Materials and Methods. The Xcelligence system was used to profile a series of 3 IBC and 3 subtype-matched nIBC cell lines for the cell motility inducing capacity of a panel of chemokines: TGFα, TGFβ, EGF, FGF, HGF, IGF, PDGF, CCL2, CCL5, CCL12 and CXCL21. Significant results were confirmed using classical wound healing assays (WHA). A series of 79 IBC and 133 nIBC patient samples was evaluated for nuclear SMAD2, -3 and -4 protein expression using immunohistochemistry (IHC). In a subseries of 14 IBC and 21 nIBC patient samples, protein expression and Affymetrix gene expression data were integrated and Expression2Kinases was used to identify key components of TGFβ signalling in IBC.

Results. Whereas TGFβ induced cell motility in all nIBC cells, we noted a 18-fold reduction of cell motility in IBC cells. Classical WHA showed a near complete wound closure (90% reduction of the wound area) after 24hrs of TGFβ treatment in nIBC cells. Under similar conditions in IBC cells, the reduction of the wound area was less than 10%. IHC on patient samples revealed increased nuclear SMAD2 protein expression in combination with attenuated nuclear SMAD3 protein expression in IBC, independent of classical clinicopathological variables. Integration of protein and gene expression data demonstrated that nuclear SMAD2 expression in IBC is mediated through the canonical TGFβ signalling pathway, whereas the absence of nuclear SMAD3 expression is due to impaired non-canonical, p38MAPK/ATF2-dependent TGFβ signalling. Of note, ATF2 expression is specifically attenuated in IBC.

Discussion. This study provides the basis for continued research into the role of TGFβ in IBC. We show that, unlike nIBC cells, IBC cells do not increase cell motility in response to TGFβ stimulation. This observation can be explained by impaired non-canonical p38MAPK/ATF2-dependent TGFβ signalling in IBC, which is essential for SMAD3-driven epithelial to mesenchymal transition (EMT). SMAD2 on the other hand is a proven driver of EMT-independent modes of cell motility. Our results strengthen the vision that EMT is not required for IBC cell invasion.
Title: Human mammary tumor virus, HMTV, is an MMTV-adapted human pathogen


Body: Forty percent of American women's breast cancers contain HMTV, a betaretrovirus 90-98% homologous to MMTV, the causative agent for breast cancer in mice. HMTV is reverse-transcribed and integrated into chromosomal DNA of human breast cells. MMTV contamination of our analyses has been definitively excluded. In 8% of unselected American mothers HMTV is found in cells in milk, but in 21% of milks from women previously biopsied for unconfirmed cancer suspicion. In breasts biopsied for suspicion of cancer before a later diagnosis of breast cancer, we see suggestive morphologic changes if the subsequent breast cancer is HMTV+. HMTV is infectious in vitro for B and T lymphocytes, dendritic cells, and human mammary epithelial cells. Infecting MCF-10 breast cell lines with HMTV produces molecular changes of epithelial-mesenchymal transition with upregulation of vimentin, and downregulation of E-cadherin. The excess of breast cancer in western European countries and their former colonies (age standardized rates of 47 to 92 per 100,000 per year) compared to Asian incidence (29 to 43 ASR/y) can be explained by excess HMTV-related breast cancer incidence. In 7 West European, American and Oceania countries, 30 to 60% contain HMTV, while in 5 Asian nations HMTV in breast cancers ranges from 0 to 22% Different indigenous murine species with disparate MMTV burdens parallel these findings: mus domesticus in the West with much MMTV in its genome, and m. castaneus or m.musculus in the East with less. Ancient contamination of the food chain, and current lactational transfer perpetuate infection. HMTV poses a new and challenging dimension to breast cancer research involving diagnosis, molecular mechanisms, epidemiology, therapy and prevention.
Title: DNA repair deficiency enhances immune response and correlates with excellent clinical outcome in triple negative breast cancer

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Body: Background: Mutations or epigenetic silencing of BRCA1/2 genes result in DNA repair deficiency in a large proportion of TNBC cases. Yet it is unclear whether this deficiency is associated with increased chemosensitivity and improved benefit from standard-of-care chemotherapy. We systematically evaluated BRCA deficiency in TNBC using integrated DNA and RNA sequencing data and its association clinical outcome, exploring the potential role of tumor immune response.

Patients and Methods: Whole-exome, DNA methylation, copy number variation, and RNA sequencing data from 102 stage I-III TNBCs were retrieved from The Cancer Genome Atlas (TCGA). Almost all patients received adjuvant taxane-anthracycline-cyclophosphamide (T-AC) chemotherapy and had >30 days of follow-up. The number of predicted neoantigens and an estimate of the level of immune cell activity for 77 of these tumors were previously published. Deleterious germline or somatic BRCA1/2 mutations were identified by majority voting on predictions of 5 variant scoring algorithms. Definition of BRCA1/2 deficiency (BRCA-D) included carrier of deleterious BRCA1/2 mutations or BRCA1/2 normal (BRCA-N) with wild type BRCA1/2 expression less than the maximum observed in mutation carriers. Normalized genomic mutation rate and mutant allele tumor heterogeneity (MATH) were computed as broad measures of genomic instability. Characteristics of BRCA-D vs N tumors were compared using the Wilcoxon rank test.

Results: Twenty tumors (19.6%) had mutations in BRCA1, 6 (5.8%) in BRCA2, and 2 (1.9%) in both. Based on the expanded definition, 39 cases (38%) were characterized as BRCA1 deficient, 5 (4.9%) as BRCA2 deficient and 4 (3.9%) as deficient in both. BRCA-D tumors (47%) were associated with a significantly higher mutation rate (P=8x10-4) but had similar clonal heterogeneity (P=0.55) as BRCA-N tumors. BRCA-D tumors had excellent 4-year overall survival (100%) compared to 79.5% (95%CI: 66.6-94.9) for BRCA-N tumors (log-rank P=0.02). BRCA-D tumors also presented a significantly higher number of predicted neoantigents (P=0.003), which resulted in increased level of immune cell activity. In contrast, low immunogenic TNBC tumors were underrepresented in BRCA-D (p=0.05) and showed potential signs of immuneediting (observed/expected number of predicted neoantigens < 1; p=0.07).

Conclusions: Deleterious mutations in BRCA1/2 genes occur in 25% of TNBC tumors, but parallel quantification of wild-type BRCA1/2 expression identifies 47% of TNBC samples with double strand break DNA repair deficiency. These BRCA-D TNBC tumors are characterized by a significantly higher mutation rate and present a significantly greater number of neoantigens that result in increased immune cell activity. Our analysis suggested that enhanced immune activation could explain to a large extent the excellent clinical outcome in patients with BRCA-D tumors treated with standard-of-care T-AC chemotherapy.
Title: Targeted therapies for TNBC: Exploiting vulnerabilities that arise from DNA damage repair pathway dependencies

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Body: We examined the synergistic effects of DNA damage, Chk1 inhibition and poly(ADP-ribose) polymerase (PARP) inhibition in TNBC. This combinatorial targeting allows us to exploit vulnerabilities in two pathways that are often deregulated in TNBCs: DNA damage checkpoint defects due to TP53 deficiency and DNA repair defects due to alterations in homologous recombination repair (HRR). TP53 maintains genome integrity by inhibiting cells that are experiencing genotoxic stress from progressing through the cell cycle, or by inducing apoptosis or senescence. In response to DNA damage, p53 activates gene expression to arrest cells in the G1 phase of the cell cycle and to reinforce the S- and G2-checkpoints. Thus, p53-deficient cells lack a G1 checkpoint and are impaired in their ability to sustain S- and G2-checkpoints. This makes p53-deficient tumors particularly sensitive to agents that abrogate these checkpoints. Because Chk1 inhibitors abrogate both S- and G2-checkpoints, combining Chk1 inhibitors with agents that induce genotoxic stress provides a rational therapeutic strategy for killing p53-deficient TNBC.

Loss of HRR increases dependence of cells on a class of enzymes called PARPs, and Chk1 has also been shown to be important for efficient HRR. Thus, by interfering with HRR, Chk1 inhibitors are predicted to sensitize TNBC cells to PARP inhibitors. We tested the hypotheses that by impairing HRR, Chk1 inhibitors will sensitize TNBCs to PARP inhibitors, and that therapies that combine Chk1 inhibitors with PARP inhibitors will be effective at killing TNBCs because they will simultaneously induce checkpoint bypass and block DNA repair. We generated a set of isogenic TNBC cell lines that are p53-proficient (p53WT) or p53-deficient (p53KD), and evaluated their sensitivity to Chk1 inhibitors (LY2606368) and DNA damaging agents (cisplatin). Loss of p53 conferred a dramatic increase in sensitivity to treatment with cisplatin + LY2606368. Surprisingly, inhibition of PARP1 (BMN673) did not increase sensitivity to Chk1 inhibitor ± cisplatin. To determine why Chk1 inhibition did not sensitize cells to PARP inhibition, we evaluated the effect of Chk1 inhibition on the ability of cells to recruit HRR proteins to sites of DNA damage. In line with CHK1 regulating HRR, Chk1 inhibition was associated with an inability of Rad51 to localize to sites of DNA double strand breaks. Interestingly, we also found that upstream of Rad51, there was a significant alteration in the formation of phospho-RPA2 foci in cells treated with the Chk1 inhibitor. On-going studies are evaluating whether there are changes in the kinetics of formation and/or resolution of Rad51 and phospho-RPA2 foci in response to Chk1 inhibition.
Title: PARP1/2 inhibition in a subset of triple negative breast cancer (TNBC) patient-derived tumor xenografts (PDX) identifies predictive biomarkers of response

Serra V, Cruz C, Bruna A, Ibrahim YH H, Vivancos A, Vivancos A, Nuciforo P, Bellet M, Gómez P, Pérez JM M, Saura C, Vidal M, Serres X, Rueda OM M, Peg V, Caldas C, O'Connor MJ J, Baselga J and Cortés J. Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Cancer Research UK, CI (CRUK), Cambridge, United Kingdom; Vall d'Hebron University Hospital (VHUH), Barcelona, Spain; Astra Zeneca (AZ), Macclesfield, United Kingdom; Netherlands Cancer Institute (NKI), Amsterdam, Netherlands and Memorial Sloan Kettering Cancer Center (MSKCC), NY.

Body: Background: BRCA1/2 mutation carriers (gBRCA) have a higher risk of breast or ovarian cancer, since BRCA1/2 mutation results in impaired high-fidelity DNA repair by homologous recombination (HR) and subsequently genetic instability. In non-gBRCA TNBC, HR deficiency occurs at the somatic level, by means of BRCA1 mutation, BRCA1 epigenetic loss or mutation in other HR-associated genes. Because PARP1/2 inhibitors (PARPi) are well-tolerated and active anti-cancer agents in the advanced setting of gBRCA tumors, we sought to expand their applicability by identifying response biomarkers in TNBC.

Methods: We have assessed the antitumor response of the PARP1/2 inhibitor olaparib as single agent in a panel of 12 primary and advanced TNBC PDX models. On PDXs exhibiting primary sensitivity to olaparib, we have developed models of acquired resistance by continuous exposure to the drug and identifying progression on treatment. We have characterized the models through targeted sequencing and the analysis of the hypermethylation and expression levels of BRCA1 transcript to find potential correlates of drug-sensitivity.

Results: Three out of 12 PDXs (25%) treated with single agent olaparib, exhibit tumor regression or disease stabilization. BRCA1 is hypermethylated in two of these PARPi-sensitive TNBC PDX models and is associated with loss of BRCA1 mRNA expression. The third PARPi-sensitive TNBC PDX harbors a frameshift, heterozygous PALB2 mutation, which is no longer detected in the acquired resistance PDX model. Acquired resistance in the hypermethylated PDXs is under study as well as the duration of response compared to gBRCA PDX models.

Conclusions: Our study highlights that somatic HR-deficiency is frequent in TNBC and provides the basis of sensitivity to PARPi.
Title: PARP inhibition in breast and ovarian patient-derived tumor xenografts (PDX) harboring germline BRCA1/2 mutations unveils mechanisms of primary and acquired resistance that restore homologous recombination (HR)

Cruz C, Bustos Ld de, Gris A, Palafox M, Castroviejo M, Llop A, Morancho B, Diez O, Gutiérrez S, Caratú G, Prudkin L, Bruna A, Caldas C, O'Connor MJ J, Rubio IT T, Arribas J, Baselga J, Cortes J, Serra V and Balmaña J. Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Cancer Research UK, CI (CRUK), Cambridge, United Kingdom; Astra Zeneca (AZ), Macclesfield, United Kingdom and Memorial Sloan Kettering Cancer Center (MSKCC), NY.

Body: Background: PARP1/2 inhibitors (PARPi) are active anti-cancer agents in BRCA1 or BRCA2 mutation carriers (BRCA) with advanced breast or ovarian cancer. However, not all BRCA-tumors respond to PARP blockade, and eventually all develop acquired resistance. Little is known about clinically relevant mechanisms of PARPi resistance in BRCA-breast cancer. Here, we sought to identify biomarkers correlating with primary and acquired resistance to PARPi using PDX derived from both the early disease and the metastatic setting.

Methods: We have developed a panel of PDX from patients harboring germline BRCA1 or BRCA2 mutations, namely from 12 primary and advanced breast cancer and 1 high-grade serous metastatic ovarian cancer (HGSOC). The antitumor activity of the PARP1/2 inhibitor olaparib as single agent (50 mg/kg) was assessed in all models. To study the mechanisms of acquired resistance, the olaparib-sensitive PDXs were exposed to olaparib for >100 days, until individual tumors regrew. The tumor's capacity to repair DNA double strand breaks was estimated by quantification of the BRCA1 and RAD51 nuclear foci in the S-phase of the cell cycle. We investigated the correlation between the tumor's BRCA1/RAD51 foci formation and sensitivity to olaparib, and also identified potential genetic modifiers of PARPi sensitivity by targeted sequencing.

Results: Four out of 13 PDX (31%) treated with single agent olaparib exhibited tumor regression or disease stabilization. Nuclear BRCA1/RAD51 foci formation correlated with PARPi resistance in six BRCA1 PDX models investigated, either with primary or acquired resistance. No reversions in BRCA1/2 mutations were identified as the mechanism of olaparib resistance. We identified four potential genetic modifiers of PARPi sensitivity and the corresponding validating studies will be presented.

Conclusions: Among our BRCA PDX, reactivation of HR functionality is a frequent event that is associated with PARPi resistance and seems to occur through mechanisms other than secondary mutations in BRCA1/2 in contrast to what it has been reported for HGSOC.
Title: Imipramine Blue - A safe and potent therapeutic regimen that suppresses breast cancer growth and progression by targeting DNA damage surveillance pathway

Rajamanickam S, Subbarayalu P, Timilsina S, Gorthi A, Drake MT T, Chen Y, Vadlamudi R, Bishop AJR JR, Arbiser JL L and Rao MK K. University of Texas Health Science Center at San Antonio, San Antonio, TX and Emory University, Atlanta, GA.

Body: Despite improvement in overall survival of breast cancer patients, many women don't survive this disease. Moreover, the quality of life for patients who do survive is often substantially reduced due to the toxicity associated with the chemotherapy. Here, we report that imipramine blue (IB), a novel analogue of anti-depressant imipramine that we recently synthesized, may serve as a safe and potent therapeutic agent for treating breast cancers. We show that IB reduced cell growth, migration and invasion of breast cancer cells. Systemic delivery of IB using nanoparticle-based drug delivery approach suppressed breast cancer growth and metastasis without inducing any toxicity in pre-clinical orthotropic mouse models. Notably, using ex-vivo model of tumor explants from breast cancer patients, we demonstrated that IB inhibited breast cancer growth without affecting normal mammary epithelial cell proliferation. Furthermore, IB improved the sensitivity of breast cancer cells to chemotherapy drugs paclitaxel and doxorubicin. Our results revealed that IB mediated its anti-tumor effect by targeting genes involved in cell cycle progression, microtubule dynamics and DNA damage surveillance pathway including Forkhead Box M1 (FOXM1), stathmin1, S-phase kinase-associated protein 2 (Skp2) and XRCC3, which we show to be highly expressed in breast cancer patients. Importantly, we demonstrated that IB inhibited breast cancer cell's ability to repair DNA strand breaks by impairing homologous recombination events. These findings highlight the potential of IB to be used as a potent therapeutic regimen for treating breast cancer patients. Since IB-1 is derived from a FDA approved drug it has potential to be rapidly translated to the clinic.
Title: Evaluating Rad51/geminin protein expression as an indicator of homologous recombination deficiency in breast cancer models


Body: Background: Homologous recombination deficiency (HRD) in cancer cells can occur due to mutations (germline or sporadic), methylation or other epigenetic causes. HRD leads to a defect in the conservative, error-free DNA repair mechanism and is associated with enhanced susceptibility to DNA targeting chemotherapy. Currently functional HRD assays are not broadly available for clinical use. Many of the HRD assays used in the experimental setting require fresh frozen tissue for optimal results, or require specialized expertise to interpret the results. We evaluated an immunohistochemical (IHC) assay using formalin fixed paraffin embedded (FFPE) tissue to measure protein expression of Rad51 and geminin, a cell proliferation marker, to assess HRD in breast cancer cell line models and clinical breast cancer samples. We hypothesize that Rad51, which is involved in the later stages of HR, can serve as a functional marker of HRD.

Methods: The MCF-7 human breast cancer cell line was used as a model with intact HR. Western blotting of total cell lysates from cells grown in culture was performed to confirm HR response following treatment with DNA damaging chemotherapeutic agents, cisplatin and doxorubicin. Paclitaxel, a microtubule targeting agent, was used as a negative control. Mice with MCF-7 xenograft tumors were also treated with cisplatin, or doxorubicin at two dose levels (low and high) and various time points post treatment to assess the dose and time response to HR markers. Tumors from mice treated with paclitaxel were used as a negative control. Xenograft tumors were fixed and analyzed by IHC using an antibody specific for total Rad51 and geminin expression. DNA damage was also assessed in a portion of the tumor using a pulse gel electrophoresis assay. We also analyzed FFPE breast cancer clinical samples from patients with BRCA1 mutations for Rad51 and geminin expression.

Results: In MCF-7 grown in vitro, total Rad51 was elevated as soon as 4 hours following exposure to doxorubicin and cisplatin, but not in response to paclitaxel treatment. In xenograft tumors, baseline Rad51 and geminin expression were relatively high illustrating proficient HR in an actively proliferating tumor model. Rad51 expression increased post treatment with cisplatin and doxorubicin as early as 6hrs and peaked at 16-24hrs. Geminin expression correlated well with expression of Rad51 at baseline and in time response to treatment. Pulse gel electrophoresis in paired tumor samples confirmed DNA damage was occurring compared to vehicle control treated tumors. However, this technique did not show a strong dose or time response. Five breast tumors from patients with known BRCA1 mutations were stained for Rad51 and geminin expression. High geminin expression and low Rad51 expression was noted in the majority of these tumors.

Conclusions: An IHC assay using FFPE tissue to measure Rad51/geminin is a promising method to assess HRD in breast cancer. Further analytical and clinical validation of this approach is ongoing.
Title: The prognostic significance of ataxia-telangiectasia-mutated (ATM) and p53 expression in breast cancer


Body: The purpose of this study was to investigate the correlation of ataxia-telangiectasia-mutated (ATM) protein and p53 expression with clinicopathological features and prognosis in patients with sporadic breast cancers. The expression of ATM and p53 was determined by immunohistochemistry in 420 surgically resected breast cancers. Loss of ATM was observed in 126 out of 407 evaluable cases (31.0%), and was significantly associated with aggressive features with large tumor size, lymph node metastasis, higher tumor grade, and negativity of ER and/or PR. ATM loss was associated with a significantly shorter disease-free survival (DFS) (p = 0.019). Abnormal p53 expression was found in 39.3% of tumors (157 out of 400), conferring a worse DFS as well (p = 0.002). When investigated together, combined ATM and p53 expression status were associated with a worse DFS (p = 0.002). On multivariate analysis, ATM loss and abnormal p53 expression status was an independent predictor of poorer DFS (intact ATM and normal p53 vs. ATM loss and abnormal p53, HR 3.350; 95% CI 1.496 - 7.502; p = 0.003). Furthermore, in patients treated with adjuvant anthracyclines, either p53 alone or p53 combined with ATM significantly influenced DFS (p = 0.004, p = 0.015, respectively). The present study demonstrates that expression of ATM and p53 is an independent prognostic marker in breast cancers, and might be a practical tool for predicting benefits from anthracycline-based adjuvant therapy.
Title: MYB is involved in the DNA damage response in human ER+ breast cancer cells

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Body: Aims:
Over 70% of human breast cancer cells are oestrogen receptor positive (ER+) and express MYB. MYB expression is necessary for the proliferation of ER+ breast cancer cells in vitro and for tumour development in vivo. Our previous studies found that shRNA-mediated MYB knock-down greatly sensitised breast cancer cells to chemically-induced apoptosis by down-regulating the BCL2 (a MYB target gene) (Drabsch et al., 2010). Furthermore, several published studies (Taha et al., 2004; Thomadaki et al., 2006) indicated that actinomycin D and etoposide treatment could induce DNA damage in human ER+ breast cancer (MCF-7) cells. Moreover, silencing MYB increased DNA damage-induced cell death in castration resistant prostate cancer cells by down-regulating DNA damage response genes (Li et al., 2014). However, there is very little information on MYB function in the DNA damage response of ER+ breast cancer cells. Therefore, the aim of this study was to investigate whether silencing MYB affected the DNA damage repair and resulting in cell death in MCF-7 cells.

Methods:
To achieve down-regulation of MYB expression in human ER+ breast cancer, we used doxycycline inducible shRNA lentiviral vectors (pLV711 (Drabsch et al., 2007; Brown et al., 2010)) in human MCF-7 breast cancer cells. We used PI staining plus FACS analysis for cell death assessment. We also used γ-H2AX protein expression and γ-H2AX foci counting to assess DNA damage.

Results:
We found that silencing MYB alone did not result in cell death, as reported previously (Drabsch et al., 2010). However, silencing MYB significantly increased actinomycin D-induced cell death. This similar result was also found on etoposide-induced cell death. Furthermore, we found that silencing MYB significantly increased actinomycin D or etoposide-induced γ-H2AX expression. Moreover, anti-apoptotic BCL2 expression, measured by western blotting, was dramatically reduced after the combination of MYB knock down and actinomycin D or etoposide, compared to wild type and nonsilencing controls.

Conclusions:
Silencing MYB significantly increased DNA damage-induced cell death and DNA damage. This may result from down-regulation of BCL2 expression, an effect on DNA damage response genes, or both. This requires further investigation. For example, Rad51 and γ-H2AX colocalization and 53BP1 expression in response to DNA damage will be presented.

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AMPK facilitates breast cancer cell survival by modulating microenvironmental stress

Sullivan KL L, Kopsiaftis S, Phoenix KN N, Fox MM M, Tsurutani N, Vella AT T and Claffey KP P. University of Connecticut Health Center, Farmington, CT and Wingate University, Wingate, NC.

Body: Recurrent and metastatic breast cancers are responsible for the majority of breast-cancer related deaths. These cancer cells are able to adapt to stressors within the tumor microenvironment including hypoxia, low nutrient levels, and chemotherapy-induced toxicities. Breast cancer cells can respond to these microenvironmental stressors through a variety of mechanisms, including cell cycle inhibition and metabolic alteration. Tumor cell survival is dependent on the ability to alter these mechanisms in response to stress. AMPK (AMP-activated protein kinase) is the main metabolic sensor of the cell, and both its expression and activity have been reported to be altered in breast cancer. Moreover, there are two isoforms of the catalytic subunit (α1 and α2), and differential functionality of these isoforms has been suggested. Using estrogen receptor-positive human breast cancer cell lines, we investigated the effect of differential AMPKα isoform expression on breast cancer cell survival. We found that over-expression of AMPKα2 in MCF-7 cells resulted in decreased ATP production in response to low glucose levels, while the knockdown of AMPKα2 in HCC1500 cells ablated this response to low glucose conditions. A similar difference in response was also seen when the cells were treated with a combination of nutrient stress and the estrogen receptor alpha (ERα) inhibitor, ICI182780. In response to this finding, we compared the glycolytic and oxygen consumption rates of our MCF-7 GFP and MCF-7 AMPKα2 cells. We found that in response to low glucose stress, AMPKα2 expressing MCF-7 cells maintained both a higher glycolytic rate and a higher oxygen consumption rate as compared to GFP cells. Furthermore, these cells seem to alter their cellular signaling in response to metabolic stress faster than GFP cells. To evaluate this differential response to microenvironmental stress in vivo, MCF-7 cells expressing either GFP or AMPKα2 were injected into athymic nude mice previously implanted with slow-release estradiol pellets. After one week, the estradiol pellets were removed to induce cellular dormancy for thirty days. Analysis of tumors at this time indicated that more of the AMPKα2 expressing cells survived estradiol deprivation than did the control cells. Analysis of proliferation by Ki67 staining indicated that the GFP cells maintained proliferation during deprivation, while AMPKα2 cells were largely negative for proliferation. ApoTag staining revealed a similar trend for apoptotic cells. This suggests that an inability to control cell cycle resulted in a decreased survival of the GFP cells under estradiol deprivation. Following the deprivation period, estradiol pellets were re-implanted and residual dormant tumors resumed growth. AMPKα2 tumors grew to roughly double the size of GFP tumors. Interestingly, AMPKα2 tumors had a higher number of mitotic events than did GFP tumors as visualized by Ki67 staining. This could be due to more viable cells being present following estradiol deprivation. We conclude that the expression of AMPKα2 promotes long-term breast cancer survival in estrogen-sensitive cells, due to their increased ability to sense and respond to changes in their microenvironment, which therefore increases their chances for survival.
Title: APOBEC3B upregulation by the PKC-NFκB pathway in breast cancer


Body: Overexpression of the antiviral DNA cytosine deaminase APOBEC3B has been linked to somatic mutagenesis in breast and other cancer. Human papillomavirus (HPV) infection accounts for APOBEC3B upregulation in cervical and head/neck cancers. However, the responsible mechanisms are unclear for non-viral malignancies such as breast cancer. Here, we demonstrate APOBEC3B upregulation through the PKC-NFκB pathway. PKC activation by the diacylglycerol mimic PMA causes specific and dose-responsive increases in APOBEC3B mRNA, protein, and activity levels, which are strongly suppressed by PKC and NFκB inhibition. Induction correlates with RELB (but not RELA) recruitment to endogenous APOBEC3B implicating non-canonical NFκB signaling. Relevance to tumors is supported by PKC inhibitor-mediated APOBEC3B downregulation in multiple breast cancer cell lines. These data establish the first mechanistic link between APOBEC3B and a common signal transduction pathway, suggesting that existing PKC-NFκB inhibitors could be repurposed to suppress cancer mutagenesis, dampen tumor evolution, and decrease the probability of adverse outcomes such as drug resistance and metastases.
DEAD-box RNA helicase DP103 as a novel regulator of Wnt/β-catenin signaling pathway and promotes cancer stem cell-like behavior in triple negative breast cancers

Cai W, Cheong JK, Edison E, Banerjee A, Tan TZ, Gaboury L, Yousef EM, Thiery JP, Lobie PE, Virshup DM, Yap CT and Kumar AP.  Cancer Science Institute of Singapore, Singapore; National University of Singapore, Singapore; Cancer & Stem Cell Biology Program, Graduate Medical School, Duke-NUS, Singapore; National University of Singapore, Singapore; Institute of Molecular and Cell Biology, A*STAR, Singapore, Singapore; National University of Singapore, Singapore; School of Biomedical Sciences, Faculty of Health Sciences, Curtin University, Perth, Western Australia, Perth, Western Australia, Australia; University of North Texas, Dallas, TX and Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Canada.

Body: Despite recent advances in breast cancer therapeutics, mortality of metastatic triple negative breast cancer (TNBC) subtype remains high; due to their lack of hormone receptors expression for targeted therapy. Aberrant activation of Wnt/β-catenin signaling has been associated with breast cancers; where 40% of total breast cancers have elevated β-catenin levels with increased Wnt activity. Recently, we identified DEAD-box RNA helicase DP103 as a novel prognostic biomarker and metastasis-driving oncogene; highly expressed in TNBC subtype. Interestingly, we found high DP103 expression to be positively correlated with high β-catenin expression in clinical specimens (n=400). This led us to hypothesize a possible role of DP103 in modulating the Wnt/β-catenin pathway in TNBCs. Depletion of DP103 in metastatic TNBC cells decreases Wnt/β-catenin activity and expression of downstream Wnt target genes, while overexpression of DP103 increases Wnt activity. Depletion of DP103 also decreases phosphorylation of LRP6 and several important Wnt modulators required for downstream Wnt activation. Moreover, induction of Wnt/β-catenin signaling in Wnt responsive TNBC cells also significantly increased DP103 expression, indicating a possible positive feedback loop. Both canonical and non-canonical Wnt signaling is known to independently promote stem cell growth in mammospheres. Herein, we will also provide evidence on the role of DP103 in promoting breast cancer stem cell-like properties. Collectively, our data show a novel regulatory role of DP103 in the Wnt/β-catenin signaling pathway and in promoting breast cancer stem cell-like behavior, presenting itself as a potential drug target in TNBC patients.
Title: Navigating genomic landscape to find a PI3K-signaling algorithm for a rational combination in precision medicine


Body: Background: Treatment of BC is conventionally based on the presence/absence of ER/PR or HER2 status of the primary tumor. We have enriched this approach by including major genetic and proteomic changes in tumors of individual patients in order to develop a better treatment-rationale based on an alteration driven signaling algorithm. Methods: Genomic and proteomic data from 75 BC patients seen in our center were retrospectively analyzed. Patients were re-biopsied after consultation and samples were characterized (IHC for ER, PR, and HER2; FFPE samples for genomic [Foundation Medicine] and proteomic analyses [Theranosics]). In vivo studies were conducted using xenograft models. Results: Although alterations of PIK3CA, PIK3R1, AKT, PTEN, MDM2, MDM4, TSC1, mTOR and RICTOR are most frequently observed in our patients, there is a distinct pattern of alteration(s) of the PI3K pathway genes in different subtypes of BC. A total of 76 genes were altered in 48 ER+BC patients. In 79% of ER+BC patients the above mentioned PI3K pathway genes were altered. Analyzing the set of alterations of genes in individual patients, we observed that within these 48 patients 25% exhibited alterations in more than one node of the pathway; the most common combination (alterations) being the amplification/mutation of PIK3CA with the amplification of MDM2/4 genes. The percentage of patients belonging to HER2+ & TNBC exhibiting similar alterations in the PI3K pathway genes were significantly lower (~40%). Our previous in vivo studies demonstrated that GDC-0980 and BEZ235 enhanced the antitumor activity of ABT888 plus carboplatin in TNBC or trastuzumab in HER2+ BC respectively and blocked the growth of established xenograft tumors by 80% to 90% with a concomitant decrease in tumor Ki67, pS6RP and CD31. Mechanistically the action of the PI3K-mTOR pathway targeted drug(s) was tested using cell line based models of BC subtypes pertaining to their respective genomic alterations. A combination of a pan-PI3K pathway inhibitor, GDC-0941 or isoform-specific inhibitors along with AI, trastuzumab, or HRD inhibitors (PARP) blocked proliferative signals and enhanced apoptosis (cleaved caspase3) in ER+/PIK3CA mutated, HER2+/PIK3CA mutated or PTEN-null TNBC cells respectively as demonstrated by WB, flow cytometry, cell proliferation, viability and cytotoxicity assays. A recent study demonstrated that exposure to chemotherapy induced a phenotypic shift or cell state transition towards a transient CD44Hi/CD24Hi chemotherapy-tolerant state, leading to the activation of downstream non-receptor tyrosine kinase signaling towards an emerging adaptive resistance (Goldman et al., Nature Comm. 2015). Hence drug combination(s) are being tested for their effect on CD44/CD24 expression levels, results of which will be presented in the meeting. Conclusion: Plotting the genetic alterations from the patient on the signaling landscape will be useful in cracking the code leading to improved treatment options. Patient specific in-depth plotting of genetic alterations of the PI3K-mTOR pathway and the relevance of these alterations in the context of (1) mechanisms of PI3K-mTOR pathway targeted drugs and (2) cell signaling are critical in determining choice of drugs in BC subtypes.
Title: Transfer of Trastuzumab® across the human placenta barrier

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Body: Background: Breast cancer (BC) occurs between one in 3000 to one in 10 000 pregnancies. About 20% to 44% of BC diagnosed in women younger than 30 ys is associated by Her2/Neu receptor overexpression. The prognosis of the Her2/Neu positive BC has improved fundamentally with the introduction of trastuzumab. The increasing age of child bearing mothers leads to an increased probability of the incidence of BC, and thus Her2/Neu positive BC in pregnancy as well. The administration of trastuzumab during pregnancy is not recommended and leads to the development of reversible oligo- and anhydramnios and fetal renal volume. The mechanism has not been identified yet. Trastuzumab is a humanized monoclonal IgG kappa 1 antibody, which is transported across the human placenta. Moreover, the presence of Her2/Neu receptor in the second and third trimester placenta and fetal kidneys has been described. The interaction of trastuzumab with these tissues/receptors may cause the development of oligo- and anhydramnion. Apart of the study that tested the transplacental transfer of trastuzumab in pregnant baboon (2009), to our knowledge there are no data in the literature tested the transfer of trastuzumab across the human placenta.

Objectives: We examined the transfer of Trastuzumab® in a relevant pharmaceutical concentration across the human term placenta with the ex-vivo placenta perfusion model, as well as the expression of the Her2/Neu receptor in the placenta tissue (syncytiotrophoblast).

Methods: The dual ex vivo human placental perfusion model was used to analyse the transfer of Trastuzumab® across the placental barrier (n=3). The ex vivo placenta perfusion model was performed by establishing an artificial maternal and fetal circulation system within 20 minutes after delivery. Nutrition and oxygen supply was established to keep the tissue and its barrier function under physiological conditions over several hours. Closed loops of the maternal and fetal circulations were used within the perfusion experiments. We used an antibody concentration in the maternal circulation of 50 µg/ml. This concentration is a common bolus application in therapy of Her2positive BC. Quantification of the protein was performed by ELISA. The presence of Her2/Neu in the placenta was determined by immunhistochemical stainings.

Results: The transport of Trastuzumab® in the maternal to fetal direction could not be detected over 90 minutes of placenta perfusion. An average antibody concentration decrease of about 30% was determined in the maternal circulation. The presence of the Her2/Neu receptor in the syncytiotrophoblast layer was detectable by immunhistochemical staining.

Conclusions: The results indicate that Trastuzumab® does not cross the human placenta within 90 min of placenta perfusion. Due to the presence of Her2/Neu receptor expression at the syncytiotrophoblast of the human placenta, a binding to this receptor is assumed. This may explain the trastuzumab decrease over time in maternal circulation. These findings indicate that the pregnancy complications can also be caused by a direct effect of trastuzumab on the human placenta. Our results are in contrast to published data of transplacental transfer of trastuzumab in pregnant baboon published 2009 and need to be investigated in further studies.
Modulation of hypoxia-inducible factors and the HIF transcriptional response to hypoxia by ERBB2 overexpression in the MCF7 breast cancer cell line


Objective: To explore the role of HIF2α in growth factor receptor-driven HIF modulation and investigate the relationship between growth factor- and hypoxia-driven HIF activation. HIF-mediated transcriptional activity is known to drive genes involved in various processes which are associated with cancer pathology such as glycolysis, angiogenesis and metastasis. Therefore, understanding the implications of hypoxia-independent HIF regulation for both HIF1α and HIF2α, may give new insight into the mechanisms by which HIF drives cancer pathology in vivo and a greater understanding of when HIF inhibitory agents may be effective therapies.

Methods: We used an ERBB2 overexpressing MCF7 cell line (MCF7-HER2) to investigate the effect of ERBB2 on the HIF-axis. Western blotting was used to assess protein level in these cell lines. HIF protein expression was compared with and without ERBB stimulation by ERBB3 ligand neuregulin 1β. Illumina BeadChip analysis was used to compare mRNA levels between these cell lines in normoxia (20% oxygen), acute hypoxia (0.5% oxygen for 24 hours) and chronic hypoxia (0.5% oxygen for 10 weeks). Differentially expressed genes were identified using rank products analysis with a cut-off P-value of 0.01. This allowed an in-depth comparison of hypoxia responses at the level of transcription between the cell lines to ascertain the effect of ERBB2 overexpression on hypoxia driven transcriptional changes.

Results: Immunoblotting shows that HIF1α protein level is comparable between MCF7 and MCF7-HER2 cell lines, and is inducible in normoxia by stimulation with neuregulin 1β. Conversely, HIF2α protein is unaffected, but is constitutively expressed in MCF7-HER2 only. This suggests that both HIF isoforms can be up-regulated in normoxia but by different mechanisms. Microarray data suggests that the constitutively higher HIF2α levels in the MCF7-HER2 cell line may be due, at least in part, to the increased transcription of the HIF2A gene which is higher in normoxia but in response to hypoxia when compared to wild-type MCF7. Overexpression of ERBB2 in MCF7-HER2 cells appears to prime cells for their response to hypoxia, as 14% (N= 591) of the genes which are induced in acute hypoxia are also expressed at significantly higher levels in normoxic MCF7-HER2 cells. However, only 1% are more highly expressed in wild-type MCF7 cells. For chronic hypoxic genes, 18% (N= 514) were more highly expressed in normoxic MCF7-HER2 cells and just 8% in wild-type MCF7 cells. These up-regulated genes include both HIF1 and HIF2 target genes which may have important consequences for glycolysis (ALDOC, PFKFB), tumour cell survival (E4BP4, STC2) and proliferation (FOS, KDM5B).

Conclusions: We have demonstrated that both HIF1α and HIF2α can be regulated independently of hypoxia, however these appear to be controlled through distinct mechanisms. Whilst the implications of HIF1 in breast cancer pathology have been appreciated for some time, relatively little is known about the impact of HIF2. Here we show that ERBB2 overexpression can not only increase HIF2α protein levels in normoxia, but may also prime cells for hypoxia by allowing the constitutively higher expression of HIF1 and HIF2 target genes.
**Title:** CDCP1 cleavage is necessary for homodimerization-induced migration of triple-negative breast cancer


**Body:** Triple negative breast cancer (TNBC) is a highly aggressive and metastatic form of breast cancer that lacks the estrogen, progesterone, and HER2 receptors and is resistant to targeted and hormone therapies. TNBCs express high levels of the transmembrane glycoprotein, CUB-domain containing protein 1 (CDCP1), which has been correlated with the aggressiveness and poor prognosis of multiple carcinomas. Full-length CDCP1 (flCDCP1) can be proteolytically cleaved, resulting in a cleaved membrane-bound isoform (cCDCP1). CDCP1 is phosphorylated by Src family kinases in its full-length and cleaved states, which is important for its pro-metastatic signaling. We observed that cCDCP1, compared to flCDCP1, induced a dramatic increase in phosphorylation of the migration-associated proteins: PKCδ, ERK1/2, and p38 MAPK in HEK 293T. In addition, only cCDCP1 induced migration of HEK 293T cells and rescued migration of the TNBC cell line, MDA-MB-231, expressing shRNA against CDCP1. Importantly, we found that only cCDCP1 is capable of dimerization, which can be blocked by expression of the extracellular portion of cCDCP1 (ECC), indicating that dimerization occurs through CDCP1’s ectodomain. Furthermore, we found that ECC inhibited phosphorylation of PKCδ by Src and migration of TNBC cells, indicating that the cCDCP1 dimer is an important contributor to TNBC aggressiveness. These studies have important implications for development of a therapeutic to block CDCP1 activity and TNBC metastasis.
Title: FGFR1 protein expression is associated with prognosis in primary breast cancer: A comprehensive analysis of gene copy number, mRNA and protein expression

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Body: Background: The Cancer Genome Atlas (TCGA) showed that copy number gain/amplification of FGFR1 was around 10% in primary breast cancer. FGFR1 gene amplification in breast cancer has been reported in some studies, more likely seen in ER-positive subtype. Several preclinical and clinical studies demonstrated that FGFR1 was one of novel targets of therapy for metastatic breast cancer. Previous studies suggested that aberrant FGFR1 expression was associated with poor prognosis, while there was no report that compared copy number aberration, mRNA and protein expression. The aim of this study is to analyze FGFR1 gene copy number, expression levels of FGFR1 mRNA and FGFR1 protein in ER-positive/HER2-negative primary breast cancer, and to examine the relationship between FGFR1 status and clinicopathological parameters including prognosis.

Methods: The cohort of this study included 307 ER-positive/HER2-negative primary invasive breast cancer patients treated with standard care at Kumamoto University Hospital between June 2000 and January 2011. We performed a comprehensive analysis of FGFR1 at the levels of gene copy number, mRNA and FGFR1 protein expression analyzed by qPCR, qRT-PCR and immunohistochemistry, respectively.

Results: FGFR1 gain/amplification was identified in 43 (14.0%) out of 307 patients. FGFR1 gain/amplification had significantly associated with higher nuclear grade (p=0.010). No correlations between FGFR1 mRNA expression levels and any clinicopathological factors were found. Expression levels of FGFR1 protein was positively associated with invasive tumor size (p=0.039). Modest positive correlations between these three (FGFR1 gene gain/amplification, expression levels of FGFR1 mRNA and FGFR1 protein) were found. The univariate analysis revealed that high FGFR1 protein expression was significantly related to poor prognosis (p=0.0019, HR: 2.63, 95%CI: 1.17-5.98) in terms of relapse-free survival (RFS) but not breast cancer-specific survival. The univariate analysis did not show that any factors except FGFR1 protein expression were significantly associated with RFS in this cohort.

Conclusion: Expression levels of FGFR1 protein may be an independent prognostic factor in terms of RFS for ER-positive/HER2-negative breast cancer patients receiving standard care.
Concordance in fibroblast growth factor receptor 1 (FGFR1) and 2 (FGFR2) status in breast cancer during tumor progression


BACKGROUND: In recent years, changes in HER2 and hormone receptor (HR) status between primary and metastatic breast cancer (BC) have been extensively reported. Additionally, current advances in BC biology have identified emerging biomarkers with clinical and prognostic implications, particularly the PI3K-AKT-mTOR and the FGF/FGFR pathways. FGFR1 and FGFR2 gene amplifications represent the most frequent genomic aberrations in BC. The 8p11-12 chromosomal region harboring the FGFR1 gene locus is amplified in about 10-18% of human BC, mainly in HR-positive/HER2-negative subtype, whereas the FGFR2 gene, located on chromosome 10q26, is amplified in approximately 4% of triple negative BC. To our knowledge, there is a lack of data regarding the concordance of FGFR1 and FGFR2 status between primary and metastatic tumors.

METHODS: Tumor samples from 205 and 67 advanced BC patients diagnosed at our institution between 2010 and 2014 were screened for FGFR1 and FGFR2 amplification by FISH using the ZytoLight SPEC FGFR1/CEN8 and FGFR2/CEN10 probes, respectively. FGFR1 and FGFR2 amplification were defined as a ratio of FGFR1/CEN8 and FGFR2/CEN10 ≥ 2.2. We investigated the correlation of FGFR1 and FGFR2 status between primary and metastatic tumors in 16 and 13 patients, respectively, for whom paired samples were available.

RESULTS: A total of 47 FGFR1-amplified patients (22.9%) and 3 FGFR2-amplified patients (4.5%) were identified. Patients with paired samples were classified according to FGFR1 and FGFR2 status in primary tumor. Regarding FGFR1 amplification, eight patients were FGFR1-amplified and eight were FGFR1-non-amplified. One patient in each group showed discordance in FGFR1 status in the metastatic tumor. Overall rate of concordance between primary and metastatic tumors was 87.5% (14/16). In relation to FGFR2 status, two patients were FGFR2-amplified and 11 were FGFR2-non-amplified with a 100% overall rate of concordance between paired samples (13/13). These data are summarized in Table 1.

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<th>FGFR1</th>
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<td>Non-amplified</td>
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CONCLUSIONS: Results suggest a high concordance in FGFR1 and FGFR2 status between primary and metastatic BC. The potential impact of these findings on the development of FGFR inhibitors merits further investigation.
Title: Prognostic and predictive value of COX2 in breast cancer, correlation with PIK3CA mutations


Body: Background: Cyclooxygenase-2 (COX2) is responsible for the synthesis of prostaglandins from arachidonic acid. This enzyme is weakly expressed in normal tissues and implicated in oncogenic and inflammatory processes. Treatment with a COX2 inhibitor (aspirin) increases overall survival of patients with colorectal cancer only for PIK3CA mutated tumors confirming an interaction between COX2 and the PI3K/AKT pathway. PIK3CA gene mutations are detected in 10-40% of BC depending on the molecular subtype. We hypothesized that COX inhibition could have an impact in Breast Cancer (BC) treatment.

Methods: COX2 mRNA expression levels were analyzed in 446 BC samples and 61 patient-derived xenografts (PDX) using qRT-PCR. Protein expression of COX2 was studied by immunohistochemistry (IHC) in 26 BC and 14 PDX. The prognostic impact of COX2 expression level according to PIK3CA mutation status was also analyzed in BC patients. The activity of celecoxib, a selective COX2 inhibitor, was tested in two PDX of triple-negative BC: the HBCx50 PDX (PIK3CA wild-type, expressing COX2) and the HBCx4B PDX (PIK3CA mutated, expressing COX2).

Results: COX2 transcript was under-expressed in 74% and overexpressed in 2% of the BC samples. COX2 overexpression is significantly associated with triple-negative subtype (11%, 7/68 cases, p<0.0001). Moreover, immunostaining of COX2 was well correlated with COX2 mRNA expression level. PIK3CA mutations were detected in 33% of patients. We showed that metastasis-free survival (MFS) was significantly better in patients who do not under-express COX2 (p=0.007) or in patients with PIK3CA mutation (p=0.02) regardless the BC subtype and adjuvant treatment. In the PIK3CA wild-type (wt) subgroup, MFS of patients was significantly better in patients not under-expressing COX2 than in patients under-expressing COX2 (p=0.01). In PDX, the strongest expression levels of COX2 were found in triple-negative (median 36 [0-1673]) compared to luminal (median 0 [0-202]) and HER2 positive subtypes (median 6 [0-601]). In vivo studies showed that celecoxib has no effect on the HBCx50 (PIK3CA wt) PDX growth while a significant antitumoral effect of celecoxib is observed in HBCx4B (PIK3CA mutated) PDX (tumor growth inhibition=41%, p=0.03).

Conclusion: In BC, COX2 underexpression is frequent and impact prognosis. BC overexpressing COX2 are rare and mainly belong to the triple-negative subtype. In vivo PDX studies show that the antitumoral effect of celecoxib may be restricted to BC expressing COX2 with PIK3CA mutation. Analyses of signaling pathways, expression levels of COX2 and its target proteins, tumor proliferation and apoptosis induction are ongoing on tumors and serum of mice to elucidate the antitumoral effect of celecoxib. This work could help to identify a subgroup of BC patients who may benefit from celecoxib especially as COX2 immunostaining and PIK3CA mutation status could routinely be used as COX2 inhibitor sensitivity biomarkers. These results need to be validated in a phase II clinical trial.
Title: Robust 7-gene signature for recurrence prognosis of breast cancer validated in formalin-fixed paraffin-embedded samples

Chacolla RJ J, Trevino VM M, Scott SP P, Guzman EA A and Cardona S. Instituto Tecnologico y de Estudios Superiores de Monterrey, Monterrey, Nuevo Leon, Mexico and Hospital San Jose Tec de Monterrey, Monterrey, Nuevo Leon, Mexico.

Body: BACKGROUND: Breast cancer (BC) is a heterogeneous disease with diverse biological-molecular characteristics and clinical behaviors. Traditionally, prediction of recurrence in patients recently diagnosed is a big challenge for oncologists. In the last decade, advances in gene expression profiling technologies have improved the genetic knowledge of breast cancer by reporting several prognostic gene-signatures. However, there is still a need for covering the molecular heterogeneity of this cancer. Integration of public microarray data could be used to identify better and robust predictive gene-signatures. The aim of this study was to investigate prognostic genes that might function as biomarkers to differentiate among all heterogeneous BC patients, into those with high, medium or low recurrence risk.

METHODS: We collected nine public datasets, 1574 BC patients with median follow-up time of 10 years. The data were split into train-test, 50% each. Train was subject to univariate and multivariate analysis to generate a signature, which was validated in the test data. Seven genes were identified with high accuracy for recurrence prediction. This signature was validated in 40 formalin-fixed paraffin-embedded (FFPE) Mexican BC tissues with clinical follow up of 3 to 13 years by quantitative RT-PCR assays.

RESULTS: We identified a 7-gene signature which showed an accuracy prediction measured by concordance index of 65.62% and Log-rank test p-value: 1 x 10^-15. The validation of the gene-signature level expression in 40 FFPE samples in Kaplan Meier analysis showed prediction significance by concordance index of 65.25% and Log-Rank p-value: 0.0005.

CONCLUSIONS: This 7-gene signature may provide a powerful tool to guide the adequate treatment protocols in early-diagnosed breast cancer patients.
Expression of breast cancer stem cell marker as a predictor of prognosis and response to trastuzumab in HER2-positive breast cancer

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BACKGROUND: Breast cancer stem cells (BCSCs) have been suggested to have clinical implications for cancer therapeutics because of its proposed role in chemoresistance. The aim of this study was to investigate the impact of BCSC marker expression in clinical outcome and trastuzumab response HER2-positive breast cancer.

METHODS: We analyzed the clinical significance of BCSC marker (ALDH1 and CD44+/CD24-) expression in 242 HER2-positive primary breast cancers using immunohistochemistry. In addition, we examined the relationship between BCSC marker expression, trastuzumab response and clinical outcome in 447 primary, and 112 metastatic HER2-positive breast cancer patients treated by trastuzumab.

RESULTS: In the first set, ALDH1 positivity and CD44+/CD24- phenotype were detected in 10.0% and 29.9%, respectively. ALDH1 positivity and CD44+/CD24- phenotype correlated with hormone receptor negativity. In survival analyses, CD44+/CD24- phenotype, but not ALDH-1 expression, was found to be an independent prognostic factor for poor disease-free and overall survival of patients in entire group, and in subgroup not receiving trastuzumab. In HER2-positive primary breast cancer patients treated by adjuvant trastuzumab, CD44+/CD24- phenotype was also an independent poor prognostic factor for disease-free survival. However, ALDH1 positivity and CD44+/CD24- phenotype had no effect on trastuzumab response and survival in patients with metastatic HER2 positive breast cancer.

CONCLUSION: These results suggest that CD44+/CD24- phenotype can be used as a prognostic factor for clinical outcome and a predictive factor of trastuzumab response in patients with HER2-positive primary breast cancer.
Background: Receptor activator of NF-kB (RANK) pathway regulates bone remodeling and is involved in breast cancer (BC) progression. RANK over-expression in primary BC associates with poor prognosis and metastasis development. In patients with BC, RANK and RANK-ligand (RANKL) single nucleotide polymorphisms (SNPs) have also been associated with BC risk and bone metastasis (BM)-free survival, respectively. Here we analyze the association of five RANK missense SNPs with prognosis of patients with BM from BC.

Methods: Missense RANK SNPs (rs34945627, rs12721431, rs35184120, rs35993683, rs61751992) were genotyped in germline DNA from a retrospective cohort of 76 patients with BM from BC, under bisphosphonates; and a cohort of 80 healthy volunteers (samples from Biobanco-IMM, Lisbon, Portugal). Genotypic allelic frequencies were assessed using TaqMan assays (Applied Biosystems). SNP rs34945627 was analyzed with regard to clinicopathological characteristics, skeletal-related events (SRE), bone progression-free interval (BPFi), and overall survival (OS). Univariate association with clinicopathological characteristics was performed using Fisher's exact test and Wilcoxon rank-sum test; univariate differences between survival rates were tested for significance using the log-rank test, while multivariate analysis for survival was tested using Cox proportional hazards models. Results: SNP rs34945627 had a minor allele frequency of 11.84% (n=9) in BC patients versus 1.25% (n=1) in healthy individuals, whereas the remaining SNPs had a minor allele frequency of 2.60% (n=2) in BC patients. Therefore, for inferential analysis only SNP rs34945627 was considered. No differences between alleles were observed regarding clinicopathological characteristics, including treatment. Homozygous patients (CC) had increased OS when compared to heterozygous patients (CT) controlling for age at diagnosis, visceral involvement, radiographic pattern of BM and baseline urine NTX (adjusted HR 5.76, 95% CI 2.10-15.81; p<0.001). No association could be seen with regard to SREs (adjusted HR 1.43, 95% CI 0.46-4.46; p=0.54) or BPFi (adjusted HR 0.26, 95% CI 0.06-1.17; p=0.08).

Conclusions: RANK SNP rs34945627 seems to be a marker of poor prognosis in patients with BC and BM. Further studies are required to characterize the biological and clinical significance of this finding.
Title: A novel long-non coding RNA, TSA-LINC1, is associated with poor survival and cellular growth in breast cancer

Body: Background: Genomic analyses of cancer cells can help to identify novel cancer drivers in all malignancies. Long non-coding RNAs (LINC) are an emerging class of molecules that play an important role in the pathogenesis of breast cancer. Methods: In this study we used non-adherent growing tumor spheres ("mammospheres") as a model system to identify tumor-sphere associated (TSA) gene expression patterns. We used microarrays to profile different breast cancer cell lines and selected the most up/down-regulated differentially expressed genes by RT-PCR. Clinical correlations including survival analysis of 784 breast cancer patients and experimental evaluation of the biological function by were performed. Results: Among several TSA-genes, one novel not yet reported long non-coding RNA, (TSA-LINC1) was significantly up-regulated in mammospheres (up to 70 fold, p<0.05). In patient samples, TSA-LINC1 was significantly up-regulated in cancer tissue compared to normal breast tissue, and high expression was associated with poor survival (p<0.05). Knock-down of TSA-LINC1 in breast cancer cell lines led to significantly altered cellular growth (p<0.05). Conclusion: This novel long non-coding RNA is involved in breast cancer progression and might be useful as a prognostic marker in breast cancer patients.
Title: miR-135a is associated with a metastatic phenotype in invasive lobular carcinoma

Howe GA A, Zhao H, Daneshmand M, Clemons M, Robertson SJ J, Arnaout A and Addison CL L. Ottawa Hospital Research Institute, Ottawa, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada and University of Ottawa, Ottawa, ON, Canada.

Body: Invasive lobular carcinoma (ILC) is the second most common type of breast cancer. Classic type ILC is generally regarded as indolent in nature with its favourable biologic characteristics such as low grade, ER positivity and luminal A subtype. Despite this, patients with ILC can develop significant distant recurrence or metastases. Thus the ability to identify those patients at highest risk of recurrence or metastasis, and identification of novel therapies for ILC are urgently required. To this end, we recently profiled the miRNA expression in primary surgical ILC specimens from patients who went on to have metastatic disease compared to those who remained tumor free long term. As miRNA are stable in formalin fixed paraffin embedded tissues1, we speculated they could be robust biomarkers. RNA was isolated from laser capture microdissected ILC tumor epithelium, and subjected to miRNome analysis using a PCR-based amplification method. Many differentially expressed miRNAs were identified, and we initially focused further validation on those which had been previously linked to metastasis. One of these, miR-135a, was elevated in tumors from ILC patients who developed metastases compared to those that did not. We utilized two representative ILC cell lines which differ in their invasive ability, MDA-MB-134VI (non-invasive) and MDA-MB-330 (invasive), to test whether miR-135a regulated ILC invasion. We found that levels of miR-135a correlated with the invasive potential of ILC cell lines and was elevated in the invasive MDA-MB-330 cells compared to less invasive MDA-MB-134VI cells. We also found that decreasing miR-135a expression using specific hairpin inhibitors in MDA-MB-330 cells resulted in decreased cell invasion. As miR-135a has been shown to regulate invasion via targeting metastasis suppressor 1 (MTSS1) mRNA for degradation2, we examined whether MTSS1 levels were inversely associated with miR-135a levels in ILC cells. As predicted, in MDA-MB-330 cells where miR-135a levels were significantly higher, levels of MTSS1 were the lowest while MTSS1 levels were higher in parallel with decreased levels of miR-135a in the non-invasive MDA-MB-134VI cells. Overexpressing miR-135a using miRNA mimics in normal mammary epithelial cells (HMEC) where miR-135a is normally low, reduced levels of MTSS1 supporting suggestions it is a direct target of miR-135a. We also confirmed reduced mRNA levels of MTSS1 in surgical specimens from ILC patients who developed metastases compared to those that did not. Taken together, our results suggest that high levels of miR-135a, and low levels of MTSS1 may be useful prognostic information to assess risk of metastasis in ILC.

References
Title: Verification of the breast cancer progression-associated miRNA hsa-miR-199a-5p using NanoString® platform

Fehm T, Schultz S, Bartsch H, Petat-Dutter K, Kahlert S, Sotlar K, Niederacher D and Neubauer H. Heinrich-Heine-University Duesseldorf; Institute of Pathology, Ludwig-Maximilians-University Munich and Ludwig-Maximilians-University Munich.

Body: A fundamental and clinically important step during breast tumourigenesis is the transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC). Improved knowledge of this transition from pre-invasive to invasive breast cancer will pave the way for novel preventative and therapeutic strategies. We have previously reported on differential expression of the miRNA hsa-miR-199a-5p in 15 matched pairs of DCIS and IDC areas isolated by laser capture microdissection (LCM) from formalin fixed and paraffin embedded (FFPE) breast cancer tissues using Illumina miRNA BeadChip microarray platform. Differential expression of hsa-miR-199a-5p was validated by quantitative RT-PCR in additional independent DCIS/IDC sample pairs from 25 breast cancer patients. Knock down of hsa-miR-199a-5p in invasive MDA-MB-231 and TMX2-28 breast cancer cells using a specific inhibitor significantly reduced invasiveness by approx. 73% and 71%, respectively (P <0.01 and P<0.05).

Now we report on experiments to validate differential expression of hsa-miR-199a-5p using a new platform – NanoString® (nCounter® miRNA Expression Assay, NanoString Technologies®). The NanoString® System is an automated, digital detection and counting system which uses a novel barcoding technology to directly profile up to 800 miRNAs simultaneously from a single sample. Total RNA from 6 DCIS/IDC FFPE tumours was used for miRNA expression analysis. This analysis resulted in 10 differentially expressed miRNAs including hsa-miR-199a-5p which is upregulated in IDC (P <0.05). Besides hsa-mir-199a-5p, hsa-miR-222 is significantly differentially expressed between DCIS and IDC which could be found in both expression data sets (Illumina® and NanoString®).

In this project we identified candidate progression-associated miRNAs which are differentially expressed between DCIS and IDC. Hsa-miR-199a-5p was validated in an independent sample cohort and its expression was further verified using the new miRNA expression analysis platform NanoString®. Hsa-miR-199a5-p is influencing in vitro cell invasiveness and may therefore be a potential drugable regulator of tumour progression and invasion in breast cancer.
Title: Epigenetically altered microRNA mediated pathway dysregulation in ER negative breast cancer

Worsham MJ J, Chen KM M, Datta I, Stephen JK K, Chitale D and Divine G. Henry Ford Health System, Detroit, MI.

Body: Background: Micro RNAs (miRNA) are endogenous, small non-coding RNAs that control gene expression by directing their target mRNAs for degradation and/or posttranscriptional repression. Compared to mRNA signatures, miRNAs have better and stronger biomarker properties with 20 times more power in biomarker studies as compared to mRNAs (when comparing 20,000 mRNAs to ~1,000 miRNAs). Emerging evidence now supports the idea that DNA methylation is crucially involved in the dysregulation of miRNAs in cancer, representing a novel class of potential biomarkers for diagnosis, prediction of treatment, or prognosis. ER-negative breast cancer (BC) is an aggressive histological subtype with limited treatment options and very poor prognosis. Our long term objective is to derive a diagnostic, prognostic, and predictive ER-negative specific miRNA panel for detection of early cancer, recurrence/metastasis, and as potential therapeutic targets for better management of ER-negative BC.

Methods: The initial discovery step profiled 39 primary ER negative and 40 ER positive BC cases using the Illumina Infinium HumanMethylation450 BeadChip followed by a subanalysis focusing on 2249 miRNA CpGs assigned to 615 unique miRNAs. T-tests were used to compare the means of the M-values for the ER-positive and ER-negative groups. The t-test p-values were used to generate adaptive FDR (aFDR) levels and aFDRs of 0.05 or lower were considered to be statistically significant (Tier 1). Tier 1 CpGs were subsequently filtered to select only those with a mean beta ratio between ER positive and ER negative of under 0.5 or over 2.0 (Tier 2). The Tier 2 CpGs were further filtered to select only those with a mean beta difference of 0.2 or more (Tier 3). Because miRNAs perform their important functions via their targets, the targets of miRNAs were assessed for functional enrichment analysis in IPA for biologic involvement.

Results: Over half of the miRNA CpGs (1224/2249, 54%) were differentially methylated between ER negative and ER positive BC with significant aFDR levels. The 1224 CpGs at Tier 1 were associated with 379 miRNAs; the 24 and 2 CpGs for Tiers 2 and 3 with 22 and 2 miRNAs, respectively. The 22 miRNA genes were assigned to 4621 targets using online databases that predict miRNA targets. The degree of confidence that a target gene is associated with a miRNA is characterized in these databases as either "experimentally observed", or just as "high" (predicted). Of these 4621 targets, 87 were designated as experimentally observed and were examined in IPA. Top pathways and networks designated by miRNA targets included the cell cycle G1/S checkpoint regulation canonical pathway, and the cell-to-cell interaction/cancer networks among others. MiRNA targets in top pathways and networks were circled back to their respective miRNAs revealing cooperatively mediated pathway dysregulation of ER negative BC.

Conclusion: Aberrantly methylated miRNAs showed perturbation of biologically significant pathways and networks, suggesting that miRNAs mediate pathway dysregulation in a coordinated manner, strengthening the case for utility of miRNAs as viable biomarkers in ER negative BC. Support: Komen Foundation: KG110218.
Title: microRNA markers and risk of recurrence in women with ductal carcinoma in situ

Liu Y, Baker C, Wang X and Colditz G. Division of Public Health Sciences, Washington University School of Medicine, St. Louis, MO; Washington University School of Medicine, St. Louis, MO and Alvin J. Siteman Cancer Center at Barnes-Jewish Hospital and Washington University School of Medicine, St. Louis, MO.

Body: Background: Women with DCIS have increased risk for invasive breast cancer. Limited abilities to accurately identify aggressive DCIS have resulted in inappropriate treatment for many women with mammographically-detected DCIS. miRNAs play a critical role in tumorigenesis. It remains unknown if miRNAs are associated with recurrence and refine risk assessment for DCIS patients.

Methods: The study included 16 DCIS cases that developed ipsilateral recurrence (cases) and 32 DCIS cases that did not develop any type of recurrence (controls). Cases and controls were treated with breast conserving surgery and matched on age, race, and year of diagnosis. Expression of 96 cancer-related miRNAs was quantified using RT-PCR.

Results: Two miRNAs were differentially expressed between cases and controls (p<0.05). A combination of the two miRNA markers was significantly associated with recurrence risk (odd ratio=2.60 per 1-unit increase, 95% confidence interval=1.14-8.13), which was independent of family history of breast cancer, radiation and endocrine therapy. The miRNA-based score (c-statistic=0.73) performed better than individual miRNAs (c-statistic=0.64-0.69) in discriminating DCIS cases who did and did not develop recurrence.

Conclusion: miRNA expression in DCIS was associated with recurrence risk, which warrants further investigations to identify new miRNA markers by including more DCIS cases and analyzing all breast cancer-related miRNAs.
RS4646 polymorphism in aromatase gene is associated with efficacy of hormone therapy in early breast cancer


Purpose The aim was to verify the potential association between CYP19A1 genetic polymorphisms and clinical outcome of hormone therapy in hormone receptor- (HR-) positive early breast cancers.

Methods Genotyping for the CYP19A1 polymorphisms rs4646 (C/A) was performed on 287 women with HR-positive early breast cancer. Associations were evaluated between CYP19A1 rs4646 genotypes and disease-free survival (DFS) as well as 5-year DFS rate.

Results Totally, women with the minor allele (AA or AC) have an improved DFS, 5-years DFS rate when compared with those carrying the homozygous common allele (CC) (62.7 months versus 55.6 months; 50.4% versus 45.0%; P = 0.04). These difference were further demonstrated by a multivariate analysis (HR, 0.681; P = 0.011). In premenopausal women, AA genotype was associated with prolonged DFS, 5-years DFS rate (AA versus CC or AC: 98.2 months versus 56.2 months; 66.0% versus 45.2%; P = 0.024). In addition, women with the A allele have an improved DFS, 5-years DFS rate when compared with those carrying the homozygous C allele (62.7 months versus 55.6 months; 51.1% versus 42.7%; P = 0.033). These findings were further confirmed by the Cox regression (HR, 0.336, 0.670; P = 0.017, 0.019). In postmenopausal women, rs4646 genotypes were significantly associated with DFS and 5-years DFS rate (AA versus AC versus CC: 32.7 months versus not reached versus 56.3 months; 0% versus 56.7% versus 45.9%; P = 0.011). Women carrying AA variant have a poorer DFS, 5-years DFS rate than those with CC or AC genotypes (32.7 months versus 70.6 months; 0% versus 52.1%; P = 0.005). Furthermore, being adjusted by the patients features in multivariate analyses, AA genotype remained an independent prognostic factor for DFS (HR, 3.614; P = 0.013).

Conclusions The homozygous minor allele (AA) of CYP19A1 rs4646 is significantly associated with improved clinical outcome of hormone therapy in premenopausal HR-positive early breast cancer patients, but with a worse outcome for postmenopausal women. Further validation of CYP19A1 rs4646 polymorphism as a predictive tool for hormone therapy in a larger independent cohort of HR-positive early breast cancer patients is warranted.
**Title:** Breast cancer recurrence risk: A role for the progesterone receptor isoforms


**Body:** Progesterone receptor (PR) is currently used as a surrogate marker for functional estrogen receptor activity in breast cancer. Two PR isoforms have been described, PRB and PRA. PRA (94 kDa) is a truncated protein that lacks the first 164 amino acids of the NH2 terminal of PRB (115 kDa), making difficult the development of antibodies that discriminate PRA from PRB by standard immunohistochemistry (IHC). There are few studies describing the expression of PR isoforms in breast cancers. While there is a general consensus that PRA is the prevailing isoform expressed in breast cancer tissues as compared with normal mammary gland, there is controversial data regarding the association between their deregulated expression and endocrine response or aggressiveness. We are currently studying the expression of the PR isoform ratio in breast cancer samples obtained during surgical resection in order to test their antiprogestin responsiveness in tissue cultures. The study has been approved by the IRB (2012-028). Selected samples were studied by RNAseq and the data was used to analyze the PAM50 genes to predict risk of recurrence, and interestingly, almost all the genes related to proliferation were up-regulated in samples categorized as having higher levels of PRB than PRA, being also these patients those with a high risk of recurrence (May and Rojas et al., ASCO Annual Meeting, poster#11016, 2015). These observations are in agreement with data obtained in hormone resistant breast cancer xenografts with higher levels of PRB than PRA (Wargon and Riggio et al, International Journal of Cancer: 2680, 2015).

The aim of this study is to evaluate a possible correlation between the PR isoform ratio, proliferation as evaluated by Ki67 or HER2 expression, and clinical outcome in selected breast cancer samples. Ki67 was evaluated by IHC using standard protocols in 80 PR+ samples. The PRA/PRB ratio was also evaluated in nuclear extracts, performed from frozen tissue, from the same patients, by Western Blot. A negative correlation was observed between the Ki67 score and the log2 value of the PRA/PRB ratio (Spearman R:-0.3418; p< 0.0029). Samples were considered PRA+ if PRA/PRB ≥ 1.2 and PRB+, if PRA/PRB ≤ 0.83. Seven out of 62 PRA+ (11.29%), and 7 out of 35 (20%) of PRB+ samples were HER2+. The differences between both groups, although not significant, correlate directly with the Ki67 evaluation. The results of this ongoing project lend support to the hypothesis that the ratio of PRA/PRB is associated with prognosis and highlight the role of PR as key players regulating breast cancer growth.
PhosphohistoneH3 as a prognostic marker in breast cancer: High expression is associated with younger age, triple negative subtype, and disease specific survival

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Body: BACKGROUND PhosphohistoneH3 (PPH3) is an emerging marker in breast cancer and has been linked to both patient survival and age. Phosphorylation of HistoneH3 is an important step during the cell cycle leading to proper compaction of the chromatin during late G2 and early mitosis. Here we assessed the use of PPH3 as a prognostic marker within a group of invasive breast cancers in the Clinical Breast Care Project (CBCP).

METHODS CBCP participants and their samples were collected following IRB-approved, HIPAA-compliant protocols. Samples from 157 CBCP patients were selected for tissue whole section immunohistochemistry (IHC), using antibodies to PPH3, ER, PR, Ki67, and Her2. For each sample, staining of PPH3 was assessed across 5 high powered microscope fields and was considered positive if there was an average >2 stained cells per field. ER and PR were considered positive when there was >5% nuclear staining, and Ki67 was positive when there was >15% nuclear staining. Her2 was considered positive with an IHC score of 3+ or 2+ with a FISH score above 2.2. The samples were subtyped as Luminal A (LA: ER+/HER2-/Ki67-), Luminal B1 (LB1: ER+/HER2+/Ki67), Luminal B2 (LB2: ER+/HER2+), Her2+ (ER-/PR-/HER2+), and Triple Negative (TN: ER-/PR-/HER2-). PPH3 was tested for associations with age and subtype using a stratified univariate Wilcoxon rank-sum analysis and a multivariate analysis controlling for subtype. To test the efficacy of PPH3 as a prognostic marker, Kaplan-Meier curves for disease specific survival were analyzed and the cox proportional hazard regression model was calculated. Further analysis addressing population demographics and additional cancer characteristics is ongoing.

RESULTS Wilcoxon analysis revealed an association between higher PPH3 levels and younger age (P=.0038). Subtype was also found to be associated with PPH3, with the TN subtype 6.26 times more likely to have higher PPH3 expression than LA (P=.005). The association with age was confirmed by repeating the analysis and stratifying into non-TN subtypes (P=.05) and TN only subtype (P=.017). Non-TN subtypes positive for PPH3 expression had median age of 53.18 at diagnosis and 63.29 for negative PPH3 expression; TN subtypes that were positive for PPH3 had a median age of 50.44 and 72.9 for negative PPH3. Multivariate analysis with age and subtype as the variables also supported these results (age P=.017; TN vs LA P=.022). Disease specific survival analysis showed that a shorter survival time was associated with positive PPH3 protein levels (P=0.03; hazard ratio=6.97).

CONCLUSIONS High expression of PPH3 is associated with a younger age, poorer survival rate, and the TN subtype. These results corroborate the use of PPH3 as a prognostic marker for breast cancer patients.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S. Government.
**Title:** BRCAness and PD-L1 expression of basal-like and not basal-like triple negative breast cancer

Mori H, Kubo M, Yamada M, Kai M, Osako T, Nishimura R, Arima N, Okido M, Kuroki S, Oda Y and Nakamura M. Graduate School of Medical Sciences, Kyushu University, Fukuoka City, Japan; Graduate School of Medical Sciences, Kyushu University, Fukuoka City, Japan; Breast Center, Kumamoto Shinto General Hospital, Kumamoto City, Japan; Kumamoto City Hospital, Kumamoto City, Japan; Hamanomachi Hospital, Fukuoka City, Japan and Kuroki Breast Clinic, Fukuoka City, Japan.

**Body:**

Background: Triple Negative Breast Cancer (TNBC) subtype occurs in approximately 20% of all patients with breast cancer and is associated with rapid growth, early metastasis and poor prognosis compared with other subtypes. TNBCs are a heterogeneous disease entity and further subclassification is needed, but still ongoing. In this study, we assessed BRCAness, defined as shared characteristics between sporadic and BRCA1-mutated tumors, in a cohort of basal-like and non-basal-like TNBCs.

Patients and Methods: DNA was isolated from formalin-fixed paraffin-embedded tumor tissues and BRCAness status was analyzed in 262 patients with primary TNBCs resected at our three hospitals between 2004 and 2014. Classification of BRCAness was performed by using Multiple Ligation-dependent Probe Amplification (MLPA) with the probemix P376 BRCA1ness by MRC (Amsterdam, Holland). The tumor subtypes were routinely determined immunohistochemically by using resected specimens. Basal-like phenotype was defined as being positive for Epidermal Growth Factor Receptor (EGFR) and/or Cytokeratin 5/6 (CK5/6). Moreover, TNBCs were stained and analyzed for programmed cell death ligand-1 (PD-L1) expression as a target of new immune therapies.

Results: Of 262 TNBCs, 232 tumors (88.5%) was a basal-like phenotype. The results of MLPA assay showed that 159 (68.5%) of 232 tumors had a BRCAness profile. Patients with basal-like BRCAness tumors were younger than patients with basal-like non-BRCAness tumors (p<0.0001). There was no significant difference between the two groups regarding pathological stage. The basal-like BRCAness group had shorter relapse-free survival (RFS) and overall survival (OS) than the basal-like non-BRCAness group (p=0.028 and p=0.13, respectively), and anthracycline-based regimens provided greater benefit to the basal-like BRCAness group significantly (p=0.01 in RFS and p=0.007 in OS). PD-L1 was expressed in 71 (44.7%) of 159 basal-like TNBCs with BRCAness.

Conclusion: We reported the majority of basal-like TNBCs showed a BRCAness profile and PD-L1 expressed in approximately 50% of BRCAness tumors. It is known that about 30% of BRCAness tumors are BRCA1-mutated tumors. Those biomarkers are essential for subclassification of TNBCs and may offer not only platinum-based chemotherapy but also novel therapies, such as immune-targeted therapies of PD-1/PD-L1 inhibitors and PARP inhibitors, to patients with basal-like TNBCs with BRCAness.
Title: A monoclonal antibody with exceptional specificity across major breast cancer subtypes

Das Roy L, Zhou R, Dillon L, Moore LJ J, Puri R, Marks JR R, Lyerly HK Kim and Mukherjee P. OncoTab Inc, Charlotte, NC; University of North Carolina at Charlotte, Charlotte, NC; OncoTab Inc, Charlotte, NC; University of North Carolina at Charlotte, Charlotte, NC; OncoTab Inc, Charlotte, NC; Duke University School of Medicine, Durham, NC; Duke University School of Medicine, Durham, NC and OncoTab Inc, Charlotte, NC.

Body: Background: Breast cancer (BC) remains the second leading cause of cancer-related deaths for women in the United States and is recognized to be a heterogeneous disease. Advances in technologies such as whole genome sequencing are leading the way to precision medicine and the leading researchers are envisioning personalized therapies in the not too distant future. However, given the diversity of cancer cell populations, that remains a challenging task at best. The tumor form of MUC1 (designated tMUC1), a transmembrane glycoprotein, is aberrantly glycosylated and overexpressed in $\sim95\%$ of BC. We have developed an antibody (TAB004) that specifically recognizes tMUC1 across all major subtypes of BC and importantly does not recognize normal breast epithelia. This is a significant development in light of the challenges faced in treating triple negative BC.

Methods: A panel of thirty BC cell lines was obtained from ATCC. The following techniques were used to assess the specificity of TAB 004 to the major subtypes based on ER, PR and Her2 expression: 1) Flow cytometry to quantify membrane bound expression of tMUC1 using Cy7-conjugated TAB004; 2) Western blotting to detect molecular weight patterns of tMUC1 in whole cell lysate; 3) A TAB004 based GMP-grade ELISA kit to measure shed tMUC1 in the supernatant and 4) In vivo imaging of tumors in mice using TAB 004 conjugated to Indocyanine Green (ICG). Specificity and sensitivity was further confirmed using primary human serum and tissue samples from all major BC subtypes obtained from bio-repositories at Duke University Cancer Center, Fox Chase Cancer Center and Carolinas Health Care System. Shed tMUC1 in serum samples were tested using the TAB 004 ELISA kit and tissue sections were analyzed using immunohistochemical (IHC) staining with TAB 004 conjugated to HRP.

Results: 1) Flow cytometry data shows that TAB 004 recognized tMUC1 on all major BC subtypes: 25 out of 30 BC cell lines tested had higher expression than a normal epithelial breast cell line; 2) Western blotting also detected tMUC1 on all BC subtypes with distinct molecular weight patterns; 3) ELISA showed high levels of shed tMUC1 in the supernatant and 4) In vivo imaging shows clear localization of TAB004-ICG to the tumors expressing tMUC1. Primary human breast cancer patient data shows that shed tMUC1 was detected in the serum obtained from all major BC subtypes and showed statistically significant differentiation from normal/benign. IHC results show strong tMUC1 expression in malignant tissue with excellent differentiation from adjacent normal tissue.

Conclusion: TAB004 antibody's extraordinary specificity across major BC subtypes has been confirmed with flow cytometry, western blotting, ELISA and Immunohistochemistry. A number of clinical applications are under development: (a) An ELISA test as a supplement to mammography for the early detection of BC in women with dense breasts; (b) serum monitoring during treatment and to detect disease recurrence; and, (c) targeted antibody-drug/antibody-imaging agent based therapies and imaging modalities particularly for triple negative BC.
Wnt5a expression is associated with high-grade malignancy in ER-positive breast cancer


Background: Wnt5a is a representative ligand that activates the β-catenin-independent pathways. The purpose of our study is to elucidate the implication of Wnt5a expression in breast cancer.

Materials and methods: One hundred seventy eight breast cancer patients (mean age ± SD: 59.6 ± 13.2 years) with clinical Stage I∼III between January 2011 and February 2014, were prospectively evaluated. Patients who underwent operation without neoadjuvant therapy were enrolled to this study. The immunohistochemical analyses of Wnt5a protein was performed to evaluate relationships between Wnt5a expression and clinicopathological factors. MCF7 cells that stably express Wnt5a were generated and used for cDNA microarray analyses to investigate Wnt5a-dependent gene expression.

Results: Wnt5a expression was significantly more frequent when estrogen receptor (ER) was present, 68/153 (44%) than when ER was absent, 1/25 (4%) (p<0.001). Wnt5a expression was also related with progesterone receptor (PgR) (P<0.001), but not with HER2 status (P=0.496). In ER-positive breast cancer, a significant interaction between expression of Wnt5a with lymph node metastasis (P<0.001), nuclear grade (P=0.004), lymphatic invasion (P=0.002), vessel invasion (P=0.050), and pStage (P<0.001). Microarray analyses identified several genes induced by Wnt5a (>3.0 fold), involving activated leukocyte cell adhesion molecule (ALCAM). ALCAM is known to be related with apoptosis, invasion and prognosis of breast cancer. Wnt5a expression levels correlated with those of ALCAM in ER-positive tumor samples from patients by immunohistochemical analyses (P<0.001).

Relationship between Wnt5a expression and clinicopathological feature

<table>
<thead>
<tr>
<th>Clinicopathological feature</th>
<th>total (n=153)</th>
<th>Wnt5a expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n=85)</td>
<td>Positive (n=68)</td>
<td></td>
</tr>
<tr>
<td>Age (median, range)</td>
<td>63, 35-86</td>
<td>57.5, 34-87</td>
<td>0.065</td>
</tr>
<tr>
<td>Age, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤45</td>
<td>28 (18)</td>
<td>13 (46)</td>
<td></td>
</tr>
<tr>
<td>&gt;45</td>
<td>125 (82)</td>
<td>72 (58)</td>
<td>0.282</td>
</tr>
<tr>
<td>Menopausal status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>58 (38)</td>
<td>27 (47)</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>95 (62)</td>
<td>58 (61)</td>
<td>0.080</td>
</tr>
<tr>
<td>Tumor size, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1 ≤20mm</td>
<td>104 (44)</td>
<td>63 (61)</td>
<td></td>
</tr>
<tr>
<td>pT2/pT3 &gt;20mm</td>
<td>49 (56)</td>
<td>22 (45)</td>
<td>0.069</td>
</tr>
<tr>
<td>Lymph node metastasis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>103 (67)</td>
<td>72 (70)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50 (33)</td>
<td>13 (26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nuclear grade, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>85 (56)</td>
<td>56 (66)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>68 (44)</td>
<td>29 (43)</td>
<td>0.004</td>
</tr>
<tr>
<td>Lymphatic invasion, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>101 (66)</td>
<td>65 (64)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>52 (34)</td>
<td>20 (38)</td>
<td>0.002</td>
</tr>
<tr>
<td>Vessel invasion, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions: Wnt5a express in ER-positive breast cancer and are associated with high-grade malignancy. Wnt5a could be a prognostic factor of ER-positive breast cancer. These results have implications that Wnt5a may become a preoperative and postoperative assessment tool for tumor malignancy grade and a potential therapeutic target except endocrine therapy in ER-positive breast cancer. In future studies, further research on Wnt5a are required to develop a novel treatment for more improved outcomes in a great variety of breast cancer.
Title: Characterization of circulating myeloid derived suppressor cells and cytokines in patients undergoing neo-adjuvant chemotherapy for breast cancer

Body: Myeloid derived suppressor cells (MDSC) are immature immune cells that expand in patients (pts) with cancer and suppress anti-tumor immunity. MDSC are also known to support angiogenesis. Higher circulating MDSC levels are seen in patients with greater tumor burden. Therefore, circulating MDSC levels could be affected by chemotherapy and could correlate with response. In this prospective pilot trial, peripheral blood (PB) levels of granulocytic (G-MDSC) and monocytic (M-MDSC) MDSC were measured in pts with operable breast cancer (BC) treated with neo-adjuvant chemotherapy (NAC) to study their association with pathologic complete response. It was hypothesized that MDSC % would show an association with complete pathologic response (pCR). The association of 10 different cytokine levels (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, TNF-α) with pCR was also explored. Linear mixed models tested the associations between MDSC % or cytokines across time points with pCR. Levels of MDSC were measured by flow cytometry as a % of PB mononuclear cells prior to cycle (C) 1 and 2 of doxorubicin and cyclophosphamide (AC) and 1st and last administration of paclitaxel (T) or T and anti-HER2 therapy (in HER2+ pts). For other regimens, MDSC were measured prior to 1st, 2nd and last cycle. MDSC were defined as HLA-DR-, CD11b+, CD33+ cells with G-MDSC and M-MDSC cells expressing CD15 and CD14, respectively. Plasma cytokine levels were measured using a multiplex assay (Bio-Rad). Of 24 enrolled pts, 1, 20 and 3 had clinical stage I, II, IIIA, respectively. Median age was 48 (range 32-70). 11, 6 and 7 pts were triple negative (TN), HER2+ and hormone receptor (HR)+, respectively. PCR rate was 45.8% (46%, 50%, 43%, 20% for TN, HER2+, HR+ and >10% HR+ pts). Rate of residual cancer burden (RCB) class 0-1 was 58.3% (63.6%, 50%, 57.1%, 40% in TN, HER2+, HR+ and >10% HR+ pts). Mean M-MDSC % were <1 at all time points. Mean G-MDSC % and 95% confidence intervals (CI) were 0.88 (0.23-1.54), 5.07 (2.45-7.69), 9.32(4.02-14.61) and 1.97 (0.53-3.41) at times 1-4. The increase in MDSC by C1 of T was significant (p<0.0001) in all BC types. Baseline G-MDSC % did not differ in pts with or without pCR. G-MDSC levels at the last time point were also not statistically different but were numerically slightly lower in pts with pCR (1.15; 95%CI 0.14-2.16) versus pts with no pCR (2.71; 95%CI 0-5.47). Levels of all 10 cytokines were measurable in pts throughout NAC. The mean levels of IL-1β, IL-2, IL-4, IL-13 and IFN-γ peaked by C1 of T, while levels of IL-5, IL-6, IL-10, IL-12 and TNF-α were the highest at draw 1 and decreased during NAC. This pilot study confirmed feasibility of measuring circulating MDSC and cytokines in breast cancer pts receiving neo-adjuvant chemotherapy. The results showed that G-MDSC % increase during AC and then decrease during T and that a mixture of Th1 and Th2 cytokines peak during treatment. Levels of MDSC and cytokines did not significantly differ between pts with or without a pCR. However, a larger study with greater power to detect smaller differences and evaluate association between MDSC levels and pCR in different BC subtypes is needed.
**Title:** Comprehensive multiplatform molecular profiling identifies potentially targetable biomarkers in malignant phyllodes tumors of the breast

Gatalica Z, Vranic S, Ghazalpour A, Xiu J, Ocal I, McGill J, Bender R, Discianno E, Sanati S, Reddy S and Pockaj B. Caris Life Sciences, Phoenix, AZ; Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina; Mayo Clinic Arizona, Phoenix, AZ; Miraca Life Sciences, Phoenix, AZ and Washington University School of Medicine, Saint Louis, MO.

**Body:** Introduction: Malignant phyllodes tumors are rare breast malignancies (0.1% of all breast tumors) with limited effective treatment options for recurrent and metastatic disease. Recent trials indicated a potential for anti-angiogenic therapy in soft tissue sarcomas, which led us to investigate these pathways.

**Materials and Methods:** Thirty-five malignant phyllodes tumors (including two cases with matched primary and metastatic tumors) were profiled using gene sequencing (Next-generation and Sanger), gene copy number analysis (in-situ hybridization), whole genome RNA expression, and protein expression (immunohistochemical assay).

**Results:** RNA microarray assay showed consistent over-expression of genes involved in angiogenesis including VEGFA, Angiopoietin2, VCAM1, PDGFRA, PTTG1, and CYP3A5 in all cases analyzed (n=5). No mutations in KDR (VEGFR2) were detected (0/26). EGFR protein overexpression was observed in 25/26 (96%) of cases with amplification of the EGFR gene in 8 cases (33%). EGFR gene mutations were identified in 2 cases (8%) including one case with presumed pathogenic V774M mutation and one case with EGFRvIII mutation. The most common mutations included those of TP53 (50%) and PIK3CA (15%) while other mutations (BRCA1, BRCA2, RET, CDH1, MLH1, ATM) were rare affecting single phyllodes cases. Two cases with matched primary and metastatic cancers harbored the same mutations in both sites (PIK3CA/KRAS and RB1 gene mutations, respectively).

**Conclusions:** Comprehensive multiplatform profiling approach to phyllodes tumors identifies various molecular alterations of which some are potentially actionable. Our data suggests that anti-angiogenic therapy may also be effective in patients with malignant phyllodes tumor. Evaluation of EGFR pathway discovered consistent protein over-expression but rare activating mutations, which necessitates refinement in patient selection targeting these pathways.
Title: Plasma DNA as a surrogate for tumor biopsy to identify genetic alterations in patients with metastatic breast cancer


Body: Precision medicine requires that a patient's tumor be accurately genotyped to identify a potentially effective targeted therapy. However, genotyping a tumor in patients with oligometastatic disease is complicated by the potential for intratumor and intertumor heterogeneity, and the requirement for sufficient tumor tissue obtained by invasive biopsy for genetic profiling. We sought to determine whether circulating tumor DNA in plasma provides a surrogate for solid tumor biopsy, and captures the genetic heterogeneity of tumors in patients with metastatic breast cancer. We hypothesized that genetic mutations detected in plasma DNA are reflective of the genetic mutations present in all tumors within a patient.

Eight patients with advanced/metastatic breast cancer have thus far been enrolled in an ongoing clinical study (NCT01836640). Tumor specimens from two (n=4) or three (n=4) tumor sites and blood were obtained with one month. Blood was separated into plasma and buffy coat fractions. DNA extracted from tissue, buffy coat, and plasma samples was used for massively parallel DNA sequencing using the Ion Proton platform with a custom TargetSeq capture probe set covering all exons of 196 genes (4.1 Mb). All tumor and buffy coat samples, and plasma samples from three patients have thus far been analyzed. Tumor mutations were identified by comparison to buffy coat DNA sequences. We achieved sequencing coverage of ∼100-fold for tumor and buffy coat DNA samples, and ∼1,000-fold for plasma DNA samples. In Patient #1, we obtained 14 tumor nodules from a mastectomy specimen and used 3 nodules for DNA sequencing; Among the 73 point mutations detected in DNA from at least one tumor nodule, 29 mutations (40%) were detected in plasma DNA, and 10 mutations were found in plasma but not in tumors. In Patient #5, we analyzed bilateral breast tumors and a brain metastasis; among 151 mutations detected in at least one tumor, 80 (53%) were found in plasma, and an additional 18 mutations were found in plasma but not tumors; mutations specific to the brain tumor were less likely to be found in plasma; interestingly, the bilateral breast tumors showed genetic and histologic similarity, and so were likely derived from a single clone. Patient #6 had only one lung metastasis evaluable by DNA sequencing; 64/125 (51%) tumor-derived mutations were detected in plasma, and an additional 26 mutations were found in plasma but not the tumor.

Preliminary Results

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Tumor</th>
<th>Plasma (Plasma only)</th>
<th>Total</th>
<th>Plasma concordance with tumor</th>
<th>Plasma concordance with total</th>
<th>Tumor concordance with total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient #1</td>
<td>73</td>
<td>29 (10)</td>
<td>83</td>
<td>39.7%</td>
<td>46.9%</td>
<td>87.9%</td>
</tr>
<tr>
<td>Patient #5</td>
<td>151</td>
<td>80 (18)</td>
<td>169</td>
<td>52.9%</td>
<td>57.9%</td>
<td>89.3%</td>
</tr>
<tr>
<td>Patient #6</td>
<td>125</td>
<td>64 (26)</td>
<td>151</td>
<td>51.2%</td>
<td>59.6%</td>
<td>82.8%</td>
</tr>
</tbody>
</table>

These data suggest that, although challenging to get multiple biopsies for comparison, plasma is a promising surrogate for solid tumor biopsy to identify potentially targetable mutations. However, the ability of plasma DNA to genetically reflect all tumors in a patient with oligometastatic disease remains to be clarified through further analysis.
Title: Dual expression of aquaporin 3 and 5 in patients with early breast cancer

Lee SJ, Chae YS, Kwon TJ, Chung JH, Lee J, Jung JH, Kim WW, Park HY, Jeong JY, Park S-H and Park S. Kyungpook National University Medical Center, Daegu, Korea; Kyungpook National University Medical Center; Kyungpook National University Medical Center and Kyungpook National University Medical Center.

Body: Background: We reported separately that AQP5 or AQP3 expression in tumor tissue may predict survival after surgery for the specific types of early breast cancer (EBC). However, there is no definitive evidence for the role of dual expression of the two AQPs. Therefore, the current study focused the association and its prognostic impact of their tumoral expressions in the same patients.

Patients and Methods: AQP3 and AQP5 expressions were investigated on the basis of the immunohistochemistry of tissue microarray specimens from 447 EBC patients who underwent surgery between 2003 and 2008 as described in previous studies. Patients were divided into 4 subgroups based on AQP3 and AQP5 expressions: group1 for (-/-), group2 for (-/+), group 3 for (+/-), and group 4 for (+/+), respectively.

Results: Among 477 patients, the number of patients for each group was as follows: group 1 (n=193, 43.2%), group 2 (n=74, 16.6%), group3 (n=110, 24.6%) and group 4 (n=70, 15.7%), respectively. In the current study a positive correlation was identified between AQP3 and 5 expressions (P=0.017 by a χ²- test) in particular for HER2- overexpressing and ER-positive tumors (P=0.009 and 0.044, respectively). Multivariate survival analysis showed that dual expression of AQP3 and AQP5 was a negative prognostic factor for relapse-free or distant disease-free survival for patients with HER2-overexpressing EBC (HR=3.107 and 3.683; P=0.043 and 0.027, respectively), statistically more prominent compared in case with AQP3 expression alone (HR=3.137 and 2.784; P=0.036 and 0.070, respectively).

Conclusion: Dual expression of AQP3 and AQP5 in tumor tissue may be considered as a potential prognostic marker in patients with HER2-overexpressing EBC after curative surgery.
Title: Quantitative immuno-fluorescent evaluation of Her2 expression levels in a prospectively collected cohort of breast cancer cases: Comparison to conventional IHC scoring and FISH

Neumeister VM M, Yan SS S, McGuire JA A, Carvajal DE E, Prasad ML L and Rimm DL L. Yale University, School of Medicine, New Haven, CT.

Body: Background: According to the 2013 guidelines breast cancers are defined as Her2 positive if there is evidence of protein expression in at least 10% of tumor cells by IHC and/or gene amplification by FISH. Nevertheless, there are still IHC 2+ and FISH equivocal breast cancers resulting in repeat testing. It is also known that not all Her2 positive breast cancers respond to Trastuzumab, while up to 8% of Her2 "negative" classified patients benefit from Her2 targeting regimens. Toward the goal of generating a more accurate test, we report in situ quantification of Her2 protein levels on a prospectively collected cohort of breast cancers and comparison to conventional IHC and FISH evaluation.

Materials and Methods: A prospectively designed study was initiated at Yale University, comparing quantitative, in situ measurement of Her2 protein levels with conventional IHC and FISH evaluation. All breast cancer specimens were analyzed by IHC and FISH in our routine clinical laboratory, read and signed out by the breast pathologists. Serial sections were then stained and quantified for Her2 expression levels using the AQUA method of quantitative immunofluorescence (QIF). Data for all assays were obtained on 120 samples over a period of 6 months. Staining was performed using the DAKO Herce test and the Epitomics EP3 Her2 antibody for IHC, the DAKO rabbit polyclonal antibody for QIF. The 30 highest cases were then retested for QIF and IHC using the Biocare c-erbB-2 (CB11) antibody. Each staining run included an index tissue microarray (TMA) consisting of 80 cases, cell lines and normal tissue for quality control, assay reproducibility and threshold definition of AQUA scores correlated to HER2 overexpression/amplification.

Results: Out of 120 specimens analyzed for HER2, 13 were diagnosed as IHC 2+/3+, FISH amplified, 1 case had an equivocal score, 14 cases were IHC 2+/non amplified, 2 cases IHC 1+/FISH amplified and 89 specimens IHC 0/1+ non amplified. The continuous AQUA scores for Her2 expression of the samples significantly correlate with traditional clinical Her2 scoring. However, 5 IHC 0/1+, non amplified cases revealed high AQUA scores in the range of HER2 overexpression/amplification. Repeat testing of these by both QIF and IHC showed reproducibility of the results. AQUA scores of one IHC 3+/amplified sample were lower than the threshold of HER2 overexpression/amplification.

Conclusions: QIF measurement of HER2 protein levels in a prospectively collected cohort of 120 breast cancer specimens reveals significant association between continuous HER2 protein levels and the ordinal conventional scoring system. However, five discordant cases that were above the threshold for HER2 protein by QIF, were classified as negative by conventional methods. Given the accuracy and reproducibility of the QIF test, it raises the possibility that some of these patients might benefit from HER2 targeted therapy. In summary, while continuous scoring of HER2 protein correlates well with conventional methods, it identifies a subset of patients that are discordant with current methods. Further comparative studies in a patient cohort with response to targeted therapy need to be evaluated.
Gene expression signatures of microcalcifications among Taiwanese breast cancers

Huang C-C, Huang C-S, Tu S-H and Tsai M-L. Cathay General Hospital, Taipei City, Taiwan; Fu-Jen Catholic University, New Taipei City, Taiwan and Taipei Medical University, Taipei City, Taiwan.

Introduction: Microcalcification is one of the most common radiological and pathological features of breast ductal carcinoma in situ (DCIS), and to a lesser extent, invasive breast cancer. In current study, we evaluated the transcriptional profiles associated with the phenomenon of ectopic mammary mineralization and a gene expression signature is derived.

Materials and methods: A total of 109 consecutive breast invasive cancers were prospectively collected and assayed with Affymetrix Human Genome U133 Plus 2.0 microarrays. The presence of microcalcification was confirmed by histopathological examinations as well as reviews of pre-operative mammography. The associations of gene expression profiles with microcalcifications and relevant clinical features such as DCIS including both comedo/high-grade and non-comedo subtypes were tested.

Results: Microcalcifications were presented in 84 (80%) of the study population as confirmed by pathological examination. Of these 84 patients, 81 (96%) were grown with coexistent DCIS microscopically, while only 8 (38%) of the 21 patients without concurrent microcalcifications, the invasive tumors were accompanied with DCIS (Chi-square test, P<0.001). In addition, high-grade (comedo) type DCIS were presented in 44 (54%) of the 81 cancers with microcalcifications whereas only 15% (n=2) of tumors with DCIS but without microcalcification were of high-grade (comedo) type. There were 69 genes differentially expressed between breast cancers with and without microcalcifications (nominal P<0.001 with 10,000 random permutations), and 11 were associated with high-grade (comedo) type DCIS including APOD, CCDC183, SLMO1, SLC6A5, FMO1, QPRT and CES4A. The enriched Gene Ontology categories encompasses glycosaminoglycan, aminoglycan metabolic processes, Golgi apparatus cellular component and protein ubiquitination, indicating an active secretory process. The intersect (18 probesets) of microcalcification and DCIS-associated genes provided the best predictive accuracy of 82% with Bayesian compound covariate predictor.

Performance of compound covariate predictor classifier:

<table>
<thead>
<tr>
<th>Class</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>With microcalcification</td>
<td>0.826</td>
<td>0.217</td>
<td>0.798</td>
<td>0.25</td>
</tr>
<tr>
<td>Without microcalcification</td>
<td>0.217</td>
<td>0.826</td>
<td>0.25</td>
<td>0.798</td>
</tr>
</tbody>
</table>

Compared with mammography alone, the diagnostic accuracy of gene expression-based signature is much improved (cross-validated ROC AUC: 0.738).

Discussion: Our study suggested that mammary microcalcification is not only the earlier detectable radiological finding for disease screening but the phenomenon itself resulted from distinct biological processes that constituted the molecular heterogeneity of human breast cancers. Further studies to evaluate the prognostic significance of microcalcifications are warranted.
CD163 expression is associated with young age, triple negative subtype, and poor outcome in breast cancer


**BODY**

**BACKGROUND** CD163 is a scavenger receptor specifically expressed by cells of monocyte/macrophage lineage. It is a biomarker in many clinical conditions, including coronary artery disease, anti-inflammatory response, and cancers. In breast cancer, high numbers of CD163-positive macrophages correlated with unfavorable outcome. Expression of CD163 in breast cancer was also related to early distant recurrence and poor survival. In this study we evaluated whether CD163 expression was associated with aggressive breast cancers from patients enrolled in the Clinical Breast Care Project (CBCP).

**METHODS** Patients were enrolled into the CBCP following IRB-approved, HIPAA-compliant protocols. The study focused on 129 invasive breast cancer samples with CD163 immunohistochemistry (IHC) results. Expression of CD163, ER, PR, HER2, and Ki67 were assayed by IHC. CD163 was positive if IHC>0. ER and PR were positive if there was >5% nuclear staining. HER2 was negative if IHC=0 or 1+ and positive if IHC=3+. For HER2 IHC=2+, HER2 was negative if FISH was <1.8 and positive if FISH was >2.2. Ki67 was positive if there was ≥15% nuclear staining. For subtyping, Luminal A (LA) was ER+/HER2-/Ki67-, Luminal B1 (LB1) was ER+/HER2-/Ki67+, Luminal B2 (LB2) was ER+/HER2+, Her2+ was ER-/PR-/HER2+, and triple negative (TN) was ER-/PR-/HER2-. Associations of CD163 IHC score with age, race, and IHC subtype were examined by Wilcoxon rank-sum tests and/or Fisher’s exact tests. Prognoses of CD163 in overall survival (OS), disease specific survival (DSS), disease free survival (DFS), and recurrence were studied using univariable and multivariable Cox proportional hazards regression models. CD163 score, age, race, AJCC stage, and subtype were included in the multivariable model.

**RESULTS** CD163 IHC score displayed a significant negative correlation with age (R=-0.20, P=0.022). Patients with a CD163 score of 3+ were significantly younger than those with a score of 0 (P=0.019). CD163 score distributions were not statistically different between white and African American patients. CD163 scores of LA tumors were significantly lower than those of the tumors with all other subtypes except Her2+. Similarly, the CD163 scores of TN tumors were significantly higher than those of the tumors with all other subtypes but LB2. A higher CD163 score predicted worse DSS (HR=3.87 & P=0.0020 in univariable model; HR=4.21 & P=0.033 in multivariable model) and higher risk of recurrence (HR=2.85 & P=0.00016 in univariable model; HR=2.81 & P=0.012 in multivariable model).

**CONCLUSION** Higher CD163 expression in breast cancer was significantly associated with younger age, the TN subtype, worse DSS, and higher risk of recurrence. These results highlight CD163 as a prognostic marker for breast cancer.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S. Government.
Title: Exploring immunomodulatory effects of zoledronic acid in breast cancer from clinical trial result of neoadjuvant chemotherapy with zoledronic acid: JONIE-1 study

Sangai T, Sato E, Ishikawa T, Kaise H, Hasegawa Y, Miura D, Takao S, Suzuki M, Tanino H, Horiguchi J, Akazawa K, Yamada A and Kohno N. Chiba University, Chiba, Japan; Tokyo Medical University, Tokyo, Japan; Hirosaki Municipal Hospital, Aomori, Japan; Toranomon Hospital, Tokyo, Japan; Hyogo Cancer Center, Hyogo, Japan; National Hospital Organization, Chiba Medical Center, Chiba, Japan; Kitasato University School of Medicine, Kanagawa, Japan; Gunma University Hospital, Gunma, Japan; Niigata University Medical and Dental Hospital, Niigata, Japan; Yokohama City University Medical Center, Kanagawa, Japan and Kobe Kaisei Hospital, Hyogo, Japan.

Body: Background: We previously reported that the addition of zoledronic acid (ZOL) to neoadjuvant chemotherapy (CT) is potentially beneficial in postmenopausal patients with triple-negative breast cancer (BC) in JONIE-1 Study (0% pCR rate in CT versus 50% in CT combing ZOL [CTZ], p=0.029). In order to find biomarkers of response to ZOL, a pilot study was performed using 30 core needle biopsy (CNB) samples at diagnosis from JONIE-1 study. We found that Src activation was highly associated with pCR compared to non-pCR especially in CTZ group. CD8+ TIL was also highly associated with pCR compared to non-pCR. None of CD8+ TIL low patients resulted in pCR in CT group but four out of ten CD8+ TIL low patients experienced pCR in CTZ group. From the result of pilot study, we consider Src activation as a promising biomarker for response to chemotherapy especially in combination with ZOL; however, Src inhibition by ZOL in vivo and the mechanism to explain tumor inhibition is unknown. Because ZOL was effective even in CD8+ TIL low group, the possibility of immune response alteration by ZOL still remains. Not all tumors with Src activation and CD8+ TIL had benefit from ZOL, emphasizing the need for additional markers of response. In the present study, we focused on Src and multiple molecules relating to cancer immune response and analyzed associations with pCR.

Patients and Methods: We investigated the relationship between clinicopathological features and tumor shrinkage in Stage IIA-IIIB HER-2-negative BC patients from the JONIE-1 adjuvant phase III trial comparing CT (FEC100 q3w × 4 cycles followed by weekly paclitaxel for 12 cycles) versus CT combining ZOL (4mg q3-4w). To access Src activation, HLA class I expression, PD-1 and PD-L1 expression, we performed immunohistochemistry (IHC) and stained slides were evaluated by pathologists. Infiltration of immature dendritic cells, macrophages, cytotoxic T cells, and regulatory T cells in the tumor were assessed by IHC as well. Formalin fixed, paraffin embedded CNB sample at diagnosis and surgical specimen after neoadjuvant CT were processed for HE staining and IHC using primary antibodies as follows; anti-Src Ab (36D10), anti-pan HLA-class I Ab (EMR8-5), anti-CD8 Ab (Clone C8/144B), anti-FOXP3 Ab (Clone 236A/E7), anti-CD68 Ab (Clone PGM1), anti-CD1a Ab (Clone O10).

Results: All IHC were successfully performed. Stained slides are now under evaluation by pathologist. Associations between clinicopathological features and the effect of CT with ZOL will be under investigation.

Discussion: Src activation was observed in more than 70% of triple negative BC. Multiple cellular functions of Src are mediated by Ras which is the main target of ZOL in osteoclasts. It has been reported that ZOL reverted tumor-infiltrating macrophages (TAM) phenotype from M2 to M1. Moreover, recent study showed that the activation Src and MAPK in melanoma cells promotes PD-L1 expression. Therefore the addition of ZOL might help cytotoxic T cells infiltrate the tumor for inhibiting tumor growth. We will present the results and discuss antitumor effects of ZOL through immunomodulation at the meeting.
Title: The difference between metachronous and synchronous bilateral breast cancer in terms of clinical features and biology

Arima N, Nishimura R, Osako T, Nishiyama Y, Fujisue M, Okumura Y, Murakami K and Toyozumi Y. Kumamoto Shinto General Hospital, Kumamoto, Japan and Kumamoto City Hospital, Kumamoto, Japan.

Body: Introduction
The recent diagnostic modality such as MRI can diagnose a tiny breast lesion even in healthy contralateral breasts. Recently, the rate of contralateral prophylactic mastectomy (CPM) is on the rise in the United States. In Japan, the CPM has been started for selected patients with a high risk. In this study, we divided bilateral breast cancers into the synchronous and the metachronous group and then compared the clinical features and biology.

Patients and Methods
Out of 216 bilateral breast cancer patients who underwent surgery between 1995 and March 2015, there were 101 synchronous breast cancer cases and 115 metachronous breast cancer cases (interval to the second tumor > 1 year). The items examined were age, tumor size, lymph nodal status, histological type, and biological markers (ER, PgR, HER2, p53 and Ki-67 index values) in the cases with paired data.

Results
1. The incidence of metachronous tumors was relatively stable at 2.1–2.7% throughout the period. On the other hand, the incidence of synchronous tumors has increased to 4.4% in the most recent 5-year period and the median interval was 7.1 years.
2. There was no difference in the tumor size of both tumors in the synchronous group, but the second tumor was significantly smaller than the first tumor in the metachronous group (2.2cm to 1.7cm). The node negative rates showed no difference between two groups.
3. The cases with DCIS were seen in 20% and 25% of the synchronous group and 12% and 16% of the metachronous group. Most of the patients (94.3%) with invasive cancer received systemic adjuvant therapy in the metachronous group.
4. The ER positive rates of both tumors were 87.1% and 88.1% in the synchronous group and 71.6% and 68.4% in the metachronous group, respectively. The concordance rates were higher in the synchronous group (p=0.02). Moreover, there was a significant difference in ER positive rates between the two groups (p=0.01) and the ER negative tumors were more frequent in the second tumor of the metachronous group. The PgR negative tumors increased in the second tumor of metachronous cases. The shorter the interval (< 5years), the more the ER positive rate decreased (p=0.002). However, the longer interval did not correlate with the change of receptor status.
5. The Ki-67 index values significantly increased in the second tumor of the metachronous group, especially in the cases with a shorter interval. However, there was no difference in the synchronous group. The p53 overexpression rates significantly increased in the cases with a shorter interval.
6. The postoperative prognosis for the first tumor did not differ in both groups.

Conclusion
The incidence of synchronous bilateral breast cancer cases have increased but have remained relatively stable in the metachronous group. The concordance rates of the ER, PgR, Ki-67 and p53 status were higher in the synchronous group but the cases with negative ER, negative PgR, higher Ki-67 values and positive p53 increased in the second tumor of the metachronous group. These findings suggest that adjuvant systemic therapy played a important role in the treatment of bilateral breast cancer but the secondary tumor was more aggressive in the metachronous cases.
Body: Background: Triple-negative breast cancer (TNBC) is an aggressive histological subtype with high rates of recurrence and metastatic disease. Intrinsic or acquired multidrug resistance facilitated by the over-expression of drug efflux pumps (ABC transporters: BCRP [ABCG2], MRP1 [ABCC1] and PGP [ABCB1]), may contribute to the aggressive nature of this disease. We examined the expression patterns of drug efflux pumps for insight on their potential role in chemoresistance of TNBC.

Methods: 1393 TNBC patients molecularly profiled with a commercial assay (Caris Life Sciences) were evaluated retrospectively for expression of BCRP, MRP1 and PGP by immunohistochemistry. Antibodies used: PGP (C494), BCRP (6D171) and MRP1 (33A6). IHC threshold: positive = ≥1+ and ≥10%). This data set also included metachronous paired samples from 71 TNBC patients. JMP was used to ascertain distributional differences.

Results: PGP and MRP1 positive expression rates were 8.4% (117/1393) compared to 78.2% (248/317), respectively, displaying an inverse association (p=0.0001). BCRP was over-expressed in 52% (90/173). Co-expression data for all three transporters was available for 153 patients. BCRP/MRP1 co-expression was most abundant, 39% (59/153), followed by MRP1/PGP at 4% (6/153) and BCRP/PGP at 2% (3/153). Furthermore, 12% (18/153) of TNBC exhibited positive expression for all three drug pumps (16/18 or 89% were from patients with metastatic disease) and 14% (22/153) exhibited negative expression for all three drug pumps (15/22 or 68% were from patients with metastatic disease). Interestingly, positive MRP1 status, but not BCRP or PGP, correlated with metastatic disease (p=0.0061). In the paired data analysis, PGP expression was absent in 77% (55/71) and retained lack of expression in subsequently profiled specimens, 3% (2/71) retained positive PGP status, 13% (9/71) lost expression of PGP and 7% (5/71) gained expression of PGP. 36/40 patients that lacked PGP expression and 8/9 patients that "lost" PGP expression, exhibited and retained expression of MRP1 in the initial and subsequently-profiled specimens.

Conclusion: Biomarker expression patterns of drug efflux pumps may provide insight to the chemoresistance phenotype observed in TNBC. Expression of MRP1 is favored in TNBC and is correlated with metastatic disease status. Although PGP expression may be absent or lost during TNBC progression, MRP1 (and BCRP) expression are almost always retained. Utilization of chemotherapies that are substrates of PGP, but not MRP1, (based on expression status) may be worthy of future investigation.
Title: Abstract Withdrawn
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-10-02

Title: Cancer team approach for implementing survivorship care plans in the breast cancer survivorship clinic

Danciu OC C, Bharadwaj SN N and Hoskins K. University of Illinois at Chicago, Chicago, IL.

Body: Background: Recommendations from the Institute of Medicine are that cancer patients receive individualized survivorship care plan (SCP) and treatment summary. SCP includes guidelines for monitoring and maintaining health and is a communication tool shared with families and health care providers. Offering SCP and treatment summary to cancer survivors remains challenging due to time and resource limitations, inadequate reimbursement and survivor access.

Methods: Survivorship starts when completing the initial treatment (surgery, chemotherapy or radiation therapy). A team of medical oncologists, nurse practitioner and patient navigator created a process of pre-screening and identifying breast cancer (BC) survivors. SCP and treatment summary were pre-populated, individualized for each patient, then finalized and discussed with the patients during their medical oncology clinic visit. Pre intervention data was retrospectively collected, including all BC cases from March 2014 to March 2015. Post intervention data was prospectively collected over eight weeks. Pre and post intervention SCP completion rates were compared with chi square analysis.

Results: A baseline one year review of 1124 encounters noted 23 of 90 (25%) BC survivors received SCP. Ninety-six encounters occurred during the 8 week pilot period. Sixteen (16.6%) cases met the definition of BC survivor. During the pilot period, 15 out of 16 (93.7%) survivors received the SCP and treatment summary (p <0.0001). After the pilot period, 96.4% of BC survivors were seen in the BC survivorship clinic.

Conclusions: We successfully piloted the implementation of SCP for BC survivors. Our team found that using clinic visit screening and pre-identifying patients that transition into the survivorship program resulted in improvement of compliance with survivorship measures. In BC survivorship clinic we address specific survivorship issues and review SCP and treatment summary.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-10-03

Title: Compliance improvement with enhanced, patient-specific breast clinic infrastructure

Carandang MI I, Wagman LD D and Babaran W. The Center for Cancer Prevention and Treatment at St. Joseph Hospital, Orange, CA.

**Body: Background**: NCCN provides guidelines for breast cancer post-therapy surveillance (clinic appointments, mammograms, and anti-hormone therapy use). The multidisciplinary breast care clinic (MDC) was designed with a specific infrastructure to serve women with state-federal safety net funding. The purpose of this study was to determine patient compliance in this population.

**Methods**: A retrospective chart review was performed on 82 previously diagnosed breast cancer subjects seen at the MDC during 2011-13. Post-therapy surveillance compliance for clinic appointments (CA, n=82), annual mammography for subjects with one or both breasts intact (MAM, n=75), and yearly evaluation for subjects prescribed anti-hormone therapy (AHT, n=61) was assessed. Compliance was compared based on subject characteristics: age, stage, distance from home to MDC, insurance carrier, race, and ethnicity.

**Results**: The per patient average combined compliance for all post-therapy surveillance was 87.7% at 12 months, 57% at 18 months, and 76.9% at 60 months. There were no trends in the analysis of average of CA kept based on insurance carriers. HMO participants had the highest percentage compliance at (95.0%; n= 2). After that, the ranking was as follows: 73.7% of those who are under the Breast Cancer Early Detection Program (BCEDP) (n=9), 68.6% under Cal Optima (n= 63), 68.2% straight Medicare (n= 2), and 63.1% for subjects with MediCal (n= 6). While no trend was identified for MAM by insurance carrier, the compliance was overall higher (average of 90%) when compared to CA (average of 74%) and AHT compliance (average of 87%). The overall cumulative percent compliance by test category and characteristic groups was:

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Stage</th>
<th>Distance (in miles)</th>
<th>Ethnicity</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39: 70</td>
<td>0: 59.3</td>
<td>0-9.9: 70.6</td>
<td>White: 67.8</td>
<td>Non-Spanish: 70.7</td>
</tr>
<tr>
<td>40-49: 71.5</td>
<td>1: 71.2</td>
<td>10-19.9: 69.4</td>
<td>Asian: 72.3</td>
<td>Spanish: 65.2</td>
</tr>
<tr>
<td>50-59: 66.7</td>
<td>2: 64.4</td>
<td>20+: 67.6</td>
<td>Other: 68</td>
<td></td>
</tr>
<tr>
<td>60-69: 70.4</td>
<td>3: 80.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4: 77.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39: 90</td>
<td>0: 87.7</td>
<td>0-9.9: 88</td>
<td>White: 87</td>
<td>Non-Spanish: 87.6</td>
</tr>
<tr>
<td>40-49: 80.1</td>
<td>1: 90.6</td>
<td>10-19.9: 93.5</td>
<td>Asian: 90.1</td>
<td>Spanish: 90.1</td>
</tr>
<tr>
<td>50-59: 92</td>
<td>2: 85.1</td>
<td>20+: 81</td>
<td>Other: 86.7</td>
<td></td>
</tr>
<tr>
<td>60-69: 86.7</td>
<td>3: 89.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4: 91.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39: 87.5</td>
<td>0: 73.1</td>
<td>0-9.9: 80.3</td>
<td>White: 78</td>
<td>Non-Spanish: 78.4</td>
</tr>
<tr>
<td>40-49: 78.3</td>
<td>1: 78.6</td>
<td>10-19.9: 75.2</td>
<td>Asian: 80.4</td>
<td>Spanish: 76.5</td>
</tr>
<tr>
<td>50-59: 74.2</td>
<td>2: 76.2</td>
<td>20+: 77.5</td>
<td>Other: 52.5</td>
<td></td>
</tr>
<tr>
<td>60-69: 82.6</td>
<td>3: 81.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4: 83.3</td>
<td></td>
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</tbody>
</table>

**Conclusions**: Given the perceived challenge of consistent, reliable follow up in this patient population, the results of this analysis are quite encouraging with averages for mammogram at 87%, AHT compliance of 76%, and CA at 68%. The compliance patterns
discovered in this evaluation document the expectations and serve as a real-life practical, community standard. Mammography emerged as a driving component of compliance. The overall average combined compliance reduction at 18 months may be associated with possible visits to non-MDC facilities when mammography was not performed. An attempt to define outlier groups for potential infrastructure modification, based on unique group characteristics, was unsuccessful. While this may be attributed to the small n values, an alternate explanation is that the consistent infrastructure designed for a diverse, underinsured population resulted in a lack of variation.
Employment trends in young women following a breast cancer diagnosis

Rosenberg SM M, Rajagopal PS S, Ruddy KJ J, Tamimi RM M, Schapira L, Come S, Borges V, Gelber S and Partridge AH H. Dana-Farber Cancer Institute; University of Pittsburgh Medical Center; Mayo Clinic; Channing Division of Network Medicine, Brigham and Women’s Hospital; Massachusetts General Hospital; Beth Israel Deaconess Medical Center and University of Colorado Cancer Center.

Body: Background: Workplace concerns are particularly salient for young women with breast cancer (BC), and a cancer diagnosis (dx) and treatment may affect their careers. We sought to evaluate the perceived impact of dx on employment, describe job changes, and identify factors associated with transition out of the workforce after dx of BC at a young age.

Methods: As part of an ongoing, multi-center cohort of young women diagnosed with BC at age ≤ 40, we surveyed women with early-stage BC about their pre- and post-dx employment status. Additional items assessed socio-demographic and treatment information; tumor characteristics were ascertained via pathology and medical record review. We used logistic regression to identify predictors of transitioning from pre-dx employment to unemployment at 1 year after dx. Among women employed 1 year after dx, we evaluated job satisfaction, perceived impact of dx on job performance, accommodations made by employers, and perceived likelihood of employment in the future.

Results: 76% of women (555/730) were employed both before dx and at 1 year; 13% were not employed at either time point; 7% were employed pre-dx but unemployed at 1 year; 4% were not employed prior to dx but reported employment at 1 year. Among women employed 1 year after dx, 74% (427/581) were somewhat or completely satisfied with their job. Only 6% said cancer or treatment limited their ability to perform their job quite a bit or very much; 38% said their ability was affected a little bit. Most (63%) said their employers had made accommodations for them, and almost all women (93%) said it was very likely they would be working in 1 year. In multivariable analyses (Table 1), women with stage 3 disease (vs. stage 1), were more likely to transition out of the workforce following dx, while women with a college or graduate degree (vs. no college degree) were less likely to transition out.

Conclusion: Most young women with early stage BC remain employed and report a willingness by their employer to make accommodations following a breast cancer dx. While few women reported that their dx or treatment limited their job performance, the finding that women with more advanced disease were more likely to transition out of the workforce suggests an impact of dx/treatment burden on employment. Women without a college degree were also at risk for unemployment post-dx, suggesting that job type, socioeconomic status, and environment affect employment outcomes. Attention to these subgroups of women is warranted to ensure that they are sufficiently supported given the potential adverse psychosocial and financial impacts of unemployment on patients, families, communities, and society.

Table 1. Multivariable analysis of factors associated with transition out of workforce 1 year post-dx (N=634)

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage (ref=1)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.52 (0.60-33.85)</td>
</tr>
<tr>
<td>2</td>
<td>1.11 (0.48-2.58)</td>
</tr>
<tr>
<td>3</td>
<td>4.05 (1.53-10.72)*</td>
</tr>
<tr>
<td>White non-Hispanic (ref=non-WNH)</td>
<td>1.47 (0.56-3.81)</td>
</tr>
<tr>
<td>College graduate (ref=no college degree)</td>
<td>0.44 (0.22-0.90)*</td>
</tr>
<tr>
<td>Married/Living as married (ref=unmarried)</td>
<td>0.95 (0.43-2.08)</td>
</tr>
<tr>
<td>Parous (ref=nulliparous)</td>
<td>1.75 (0.83-3.69)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>0.98 (0.90-1.06)</td>
</tr>
<tr>
<td>Mastectomy (ref=lumpectomy)</td>
<td>1.74 (0.75-4.05)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Endocrine therapy (ref=none)</td>
<td>0.75</td>
</tr>
<tr>
<td>Chemotherapy (ref=none)</td>
<td>5.20</td>
</tr>
<tr>
<td>Radiation (ref=none)</td>
<td>1.38</td>
</tr>
</tbody>
</table>

*p<0.05
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-10-05

Title: Changes in metabolic syndrome components in breast cancer patients receiving neoadjuvant or adjuvant chemotherapy

Dieli-Conwright CM M, Wong L, Waliany S, Bernstein L, Salehian B and Mortimer JE E. University of Southern California, Los Angeles, CA and City of Hope, Duarte, CA.

Body: Purpose. We sought to determine the impact of (neo)adjuvant chemotherapy on metabolic syndrome (MetS) components and related anthropometric and metabolic biomarkers among premenopausal and postmenopausal early stage breast cancer patients.

Methods. Eighty-six women with early stage (I-III) breast cancer who were free from clinically diagnosed MetS (defined as 3 out of 5 components of MetS) and were planning to undergo chemotherapy, enrolled in the study. Participants were tested for MetS (blood pressure; BP, waist circumference; WC, fasting blood glucose; FBG, high-density lipoprotein cholesterol; HDL-C, and triglycerides; TG), anthropometrics (body weight; BW, percent body fat; BF, fat mass; FM), lipid profile (total cholesterol; TC, low-density lipoprotein cholesterol; LDL-C), glucose metabolism (insulin, homeostatic model- insulin resistance; HOMA-IR, glycosylated hemoglobin; HbA1c), and inflammation (C-reactive protein; CRP) within one week before and following the completion of chemotherapy. Fasting (12-hour) venous blood samples of the antecubital vein were drawn to measure glucose, insulin, lipid profile (TC, HDL-C, LDL-C, and TRI), HbA1c, and CRP. Blood samples were analyzed at the City of Hope Clinical Pathology Laboratory. Insulin resistance was calculated using the HOMA index: [(fasting glucose (mg/dL) x fasting insulin (mg/dL))/405]. Height, BW, and BP measurements were obtained by the nursing staff during pre-chemotherapy physical exams. Body composition (BF and FM) was measured using a portable hand-held bioelectrical impedance device (Omron®; Hoffman Estates, IL). WC was measured, using a fabric measuring tape, as the distance around the waist using the umbilicus as the reference point.

One-way analysis of covariance (ANCOVA) using SPSS version 18.0 was used to compare means adjusting for covariates such as age, race, type of chemotherapy, duration of chemotherapy, BMI at baseline, and menopausal status.

Results. The majority of the 86 women enrolled were Caucasian (44%) or Hispanic (30%), nonsmoking (96%), employed (84%), and well-educated (90%), with a mean age of 48.2 years. Women were most commonly undergoing Cytoxan/Adriamycin + Taxol (42%) or Taxotere/Cytoxan (36%) chemotherapy regimens, lasting on average 15.3 (±2.7) weeks. Overall the population was sedentary, averaging 7.2 (±5.8) minutes of physical activity/week.

Following chemotherapy, all MetS components and overall MetS score (out of 5) significantly increased (p<0.01). Additionally, BW, BF, FM, lipids (TC, LDL), glucose metabolism (HOMA-IR, insulin, HbA1c), and inflammation (CRP) significantly increased (p<0.01).

Conclusion. In women without MetS, (neo)adjuvant chemotherapy negatively altered MetS components, related anthropometrics, and biomarkers of glucose metabolism and inflammation, within 12 -18 weeks. Studies that test the impact of lifestyle interventions, such as diet and exercise, should be explored in this population of breast cancer patients to reduce the onset of MetS.
Title: Abstract Withdrawn

Body:
Genetic predictors of chemotherapy-related amenorrhea

Ruddy KJ J, Rack B, Schwitulla J, Lambrechts D, Haeberle L, Schramm A, Trapp E, Scholz C, Beutler AS S, Ginsburg E, Couch F, Partridge AH H, Wang L, Weinshilboum RM M, Janni W, Vachon C and Fasching P. Mayo Clinic, Rochester, MN; Ludwig-Maximilian University, Munich, Germany; University of Erlangen, Erlagen, Germany; University of Leuven, Leuven, Belgium; University Hospital Erlangen, Erlangen, Germany; University of Ulm, Ulm, Germany and Dana-Farber Cancer Institute, Boston, MA.

Background: Chemotherapy-related amenorrhea (CRA) impacts quality of life and reproductive options, and may play a role in treatment decision-making for young breast cancer patients. Although age, increasing doses of alkylating agents, and tamoxifen are known to increase the risk of chemotherapy-related amenorrhea (CRA), it is difficult to predict which women will develop CRA in order to inform fertility preservation and treatment decisions. We sought to investigate whether single nucleotide polymorphisms (SNPs) that have been associated with a younger age at natural menopause and ovarian reserve in non-oncologic populations were predictive of CRA in two large German clinical trials that administered gonadotoxic chemotherapy.

Methods: A total of 1322 premenopausal participants under age 50 who enrolled in SUCCESS B (a phase 3 trial of FEC-docetaxel-trastuzumab +/- gemcitabine for high risk early stage breast cancer) or SUCCESS C (a phase 3 trial of FEC-docetaxel-cyclophosphamide vs. docetaxel-cyclophosphamide that also tested the efficacy of a weight loss intervention in early stage breast cancer patients) were eligible for this study. We excluded patients who were receiving ovarian suppression. On these trials, menstrual status was self-reported as amenorrheic or not every three months after chemotherapy. Bloods were stored and patients provided consent for DNA analysis. We genotyped nine candidate SNPs in six genes (rs2002555 and rs11170547 in AMHR2, rs6166 in FSHR, rs10852344 <60kb from TNFRSF17/RUNDC2A/GSPT1, rs12461110 in NLRP11, rs1801133 in MTHFR, rs2066470 in MTHRF, rs3810682 in BMP15, and rs615942 in COASY), and examined their association with CRA at 12-month and 24-month time points using logistic regression adjusted for age, BMI, and tamoxifen. Results: Of the 554 eligible women with SNP data and menstrual data at 12 months, 70% reported CRA; of the 458 eligible women with SNP data and menstrual data at 24 months, 69% reported CRA. The two SNPs in AMHR2 showed suggestive associations with CRA at 12 and 24 months. [Final results pending].

Conclusions: Genetic variation in AMHR2 may be associated with CRA. Additional research will be needed to validate these findings in other breast cancer survivors and to identify additional SNPs that may be associated with CRA. Together with other clinical factors, genetic variation may improve our ability to predict who will experience CRA, and may inform reproductive and treatment decision-making in cancer patients.
Title: Can a diagnosis of invasive breast cancer effectively motivate patients to follow healthy lifestyles?

Ellsworth RE E, Costantino N, Toro AL L, Shriver CD D and Ellsworth DL L. Windber Research Institute and Murtha Cancer Center.

Body: Background: Survival rates for patients diagnosed with invasive breast cancer have increased dramatically, yet survivors often face a host of adverse health effects. Factors such as obesity, physical inactivity and tobacco use may contribute to decreased survival and quality of life. Behavioral risk factors in patients with and without breast cancer were evaluated to determine whether a diagnosis of invasive disease was sufficient motivation to modify lifestyle choices.

Methods: The dataset included female patients diagnosed between 2001-2011 with malignant (n=421) or benign (n=230) breast disease and who had baseline and >1-year follow-up information available. Changes in body mass index (BMI), fat intake, exercise frequency, alcohol and tobacco use, caffeine consumption, hormone replacement therapy (HRT) use and frequency of breast self-exam (BSE) were assessed. Random coefficients models were used to examine longitudinal effects of an invasive diagnosis on healthy behaviors. P<0.05 was used to define significance.

Results: At diagnosis, patients with invasive cancer were significantly older (59 years), more likely to consume >7 glasses of alcohol/week (7%) but less likely to be using HRT (3%) than those with benign disease (50 years, 3% and 11%, respectively). At baseline, a majority of both invasive and benign patients were overweight, non (current) smokers, and consumed a high fat, highly caffeinated diet and exercised <90 minutes/week, and >50% of both groups performed BSE at least once/month. Exercise, BMI, and caffeine, alcohol and fat intake did not change over time in either invasive or benign groups. Smoking decreased to a similar extent in both invasive and benign patients. In contrast, compliance with monthly BSE increased and HRT use decreased significantly at each time point in the invasive patient group, with no corresponding changes in patients with benign disease.

Conclusions: These data support the critical importance of providing education and recommendations about engaging in healthy behaviors by the clinical staff. The two behaviors that improved significantly in patients with invasive breast cancer are both addressed by the clinical staff at Walter Reed National Military Medical Center at the time of diagnosis: with nurse navigators providing education about proper BSE and physicians recommending discontinuation of HRT. Failure to significantly change other behaviors suggests that a diagnosis of breast cancer is not a sufficient motivating factor for the patient to adopt healthier lifestyle choices without provision of education and resources. Our data suggest a need for increased health-related behavioral counseling and support systems to successfully modify personal behaviors, thus improving the health and quality of life of breast cancer survivors.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-10-09

Title: Relapse-free survival of triple negative breast cancer long term survivors and characterization of late events in MD Anderson experience

Reddy SM M, Sinha A, Syed M, Barcenas C and Valero V. MD Anderson Cancer Center, Houston, TX.

Body: Background:
Stage I-III TNBC patients have a high risk of disease relapse during the first 5 years after diagnosis. However, there is limited data on the risk of late relapse in TNBC survivors who are disease free at 5 years or more from diagnosis. We sought to characterize this risk in a cohort of TNBC long-term survivors from a large institutional database.

Methods:
The MD Anderson Breast Cancer Management System database was queried for TNBC survivors who were disease free 5 years or more from diagnosis. Demographic, tumor, and treatment data was extracted. Electronic medical records were searched to confirm pathology reports for invasive breast cancer diagnosis, triple negative receptor status, and hormone receptor percentage (%). The primary and secondary outcomes of interest were relapse free survival (RFS) and distant relapse free survival (DRFS). Patients were censored at time of developing a second primary breast cancer or at last follow-up time for those who were alive during the study. We used ACP-ASCO definition of ER and PR <1% and HER2/neu negative (IHC 0-1 or ratio <2 and average copy number <4) but also included patients with low ER/PR(1-9%) and HER2 normal. Kaplan-Meier analysis was performed to compare RFS and DRFS for the overall population and categorized by ER/PR <1%, and ER/PR 1-9%.

Results:
We identified 1038 patients who had a median follow-up of 8.0 years. Receptor % information was available on 69% of patients, with 78% of them meeting current TNBC definition. From the total cohort of 130, 12.5% suffered event(s) that occurred after 5 years from diagnosis, with 86.2% of them occurring within 5-10 years of diagnosis. The event rate was 16.4% among patients with ER/PR 1-9% versus 11.3% among patients with ER/PR <1%. Table 1 shows RFS and DRFS by year from diagnosis for the entire cohort and categorized by % receptor. 18 patients developed second primary breast cancer as first event and were censored. Of total events recorded, 53(40.8%) were deaths and 77(59.2%) were recurrences, of which 51(66%) were distant and 26(34%) local, of whom 12(46.2%) subsequently developed distant metastases. Among patients who initially presented with distant recurrence, frequencies of initial sites of metastases are shown in Table 2.

Conclusions:
TNBC long term survivors are still at risk for relapse events after 5 years from diagnosis, and it is important to quantify this risk when counseling our patients. Frequency of late events was higher among patients with low hormone receptor positivity. Multivariate modeling of predictors of late recurrence is ongoing.

Table 1: RFS and DRFS by Year from Diagnosis

<table>
<thead>
<tr>
<th>Year From Diagnosis</th>
<th>All Patients</th>
<th>ER/PR &lt;1%</th>
<th>ER/PR 1-9%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RFS</td>
<td>DRFS</td>
<td>RFS</td>
</tr>
<tr>
<td>4-5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5-6</td>
<td>0.95</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td>6-7</td>
<td>0.93</td>
<td>0.94</td>
<td>0.93</td>
</tr>
<tr>
<td>7-8</td>
<td>0.90</td>
<td>0.92</td>
<td>0.90</td>
</tr>
<tr>
<td>8-9</td>
<td>0.86</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>9-10</td>
<td>0.84</td>
<td>0.87</td>
<td>0.84</td>
</tr>
<tr>
<td>10-12</td>
<td>0.81</td>
<td>0.84</td>
<td>0.81</td>
</tr>
<tr>
<td>12-15</td>
<td>0.73</td>
<td>0.78</td>
<td>0.73</td>
</tr>
</tbody>
</table>
Table 2: Site of Initial Distant Recurrence

<table>
<thead>
<tr>
<th>Site</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung/Pleura</td>
<td>28 (54.9)</td>
</tr>
<tr>
<td>Bone</td>
<td>19 (37.3)</td>
</tr>
<tr>
<td>Distant Lymph Nodes</td>
<td>19 (37.3)</td>
</tr>
<tr>
<td>Liver</td>
<td>11 (21.6)</td>
</tr>
<tr>
<td>Brain/Spinal Cord</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>Colorectal/Pancreas/Kidney/Adrenal</td>
<td>6 (11.7)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (3.9)</td>
</tr>
</tbody>
</table>

*Patients presenting with multiple sites of distant recurrence are counted in each category.*
The City of Hope breast cancer survivorship study: A longitudinal look at symptoms


BACKGROUND: Breast cancer survivors report many symptoms post treatment. However, longitudinal data, including pre-treatment measures, are scarce making it difficult to accurately attribute symptoms, as patients experience many changes; hormonal fluctuation, chemotherapy, additive endocrine therapy. Therefore, we undertook this longitudinal study to distinguish the impact of these different treatments along the survivorship time course, starting with time at diagnosis.

METHODS: Female, breast cancer survivors completed symptom questionnaires (Qx) pre-treatment (pre-tx), at 6-months follow-up (during-tx) and 12-months (post-tx) follow-up. Women rated symptoms in the past week as not at all, a little bit, somewhat, quite a bit, and very much. Symptoms included, hot flashes, vaginal discharge, vaginal itching, vaginal dryness, pain during intercourse, loss of interest in intercourse, weight gain, dizziness, vomiting diarrhea, headaches, abdominal bloating, breast sensitivity, mood swings, irritability, and joint pain. Additional measures included view of overall health, overall pain in the past month, and average fatigue in the past week. Chi-square tests were conducted across time points and stratified by menopausal status and type of treatment. Symptom changes from baseline, clustered by subject, were entered into a generalized estimating equation (GEE) models.

RESULTS: Of the 237 breast cancer survivors (median pre-tx age: 53 years, range: 24-70), who completed the pre-tx Qx, 112 completed the 6-month Qx and 95 completed the 12-month Qx. Women reported an increase in hot flashes from pre-tx to 6-months (p<0.001) and pre-tx to 12-months (p=0.04), with a slight decrease from 6 to 12 months (p=0.19). An increase in vomiting was observed from pre-tx to 6-months (p=0.04). Women reported an increase in vaginal discharge from pre-tx to 12-months (p=0.04), and a decrease in average fatigue in the past week from pre-tx to 12-months (p=0.04). Both overall pain in the past month and joint pain in the past week increased from pre-tx to 6-months and then decreased below pre-tx levels at 12-months (p=0.03 and p=0.12, respectively). Changes in hot flashes, vaginal dryness, pain during intercourse and weight gain were only observed among premenopausal women, while changes in vaginal discharge were only observed among postmenopausal women. GEE modeling showed associations between the use of endocrine therapy and increased hot flashes (Odds Ratio [OR]: 2.60, 95% Confidence Interval [CI]: 1.97-3.42), vaginal discharge (1.38; 1.15-1.66), pain during intercourse (1.34; 1.05-1.72), weight gain (1.61; 1.22-2.12) and joint pain (1.90; 1.48-2.45). By 12-months, severity of all symptoms decreased, except for vaginal discharge. Associations were observed between the use of chemotherapy and decreased weight gain (OR: 0.66, 95% CI: 0.48-0.91) and decreased breast sensitivity (0.67; 0.49-0.93).

CONCLUSIONS: Several symptoms thought to be related to treatment may actually be present at time of diagnosis and many treatment-related symptoms appear to decrease by 12-months. Severity appears to be modified by menopausal status and type of treatment. Our results give crucial insight for the development of effective symptom-based management and intervention along the treatment time course.
Title: Comparability of computerized and paper-pencil patient reported outcome (PRO) assessments – Does it matter how they are administered?


Body: Introduction:
There is an increased need to monitor and intervene to assist breast cancer (BC) survivors overcome the long-term and late effects of treatment. Many institutions are moving toward computerized assessments (CA) of PROs such as symptoms and concerns in place of more traditional paper and pencil administration, but little work has been performed to demonstrate that these very different methods of administration produce comparable results. Our goal was to evaluate the outcomes produced by these methods by comparing two similar samples of breast cancer survivors, one of which completed a PROs assessment using paper and pencil (PP), the other of which was assessed using a computerized system that links to the patient portal in use at our facility. Data collection of the CA of PROs is ongoing.

Method:
Women were eligible if they had a confirmed diagnosis of Stage 0-III BC, were within one year of completion of primary therapy, and were scheduled for a survivorship-focused end of treatment visit. As this was a naturalistic cohort study, no randomization was undertaken. The PRO assessment covered 19 common long-term or late effects of treatment, inquiring about their occurrence and severity in the previous month. On the day of the visit, participants in the PP cohort were provided with the questionnaire packet to complete prior to meeting with their provider. The women who completed the CA were either already enrolled in the patient portal or enrolled at the time of recruitment and sent an online version of the same questionnaire. Reminder calls and/or emails were sent to the CA participants to improve compliance.

Results:
164 BC survivors completed the PP questionnaire, and 62 women completed the CA. Racial make-up, marital status, and education, were similar between groups. Women in the PP group were older than those in the CA group (55.45 vs. 51.23 yrs, p < 0/05) and those in the PP group were marginally more likely than those in the CA group to have been menopausal prior to treatment (50% vs. 35%, p = 0.05).

With respect to PROs, there were no significant differences between groups in either the proportion of women endorsing a given symptom/concern or in the mean severity rating for any symptom/concern. The five most commonly reported concerns did differ somewhat between groups, with PP reporting Fatigue, Insomnia, Hot Flashes, Aching Joints, and Memory Difficulties, respectively, and CA reporting Fatigue, Anxiety, Body Image Problems, Memory Difficulties, and a tie between Insomnia and Depression, respectively.

Conclusion:
The results of the PRO assessment can be assumed to be comparable whether the method of administration is either PP or computerized. Differences found between groups in the most commonly endorsed symptoms likely reflected differences in age and menopausal status.
Title: Psychosocial health of disease-free breast cancer survivors compared with cancer-free general population: Korean health examinee cohort study

Lee MH, Park B, Song EJ, Park SJ, Kong S-Y and Lee ES. Research Institute and Hospital, National Cancer Center, Goyang, Republic of Korea.

Body: Background/purpose: The number of long-term breast cancer survivors is increasing rapidly due to the growing rates of detection, incidence and improved survival. Quality of life (QOL) of physical and psychosocial health outcomes vary across the breast cancer survivors including diagnosis at different stages of breast cancer. There is little data regarding the psychosocial health of disease-free breast cancer survivors compared with those of general population. We conducted to assess the QOL, especially psychosocial health, of disease-free female survivors over 2 year after breast cancer diagnosis compared with cancer-free control women.

Methods: We used baseline data from the health examinee cohort, a part of the Korea Genome Epidemiology Study which is a large scaled cohort study established since 2001. This cohort has collected data of past medical history, socioeconomical factors (income, education, marital status and current employment status) and health behavioral factors (smoking, drinking, physical activity, BMI, menopausal status and subjective health status) of participants while they underwent regular health examination. The disease-free breast cancer survivors were defined as those who answered that they were ≥2 years from the initial diagnosis of breast cancer without recurrence and current treatment. Among the participants female subjects without history of any type cancer were randomly selected at 1:4 ratio by 5-year age groups, educational attainment level and household income as a comparison group. We analyzed Psychosocial Well-being Index-Short Form (PWI-SF) in these two groups. Subjects with score ≤8, 9–26, and 27 were classified as the ‘healthy group’, ‘latent stress group’, and ‘stress group,’ respectively.

Results: Total 347 survivors of breast cancer and 1,388 matched participants without cancer history were selected for analysis. Even after being matched for education and household income status, breast cancer survivors showed better psychosocial health status and health behaviors compared with matched comparison group. The prevalence of latent stress and stress group (vs healthy group) by PWI-SF score was 88.2% in breast cancer survivors and 89.9% in the matched female controls, showing borderline significant differences (p = 0.057). The prevalence rates of ever drinkers and smokers or obese women were lower and of those who exercised ≥150 min/week were higher in breast cancer survivors (p < 0.05). The total PWI-SF score was lower in breast cancer survivors, suggesting lower level of psychosocial stress level in breast cancer survivors. After adjusting for effects of other sociodemographic variables, breast cancer survivors were less likely to be included in stress group by 36% (OR = 0.64; 95% confidence interval [CI], 0.42–0.98).

Conclusions: The disease-free breast cancer survivors with regular health examination showed better psychosocial health status compared with matched general population. The better health behaviors in cancer survivors such as less alcohol drinking, low BMI, less history of smoking and more regular exercise, which have been identified in several previous cancer survivors studies might be attributed to their better psychosocial health status.
Follow-up care of breast cancer patients who were treated in a German breast cancer centre - Survey of patients and attending physicians and analysis of treatment data


Body: Introduction: Breast cancer treatment leads to long-lasting impairments which, according to international guidelines, have to be identified and treated in follow-up care. It remains unclear how follow-up care is perceived by patients and if all needs are met in routine care.

Methods: All breast cancer patients who underwent surgery in a German breast cancer centre from 2007 to 2013 were asked to fill out a standardized scanner-readable questionnaire. Medical data were retrieved from their charts and statistically analyzed together with the questionnaire responses. Physicians who could possibly care for breast cancer patients after primary therapy were invited to fill out a standardized scanner-readable questionnaire as well.

Results: 920 questionnaires were filled out and returned (response rate: 61%) by patients. Median age at the time of the survey was 65 years (32-95). 58% of patients still received some form of therapy, 94% of them hormonal therapy. 94% were still in follow-up care, 5% stopped and 1% never went. Intervals of follow-up visits suggested by international guidelines were assessed as “quite right” in 93%. The following examinations were conducted throughout the whole follow-up period at least once: physical examination (93%), mammography (90%), sonography of breast (81%) and liver (22%), laboratory (56%), tumor marker (23%), bone scan (21%), MRI (20%) and CT (15%). Different items were rated on a 6-point scale ranging from ”0” ”not true at all” to ”5” ”completely true”. Follow-up care was regarded as very important for the own health (4.7), reassuring and calming (4.5), well-being to be looked after (4.4) and well cared for (4.4). A continuous contact between patient and doctor was appreciated (4.4). Visits were connected only to a part with distress (2.1), the median score on the NCCN distress thermometer was 4 (0-10). 105 questionnaires were answered by healthcare professionals (response rate 12%), most of them general practitioners (51%) or gynecologists (30%). Doctors carried out or referred asymptomatic patients most often to the following examinations: medical history taking (92%), physical examination (87%), blood chemistry (63%) and tumor markers (40%). Mammography was mentioned in 45%, sonographic examinations of breast, liver and axilla in 49%, 45% and 38%, respectively. 55% were (very) satisfied with international guidelines on follow-up care. Intervals and duration of follow-up visits were assessed as ”quite right” in 88% and 60%, respectively. Different items were rated on a 6-point scale ranging from ”0” ”not important at all” to ”5” ”very important”. Detection of disease recurrence and secondary tumors (4.8), reassurance of patients (4.7) and detection of treatment toxicities (4.5) were assessed as most important aims in follow-up care.

Conclusions: An overwhelming majority of patients makes use of follow-up care. Most important qualities from the patient's perspective are reassurance, a feeling of security, calming and continuous care by their doctor. Examinations which are not recommended in international guidelines are used by a considerable amount of healthcare providers.
Breast reconstruction changes coping mechanisms in breast cancer survivorship

Lake B, Fuller HR R, Rastall S and Usman T. Shrewsbury & Telford NHS Trust, Telford, United Kingdom and School of Postgraduate Medicine, Keele University, Keele, United Kingdom.

Introduction
Cancer survivorship is the process of living through and beyond cancer; a key part is how a patient copes with their diagnosis. Breast cancer is the most common malignancy of women worldwide and is known to be a severe stressor. Research has determined that the coping strategies used by women with breast cancer are vital to adjustment to their disease. Immediate breast reconstruction at the time of mastectomy with preservation of the breast form has been shown to be a positive influence on breast cancer patients however there are currently no studies to show whether breast reconstruction changes mechanisms of coping for such patients. The aim of this study, therefore, was to conduct a prospective cohort study to determine whether immediate breast reconstruction following mastectomy changes the way women with breast cancer cope with their diagnosis, compared to those who have mastectomy alone.

Method
A standardised questionnaire, the Brief Cope Scale was sent to two cohorts of patients who had a mastectomy and immediate reconstruction or mastectomy alone over an 11 year period 2003 to 2014 in Shropshire, England. It is a 28-point item with a four point Likert scale, which measures 14 different coping mechanisms: self-distraction, active coping, denial, substance use, use of emotional support, use of instrumental support, behavioral disengagement, venting, positive reframing, planning humour, acceptance, religion and self-blame. The inclusion criteria for this study was all woman who had mastectomy with immediate breast reconstruction in Shropshire between 2003 and 2014 for either Ductal carcinoma in situ (DCIS) or breast cancer which was node negative (cohort 1). The principle exclusion criteria were: men, node positive cancer, prophylactic mastectomy and breast reconstruction. Each index patient was matched for year of diagnosis, adjuvant therapy and age to woman who had mastectomy alone for DCIS or breast cancer which was node negative (cohort 2). An anonymous questionnaire was sent out to all patients identified who were still living, with a reminder letter at six weeks.

Results
Questionnaires were sent to a total of 234 patients; 117 patients in each cohort. Preliminary results indicate a response rate of 46%, with 60 responses from reconstruction cohort and 48 from mastectomy. The mean age was 50, with range 29 to 70 for reconstruction cohort, and the mean age of mastectomy cohort was 52, with range 32 to 70. Common coping styles for the reconstruction cohort were acceptance, active coping and use of emotional support. Common coping styles for mastectomy cohort were acceptance, use of emotional support and positive reframing. Significantly more patients from the reconstruction cohort coped by active coping (T value 1.88 at P value 0.02). Significantly less patients coped by active venting in reconstructive cohort compared to mastectomy cohort; (T value 1.91 at P value 0.03).

Conclusion
Breast reconstruction alters coping mechanisms in breast cancer patients allowing less venting coping style and more active coping. Understanding how breast surgery changes coping mechanisms allows clinicians to understand cancer survivorship in breast cancer patients and helps to provide needed support.
Title: A cross-sectional study of childbearing desires of young Chinese breast cancer women

Zhang L, Yuan P, Zhu A and Xu B. Cancer Hospital, Chinese Academy of Medical Sciences.

Objective: The aim of this study is to investigate whether young breast cancer women in China want to have children after diagnosis, factors that affect their childbearing desires, pregnancy after breast cancer, outcome of pregnancy and fertility counseling with clinicians.

Patients and Methods: This study is a single-center, random sampling and retrospective investigation. We selected patients from Cancer Hospital, Chinese Academy of Medical Sciences. Young breast cancer women (initial diagnosis at age no more than 40 years old) answered a questionnaire developed by the investigators. SPSS 20.0 statistical software was used to set up database and complete statistical analysis.

Results: 308 young breast cancer women answered the questionnaire. 81 (26%) patients wanted to have children after breast cancer diagnosis and treatment. Of them, 48 wanted to have their first child, 33 wanted to have a second child. Of the women who had childbearing desires, 6 have taken measures to preserve fertility, all of them had used GnRHa during chemotherapy; of the 6 patients, 1 cryopreserved her oocytes before chemotherapy. 8 patients tried to get pregnant, 7 was successful; 4 women delivered their babies, 3 women had induced abortion.

Pregnancy after breast cancer

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>Age at pregnancy</th>
<th>Chemo regimen</th>
<th>Fertility advice</th>
<th>Fertility preservation</th>
<th>Mode of pregnancy</th>
<th>Pregnancy Outcome</th>
<th>Disease Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>33</td>
<td>AC<em>4-T</em>3</td>
<td>Y</td>
<td>Nil</td>
<td>Natural</td>
<td>Baby birth</td>
<td>Nil</td>
</tr>
<tr>
<td>26</td>
<td>34</td>
<td>AT*6</td>
<td>Y</td>
<td>Nil</td>
<td>Clomiphene</td>
<td>Baby birth</td>
<td>Nil</td>
</tr>
<tr>
<td>27</td>
<td>30</td>
<td>AT*3</td>
<td>Y</td>
<td>Nil</td>
<td>Natural</td>
<td>Baby birth</td>
<td>Suspected recurrence</td>
</tr>
<tr>
<td>30</td>
<td>33</td>
<td>AT*4</td>
<td>Nil</td>
<td>Nil</td>
<td>Natural</td>
<td>Baby birth</td>
<td>Recurrence</td>
</tr>
<tr>
<td>33</td>
<td>36</td>
<td>AC<em>4-T</em>3</td>
<td>Y</td>
<td>Nil</td>
<td>Natural</td>
<td>Induced abortion: embryo damage</td>
<td>Nil</td>
</tr>
<tr>
<td>34</td>
<td>35</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Natural</td>
<td>Induced abortion: fear of recurrence</td>
<td>Recurrence</td>
</tr>
<tr>
<td>36</td>
<td>38</td>
<td>AC-T*7</td>
<td>Nil</td>
<td>Nil</td>
<td>Natural</td>
<td>Induced abortion: fear of birth defect</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Younger age, higher educational level, having received breast conserving surgery and not having children at the time of cancer diagnosis were associated with stronger childbearing desires. Reasons for not wanting to have children after breast cancer mainly included already having children, single-child policy, fear of disease recurrence, already having recurrence and fear of losing fertility after treatment.

Reasons for not trying for pregnancy

<table>
<thead>
<tr>
<th>Reason</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed family</td>
<td>196</td>
</tr>
<tr>
<td>Restricted by single-child policy</td>
<td>53</td>
</tr>
<tr>
<td>Fear of recurrence</td>
<td>48</td>
</tr>
<tr>
<td>Recurrence/disease progression</td>
<td>42</td>
</tr>
</tbody>
</table>
Fear of loss of fertility 19
Fear of disease inheritance 15
Fear of birth defect 13
Economic reasons 10
Fear of leaving child w/o parent 8
Felt unwell 7
Not in relationship 5
Fear of body discomfort 4
Did not want children 3
Fear of breastfeeding issue 2

In some cases reasons were multiple.

There were 72 (23%) patients in total that received fertility counseling from clinicians after breast cancer diagnosis. Conclusion: This is the first study in which the childbearing desires, the pregnancy rate and fertility consultations of young breast cancer women were investigated in China. The childbearing desires of young Chinese breast cancer women are intense. However, women have various concerns facing the fertility issue. Besides, there is a lack of fertility consultations given by the clinicians. Therefore, the number of women who get fertility preservation and give birth after breast cancer is small.
Long term effects of trastuzumab on cardiopulmonary and left ventricular function in women with HER2 overexpressing breast cancer

Pituskin E, Paterson I, Ghosh S, Mackey JR R and Haykowsky MJ J. University of Alberta, Edmonton, AB, Canada; Cross Cancer Institute, Edmonton, AB, Canada and Mazankowski Alberta Heart Institute, Edmonton, AB, Canada.

Background: Adjuvant trastuzumab (TRZ) is the standard of care for HER2-overexpressing (HER2+) early stage breast cancer (EBC) patients (PTS), with five-year survival rates exceeding 90%. However, significant cardiac toxicities are observed, with a fivefold increase in clinical heart failure (HF). Left ventricular (LV) remodeling (increased heart size and mass) is an early indicator of cardiac injury, progressing to further LV dysfunction, reduced exercise tolerance and overt HF. Therefore, effective prevention of such negative sequelae is of enormous clinical interest. As the pivotal TRZ trials assessed cardiac function with MUGA or echocardiography, insensitive modalities to evaluate LV remodeling, the long-term sequelae of TRZ remain unknown.

Objective: to determine the long term effects of trastuzumab on cardiopulmonary and left ventricular function in women with HER2 overexpressing breast cancer. Additionally as aerobic training is an effective intervention in HF PTS who adhere to prescribed exercise, a sub-analysis compared those who adhered during a 4 month exercise intervention (AEX) vs those who did not adhere (NEX).

Methods: 16 PTS (mean age 58 ± 7) who participated in an exercise intervention study during the first 4 months of TRZ therapy were recruited, with an average of 4 years elapsing since TRZ completion. Cardiopulmonary exercise ($VO_{2peak}$) testing and resting cardiac MRI (CMR) were performed and compared with baseline and 4 month assessments. Adherence to exercise intervention was defined as attendance ≥80% prescribed sessions.

Results: All 16 PTS reported independent living with no limitations to ADLs. At 4 years, mean $VO_{2peak}$ for all PTS was 22.4 ml/kg/min (20.0 at baseline and 22.0 at 4 months). In AEX PTS, higher $VO_{2peak}$ persisted 4 years after cessation of therapy, 4.1 mL/kg/min higher than NEX PTS (24.9 and 20.8 mL/kg/min, respectively ). Mean LVEF for all PTS was 60 ± 6%, not significantly different from baseline or 4 months (61 ± 5 and 55 ± 4%, respectively). Statistically significant interactions of exercise adherence to other CMR metrics were not observed.

Conclusions: Clinically significant impairment of cardiopulmonary function (equal to 14 years of aging) are present before therapy and persist in PTS four years following exposure to TRZ-based chemotherapy. This observation is consistent with our other work, and occurs on a background of normal LVEF, implying additional negative effects to other components of the oxygen cascade. As mortality risk has been shown to decrease by 17% for every 3.5 mL/kg/min difference in aerobic capacity in healthy females, these findings indicate adherence to exercise interventions during TRZ-based therapy has potentially important long-term implications.
Title: Changes of bone turnover markers during perioperative anthracycline- and/or taxane-based chemotherapy in pre- and postmenopausal patients with primary breast cancer


Body: Background: Loss of bone mineral density (BMD) is among the well known sequelae of pharmacological therapy of patients (pts) with primary breast cancer (PBC). Cancer therapy induced bone loss (CTIBL) progresses more rapidly as compared to normal age-related changes of BMD and is best known to be associated with aromatase inhibitors in postmenopausal pts. Chemotherapy (Ctx) may also lead to a deterioration of BMD but in contrast to endocrine Tx, this phenomenon is by far less elucidated and, at least in younger pts, mostly interpreted as a secondary effect following Ctx induced ovarian failure. Previous investigations focused on the classical CMF scheme, whereas conclusive data regarding direct effects of more recent Ctx protocols on the bone metabolism of PBC pts are still lacking. This translational project was initiated to gain detailed insights into the influence of anthracycline (A)- and/or taxane (T)-based Ctx on bone turnover of both pre- and postmenopausal PBC pts in the clinical routine. Methods: Data of 109 pts (premenopausal: 49; postmenopausal: 60) with non-metastatic Ctx-naïve PBC exposed to neoadjuvant or adjuvant Ctx were analyzed. 84 pts (75%) had estrogen receptor-positive (ER+) disease, HER2-overexpression was found in 18 pts (17%). 16 pts (15%) received A-based Ctx, 34 pts (31%) received T-based Ctx, and 59 pts (54%) received A/T-based Ctx. Trastuzumab was given to 17 pts (16%) with HER2-positive disease. Serum bone markers including the C-telopeptide of type I collagen (ICTP) indicating osteoclast activity, the N-propeptide of type I collagen (P1NP) measuring osteoblast activity, and alkaline phosphatase (AP) were determined at baseline and prior to each subsequent Ctx cycle (C) up to C6. Changes of ICTP, P1NP, and AP over time were analyzed by repeated-measure ANOVA. Results: 600 Ctx cycles were analyzed. Baseline levels of ICTP (p = 0.0027), P1NP (p = 0.0063), and AP (p = 0.0007) were significantly higher in post- versus premenopausal pts. AP levels remained largely unchanged during Ctx. Trends showing an increase of ICTP from baseline until C6 in premenopausal pts and a decrease in postmenopausal pts did not reach statistical significance. In contrast, P1NP significantly declined in postmenopausal pts from baseline to C6 (p = 0.0152). In premenopausal pts, P1NP declined from baseline to C3 and thereafter increased to C6. These changes were highly significant (p = 0.0024). Conclusions: Our study represents one of the first systematic evaluations of bone turnover in pts exposed to modern A- and/or T-based Ctx for PBC in the clinical routine. Postmenopausal pts presented with higher baseline levels of all three markers which may be attributable to an enhanced bone turnover related to the loss of ovarian function prior to the initiation of Ctx. In postmenopausal pts, Ctx was associated with a sustained suppression of osteoblast activity whereas osteoblast suppression recovered until the end of Ctx in premenopausal pts. Whether these effects will translate into an increased risk of CTIBL remains a matter of further investigations which should clearly focus on the individual menopausal status.
Factors influencing patient reported cosmetic outcome: Results of the Young Boost trial

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Purpose
The Young Boost trial (YBT), a multicenter RCT (NCT00212121), investigates whether a higher boost dose leads to a lower recurrence rate in young patients treated with breast conserving therapy. Cosmetic outcome is the secondary objective. The aim of the current analysis is to investigate which factors influence the patients’ opinion about cosmesis.

Patients & methods
From 2004-2011, 2421 breast cancer patients ≤ 50 yrs were included in The Netherlands, France, and Germany. All patients were treated with lumpectomy, followed by 50 Gy whole breast irradiation. Patients were randomized to receive a standard 16 Gy (n=1211) or a high 26 Gy boost (n=1210) to the tumour bed. Cosmesis was scored prior to radiation therapy, at 1 year and at 4 years follow-up according to the following three scoring systems:

1. BCCT.core: Digital photographs were analyzed using a software program to extract an overall cosmetic score: excellent, good, fair or poor. This score is based on symmetry, skin colour and scar visibility. The 7 features of symmetry in the BCCT.core program are:
   - pBRA (nipple position)
   - pLBC (level of lower breast contour)
   - pUNR (level of nipple)
   - pBCE (distance from nipple to inframammary fold)
   - pBCD (length of breast contour)
   - pBAD (area of the breast)
   - pBOD (non overlapping area between left and right breast)

2. Physician’s score: excellent, good, fair or poor

3. Patients’ score using a validated patient's questionnaire about the breast appearance, including an overall score: very satisfied, satisfied, not dissatisfied, dissatisfied or very dissatisfied. Very satisfied and satisfied were grouped as satisfactory.

At the same time points fibrosis was scored by the physician on a 4-point scale. The presence of rib pain was scored as well.

First, we analyzed the correlation between the 3 scoring methods. Secondly, we analyzed the 7 features of BCCT.core in a proportional odds model, to investigate which parameters are related to the patients’ opinion about cosmesis at 4 years. Also, we analyzed whether fibrosis score or presence of rib pain are related to the patients' opinion on cosmetic outcome.

Results
At 4 years, the agreement between the different scoring methods was low. The agreement between the physician and the patient was the highest (kappa 0.42), compared to the agreement between the patient and BCCT.core (kappa 0.26) or between BCCT.core and physician (kappa 0.39).

Of the 7 BCCT.core parameters, pBCE and pBCD are significantly related to patients' score at 4 years (table 1). Further, patients with any fibrosis interpret their cosmesis worse than patients without fibrosis, even when the objective score (i.e. BCCT.core) was similar. This effect was larger by increasing grade of fibrosis. The presence of rib pain had no influence.

Conclusion
The patients’ opinion on cosmetic outcome was significantly related to the score on pBCE and pBCD, and to the severity of fibrosis. The correlation between objective score and subjective scores (patient and physician) was low.

<table>
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<td>pBCD</td>
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<tr>
<td>pBOD</td>
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Title: Endocrine-related symptoms during neoadjuvant endocrine therapy for breast cancer: Agreement between patient and physician reporting in a prospective clinical trial

Fujisawa T, Iwata H, Sakai T, Nakamura R, Hasegawa Y, Ohtani S, Kashiwara M, Taira N, Toyama T, Masuda N, Yamamoto Y, Kihara K, Shimozuma K, Ohashi Y and Mukai H. Gunma Prefectural Cancer Center, Ota, Gunma, Japan; Aichi Cancer Center Hospital, Nagoya, Aichi, Japan; Cancer Institute Hospital, Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan; Chiba Cancer Center Hospital, Chiba, Japan; Hiroasaki Municipal Hospital, Hiroasaki, Aomori, Japan; Hiroshima City Hospital, Hiroshima, Japan; Iwate Medical University, Morioka, Iwate, Japan; Okayama University Hospital, Okayama, Japan; Nagoya City University Hospital, Nagoya, Aichi, Japan; National Hospital Organization Osaka National Hospital, Osaka, Japan; Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; The University of Tokyo, Bunkyo-ku, Tokyo, Japan; Ritsumeikan University, Kusatsu, Shiga, Japan; Chuo University, Bunkyo-ku, Tokyo, Japan and National Cancer Center Hospital East, Kashiwa, Chiba, Japan.

Body: Background: There is a high risk of under-reporting subjective toxicities by physicians, even when collected prospectively in clinical trials. It has been recommended to include patient reported measures regarding symptoms in prospective clinical comparative effectiveness trials. However, there have been few reports of agreement in endocrine related symptoms between patient and physician reporting.

Patients and Method: The National Surgical Adjuvant Study of Breast Cancer 06 (N-SAS BC 06) is a multicenter, randomized clinical trial of postmenopausal, hormone receptor-positive breast cancer patients, with a two-stage (preoperative and postoperative) enrollment, and intervention. The primary aim was to evaluate the need for adjuvant chemotherapy in the treatment of postmenopausal breast cancer patients who responded to neoadjuvant treatment with Letrozole (LET) for 24-28 weeks. After surgery, responders were randomized into two arms receiving either chemotherapy plus LET, or LET alone. The primary endpoint was disease-free survival, and the secondary endpoints included adverse events, quality of life and health economic evaluation. This study enrolled 497 subjects from the N-SAS BC 06 who were evaluated by Patient Reported Outcomes (PROs). The concordance rate between Clinician Reported Outcomes (CROs) and PROs in their endocrine symptoms during neoadjuvant endocrine therapy was examined. Symptoms were collected prospectively by physicians using the Common Toxicity Criteria for Adverse Events at enrollment, i.e., baseline, and 4 and 16 weeks after starting neoadjuvant LET. Patients also completed the FACT-G (General), B (Breast), ES (Endocrine Symptoms), and HADS. The endocrine symptoms according to the PROs, included nausea, hot flushes, cold sweats, headaches, and HADS-Depression score. In FACT, "Not at all" was used to express the absence of the symptoms, and "A little bit", "Some-what", "Quite a bit", and "Very much" were used to express the presence of symptoms. The HADS-Depression score threshold was 10/11. According to the CROs, grade 0 was defined as the absence of symptoms and grade 1 or more was defined as the presence of symptoms. Cohen's kappa was used to determine the concordance between CROs and PROs. The sensitivity of CROs was also calculated.

Results: The calculated point estimates of Cohen's kappa at Weeks 4 and 16 after starting neoadjuvant LET were 0.12 and 0.01 for nausea, 0.16 and 0.18 for hot flushes, 0.12 and 0.09 for cold sweats, 0.03 and 0.02 for headaches, and 0.11 and 0.11 for dysthymia/depression, respectively; the concordance was quite low. The sensitivity of CROs at Weeks 4 and 16 after starting neoadjuvant LET was 0.07 and 0.03 for nausea, 0.16 and 0.17 for hot flushes, 0.1 and 0.08 for cold sweats, 0.03 and 0.03 for headaches, and 0.11 and 0.1 for dysthymia/depression, respectively; the sensitivity was quite low.

Conclusion: This study showed that there were big differences between CROs and PROs in endocrine symptoms associated with endocrine therapy for breast cancer and that physicians could not obtain sufficient information on the endocrine symptoms. It is recommended that PROs be used to evaluate adverse events caused by endocrine therapy.
Title: The impact of extended endocrine therapy on symptom burden and health-related quality of life in patients with early-stage breast cancer

Fisher MD D, Schroeder BE E, Miller PJ J, Schnabel CA A, Schwartzberg L and Walker MS S. Vector Oncology, Memphis, TN; BioTheranostics, Inc., San Diego, CA and The West Clinic, Memphis, TN.

Body: Background: Extended endocrine therapy (> 5 years; EET) is recommended for many ESBC patients following the results of the MA.17, ATLAS, and aTTom clinical trials. Clinical practice guidelines recommend consideration of 10 years of endocrine therapy; however, they note the challenging risk vs benefit profile given the modest benefit of EET in terms of preventing disease recurrence (~3-5%) and the potential for adverse effects and tolerability challenges. Studies examining the long-term impact of EET are lacking. The objective of this study was to assess the impact of EET in ESBC patients on symptom burden and health-related quality of life (HRQoL).

Methods: Retrospective review of existing medical records for patients (N=308) with ER+ ESBC. Eligible patients had completed 5 years of adjuvant endocrine therapy without disease progression, minimum of 1 year additional follow-up, and at least one Patient Care Monitor (PCM) survey, a validated 86-item, patient reported outcomes measure that assesses symptoms common in patients undergoing cancer treatment, during the 1 to 3 year follow-up period. Primary analysis included 6 PCM index scores and 12 PCM items representing symptoms of particular interest. Patients were classified as having received EET (minimum 8 months) vs. Control (no extended therapy). Linear mixed models were employed to examine differences in symptom burden between EET and Control groups during the 3-year follow-up period, including differences in change over time across groups, and whether patterns of symptoms lead to discontinuation of EET.

Results: This analysis included 156 EET and 152 Control patients [75.0% Caucasian, 22.7% African American, with mean age of 61 (±11) years, and predominantly from the Southern US (93.8%)]. The sample was 40.9% Stage I at diagnosis, 48.4% stage II, and 10.1% stage III. EET patients were younger (59 vs. 63 years, p = .0008), and more likely to have stage III disease (p =<.0001). Results from preliminary interim analyses indicate that EET vs no EET was associated with statistically significant differences in symptom burden in certain PCM items (eg, increased vaginal dryness, reduced sexual enjoyment). Final analyses will be available on Sept 1st and the abstract will be updated at that time.

Conclusions: Based on interim analyses from this study, EET may be associated with continued symptom burden and impact on HRQoL. These results suggest that the decision whether to extend endocrine therapy in patients with ESBC should be multi-faceted, including discussion of the potential benefit of extended therapy, risk of ongoing/worse symptomatology, and long-term impact on patients QoL.
Objectives: To evaluate the effectiveness of simple sexual/vaginal health treatment strategies in breast cancer patients/survivors seeking treatment at a female sexual medicine program, and to evaluate compliance and clinical outcomes.

Methods: Demographics, medical information, and clinical assessments from 94 new visits with at least 1 follow-up from 09/12–10/14 were analyzed. The assessment form consists of a clinician evaluation with the Vaginal Assessment Scale (VAS) and Vulvar Assessment Scale (VuAS), and patient-reported outcomes (PROs) including the Sexual Activity Questionnaire (SAQ), Female Sexual Function Index (FSFI), and exploratory items. Compliance with treatment recommendations at the last visit were summarized. Changes from the first to last visit were compared with regard to clinical outcomes (e.g., vaginal pH, moisture), the VAS and VuAS, and the PROs (FSFI, SAQ, and confidence about future sexual activity).

Results: The mean number of visits was 3 (range, 2-7). Mean age was 55.2 years (range, 29-48), 67% (n=63) were married or in an intimate relationship, and 94% (n=88) were menopausal. Seventy-seven percent (n=72) were on some form of active treatment, such as endocrine therapy (89%, 64/72). Only 15% (11/72) reported any hormonal supplementation (e.g., Vagifem). Treatment strategies included the use of vaginal moisturizers, lubricants with sexual activity, pelvic floor exercises, and dilator therapy. At last visit, 94% (80/85) of the women had complied with the clinical recommendation to moisturize 2 to 5+ times per week. Vaginal pH scores >6.5 declined from 31% (29/93) at Visit 1 to 20% (19/93) at the last visit (p=0.049). Vaginal (VAS) symptoms improved from the first to last visit (mean 1.2 to 0.59, p<0.001) as did the Vulvar (VuAS) symptoms (mean 0.78 to 0.57, p=0.007). Only 17% (15/19) had normal vaginal moisture at Visit 1, compared to 29% (26/90) at the last visit (p=0.04). Sixty-seven percent (59/88) reported performing pelvic floor exercises a few times per week to daily at their last visit. FSFI arousal mean scores improved from the first (mean=2.30) to last visit (mean=2.93) (p=0.003), and urinary incontinence rates declined from the first (58%, 32/55) to last visit (24%, 13/55).

Conclusions: Breast cancer patients/survivors attending our female sexual medicine program reported improvement of vaginal and vulvar symptoms, sexual function and activity, and had clinical improvement over time on vaginal pH and moisture. Preliminary findings suggest that simple strategies, education, and support can improve vaginal/sexual health concerns in cancer survivorship.
Title: Safety of fertility preservation indicated by a diagnosis of breast cancer: A Swedish registry-matched cohort study


Body: Purpose
The number of women that perform fertility preservation (FP) before initiating chemotherapy for treatment of breast cancer is increasing. Our purpose was to investigate the safety of performance of fertility preservation with and without hormonal stimulation in the incidence rate of breast cancer relapse.

Patients and methods
The study was designed as a matched cohort study. Women who had undergone FP at the Reproductive Medicine clinic of Karolinska University Hospital (N=187), irrespective of whether hormonal stimulation was required or not, were considered exposed. For all exposed women, two age-matched women who had not undergone fertility preservation were identified in the Regional Breast Cancer Registry Stockholm-Gotland and the Swedish National Quality Registry for Breast Cancer (N=319). The proportional hazards assumption was evaluated by applying the Grambsch-Therneau test on the Schoenfeld residuals obtained from each model, respectively. When interpreting the test results, a significance level of 5% was used to determine statistical significance.

Results
In the exposed cohort, a higher proportion of women underwent FP with hormonal stimulation aiming at freezing eggs or embryos (81%, N=142), whereas only 19% underwent freezing of ovarian tissue or attempted egg retrieval without hormonal stimulation (N=35). The mean follow up time was 6.3 years (range: 1.5-17.8 years), and median follow-up: 5.7 years.

There was no evidence of non-proportional hazards with respect to the effects of fertility preservation on the risk of relapse in the whole FP cohort, irrespective if the women underwent hormonal stimulation or not for FP, or of receptor status or tumor size at diagnosis. A stratified Cox regression model that allowed separate baseline hazard functions for each level for the investigation of the effect of number of involved lymph nodes was performed. In this model, the effect of fertility preservation on the risk of relapse was virtually unchanged (IRR: 1.02, 95% CI:0.56-1.84).

Conclusion
Fertility preservation either using hormone stimulation or not is unlikely to cause substantially increase recurrence risk of breast cancer, and irrespective of the receptor status, tumor size of lymph node compromise. The incidence of breast cancer relapse after hormonal or non-hormonal fertility preservation was not different from that of matched controls from the Regional Breast Cancer Registry in this large cohort study.
**Title:** Feasibility of a non-hormonal vaginal moisturizer in postmenopausal cancer survivors


**Body:**

Objectives: This is a single-arm prospective longitudinal clinical trial investigating the feasibility of using a non-hormonal hyaluronic acid (HLA) vaginal gel (Hydeal-D) to improve estrogen deprivation vaginal health symptoms in postmenopausal women with a history of hormone receptor-positive cancer.

Methods: Preliminary data from an ongoing clinical trial were examined. Demographics, medical information, and clinical assessment from breast cancer patients enrolled on study at baseline (n=23) and at 4-6 weeks (n=18) are presented. Eligible participants included those with a history of breast cancer receiving treatment with an aromatase inhibitor (AI) at the time of enrollment. Furthermore, participants could not have evidence of disease and had to have completed treatment for at least 3 months and no longer than 5 years (excluding AIs). Study participants were instructed to use HLA daily for 2 weeks, then 3 times per week for 12-14 weeks. Study outcomes include: pelvic exam results as recorded on a clinician evaluation form with the Vaginal Assessment Scale (VAS); patient-reported outcomes (PROs) of the Sexual Activity Questionnaire (SAQ), Sexual Self-Schema Scale, and Female Sexual Function Index (FSFI); PROMIS sexual function items; and exploratory items.

Results: The mean age was 56 years (range, 42-75). Seventy-four percent (17/23) were married or living with a partner. Fifty-seven percent (13/23) reported sexual activity with a partner at baseline, which was 72% (13/18) at 4-6 weeks. On the VAS, 65% (15/23) reported symptoms of severe dryness at baseline and 61% (14/23) reported severe dyspareunia; these reported symptoms decreased to 6% (1/18) and 6% (1/18), respectively, at 4-6 weeks. Vaginal pH scores were greater than 6.5 in 30% (7/23) at baseline; by 4-6 weeks, only 22% (4/18) had a pH in this elevated range. At baseline, 78% (18/23) had minimal moisture and 22% (5/23) had no vaginal moisture seen on exam; by 4-6 weeks, 11% (2/18) had normal moisture and 89% (16/18) had minimal moisture. Pain with pelvic exams declined over time—87% (20/23) had pain at baseline, with 22% (5/23) rating it as severe, and 78% (14/18) had pain at 4-6 weeks, with none of the women rating their pain as severe. Forty-eight percent (11/23) indicated confidence about future sexual activity at baseline, which was 56% (10/18) at 4-6 weeks. Level of concern about sexual/vaginal health was measured on a scale of 0-10, with greatest concern rated as a 9 or 10. Sixty-one percent (14/23) of the women fell into this range at baseline; the percentage decreased to 28% (5/18) at 4-6 weeks.

Conclusions:

Preliminary findings suggest that an HLA vaginal gel may improve vaginal/sexual health issues and concerns of breast cancer survivors both in their perceived symptoms and on clinical exam; however, further study is needed to examine if these promising trends continue over time and to determine the ideal frequency of product administration.
Title: Abstract Withdrawn

Body:
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-11-08

Title: Impact of treatment on quality of life (QOL) in the BEACON study, a randomized phase III trial of etirinotecan pegol (EP) versus treatment of physician's choice (TPC) in patients (pts) with advanced breast cancer (aBC) whose disease has progressed following anthracycline (A), taxane (T) and capecitabine (C)

Cortes J, Awada A, Rugo HS S, Twelves C, Im S-A, Zhao C, Hoch U, Ney J, Hannah AL L and O'Shaughnessy J. Vall D'Hebron University Hospital, Barcelona, Spain; Jules Bordet Institute, Université Libre de Bruxelles, Brussels, Belgium; Mayo CLinic, Jacksonville, FL; University of California, San Francisco, San Francisco, CA; University of Leeds, Leeds, United Kingdom; Seoul National University College of Medicine, Seoul, Korea; Nektar Therapeutics, San Francisco, CA; Consultant, Sebastopol, CA and Baylor Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX.

Body: Background: The need remains for novel agents that prolong survival and/or improve QOL in women with aBC. EP is a long-acting topoisomerase 1 inhibitor engineered to produce sustained exposure to irinotecan and its active metabolite SN38. Given previous efficacy seen in an earlier phase II trial in MBC, EP 145 mg/m2 every 3 weeks was compared to TPC (one of 7 single-agent regimens) in the randomized phase 3 BEACON study (NCT01492101). As reported at ASCO 2015 (abstract 1001), EP prolonged median overall survival by 2.1 months, although this did not reach statistical significance (12.4 vs 10.3 months; HR 0.87, p=0.08). Grade ≥ 3 adverse events were significantly less common with EP (48% vs 63% with TPC, p<0.001). We now present results of the QOL analyses.

Methods: Patients completed validated health-related QoL (HRQoL) questionnaires, EORTC QLQ-C30 (version 3.0) and breast cancer-specific QLQ-BR23, pretreatment and every 8 weeks until progression, death or withdrawal of consent. Questionnaires were scored according to the EORTC manual. For each scale, raw scores were standardized via a linear transformation to a range from 0 to 100. Absolute scores and changes from baseline were analyzed longitudinally and categorically using a 5-point difference calculated by treatment group. Comparisons between treatment groups were conducted to evaluate the differences in global health status, functional scores and symptoms over time.

Results: The majority of patients who were randomized (total: 733/852 [86%], EP: 378/429 [88%], TPC: 355/423 [84%]) completed at least one post-baseline HRQoL questionnaire. In the EORTC QLQ-C30, grade ≥ 3 AEs significantly impacted HRQoL measured by global health status and 5 additional functional domains. Of the six domains, compared to TPC in a longitudinal analysis, EP was statistically superior in the mean treatment effect through Week 32 in global health status p=0.02 and physical functioning scale p=0.01. EP was also numerically superior in all other scales. In EORTC QLQ-C30 and BR-23, a total of 13 symptoms were measured and categorically analyzed. There were no treatment differences in 7 of 13 symptom scales. EP was associated with worsening of 3 symptom scales: appetite loss, nausea/vomiting, and diarrhea. TPC was associated with worsening of 2 symptom scales: dyspnea and systemic side effects.

Conclusions: In the phase 3 BEACON trial comparing EP to TPC, the more favorable toxicity profile of EP resulted in an improvement in global health status and physical function (results of the symptom scales confirmed the different toxicities of the two arms). EP remains a promising investigational therapy for aBC.
A randomized controlled trial of postoperative adjuvant therapy for elderly breast cancer patients: Comparison of health-related quality of life between clinical trial participants and decliners

Saito T, Sawaki M, Hozumi Y, Sagawa N, Iwata H, Kashiwaba M, Kawashima H, Kobayashi K, Taira N, Takashima T, Takahashi M, Tsuneizumi M, Nakayama T, Baba S, Bando H, Mizuno T, Yamaguchi M, Yamamoto Y, Uemura Y, Ohashi Y and Mukai H. Saitama Red Cross Hospital, Saitama, Japan; Aichi Cancer Center Hospital, Nagoya, Aichi, Japan; Jichi Medical University Hospital, Shimotsuke, Tochigi, Japan; Kameda Medical Center, Kamogawa, Chiba, Japan; Iwate Medical University, Morioka, Iwate, Japan; Aomori City Hospital, Aomori, Japan; Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan; Okayama University Hospital, Okayama, Japan; Osaka City University Graduate School of Medicine, Osaka, Japan; NHO Hokkaido Cancer Center, Sapporo, Hokkaido, Japan; Shizuoka General Hospital, Shizuoka, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; University of Tsukuba Hospital, Tsukuba, Ibaraki, Japan; Meie University Hospital, Tsu, Mi, Japan; JCHO Kurume General Hospital, Kurume, Fukuoka, Japan; Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; Tokyo University Hospital, Bunkyo-ku, Tokyo, Japan; Chuo University, Bunkyo-ku, Tokyo, Japan; National Cancer Center Hospital East, Kashiwa, Chiba, Japan and Sagara Hospital, Kagoshima, Japan.

Background: Health-related quality of life (HRQoL) is one of the important outcomes in cancer control trials and has increasingly become one of the primary foci. Obtaining informed consent from participants is essential for participation in randomized controlled trials (RCTs), but the participation in these RCTs may directly influence HRQoL, because treatment options are determined according to the allocation schedule. To date, only a few studies have compared HRQoL between clinical trial participants and decliners.

Patients and Method: The National Surgical Adjuvant Study of Breast Cancer 07 (N-SAS BC 07) is a randomized controlled trial in women with HER2-positive primary breast cancer who are over 70 years of age. The primary aim was to investigate the benefit of trastuzumab monotherapy compared with combination therapy using trastuzumab and chemotherapy. The study concept and design were published in concept paper (Sawaki M. et al., Jpn J Clin Oncol. 2011). In this study, patients were randomized to receive either trastuzumab plus chemotherapy or trastuzumab monotherapy. The primary endpoint was disease-free survival, and the secondary endpoints were overall survival, relapse-free survival, safety, HRQoL, comprehensive geriatric assessment (CGA) and cost effectiveness (protocol ID; NCT01104935).

HRQoL and CGA were assessed at registration (baseline), 2 month, 1 year, and 3 years after the start of protocol treatments using the Functional Assessment of Cancer Therapy-General (FACT-G), Hospital Anxiety and Depression Scale (HADS), EuroQol 5 Dimension (EQ-5D), Tokyo Metropolitan Institute of Gerontology (TMIG) index of competence, and the Philadelphia Geriatric Center (PGC) Morale Scale.

The patients who declined to participate in N-SAS BC 07 were registered in a cohort study to prospectively evaluate the subsequent treatment options and prognosis (07-Cohort). The same questionnaire that was used in N-SAS BC 07 was used in 07-Cohort to evaluate HRQoL and CGA at entry.

Results: Patients were enrolled from October 2012 to October 2016. During this period, 275 and 123 patients were registered in N-SAS BC 07 and 07-Cohort, respectively. The mean age at entry of the patients in the N-SAS BC 07 and 07-Cohort groups was 73.9 and 74.6 years, respectively. The questionnaire response rates at baseline in the patients in N-SAS BC 07 and 07-Cohort groups were 89% and 82%, respectively. There were no significant differences in FACT-G, HADS, EQ-5D, or TMIG index of competence at baseline between the groups, but the mean (standard deviation) scores of PGC Morale Scale in N-SAS BC 07 and 07-Cohort groups were 10.8 (3.3) and 9.9 (3.7), respectively, with the scores being significantly greater in the N-SAS BC 07 group (p=0.020, t-test).

Conclusion: The PGC Morale Scale provides a multidimensional approach to assess the psychological state of older people. This study indicated that participation in the RCT did not affect the baseline QoL of elderly patients but suggested that the baseline QoL of the RCT participants was better than decliners.
Title: Breast cancer patients' preferences for adjuvant radiotherapy post-lumpectomy: Whole breast irradiation versus partial breast irradiation—single institutional study

Szumacher EF F, McGuffin M, Presutti R, Pignol IP, Harth T, Meschi A, Feldman-Stewart D, Chow E, DiProspero L, Vespriini D, Rakovich E, Lee J, Doherty M, Soliman H, Ackerman I, Cao X and Kiss A. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Sunybrook Health Sciences Centre, Toronto, ON, Canada and Division of Cancer Care and Epidemiology, QCRI, Kingston, ON, Canada.

Body: Background
The standard regimen of radiotherapy following lumpectomy consists of whole breast irradiation (WBI) to the entire breast including the lumpectomy cavity and all the surrounding normal breast tissue. Recently, there has been increased interest in partial breast irradiation (PBI) as an alternative to WBI. However, the preferences of patients with early breast cancer as to what type of radiotherapy regimen post lumpectomy they would prefer and why is unclear in the literature. This study was conducted to determine whether patients with early stage breast cancer would prefer PBI or WBI and to identify important factors for patients when making their treatment decision.

Methods
Based on our previous study of early stage breast cancer patient information needs, the relevant literature and the ASTRO consensus statement guidelines, an educational tool and questionnaire were developed. New patients with early breast cancer who were referred for adjuvant radiotherapy at the large academic cancer center were invited to participate. Women ≥40 years of age with a new histological diagnosis of ductal carcinoma in-situ or invasive breast carcinoma treated with breast conserving surgery showing clear margins for non-invasive and invasive disease and negative axillary nodes were eligible. Descriptive statistics were calculated for all variables of interest. Survey question responses were compared between those preferring WBI or PBI using chi-square analyses or Fisher's exact tests.

Results
Ninety /126 patients who were approached about this study completed the survey, 27(30%) preferred PBI and 55(62%) preferred WBI. Four patients (4%) required more information to choose between WBI vs PBI, and 3 patients (3%) had no preferences. From patients who choose WBI,32(58%)patients preferred hypofractionated RTvs 14 (25%)conventional RTregimen. Factors rated as important by patients in making their decision included convenience [PBI=18/26(69%), WBI=36/54(67%)], financial factors [PBI=14/26(53%), WBI=21/55(38%)], radiation dose to the breast [PBI=20/26(80%), WBI=46/55(83%)], invasiveness [PBI=18/26(69%), WBI=43/53(81%)], recurrence rate [PBI=26/26(100%), WBI=55/55(100%)], survival [PBI=26/26(100%), WBI=54/55(98%)], side effects PBI 21/26 (81%) WBI 47/55(85%) effectiveness [PBI=25/26(96%),WBI=54/55(100%)], standard method of treatment [PBI=16/26(61%), WBI=52/54(96%), p=0.001] and radiation dose to surrounding organs [PBI=23/26(88%), WBI=52/54(95%)].

Conclusions
Our study shows that patients with early breast cancer prefer WBI as an adjuvant treatment post lumpectomy. There is significant association between preference of treatment and importance of standard treatment. Patients preferring WBRT were more likely to consider standard treatment as more important than those preferring PBI. There was a marginally significant association between marital status and preference of radiotherapy (p=0.0773) and employment (p=0.0667). Those currently not employed were marginally more likely to prefer WBI than those currently employed. A detailed analysis of all decisional preferences between WBI and PBI will be presented at the meeting.
**Title:** Patient preferences for minimally invasive and conventional locoregional treatment for early-stage breast cancer; A utility assessment

Knuttel FM M, van den Bosch MMAJ AAJ, Young Afat DA A, Emaus MJ J, van den Bongard DHJG HJG, Witkamp AJ J and Verkooijen HM M. University Medical Center Utrecht, Utrecht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands and University Medical Center Utrecht, Utrecht, Netherlands.

**Body: Introduction**

Breast cancer (BC) is increasingly diagnosed in an early stage due to improved imaging techniques and mammography screening. Non- or minimally invasive treatments, such as radiofrequency ablation (RFA) and MR-guided high-intensity focused ultrasound (MR-HIFU) ablation are currently being developed as alternatives to breast surgery. Patients' preferences with regard to these new treatments have not been investigated. The aim of this study is to assess the preferences of BC patients and healthy women regarding these new techniques, compared to conventional locoregional treatments.

**Materials and methods**

Six health states containing descriptions of BC treatments with their clinical consequences were developed based on literature and clinical expertise. Six treatment scenarios for treatment of early-stage BC without indication for adjuvant systemic therapy were proposed: 1) mastectomy with sentinel lymph node biopsy (SLNB); 2) mastectomy followed by direct reconstruction and SLNB; 3) breast conserving surgery with SLNB and whole breast radiotherapy; 4) radiofrequency ablation preceded by SLNB and followed by whole breast radiotherapy; 5) MR-HIFU ablation preceded by SLNB followed by whole breast radiotherapy; and 6) ablative partial breast radiotherapy (single dose) preceded by SLNB. Patients and healthy volunteers rated each scenario and corresponding health state by both visual analogue scale (VAS) and time trade-off (TTO). Eligible subjects are female BC patients who completed treatment > 12 months ago and women aged > 40 years without BC history. Patients were recruited from the UMBRELLA cohort at the department of Radiation Oncology of the University Medical Center Utrecht (The Netherlands). Healthy volunteers were friends or relatives of the BC patients enrolled in the study. Mean and standard deviation of VAS and TTO derived utility scores were calculated and health states were ranked by preference. Repeated measures ANOVA was used to test whether difference between ratings was significant. Spearman correlation coefficient was used to compare VAS and TTO scores.

**Results**

In a preliminary analysis in 23 women (18 patients and 5 healthy volunteers) mean age was 59±10.6 years. Responders rated breast conserving surgery as most preferred treatment (VAS 0.88±0.1, TTO 0.91±0.08). VAS scores were strongly correlated to TTO scores ($\rho=0.74$, p-value < 0.005). Updated and more detailed results on 150 patients and healthy women will be presented in December.

**Conclusion**

We are performing a utility analysis of new and existing breast cancer treatments in breast cancer patients and healthy volunteers. This study will yield important information to guide the development and innovation of breast cancer therapies in the future.
Title: Quality of life, anxiety and depression during treatment of ductal carcinoma in situ and invasive breast cancer

Young-Afat DA A, Gregorowitsch ML L, Pignol J-P, van Gils CH H, van Vulpen M, van den Bongard DJ J and Verkooijen HM M. University Medical Center, Utrecht, Netherlands and Erasmus Medical Center, Rotterdam, Netherlands.

Body: INTRODUCTION
Patients with ductal carcinoma in situ (DCIS) have excellent overall survival rates. Yet, previous studies suggested that quality of life (QoL) between patients with DCIS and patients with early-invasive breast cancer (early-IBC) are similar after treatment. We compared anxiety, depression and quality of life of patients with DCIS and patients with early-IBC during treatment, at the initiation of postsurgical radiotherapy.

METHODS
We conducted this study within a prospective observational cohort of breast cancer patients indicated to receive adjuvant radiation treatment at the department of Radiation Oncology at the University Medical Center Utrecht, the Netherlands (the UMBRELLA cohort). At the time of inclusion all cohort participants consented to the collection of clinical and patient reported outcomes (PROMs) at regular intervals. Patient reported outcomes on QoL (i.e. EORTC QLQ-C30) and anxiety and depression (i.e. HADS) were collected at the start of postsurgical radiotherapy. All patients who were diagnosed between October 2013 and January 2015 with DCIS or early-IBC (i.e. pT1 and pT2 without lymph node involvement) were included in this analysis.

To analyze differences in mean levels of PROMs (i.e. anxiety and depression, QoL) between patients with DCIS and early-IBC, two sample t-tests were used. Differences in proportions of patients with high anxiety or high depressive scores (i.e. scores ≥11) were analyzed with the Pearson-Chi square test. We compared PROMs of DCIS and early-IBC patients with those of patients with advanced-invasive breast cancer from the UMBRELLA cohort using analyses of variance (ANOVA).

RESULTS
Forty-six patients were diagnosed with DCIS and 227 with early-IBC. DCIS and early-IBC patients did not show statistically significant differences in levels of anxiety (mean DCIS 4.5, early-IBC 5.2, p=0.18), depression (mean DCIS 2.6, early-IBC 3.0, p=0.73) or QoL (mean DCIS 78.3, early-IBC 74.7 p=0.70). Seven percent of women with DCIS women reported severe anxious symptoms, compared to 8% in women with early-IBC (p=0.22). Severe symptoms of depression were seen in 2% of DCIS patients and 4% of early-IBC (p=0.30).

Patients with advanced invasive breast cancer (n=118) reported significantly higher anxiety (mean 6.3, p<0.005) and depression (mean 4.6, p<0.001) scores and poorer QoL levels (69.9) as compared to patients with DCIS and early-invasive breast cancer.

CONCLUSION
Despite excellent survival probabilities and less invasive treatment, women with DCIS report similar levels of anxiety, depression and quality of life during treatment as compared to women with early-invasive breast cancer.
Cancer-specific distress, life satisfaction and parenting concerns in young breast cancer survivors

Burgmann M, Hermelink K, Farr A, Heiduschka A, van Meegen F, Engel J, Harbeck N and Wuerstlein R. Breast Center, CCC of LMU University Hospital of Munich, Munich, Germany; Munich Cancer Registry (MCR) of the Munich Tumour Centre at the Institute for Medical Information Processing, Biometry and Epidemiology (IBE), University Hospital of Munich (LMU), Munich, Germany and Breast Center of Medical University of Vienna-General Hospital, Vienna, Austria.

Body: Introduction
It is known that young breast cancer (BC) patients tend to suffer more psychological stress and have lower quality of life than older women, are less sexually active and have more body image issues than healthy women of the same age. They may also be challenged by reproductive concerns. To our knowledge, no data exist about the effect of the disease on the life satisfaction of premenopausal BC patients. This study tries to elucidate life satisfaction in several aspects of daily life in this special cohort as well as aspects of cancer-specific distress and parenting concerns in order to improve our support strategies.

Materials and methods
In a cross-sectional study design, all patients with < 40 years at primary BC diagnosis treated at Breast Center, CCC LMU Munich (Germany) between 2006 and 2013 were eligible for participation. Standardized questionnaires assessing life satisfaction (Life Satisfaction Questionnaire; Fahrenberg et al, 2000) and cancer-specific distress (Questionnaire on Stress in Cancer patients; Herschbach et al, 2003), as well as a self-developed questionnaire on partnership, employment situation, family planning, demographic and medical data were mailed in 2014.

Results
88 patients responded (55%). Compared with population data stratified for age and sex, patients showed significantly less satisfaction in the domains of health (p<0.001) and sexuality (p=0.002) but not in any other domains or overall life satisfaction. The patients' most pronounced cancer-specific problems were fear of cancer recurrence and fear of further hospital stays, diminished sexual activity, and psychosomatic problems like nervousness, fatigue and insomnia. Of those patients who retrospectively evaluated their decision for or against fertility preservation, 76.4% were satisfied with their choice. Current desire to have children was reported by 45.8% of patients and another 15.6% were uncertain, but only 21.7% actually planned to have children. The most frequently reported reasons to refrain from childbearing were shortened life expectancy, negative impact of pregnancy on prognosis, and treatment-related infertility.

Discussion
In our cohort, the general life satisfaction of young breast cancer survivors showed no difference from women without cancer, but these patients were not satisfied with their general health status and with their sexuality. Also, cancer-related fears and psychosomatic problems considerably stress young patients and thus need to be addressed by supportive care programs. There is also a need for counselling regarding childbearing after BC treatment. In contrast, fertility preservation seems to be well established in medical consultations. In conclusion, tailored supportive care programs have to be realized to respond the unique needs of young BC patients.
Title: Content validation and modification of the treatment satisfaction questionnaire for medication (TSQM) for breast cancer


Body: Background
With the growing number of treatment options for metastatic breast cancer, patient (pt) perspective on treatment and treatment satisfaction is increasingly important to assess. A literature search was conducted to identify commonly utilized measures of pt preference. The Treatment Satisfaction Questionnaire for Medication (TSQM) was identified as the most comprehensive measure to assess patient-reported treatment effectiveness, side effects, convenience, and overall satisfaction with medication for a wide variety of medical conditions. The broad nature of the TSQM allows for assessment of treatment satisfaction across indications; however it has not been evaluated for specific use in populations of cancer patients. We assessed the content validity of the TSQM v1.4® with previously treated breast cancer (BC) patients to determine if any modifications were necessary to ensure relevance in this population.

Methods
This non-interventional, cross-sectional qualitative study used individual semi-structured cognitive interviews to: (1) assess content of the TSQM and relevance for BC, and (2) identify necessary modifications as based on patient interviews. 15 BC pts with a mix of receptor types [hormone receptor positive (HR+), HER2+, triple negative (TNBC)], age > 18 yrs and currently receiving or recently completed first-, second-, or third-line therapy for Stage IIIA to IV were included to ensure applicability across BC. Pts meeting inclusion/exclusion criteria were identified through database review and recruited. Interviews were conducted in three waves of five pts and necessary modifications were made to the TSQM after each wave. Interviews were audio recorded and transcribed.

Results
Mean age of participants was 53 years; 6 patients were HR+/HER2+, 2 patients were HR+/HER2-, 2 patients were HR-/HER2+, 5 patients were TNBC. Pts reported that the three items comprising the effectiveness scale were not relevant in BC and are difficult to answer. Specifically, pts felt the first item ('ability of medication to treat/prevent condition') would be highly dependent on scan results, and indicated that the term 'prevent' is not relevant to the experience of BC pts with a metastatic diagnosis. With the second item ('relieves symptoms'), most pts reported that they did not experience symptoms related to their cancer and thus were unsure how to respond. Pts were confused by the term 'symptoms' and interpreted it as 'side effects from treatment' rather than disease-related. For the third item ('time it takes medication to start working'), pts noted the only way to definitively know if a cancer medication has started working is with scan results, and they would not be able to assess this.

Pts found the remaining TSQM items to be relevant, clear, and easy to understand, and offered a few suggestions for improvement that can be evaluated in future research.

Conclusion
BC pts did not find the TSQM effectiveness scale items relevant to the assessment of their treatment experience, and as a result these items were eliminated in the modified TSQM for Breast Cancer (TSQM-BC). The TSQM-BC is content valid for use in breast cancer studies. Psychometric performance of this version of the TSQM-BC will be evaluated in future research.
Title: Omani women's awareness of breast cancer and early detection practices

Al-Khasawneh EM M, Seshan V, Al-Farsi Y, Siddiqui ST T and Al-Moundhri MS S. College of Nursing, Sultan Qaboos University, Muscat, Oman and College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, Oman.

Body: Background: This study assessed Omani women's awareness of breast cancer and early detection behaviour to develop an awareness intervention for empowering women with better early detection practices and improving survival rates.

Methods: A cross-sectional survey was conducted of 1372 Omani women of age ≥20 years from 5 regions, recruited using stratified random sampling. It collected demographic information, and scores for early detection behaviour and awareness of breast cancer. Mean early detection and awareness scores were calculated for age groups, education levels and income levels. The association and correlation between each demographic variable and score variables were estimated using linear regression and Pearson's correlation. The extent of belief in "evil eye" as a risk factor for breast cancer was also estimated for each demographic variable.

Results: The overall mean for early detection scores was 0.59(±0.26). The overall mean for awareness was 0.47(±0.21). The mean awareness score increased with age, levels of education and income. There was no such trend for early detection, which was highest for the 40–49 years age group, secondary education level and the OMR500–999 income level. The associations and correlations between general awareness and income level (B=0.017, R=0.147), education level (B=0.029, R=0.090) and age group (B=0.031, R=0.067) – while low in magnitude – were significant (p<0.05). Early detection had a negative significant association with income (B=-0.016), but a positive one with education (B=0.032). Overall, a majority of women (59.5%) from all demographics agreed or strongly agreed with the belief in "evil eye" as a risk factor for breast cancer.

Conclusion: Significant associations between awareness and early detection, and better socioeconomic factors demonstrate an awareness gap between the different demographics of Omani society. The overall dismal awareness and early detection results, and the survey of local beliefs highlight a severe necessity for a contextually-tailored awareness intervention programme in Oman.
Title: Long term follow up of patients (pts) with HER2positive (H+) early stage breast cancer (ESBC) treated with trastuzumab (T)


Body: Background. H+BC is an aggressive variant of BC with earlier and more frequent metastatic relapse than HER2 normal disease. Following the reports in 2005 of several large random assignment trials which showed that the anti-HER2 monoclonal antibody T improved the outcome of pts with H+ESBC receiving chemotherapy, we introduced Trastuzumab (T) as a routine component of standard adjuvant (AdjRx) and neo-adjuvant (NAdjRx) therapy for all pts with H+ESBC at our institution. Others had received it investigationally from 2000-2004. We report our 14 year experience with Adj and NAdjRx with T.

Methods. We compiled a comprehensive database of all patients ever treated at our institution with Adj or neo-Adj T for H+ESBC. All pts were cross-checked through the Pathology, Pharmacy and Med Onc datasets, and all cases were individually reviewed. Results. Out of 764 pts included in the H+BC medical oncology database, we identified 518 pts (AdjT=373, NAdjT=145) with stage I-III disease treated with T between August 2000 and October 2014. Pts characteristics: median (range) age: 54 (26-86) yrs, hormone-receptor (HR) status: HR positive (HR+) 64%/HR negative (HR-) 35%/HR unknown 1%, lymph node positive (LN+): 52% (AdjT: 44%/NAdjT 72%), systemic therapy+T: TCH [docetaxel/carboplatin/trastuzumab] 306 (59%), anthracycline and taxane regimens-85 (16%), single-agent taxane 48 (9%), other regimens or T without chemotherapy-79 (15%). The database lock out date was May 31st 2015. The median follow-up (FU) time is 50.6 (1.4-156.6) months.

The overall relapse rate (RR) in the whole population is 8.9% (AdjT 9.4%/NAdjT 7.6%) and distant RR is 8.3% (AdjT 9.1%/NAdjT 6.2%). OS rate in the whole study population is 93.4% (AdjT 92.5%/NAdjT 95.9%). RR was lower in pts with HR+ [7.2% (AdjT 7.1%/NAdjT 7.8%)] than in those with HR- [10.6% (AdjT 11.9%/NAdjT 7.4%)] disease. Among all the subgroups the lowest RR was observed in the HR+/LN- subgroup (2.7%) whilst the highest was in the HR-/LN+ subgroup (15.5%). Pts treated in the NAdjT cohort had a pathological complete response (pCR) rate of 38.6%. In the whole study population the median time to relapse (TTR) was 31.8 (7.9-97.6) months with no difference between AdjT and NAdjT cohorts. 87% of all relapses happened within 60 months of first T. Out of 6 delayed relapses (after month 60) 4 were HR+ 1was HR unknown and 1 was HR- in the form of a contralateral axillary recurrence, possibly from a second occult breast primary. Among pts treated prior to March 2010 (N=267), who have a minimum FU of 5 yrs, the RFS is 86.5% and the OS rate of 89.1%.

Conclusions. The prognosis of pts with HER2+ ESBC in the T era is excellent. Despite the large proportion of pts with LN+ disease in our database, over 90% are alive at almost 5 years. HR+ confers a better prognosis than HR- in both LN+ and LN-disease. Long-term outcome data show that nearly 90% of all relapses occur within 5 yrs from initiation of anti-HER2 therapy especially in the HR- subgroup. Late relapse is rare in HR- pts. Adjuvant hormonal therapy is likely to contribute meaningfully to the favourable outcome of HR+/HER2+ ESBC and may explain the discordance between the improved survival of ER+ Adj pts and the improved pCR reported for neoAdj T in ER+ pts.
Title: Adjuvant trastuzumab improved the prognosis of HER2-positive early breast cancer: Single institutional cohort study from clinical practice

Akiyoshi S, Ishida M, Koga C, Nakamura Y, Taguchi K, Ohno S and Tokunaga E. National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan; National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan and Breast Oncology Center, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan.

Body: Background: Trastuzumab-containing regimens for the adjuvant treatment of human epidermal growth factor receptor 2 (HER2)-positive early breast cancer significantly improved the prognosis. However, most data showing the effects of trastuzumab come from clinical trials.

Aims: To evaluate the efficacy of the adjuvant trastuzumab in the clinical practice, the prognosis of HER2-positive early breast cancer was investigated according to the adjuvant treatment with or without trastuzumab.

Methods: Among 2548 women who underwent surgery for primary breast cancer in our department between 2000 and 2011, 315 patients had HER2-positive tumors. A total of 293 patients aged 75 or younger with invasive HER2-positive breast cancer were included in this study. The associations between clinicopathological characteristics, adjuvant therapy and relapse-free survival (RFS) were examined. The RFS was estimated using the Kaplan-Meier method, and independent predictors were assessed using proportional cox hazard models.

Results: 113 (38.5%) patients were treated with chemotherapy alone (Cohort A), 100 (34.1%) patients were treated with chemotherapy and trastuzumab (Cohort B) and 80 (27.3%) patients received neither chemotherapy nor trastuzumab (Cohort C). The administration of adjuvant trastuzumab significantly increased in 2007. The prognosis of the patients treated in 2007-2011 was significantly better than that of patients treated in 2000-2006 (p=0.0011). The use of adjuvant trastuzumab was significantly associated with longer RFS (p=0.0286). The improvement of RFS by using trastuzumab was significant in node-positive patients. The patients in Cohort C had mainly node negative and small tumors. RFS of the patients treated of Cohort B was significantly more favorable than that of Cohort A. However, RFS of Cohort C was not statistically different from that of Cohort B, probably due to the early stage of Cohort C. In univariate analysis, larger tumor size (T2, 3), lymph node metastasis, lymphovessel invasion and absence of trastuzumab were related to relapse. In multivariate analysis, factors related to relapse were lymph node metastasis and absence of trastuzumab.

Univariate and multivariate analysis for relapse-free survival

<table>
<thead>
<tr>
<th>Factors</th>
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<th>Multivariate analysis</th>
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<tr>
<td></td>
<td>HR</td>
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<td>Tumor size</td>
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<tr>
<td>T2, T3 vs. T1</td>
<td>3.10</td>
<td>1.69-5.85</td>
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<td>3 vs. 1, 2</td>
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<td>0.42-1.44</td>
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<tr>
<td>Ly positive vs. negative</td>
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<td>1.87-6.25</td>
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<tr>
<td>ER positive vs. negativ</td>
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<td>adjuvant chemotherapy</td>
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<tr>
<td>adjuvant trastuzumab</td>
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</table>

Conclusions: Use of adjuvant trastuzumab improved the prognosis of HER2-positive early breast cancer in clinical practice.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-12-03

Title: Abstract Withdrawn

Body:
PALOMA3: Phase 3 trial of fulvestrant with or without palbociclib in pre- and postmenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer that progressed on prior endocrine therapy—confirmed efficacy and safety

Cristofanilli M, Bondarenko I, Ro J, Im S-A, Masuda N, Colleoni M, DeMichele AM, M, Loi S, Verma S, Iwata H, Huang Bartlett C, Zhang K, Puyana Theall K, Turner NC C and Slamon DJ J.  Thomas Jefferson University, Philadelphia, PA; Dnipropetrovsk Medical Academy, City Multiple-Discipline Clinical Hospital, Dnipropetrovsk, Ukraine; National Cancer Center, Goyang-si, Republic of Korea; Seoul National University, Seoul, Republic of Korea; NHK Osaka National Hospital, Osaka City, Japan; Istituto Europeo di Oncologia, Milano, Italy; University of Pennsylvania, Philadelphia, PA; Peter MacCallum Cancer Centre and University of Melbourne, East Melbourne and Parkville, Victoria, Australia; Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Aichi Cancer Center Hospital, Nagoya, Japan; Pfizer Inc, NY, NY; Pfizer Inc, San Diego, CA; Pfizer Inc, Cambridge, MA; Royal Marsden Hospital, London, United Kingdom and University of California, Los Angeles, CA.

Background: Selective estrogen receptor modulators and aromatase inhibitors (Al) (+LHRH agonists [premenopausal]) are standard of care (SOC) for hormone–receptor–positive (HR+) metastatic breast cancer (MBC). Many HR+ MBC patients (pts) get limited benefit from adjuvant or advanced endocrine therapy (ET) and develop endocrine resistance, refractory disease. HR+ BC growth relies on cyclin dependent kinases 4/6 that promote G1–S phase cell cycle progression. Palbociclib (PAL) with ET showed efficacy in HR+/HER2– MBC (Turner et al, 2015). We report updated safety and efficacy from PALOMA3 with longer follow-up, focusing on degrees of clinically defined endocrine resistance.

Methods: Pts with HR+/HER2– MBC that progressed on prior ET were randomized 2:1 to PAL (125 mg/d oral [3 wks drug, 1 wk off]) + fulvestrant (F, 500 mg, SOC) +/- goserelin or placebo (PLB)+F. One line of chemotherapy (CT) for MBC was allowed. Pt stratification: prior ET sensitivity; visceral metastases; menopausal status. Primary endpoint (EP) was investigator–assessed progression–free survival (PFS). Secondary EP: overall survival, response assessment, patient–reported outcomes, safety.

Results: By March 2015, median follow–up was 8.9 mo. 521 pts were randomized (PAL+F, 347; PLB+F, 174). Baseline characteristics were balanced. Median PFS was 9.5 (95% CI 9.2–11.0) mo (PAL+F) vs 4.6 (3.5–5.6) mo (PLB+F) (HR 0.46 [0.36–0.59], P<0.001). Overall response (CR+ PR) was significantly improved with PAL+F (ITT: 19% vs 8.6%. P=0.001; pts with measurable disease: 24.6% vs 10.9%, P<0.001). Clinical benefit (CBR=CR+PR+SD â“¥24wks) was 66.6% vs 39.7% (P<0.001). Benefit from PAL was confirmed in pre– and postmenopausal pts with PFS in premenopausal 9.5 vs 5.6 mo (HR=0.50 [0.29–0.87], P=0.006) and in postmenopausal 9.9 vs 3.9 mo (HR=0.45 [0.34–0.59], P<0.001). Common adverse events (AEs) for PAL+F vs PLB+F were neutropenia (80.9 vs 3.5%), leukopenia (49.6 vs 4.1%), and fatigue (39.1 vs 28.5%); febrile neutropenia occurred in 0.9% (P+ F) vs 0.6% pts (PLB+F). Discontinuation due to AEs was 4.0% on P vs 1.7% on PLB. The benefit of PAL+F vs PLB+F was compared in pts with various degrees of endocrine resistance: a) progression ≤12 mo of adjuvant ET completion, PFS 9.5 vs 5.4 mo (HR 0.55 [0.32–0.92], P=0.01); b) failed 1 line of ET, 10.2 vs 5.4 mo (HR 0.42 [0.29–0.59], P<0.001); c) failed 2 lines of ET, 9.9 vs 1.8 mo (HR=0.20 [0.10–0.39, P<0.001]; d) proven endocrine sensitive, 10.2 vs 4.2 mo (HR 0.42 [0.32–0.56], P<0.001); e) proven no prior endocrine sensitivity, 7.5 vs 5.4 mo (HR 0.64 [0.39–1.07], P=0.04) f) AI most recent therapy, 9.5 vs 3.7 mo (HR 0.42 [0.31–0.56], P<0.001).

Conclusion: Mature efficacy confirmed superior PFS and demonstrated significantly improved clinical response and CBR by the combination of ET and Palbociclib. It also consistently showed therapeutic benefit irrespective of menopausal status and various degrees of endocrine sensitivity. Safety profile is favorable. PAL+F may be an effective option for HR+ MBC pts.

Funding: Pfizer.
Body: Background
Palbociclib (P) is an oral CDK4/6 inhibitor. In PALOMA-1/TRIO-18, a randomized phase 2 trial, addition of P to letrozole (L) significantly prolonged progression-free survival (PFS) (20 mo with P+L vs 10 mo with L alone; HR = 0.488, \(P=0.0004\); Finn et al, Lancet Oncol, 2015) in postmenopausal women with estrogen-receptor-positive (ER+), HER2-negative advanced breast cancer (ABC) in the first-line setting. At the time of final PFS analysis, overall survival (OS) was immature.

Objectives
It is clinically important to understand whether patients (pts) benefit from standard of care endocrine therapy (ET) after they progressed on P+L as first-line treatment for ABC. We report patterns of post-progression treatment in the next line of therapy immediately following participation in the PALOMA-1 trial.

Methods
Postmenopausal women with ER+ and HER2- ABC who had not received any treatment for their advanced disease were randomized to receive P+L (N = 84) or L alone (N = 81) in the first-line setting. The primary endpoint was investigator-assessed PFS. Tumor assessment was performed every 8 weeks. Post-progression treatment data was captured and analyzed.

Results
As of the data cut-off (Nov 29, 2013), 40 progression events had occurred in the P+L arm and 59 in the L alone arm. 50% of pts in the P+L arm vs. 64% in the L alone arm received ET after progression on study treatment. 60% of pts in the P+L arm vs. 66% in the L alone arm received chemotherapy (CT) after progression on study treatment. The time to 1st subsequent ET/CT after progression on study treatment, duration of 1st subsequent ET/CT, and choice of 1st subsequent ET/CT are shown in Table 1.

<table>
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<tr>
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<th>P + L</th>
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<tr>
<td>Patients (pts) with Disease Progression, NN (%)</td>
<td>40 (47.6)</td>
<td>59 (72.8)</td>
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<tr>
<td>Pts who received subsequent Endocrine Therapy (ET) after progression on study treatment, n(%)</td>
<td>20 (50.0)*</td>
<td>38 (64.4)*</td>
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<tr>
<td>Time from randomization to 1st subsequent ET (days), median (range)</td>
<td>465.5 (239-1100)</td>
<td>368.5 (65-1102)</td>
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<tr>
<td>Duration of 1st subsequent ET (days), median (range)**</td>
<td>153 (24-592)</td>
<td>151 (16-1135)</td>
</tr>
<tr>
<td>Choice of 1st subsequent ET, n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>9 (22.5)</td>
<td>15 (25.4)</td>
</tr>
<tr>
<td>Exemestane</td>
<td>6 (15.0)</td>
<td>9 (15.3)</td>
</tr>
<tr>
<td>Medroxyprogesterone</td>
<td>4 (10.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Letrozole</td>
<td>1 (2.5)</td>
<td>5 (8.5)</td>
</tr>
</tbody>
</table>
Tamoxifen 0 8 (13.6)

| Pts who received subsequent Chemotherapy (CT) after progression on study treatment, n(%) | 24 (60.0)* | 39 (66.1)* |
| Time from randomization to 1st subsequent CT (days), median (range) | 388.5 (69-918) | 281 (46-1013) |
| Duration of 1st subsequent CT (days), median (range)** | 92 (1-457) | 120 (1-1143) |
| Choice of 1st subsequent CT, n(%) | Capecitabine 1 (2.5) 10 (17.0) | Mitoxantrone 13 (32.5) 1 (1.7) | Paclitaxel 0 13 (22.0) | Other 10 (25) 15 (25.4) |

*percentages are based on N as denominator; †percentages based on NN as denominator; *some patients had both ET and CT after progression; **calculated as treatment stop date minus treatment start date +1; if treatment was ongoing at time of data cut-off, stop date was imputed as Nov 29, 2013.

Conclusions
P+L delayed the time to ET/CT as compared to L alone. Pts benefited from standard of care ET/CT after they progressed on P+L as first-line treatment for ABC as demonstrated by the length of time on subsequent therapies; no difference was observed from the L alone arm.

Clinical Trial Information: NCT00721409

Funding Source: Pfizer.
Title: Updated safety from a double-blind phase 3 trial (PALOMA-3) of fulvestrant with placebo or with palbociclib in pre- and postmenopausal women with hormone receptor-positive, HER2-negative metastatic breast cancer that progressed on prior endocrine therapy

Verma S, DeMichele AM M, Loi S, Ro J, Colleoni M, Iwata H, Harbeck N, Stearns V, Cristofanilli M, Huang Bartlett C, Schnell P, Zhang K, Thiele A, Turner NC C and Rugo HS S. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; University of Pennsylvania, Philadelphia, PA; Peter MacCallum Cancer Centre and University of Melbourne, East Melbourne and Parkville, Victoria, Australia; National Cancer Center, Goyang-si, Gyeonggi-do, Republic of Korea; Istituto Europeo di Oncologia, Milano, Italy; Aichi Cancer Center Hospital, Nagoya, Japan; Brustzentrum der Universität München, München, Germany; Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD; Thomas Jefferson University, Philadelphia, PA; Pfizer Inc, New York City, NY; Pfizer Inc, San Diego, CA; Pfizer Inc, Cambridge, MA; Royal Marsden Hospital, London, United Kingdom and UCSF Medical Center at Mount Zion, San Francisco, CA.

Body: Background: Endocrine therapy (ET) resistance remains a major clinical problem for patients (pts) with hormone receptor (HR+) breast cancer (BC). In PALOMA-3, palbociclib (P) combined with fulvestrant (F) demonstrated significant prolongation of progression-free survival (PFS) vs F plus placebo (PLB) in pre/peri and postmenopausal women with HR+/HER2– metastatic BC (MBC) whose disease progressed on prior ET (median PFS 9.2 vs 3.8 m; HR=0.422, P=0.0001).

Methods: In this double-blind phase 3 study, 521 pts with HR+/HER2– MBC were randomized 2:1 to receive P (125 mg/d orally for 3 weeks followed by 1 week off) and F (500 mg given per standard of care) or PLB plus F. Pre- and perimenopausal women also received goserelin. One previous line of chemotherapy (CT) for MBC was allowed. Safety assessments occurred at baseline and D1 of each cycle; blood counts occurred every 2 wks for the first 2 cycles and on D1 of subsequent cycles. As pts may have experienced multiple episodes of neutropenia during treatment, we analyzed all episodes in aggregate based on laboratory data per CTCAE4.0.

Results: The results reported here are from the data cutoff of Dec 2014, with a median follow-up of 5.6 m. Overall rate of any grade (G) and G3/4 AEs was 98/70% of pts in P+F vs 89/18% in PLB+F. The most commonly reported AEs in P+F (≥20%) were hematologic toxicities, fatigue, nausea, and headache. Per lab data, G3/G4 neutropenia occurred in 52.2/8.2%, G3/G4 leukopenia in 39.5/1.2%, G3/G4 anemia in 20.8/2.9% and G3/G4 thrombocytopenia in 2.1/1.2% of pts on P+F. Neutropenia occurred early, with a median onset time for first episode of ≥G3 neutropenia of 15 d (13–197) and median time from first dose to the lowest absolute neutrophil count (ANC) of 29 d (13–334). The median duration of ≥G3 episode was 7 d (1–35), suggesting that most pts can resume treatment after a 1-week cycle delay. A comparable proportion of any grade neutropenia was observed in pts with or without prior CT (prior CT 88.4% vs no prior CT 85.4%). There was no difference in the rate of G3/G4 neutropenia in the older pts (>65 yrs, 51% vs ≤65 yrs, 57%) in P+F arm. Concurrent G≥3 infections occurred in 1% of pts with G≥3 neutropenia (2/192 pts). Febrile neutropenia occurred in 0.6% of pts in both arms. 21% of pts had dose reductions and 45% had dose interruption due to neutropenia. Dose intensity was maintained at 89.7% for P. Serious adverse events (SAEs) were reported in 9.6% of pts on P+F and in 14% of pts on PLB+F. The most common SAEs on P+F were pulmonary embolism (0.9%) and pyrexia (0.9%). Safety analyses with longer follow-up (data cut off, March 2015) are ongoing and will be presented.

Conclusions: Findings suggest P+F has a favorable safety profile characterized mainly by asymptomatic hematologic toxicity. Overall SAE rates were low and comparable between the 2 arms. Palbociclib-related neutropenia differs from that seen with CT, consistent with proposed mechanism of action, in that it is not commonly associated with fever, and can be effectively managed by a dose interruption or cycle delay.

Funding: Pfizer, Inc.
Title: Upregulation of cell cycle pathway genes without loss of RB1 contributes to acquired resistance to single-agent treatment with palbociclib in breast cancer


Body: Background: The oral cdk4/6 inhibitor, Palbociclib (Palbo), has been shown to prolong progression-free survival when combined with anti-estrogen therapy and have single-agent activity in metastatic breast cancer (MBC). Progressive disease (PD) on therapy does occur, however, and little is known about resistance mechanisms. Preclinical data have suggested that cell cycle gene expression changes are a potential mechanism of resistance. We performed comprehensive genomic analyses on serial tumor samples from an exceptional responder to single-agent Palbo to determine whether such changes occur in vivo.

Methods: Serial biopsies were obtained from a 67 year old patient with MBC treated on a phase II trial of single-agent Palbo at the University of Pennsylvania. Tissue was obtained from the primary lesion (1999, Stage 3, ER-/PR+/Her2+) and first recurrence (2005, contralateral breast, bone, lung; ER+/PR-/Her2+, treated with Herceptin/letrozole). At PD (2010), pt received single-agent P, 50 mg daily for 21 days each 28-day cycle, with PR for 33 months. A sample from metastatic skin lesion at PD on P (2013) was obtained. Next generation targeted sequencing was performed at Foundation Medicine using the Heme Panel. RNA was profiled using a 784-gene custom Nanostring array including cell cycle genes and ER pathway genes. Determination of pathway enrichment was performed using GSEA and the statistical significance of network neighborhood over-representation was calculated using cumulative hypergeometric distribution.

Results: There was no genetic evidence suggesting loss of RB1, or alterations in p16, cyclin D1, cdk4, PIK3CA or ESR1, and the genomic profile did not change between the primary and recurrent tissue samples. As expected, amplification of ERRB2 was present in all samples. In contrast, expression of cell cycle-regulated genes (PLK1, TOP2A, CDK1, BUB1, CDC20, CCNA2, CCNE2, CCNB1 BIRC5) increased more than two-fold at PD on Palbo compared to pre-Palbo, along with evidence of activation of the FOXM1 network.

Conclusion: Gene expression changes associated with cell cycle activation and FOXM1 activation may lead to acquired resistance to Palbociclib, despite wild-type RB1. These data demonstrate the importance of pre-/post-treatment biopsies and the feasibility of high-level genomic assessment in archival tissues to elucidate resistance mechanisms of novel therapies.
**Title:** Safety results of the US expanded access program (EAP) of palbociclib in combination with letrozole as treatment of post-menopausal women with hormone-receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC) for whom letrozole therapy is deemed appropriate

Stearns V, Smith II JW W, Patel R, Lu D, Perkins JJ J, Cotter MJ J and Brufsky AM M. Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, Baltimore, MD; Northwest Cancer Specialists PC, Portland, OR; Comprehensive Blood & Cancer Center, Bakersfield, CA; Pfizer, La Jolla, CA; Pfizer, New York City, NY and Magee-Womens Hospital of UPMC, Pittsburgh, PA.

**Body:**

**Background:** In the Phase I/II PALOMA-1 trial, a study in women with estrogen receptor (ER) positive advanced breast cancer (ABC) receiving initial therapy for their metastatic disease, combination of the CDK4/6 inhibitor palbociclib with letrozole improved progression free survival compared to letrozole. The aim of this open-label, single-arm EAP was to provide appropriate patients (pts) with ABC access to palbociclib pending marketing approval in the United States.

**Methods:** In the EAP, a total of 242 pts with HR+/HER2- ABC were treated at 42 sites in the US. Pts received palbociclib 125 mg/d (3 weeks on, 1 week off) in combination with letrozole 2.5 mg/d (continuous daily dosing) until disease progression, intolerable adverse event (AE), or commercial availability. AEs and serious AEs (SAEs) were assessed every cycle. Complete blood counts were assessed on day 1 and day 14 of the first two cycles and then at the beginning of each cycle thereafter. Tumor assessments were collected by investigators as per routine clinical practice.

**Results:** In this early analysis, we describe an initial cohort of 97 pts, with data collected during the first 3 months of study. Median duration of therapy was 31 days. Mean age was 62 yr (range 29-89). Baseline ECOG PS was 0, 1 or 2 in 36%, 49%, and 14% of pts, respectively. Common prior treatments (≥40% in any setting) included fulvestrant (59%), anastrozole (50%), paclitaxel (50%), exemestane (48%), cyclophosphamide (46%), tamoxifen (45%), doxorubicin (44%), and capecitabine (40%). Treatment-emergent AEs (TEAE; all grades) that occurred in greater than 10% of patients included neutropenia (28%), fatigue (19%), neutrophil count decreased (12%). Other hematologic TEAE rates included: anemia 9%, white blood cell count decreased 9% and thrombocytopenia 5%. All causality SAEs occurred in 6% of pts at the rate of 1 patient each for ankle fracture, constipation, disease progression, febrile neutropenia, lung infection, and pancytopenia. The rate of palbociclib dose reduction due to a TEAE was 4%. The rate of temporary delay of palbociclib due to TEAE was 36%. TEAEs leading to permanent discontinuation occurred in 1% of pts (Grade 3 nausea & vomiting). Grade 3 or 4 TEAEs were reported in 42% of pts, including neutropenia (Grade 3: 24%, Grade 4: 2%). There were no fatal outcomes due to TEAEs. This early data will be updated for final conference presentation to include the complete patient cohort and updated duration of therapy on study drug.

**Conclusions:** In this population of pts with HR+/HER2- ABC, palbociclib in combination with letrozole was well tolerated. Analysis of this early cohort indicates that the safety profile was consistent with that seen in the PALOMA-1 trial.

Clinical trial information: NCT02142868

Funding Source: Pfizer.
2015 San Antonio Breast Cancer Symposium

Title: Results of the 3rd interim analysis of the non-interventional trial BRAWO – Subanalysis of patients <70 years and ≥ 70 years

Tesch H, Grischke E-M, Fasching PA A, Decker T, Uleer C, Schneeweiss A, Salat C, Wimberger P, Mundhenke C, Förster F, Kluth-Pepper B, Schubert J, Bloch W, Jackisch C, Schütz F and Lüftner D. Onkologie Bethanien, Frankfurt, Germany; University Hospital Tuebingen, Tuebingen, Germany; University Hospital Erlangen, Erlangen, Germany; Medical Center Onkologie Ravensburg, Ravensburg, Germany; Medical Center Hildesheim, Hildesheim, Germany; National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany; Hämato-Onkologische Schwerpunktpraxis, Munich, Germany; Technical University Dresden, Dresden, Germany; University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; Poliklinik GmbH Chemnitz, Chemnitz, Germany; Novartis Pharma GmbH, Nuremberg, Germany; University Cologne, Cologne, Germany; University Hospital Heidelberg, Heidelberg, Germany and Charité University Medicine, Campus Benjamin Franklin, Berlin, Germany.

Body: Background

BRAWO is a German non-interventional study of 3000 patients (pts) with advanced/metastatic, hormone-receptor-positive and HER2-negative breast cancer treated with everolimus and exemestane (EVE+EXE). The pivotal BOLERO-2 trial demonstrated that adding EVE to EXE improved PFS over EXE and was generally well tolerated in elderly patients with HR+ advanced breast cancer (>65 years as well as >70 years). Here we describe data of elderly patients treated with EVE+EXE in daily clinical routine.

Methods

We report data of the 3rd preplanned interim analysis (IA) of the first 1300 pts documented in BRAWO. Patient and disease characteristics in elderly patients (≥ 70 years, n=485) and patients <70 years (n=813) are described. Furthermore, safety and efficacy data for both subgroups are described.

Results

At time of data cut-off, 71% pts had discontinued the study, 29% were still ongoing. Patient and disease characteristics were comparable in both groups except for: median age (60y (range: 20-69y) vs. 75y (range 70-93y)), median time since 1st diagnosis (6.4y <70y vs. 8.8y ≥70 y), ECOG performance status 0 (56.6% <70y vs. 37.0% ≥70 y), and younger pts seemed to have less comorbidities (charlson comorbidity index (CCI)=0: 80.9% vs. 67.4%). The distribution of patients by therapy line was similar as well as tumor grading, hormone receptor status, Ki67-status and metastasis localization.

More patients in the older group received fulvestrant (20.6% vs. 16.2%), in the younger group more patients received chemotherapy (20.3% vs. 14.2%) as last antineoplastic therapy. In general, more patients in the older subgroup did not receive any chemotherapy as pretreatment (53.6% vs 40.2%).

More patients in the subgroup ≥70y received 5mg EVE as starting dose (30.3% vs. 20.8%) and had 5mg as end dose (37.9% vs. 26.9%). Median PFS was 7.1 months in the overall population, 7.0 months (6.5, 8.0; 95%CI) for pts <70y and 7.3 months (6.3, 8.6; 95%CI) for pts ≥70y. Kaplan Meier estimates for median treatment duration were longer for younger pts (167.0 days (155.0, 191.0; 95%CI) vs. 128.0 days (112.0, 152.0; 95%CI)). Incidence and severity of stomatitis were comparable across subgroups (Table 1). Quality of life analysis revealed no significant differences between older and younger pts.

Table 1: Incidence and severity of stomatitis

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>&lt; 70 years (n=813)</th>
<th>≥ 70 years (n=485)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients with at least one Stomatitis Event</td>
<td>339 (41.7%)</td>
<td>200 (41.2%)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>181 (22.3%)</td>
<td>95 (19.6%)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>119 (14.6%)</td>
<td>79 (16.3%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>19 (2.3%)</td>
<td>11 (2.3%)</td>
</tr>
</tbody>
</table>
**Discussion**

The data described here show that EVE+EXE treatment is effective and safe for elderly patients in daily clinical routine. This is consistent with data from an exploratory analysis of the pivotal BOLERO-2 trial, where the same differences in baseline characteristics were observed for elderly pts compared to younger pts as in BRAWO. Efficacy was also comparable to elderly pts in BOLERO-2 (mPFS 6.8 months for EVE+EXE in pts ≥70 years).
Title: Impact of physical activity/exercise on adverse events and quality of life during treatment with everolimus and exemestane for ER+ women - Results of the 3rd interim analysis of BRAWO

Bloch W, Baumann F, Zimmer P, Grischke E-M, Fasching PA A, Decker T, Uleer C, Schneeweiss A, Salat C, Wimberger P, Mundhenke C, Förster F, Kluth-Pepper B, Schubert J, Tesch H, Schütz F, Lüftner D and Jackisch C. University Cologne, Cologne, Germany; University Hospital Tuebingen, Tuebingen, Germany; University Hospital Erlangen, Erlangen, Germany; Medical Center Onkologie Ravensburg, Ravensburg, Germany; Medical Center Hildesheim, Hildesheim, Germany; National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany; Hämato-Onkologische Schwerpunktpraxis, Munich, Germany; Technical University Dresden, Dresden, Germany; University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; Poliklinik GmbH Chemnitz, Chemnitz, Germany; Novartis Pharma GmbH, Nuremberg, Germany; Onkologie Bethanien, Frankfurt, Germany; University Hospital Heidelberg, Heidelberg, Germany; Charité University Medicine, Campus Benjamin Franklin, Berlin, Germany and Sana Klinikum Offenbach, Offenbach, Germany.

Body: Introduction
BRAWO is a non-interventional study collecting data of 3000 breast cancer patients treated with everolimus and exemestane (advanced or metastatic, hormone-receptor-positive, HER2-negative breast cancer). We introduce results of the 3rd preplanned interim analysis with data cut-off 08/01/2015, including 1300 patients. Since physical activity/exercise was reported to influence side effects as well as quality of life (QoL) of various cancer types and therapies, this analysis focuses on the impact of the physical activity/exercise history, development on adverse effects (AE) of the medical treatment and QoL.

Methods
Patients were asked to complete the EORTC QLQ-C30 QoL questionnaire and visual analogue scales (VAS-KAS) measuring their present-, past ten year- and lifetime physical activity/exercise level. To differentiate between activity/exercise levels, VAS were divided in three equal components (inactive, somewhat active, very active). Questionnaires and information about AE (e.g. stomatitis, fatigue, nausea, diarrhea, etc.) were collected before starting the medical treatment and were repeated each three months. Logistic regression model was used to estimate the impact of baseline physical activity/exercise on AE at any time point of the therapy. ANOVA models were used to calculate the impact of the baseline activity/exercise level on QoL at the last completed data set of each patient.

Results
Median age of patients was 66 years, median weight was 70 kg, median BMI was 25.9, median time since primary diagnosis was 6.2 years, and 54.4% had visceral metastases at baseline. The median PFS for the first 1300 patients was 7.1 months (95% CI, 6.5-8.0). Patients who reported to be very active (exercise) at the week prior to baseline (4.4%) showed significant lower numbers of AE compared to patients who indicate to be somewhat (14.8%) or inactive (80.8%). In contrast to the exercise level, physical activity in everyday life did not affect the AE incidence. Neither lifetime nor past ten year activity/exercise level is associated with the occurrence of AE. Regarding QoL, very active as well as somewhat active women (measured at baseline for almost each time period) showed significant higher QoL values compared to inactive women during the last assessment before death/progress.

Conclusion
Exercise prior to medical treatment with Everolimus and Exemestane may impact AE during therapy. Since physical activity did not show such a relation, this analysis highlights the importance of specific guidelines for preventive/rehabilitative exercise programs. More knowledge about dose-response relationships is needed. Furthermore a livelong healthy, "active" lifestyle may increase QoL, even in patients with advanced and terminal breast cancer disease.
**Title:** Stomatitis in patients treated with everolimus and exemestane - Results of the 3rd interim analysis of the non-interventional trial BRAWO

Schütz F, Grischke E-M, Decker T, Uleer C, Schneeweiss A, Salat C, Wimberger P, Mundhenke C, Förster F, Kluth-Pepper B, Schubert J, Bloch W, Tesch H, Jackisch C, Lüftner D and Fasching PA A. University Hospital Heidelberg, Heidelberg, Germany; University Hospital Tuebingen, Tuebingen, Germany; Medical Center Onkologie Ravensburg, Ravensburg, Germany; Medical Center Hildesheim, Hildesheim, Germany; National Center for Tumor Diseases, University Hospital Heidelberg, Heidelberg, Germany; Hämato-Onkologische Schwerpunktpraxis, Munich, Germany; Technical University Dresden, Dresden, Germany; University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; Poliklinik GmbH Chemnitz, Chemnitz, Germany; Novartis Pharma GmbH, Nuremberg, Germany; University Cologne, Cologne, Germany; Onkologie Bethanien, Frankfurt, Germany; Sana Klinikum Offenbach, Offenbach, Germany; Charité University Medicine, Campus Benjamin Franklin, Berlin, Germany and University Hospital Erlangen, Erlangen, Germany.

**Body: Background**

BRAWO is a German non-interventional study of 3000 patients (pts) with advanced/metastatic, hormone receptor positive and HER2 negative breast cancer treated with everolimus and exemestane (EVE+EXE). One of the objectives was the documentation of how stomatitis was managed and prevented in clinical routine. We report data of the 3rd preplanned interim analysis (IA) focusing on prophylaxis and management of stomatitis in daily clinical routine.

**Methods**

Here we report data of the first 1300 documented pts on efficacy and safety with focus on the adverse event stomatitis. Patient and treatment characteristics were associated with the occurrence of stomatitis. Furthermore chosen treatments for the management of stomatitis are described.

**Results**

At time of data cut-off 71% pts had discontinued the study, 29% were still under therapy. Patient and tumor characteristics were reported previously. The most commonly reported AEs of any grade were stomatitis (41.5%), fatigue (14.6%), nausea (12.2%), diarrhea (12.1%), dyspnea (11.3%). 75.2% of stomatitis events occurred during the first 5 weeks of treatment, regardless of the chosen starting dose 5 mg or 10 mg EVE per day. Median duration of a stomatitis episode was 28 days for 5 mg EVE start dose and 23 days for 10mg. However, there was a numerically lower stomatitis incidence and less severe intensity of stomatitis for a start dose of 5 mg vs 10 mg (Table 1).

**Table 1: Stomatitis incidence and severity by EVE start dose:**

<table>
<thead>
<tr>
<th>Worst intensity of stomatitis</th>
<th>Total (n=1300)</th>
<th>Start dose 5mg (n=316)</th>
<th>Start dose 10mg (n=975)</th>
<th>Other* (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%) of pts with at least one stomatitis event (any grade)</td>
<td>513 (41.5)</td>
<td>115 (36.4)</td>
<td>421 (43.2)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>276 (21.2)</td>
<td>73 (23.1)</td>
<td>202 (20.7)</td>
<td>1 (11.1)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>198 (15.2)</td>
<td>31 (9.8)</td>
<td>165 (16.9)</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>30 (2.3)</td>
<td>3 (0.9)</td>
<td>27 (2.8)</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>35 (2.7)</td>
<td>8 (2.5)</td>
<td>27 (2.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Other includes 2.5mg (n=3), 7.5mg (n=2), 15mg (n=1), 20mg(n=2), 30mg (n=1)

86.5% of pts received recommendations regarding stomatitis prophylaxis. The most frequent recommendations were: mild dental hygiene (e.g. soft toothbrush) (74.8%), avoidance of hot, sour or salty food (70.9%), rinsing with tea (61.7%), and cooling (e.g. sucking ice or frozen pineapple) (56.6%).
At least one therapeutic measure was documented for 85.5% of stomatitis events. The most common therapeutic measures were non-drug mouthwash solution (58.3%), cooling (34.7%) or drug intervention (31.9%). Temporary EVE dose adjustments due to stomatitis were done in 11.6% of stomatitis events, temporary dose interruptions in 21.8%, respectively.

Efficacy of EVE+EXE seemed to be independent of stomatitis occurrence within 8 weeks after therapy start: mPFS 6.9 months (95%CI, 6.4-8.0) without stomatitis, mPFS 7.4 months (95%CI, 6.3-8.6) with stomatitis.

**Discussion**

The percentage of patients with any grade stomatitis was lower in BRAWO (41.5%) than in the pivotal BOLERO-2 trial (59%), which might be explained by increased awareness and experience of treating physicians for prophylaxis and management of this type of adverse event under treatment with EVE+EXE. Most stomatitis events occurred during the first 5 weeks of treatment, which is consistent with data from BOLERO-2.
Clinical effectiveness of everolimus and exemestan in advanced breast cancer patients from Asia and Africa: First efficacy and updated safety results from the phase IIIb EVEREXES study

Im Y-H, Uslu R, Lee KS, Nagarkar R, Sohn J, Sevinc A, Altundag K, Chang Y-C, Abdel-Razeq H, Im S-A, Jeong J, Park HY, Arpompwirat W, Bastick P, Le TH, Ocak Arikan O, Xue HL, Canatar A, Valenti R and Kim S-B. Samsung Medical Center, Seoul, Korea; Ege University Medical Faculty, Izmir, Turkey; National Cancer Center, Gyeonggi-do, Korea; Curie Manavata Cancer Centre, Nashik, India; Severance Hospital, Yonsei University Health System, Seoul, Korea; Gaziantep University Gaziantep Oncology Hospital, Gaziantep, Turkey; Hacettepe University Medical Faculty, Ankara, Turkey; Mackay Memorial Hospital, Taipei, Taiwan; King Hussein Cancer Center, Amman, Jordan; Seoul National University Hospital, Seoul, Korea; Gangnam Severance Hospital, Yonsei University Health System, Seoul, Korea; Kyungpook National University Medical Center, Daegu, Korea; National Cancer Institute, Bangkok, Thailand; Sutherland Hospital, Caringbah, Australia; Cho Ray Hospital, Ho Chi Minh, Viet Nam; Novartis Pharma AG, Basel, Switzerland; 1Novartis Asia Pacific Pharmaceuticals Pte. Ltd, Singapore, Singapore and Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Body: Background
BOLERO-2 phase III trial established the efficacy of everolimus (EVE) plus exemestane (EXE) for the treatment of postmenopausal patients with hormone receptor (HR)-positive, HER2-negative, advanced breast cancer (aBC). However, in this study only a minority (<10%) of patients were recruited from African and Asia Pacific countries. Considering the potential effects of ethnic and cultural differences on treatment effectiveness, it remains compelling to confirm the safety and efficacy profile of EVE+EXE in these populations.

Methods
EVEREXES is an open-label phase IIIb, single arm, multi-center trial, which from March 2013 to October 2014 enrolled 232 post-menopausal, HR-positive and HER2-negative, aBC patients previously treated with aromatase inhibitors, across 13 countries in Asia Pacific, Middle East, North and South Africa, with a significant majority of patients being of Asian ethnicity (196, 84.5%). Its primary objective was to investigate the safety and tolerability profile of EVE+EXE. Secondary objectives were the evaluation of efficacy (assessed by PFS, ORR, and CBR based on RECIST 1.1 criteria) and change in ECOG performance status.

Results
At data cut off of 31st of January 2015, at a median follow up of 11.7 months, median PFS for the ITT population was 9.5 months [9.2-11.6 months], based on local assessment, with the observation of 1 (0.4%) CR and 35 (15.4%) PR. Regarding safety and tolerability, a majority (81.1%) of grade (G) 1/2 adverse events (AEs) was reported. In particular, the following pattern was observed in terms of % of patients who developed G1/G2/G3 mTOR-inhibition induced AEs: stomatitis (36.1, 13.7, 10.6), rash (21.6/6.2/0), fatigue (10.6, 4.4, 2.2), hyperglycemia (6.2, 11.5, 7.0), weight decrease (7.5, 7, 0.9), pneumonitis (5.7, 7, 0.9). No Grade 4 AEs related to EVE+EXE treatment were observed, with exception of one case of non infectious pneumonitis (0.4%). Median dose intensity of everolimus was 9.2 mg/day.

Conclusions
Efficacy and safety results from EVEREXES trial further confirm the role of EVE+EXE for the treatment of HR+/Her2- advanced BC patients in Eastern countries. Results were consistent with data previously reported in BOLERO-2 trial.
Title: Stomatitis following everolimus (EVE) plus exemestane (EXE) in patients with hormone receptor-positive (HR+), HER2– advanced breast cancer (ABC) in the BALLET trial (CRAD001YIC04)

Ciruelos EM M, Jerusalem G, Generali D, Lang I, Gavila JG G, Michelotti A, Tjan-Heijnn VCG CG, Mariani G, Conte P, Beliera A, Camozzi M, Lorizzo K and Martin M.  Breast Cancer Unit, University Hospital, Madrid, Spain; CHU Sart Tilman Liege and Liege University, Domaine Universitaire du Sart Tilman, Liege, Belgium; U.O. Multidisciplinare di Patologia Mammaria U.S. Terapia Molecolare e Farmacogenomica AZ. Istituti Ospitalieri di Cremona Viale Concordia, Cremona, Italy; National Institute of Oncology, Budapest, Hungary; Medical Oncology Unit of Fundacion Instituto Valenciano De Oncologia, Valencia, Spain; UO Oncologia Medica I, Azienda Ospedaliera Universitaria Pisana, Santa Chiara Hospital, Pisa, Italy; Maastricht University Medical Centre, Maastricht, Netherlands; Fondazione Ircs Istituto Nazionale Dei Tumori, Milan, Italy; Dipartimento Di Scienze Chirurgiche Oncologiche e Gastroenterologiche, Università di Padova, Padova, Italy; Istituto Oncologico Veneto IRCCS, via Gattamelata, Padova, Italy; Novartis Farma, Madrid, Spain; Novartis Farma, Origgio/VA, Italy and Medical Oncology Service, Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Body: Background

Stomatitis is the most common adverse event (AE) associated with mTOR inhibitors, including EVE. In the pivotal BOLERO-2 trial, stomatitis was reported for 59% vs 12% (Gr3, 8% vs <1%) of pts with HR+, HER2– ABC who received EVE + EXE vs EXE alone. Of the limited real world evidence available, the BRAWO trial showed a stomatitis incidence of 39.8% (Gr3, 3.4%) in a similar pt population. Additional data are warranted to better understand the stomatitis incidence and time-course in order to minimize treatment discontinuations.

Methods

BALLET is a Phase 3b, European, multi-center, open-label, single-arm, expanded-access study that evaluated safety of EVE (10 mg/d) and EXE (25 mg/d) in 2131 postmenopausal women with HR+, HER2– ABC that progressed on prior NSAI treatment. Primary endpoint was safety; secondary endpoint was characterization of grade 3/4 AEs. In this exploratory analysis, we split the pts in two groups based on having experienced at least one all grade stomatitis event in the first 8 wks (cut-off chosen based on reports that 89.4% of stomatitis events occurred within 8 wks of EVE initiation [Rugo, ASCO 2014]) vs none.

Results

This subgroup analysis included 919 pts with stomatitis (43.1% of the full population). Baseline pt characteristics were comparable to the overall study population, except for more comorbidity (cardiovascular and metabolic disorder) in stomatitis subset. Pts with stomatitis had a longer EVE treatment duration vs pts who didn't (5.8 mo [95%CI, 5.0-6.2] vs 4.7 mo [95%CI, 4.4-5.3]; hazard ratio, 1.121 [95%CI, 0.97-1.28] censored by pts who switched to commercial drug). The relative risk of initial onset of stomatitis at 6 wks was ~40%; median time to onset was 28 days. Majority of stomatitis events were of grade 1/2 severity (80%); grade 3/4 stomatitis was reported for 20% of pts. Most frequent reasons for treatment discontinuation in pts with stomatitis were reimbursement (37.6%), disease progression (35.0%), and AEs (16.2%). Most frequent AEs which led to treatment discontinuation in these pts were stomatitis in 3.7% (Grade 3/4, 2%), pneumonitis in 2.9% (Grade 3/4, 1.3%) and asthenia in 1.8% (Grade 3/4, 1%). Median EVE relative dose intensity in pts with stomatitis was 0.92. In the stomatitis subset, dose interruptions and reductions were required for 66.7% and 37.6% of pts, respectively; most frequent reasons for dose adjustments were AEs (65.9%) which included stomatitis (46.8%), asthenia (6.3%), and pneumonitis (5.3%). Median time to first dose modification in pts with stomatitis was 30 days; median duration of dose interruptions was 16 days. On-treatment deaths (4.4%) were due to progressive disease (2.1%), AEs (1.7%), and other reasons (0.5%).

Conclusions

These results confirmed the stomatitis time-course observed previously and that the majority of these events are of low grade. Stomatitis was the most common cause of dose reductions and interruptions due to AEs and was manageable with dose adjustments; there was a positive association between stomatitis and longer treatment duration. Therefore, proactive management, diligent monitoring and appropriate dose modifications, are recommended to keep pts on treatment.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-13-11

Title: Everolimus, letrozole and trastuzumab in estrogen receptor and HER2-positive patients with metastatic breast cancer and other solid tumors: Evaluating synergy and overcoming resistance

Janku F, Hong DS S, Atkins JT T, Karp DD D, Tsimberidou AM M, Piha-Paul SA A, Subbiah V, Naing A, Fu S, Moulder SL L, Tripathy D, Meric-Bernstam F and Wheler JJ J. MD Anderson Cancer Center, Houston, TX and MD Anderson Cancer Center, Houston, TX.

Body: Background
Combining anti-estrogen therapy with HER2 and mTOR inhibitors may be synergistic and overcome resistance to hormonal and anti-HER2 therapies in patients with estrogen receptor (ER)-positive and HER2 receptor amplified and/or mutant advanced cancers.

Patients and Methods
We evaluated the mTOR inhibitor everolimus, aromatase inhibitor letrozole and anti-HER2 antibody trastuzumab in patients with ER-positive, HER2-positive and/or mutant, solid tumors (confirmed by IHC, FISH, and/or HER2 sequencing). The primary objectives were to determine maximum tolerated dose (MTD) and toxicity and to evaluate response. Secondary objectives were to correlate activity with genomic and proteomic signatures. Next-generation sequencing (NGS) was performed either using a 50-gene panel or externally by Foundation Medicine (Cambridge, MA, USA).

Results
Nine of 10 patients enrolled are currently evaluable for toxicity and response including 7 patients with breast cancer, one patient with ovarian cancer, and one patient with cervical cancer. The median age was 57 years (range, 33-66 years) and the median number of prior therapies in the metastatic setting was 5 (range, 2-7). Of these nine patients, six were HER2+ (by IHC and/or FISH) and three had HER2 mutations (in the absence of HER2 overexpression or amplification). Dose Level (DL) 1 consisted of trastuzumab loading dose of 4mg/kg IV and maintenance dose of 2 mg/kg IV every 21 days, everolimus 5 mg PO daily and letrozole 2.5 mg PO daily. DL2 consisted of trastuzumab loading dose of 6mg/kg IV and maintenance dose of 4 mg/kg IV every 21 days, everolimus 5 mg PO daily and letrozole 2.5 mg PO daily. DL3 consists of trastuzumab loading dose of 6 mg/kg IV and maintenance dose of 4 mg/kg IV every 21 days, everolimus 7.5 mg PO daily and letrozole 2.5 mg PO daily. Currently patients are being enrolled in DL3 and no DLTs have been observed. Additional dose levels are planned with increased trastuzumab and everolimus to the FDA approved doses or until MTD is reached. The most common grade 1 and 2 treatment-related toxicities were fatigue (6 patients), anemia and constipation (2 patients each). No grade 3 or 4 toxicities have been reported. Median time to treatment failure (TTF) was 7.4 months (95% CI 5.2-9.6). One patient with breast cancer and a HER2 mutation experienced a partial response (PR) with a 32% decrease in measurable disease. This patient had no prior HER2-targeted therapy and previously progressed while on letrozole and while on exemestane and everolimus. Six additional patients experienced stable disease (SD) ≥ 6 months, 5 with breast cancer and 1 with cervical cancer. Of these 6 patients, 1 had a HER2 mutation and another had a PIK3CA mutation.

Conclusions
Combination treatment with everolimus, letrozole and trastuzumab is well tolerated and active in ER+, HER2+ and/or mutant solid tumors, including breast and cervical cancer. Clinical benefit (PR/SD≥6 months) was seen in 7 of 9 patients (78%), including patients with HER2 mutations in the absence of overexpression and/or amplification and patients with no genomic alterations in the PI3K/AKT/mTOR pathway. Enrollment is ongoing.
**Title:** Everolimus plus trastuzumab and vinorelbine for trastuzumab-resistant, taxane-pretreated, HER2+ advanced breast cancer: Overall survival results from BOLERO-3

Isaacs C, O'Regan R, Xu B, Masuda N, Arena F, Yap Y-S, Papai Z, Lang I, Armstrong A, Lerzo G, White M, Shen K, Zhang Y, Jappe A, Pacaud LB B, Taran T and Ozguroglu M. Lombardi Comprehensive Cancer Center, Georgetown, University, Washington, DC, DC; University of Wisconsin, Madison, Wisconsin; Cancer Hospital, and Institute, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; NHO Osaka National Hospital, Chuo-ku, Osaka, Japan; NYU Langone Arena Oncology, Lake Success, NY; National Cancer Centre Singapore, Singapore; Military Hospital, Budapest, Hungary; Orszagos Onkologiai Intezet, Budapest, Hungary; Christie Hospital NHS Foundation Trust, Manchester, United Kingdom; Sanatorio de la Providencia, Buenos Aires, Argentina; Monash Medical Center, Moorabbin Hospital, Bentleigh East, VIC, Australia; Cabrini Brighton Hospital, Brighton, VIC, Australia; Comprehensive Breast Health Center, RuiJin Hospital, School of Medicine, Shanghai JiaoTong University, Shanghai, China; Novartis Pharmaceuticals Corporation, East Hanover; Novartis Pharma AG, Basel, Switzerland and Istanbul University, Istanbul, Turkey.

**Body: Background**
PI3K/AKT/mTOR pathway activation due to PTEN loss may lead to trastuzumab (TRAS) resistance. mTOR inhibition has been shown to restore TRAS sensitivity in PTEN-deficient tumors. This provided the rationale for the BOLERO-3 trial which evaluated the combination of everolimus (EVE), an mTOR inhibitor, plus TRAS and a taxane in HER2+ advanced breast cancer (ABC). The addition of EVE to TRAS plus vinorelbine (VNB) led to a statistically significant prolongation of 1.2 months in median progression free survival (PFS) vs TRAS plus VNB in patients with TRAS-resistant and taxane-pretreated, HER2+ ABC (7.0 months vs 5.78 months; hazard ratio, 0.78; p=0.0067). The final overall survival (OS) analysis from this study is presented here.

**Materials and methods**
BOLERO-3 is a randomized, double-blind, placebo-controlled, phase 3 trial. Women with HER2+ ABC progressing on prior TRAS and taxane therapy were randomized (1:1) to receive either daily EVE (5 mg) or PBO plus weekly TRAS (2 mg/kg) and VNB (25 mg/m²), in 3-week cycles, stratified by previous lapatinib use. The primary endpoint was PFS by local investigator assessment. Overall survival was a key secondary endpoint.

**Results**
Overall, 569 patients were enrolled; 284 patients received EVE and 285 patients received PBO. As of April 1, 2015, after a median follow-up of 44.7 months, 388 deaths had occurred, 191 (67.3%) in the EVE arm and 197 (69.1%) in the PBO arm. The median OS in the EVE arm vs PBO arm was 23.5 months vs 24.1 months (HR = 0.96; 95% CI, 0.79-1.17; p = 0.3392). In the HR+ subgroup, the median OS with EVE was 23.5 months (vs 25.5 months with PBO; HR = 1.03; 95% CI, 0.79-1.35); in the HR subgroup, the median OS with EVE was 22.9 months (vs 23.1 months with PBO; HR = 0.86; 95% CI, 0.64-1.17). AEs leading to treatment discontinuation were reported in 81 (28.9%) vs 46 (16.3%) patients in the EVE vs PBO arms. Serious adverse events (SAEs) were reported in 122 (43.6%) vs 58 (20.6%) patients in the EVE vs PBO arms. Overall, 14 on-treatment deaths were observed, 7 (2.5%) in the EVE arm and 7 (2.5%) in the PBO arm; on-treatment deaths due to AEs were balanced between treatment arms (0.7% in each treatment arm). Types of post-progression therapies were balanced across both treatment arms.

**Conclusions**
In BOLERO-3, EVE showed a statistically significant prolongation of PFS. OS was similar in both treatment arms. The safety profile of EVE was comparable to that observed previously with EVE in breast cancer. (Funded by Novartis; BOLERO-3 ClinicalTrials.gov number, NCT01007942.)
Title: Real-world effectiveness of everolimus versus endocrine monotherapy or chemotherapy in HR+/HER2- metastatic breast cancer patients with liver metastasis or multiple metastatic sites

Li N, Hao Y, Lin PL L, Koo V, Ohashi E, Wu EQ Q and Xie J. Analysis Group, Boston, MA; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Analysis Group, NY, NY.

Body: Background:
Liver metastasis and multiple metastatic sites are associated with higher risk of progression or death among women with hormone receptor-positive, human epidermal growth factor receptor-2-negative (HR+/HER2-) metastatic breast cancer (mBC). Traditional treatments, like endocrine monotherapy (ET mono) or chemotherapy (CT), have limited effectiveness in these high-risk patients. Everolimus-based therapy (EVE) is a new treatment option with different mechanism of action. This study examined the real-world comparative effectiveness of EVE vs. ET mono or CT in patients with liver metastasis or multiple metastatic sites.

Methods:
A sample of postmenopausal women with HR+/HER2- mBC was obtained through a retrospective chart review of community-based oncology practices in the US. All patients initiated EVE, ET mono, or CT (defined as the index therapy) for mBC between July 2012 and April 2013 after the failure of a non-steroidal aromatase inhibitor. Patients with liver metastasis and those with multiple metastatic sites (i.e., ≥2 non-lymph-node metastases) at the index therapy initiation were analyzed separately. In each group, progression-free survival (PFS) and time on treatment (TOT) were compared between EVE vs. ET mono or CT, respectively, using Kaplan-Meier analyses with log-rank tests and Cox proportional hazards models adjusting for patient and disease characteristics, such as age, mBC type, performance status, tumor burden, and prior treatment. Patients without an event were censored at the last follow-up.

Results:
A total of 202 patients had liver metastasis, including 82 treated with EVE, 49 with ET mono, and 71 with CT. EVE patients had more severe mBC than ET mono patients and less severe mBC than CT patients, as indicated by proportion of patients receiving prior CT for mBC and tumor burden. Compared with ET mono, EVE was associated with significantly longer PFS (log-rank test p=0.049; hazard ratio (HR)=0.48, 95% confidence interval (CI): 0.27-0.87) and TOT (log-rank test p=0.054, HR=0.49, 95% CI: 0.28-0.86). Similarly, compared with CT, EVE was associated with significantly longer PFS (log-rank test p=0.024, HR=0.76, 95% CI: 0.44-1.32) and TOT (log-rank test p<0.001, HR=0.35, 95% CI: 0.22-0.55).

A total of 265 patients had multiple metastatic sites, including 100 treated with EVE, 79 with ET mono, and 86 with CT. Similarly, EVE patients had more severe mBC than ET mono patients and less severe mBC than CT patients, as indicated by tumor burden. Compared with ET mono, EVE was associated with significantly longer PFS (log-rank test p=0.043, HR=0.62, 95% CI: 0.41-0.95) and TOT (log-rank test p=0.054, HR=0.64, 95% CI: 0.42-0.97). Compared with CT, EVE was also associated with longer PFS (log-rank test p=0.004, HR=0.60, 95% CI: 0.39-0.92) and TOT (log-rank test p<0.001, HR=0.36, 95% CI: 0.24-0.53).

Conclusion:
In this retrospective chart review of HR+/HER2- mBC patients, EVE was associated with significantly longer PFS and TOT compared with ET mono or CT in high-risk patients with liver metastasis or multiple metastatic sites.
Title: Time on treatment of everolimus, fulvestrant, and capecitabine for the treatment of HR+/HER2- metastatic breast cancer: A retrospective claims study in the US

Li N, Hao Y, Kageleiry A, Peeples M, Fang A, Koo V and Guérin A. Analysis Group, Boston, MA; Novartis Pharmaceuticals Corporation, East Hanover, NJ and Analysis Group, Montreal, QC, Canada.

Body: Background:
Treatment guidelines for hormone receptor-positive/human epidermal growth factor receptor-2-negative (HR+/HER2-) metastatic breast cancer (mBC) recommend extending the time on treatment (TOT) of endocrine therapy (ET) prior to the initiation of chemotherapy (CT) to avoid its serious side effects and preserve patients' quality of life. Everolimus-based therapy (EVE), fulvestrant monotherapy (FUL mono), and capecitabine monotherapy (CAP mono) are among the latest ET and CT agents approved for the treatment of HR+/HER2- mBC in the US. This retrospective claims analysis compared TOT among HR+/HER2-mBC patients who received EVE versus those who received FUL mono or CAP mono respectively.

Methods:
Postmenopausal women with HR+/HER2- mBC who initiated ≥ 1 new line of therapy for mBC between 7/20/2012 (the approval date of EVE, the latest of all three therapies) and 3/31/2014 (which allowed for ≥ 3 months of potential follow-up) after a non-steroidal aromatase inhibitor were identified from the MarketScan and PharMetrics databases (2002Q1-2014Q2) using an algorithm adapted from the literature. Treatment discontinuation was defined as a treatment gap of ≥ 60 days. Patients' lines of therapies were classified into mutually-exclusive regimen groups (i.e., EVE, FUL mono, and CAP mono) and followed until discontinuation of the line of therapy, end of insurance eligibility, or data cut-off (06/30/2014). Patients who did not discontinue their treatment were censored at the end of follow-up. TOT was compared between EVE versus FUL mono and versus CAP mono using Kaplan-Meier (K-M) analyses with log-rank tests and multivariable Cox models adjusting for the line of therapy and differences in patient characteristics, including age, insurance type, de novo vs non-de-novo mBC, prior use of CT for mBC, sites of metastases (e.g., bone, brain, and visceral), and Charlson comorbidity index.

Results:
Across the first four lines of therapies for mBC, a total of 940 EVE, 953 FUL mono, and 721 CAP mono regimens were included. Based on the different lines of therapies, the K-M estimators of median TOT ranged from 5.5 to 7.2 months for EVE, 4.9 to 8.4 months for FUL mono, and 3.5 to 6.0 months for CAP mono.

Table 1. Comparison of TOT between EVE, FUL mono, and CAP mono by line of therapy

<table>
<thead>
<tr>
<th></th>
<th>Median TOT (months)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>EVE</td>
</tr>
<tr>
<td>Line 1</td>
<td>6.2</td>
</tr>
<tr>
<td>Line 2</td>
<td>6.2</td>
</tr>
<tr>
<td>Line 3</td>
<td>7.2</td>
</tr>
<tr>
<td>Line 4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*indicates p-value <0.05 for pairwise log-rank tests in comparison with EVE.

Pooling all lines of therapies, EVE was associated with significantly longer TOT compared to FUL mono (multivariable-adjusted hazard ratio [HR] = 0.87, 95% confidence interval [CI]: 0.76-0.99) or CAP mono (multivariable-adjusted HR = 0.73, 95% CI: 0.64-0.83). Similar results were observed in each line of therapy.

Conclusions:
This real-world US claims study of postmenopausal women with HR+/HER2- mBC showed that patients receiving EVE experienced significantly longer TOT than those receiving FUL mono or CAP mono, suggesting a comparative advantage of EVE in extending the duration of ET.
Dose intensity and efficacy of the combination of everolimus and exemestane (EVE/EXE) in a real world population of hormone receptor positive advanced breast cancer: A multicenter Italian experience

Forcignanò R, Petrucelli L, Cazzaniga ME E, Lupo LI I, Chiuri VE E, Cairo G, De Matteis E, Febbraro A, Giordano G, Campidoglio S, Fabi A, Giampaglia M, Bilancia D, La Verde N, Maiello E, Morriri M, Giotta F, Lorusso V, Scavelli C, Romiti S, Cusmai A, Palmiotto G, Tornesello A and Ciccarese M. “Vito Fazzi” Hospital, Lecce, Italy; “AO San Gerardo”, Monza, Italy; “Sacro Cuore di Gesù Fatebenefratelli” Hospital, Benevento, Italy; “Regina Elena” National Cancer Institute, Roma, Italy; “San Carlo” Hospital, Potenza, Italy; “AO Fatebenefratelli e Oftalmico”, Milano, Italy; “Casa Sollievo della Sofferenza” Hospital, San Giovanni Rotondo (Foggia), Italy; “Giovanni Paolo II” Institute, Bari, Italy; “S. Cuore di Gesù” Hospital, Gallipoli (Lecce), Italy; “Ospedali Riuniti” Hospital, Foggia, Italy and “Di Venere” Hospital, Bari, Italy.

BACKGROUND: Everolimus, an mTOR inhibitor, in combination with exemestane is approved for hormone receptor (HR) positive advanced breast cancer (ABC), after failure of treatment with non-steroidal aromatase inhibitor (NSAI). We assessed the toxicity of the combination and the correlation between dose intensity and response to therapy, in a real world population of ABC from 11 Italian centers. Moreover, we evaluated OS of the whole population, RR and PFS according to line of treatment (from 1rd to 3rd and from 4th on).

METHODS: 154 pts were treated with combination of everolimus 10 mg and exemestane 25 mg daily from 05/2011 today. Median age was 62 (47-82). Median time to metastatic disease was 49 months (0-269). Median number of metastatic sites was 2 (55.2% of pts visceral versus 44.8% non visceral disease). N=117 (75.9%) pretreated with HT as adjuvant; N=126 pts (81.8%) treated with HT for advanced disease prior to EVE/EXE, with a median of one line (0-5). N=102 pts (66.2%) treated with chemotherapy for metastatic disease, with a median of one line (0-6) before everolimus treatment.

RESULTS: Sixteen pts received EVE/EXE as 1st line (10.4%), 39 as 2nd (25.3%), 37 as 3rd (24%), 62 as 4th or more (40.3%). Response was evaluable in 127 out of 154 pts; CR/PR/SD respectively 5/27/56 pts. RR according to line (from 1st to 3rd vs ≥ 4th) was respectively 22.8% vs 26.4% (p=0.864). The median PFS for all population (150 pts) was 38 weeks (95% CI: 33-42). The PFS according to line (1st- 3rd vs ≥ 4th) was 38 wks in both subgroups, p=0.73. OS (126/154 pts) was 28 mths (95% CI: 31-38).

The most frequent adverse events were collected in the table.

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Overall %</th>
<th>Grade 3-4 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatitis</td>
<td>55.8</td>
<td>10.4</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>47.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>42.9</td>
<td>5.2</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>36.4</td>
<td>5.8</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>29.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Anemia</td>
<td>28.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>24.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Rash</td>
<td>23.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Increased ALT/AST/GGT</td>
<td>21.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>19.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>18.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Weight loss</td>
<td>18.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>17.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>15.6</td>
<td>1.9</td>
</tr>
<tr>
<td>Cutaneous toxicity</td>
<td>14.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Infection 14.3 3.2
Neutropenia 11.7 1.9
Nausea 11.7 0.0
Anorexia (without stomatitis) 10.4 1.3
Electrolyte alterations 9.7 1.3
Urea/creatinine increase 6.5 1.3
Vomiting 6.5 0.0
Uric acid increase 4.5 0.0

Median duration of treatment with everolimus 10 mg and 5 mg was respectively 180 (9-854) and 129 days (3-738). Fifty-eight pts (37.6%) never stopped treatment with everolimus 10 mg; 16 pts (10.4%) definitively stopped everolimus for toxicity; 80 pts (52.0%) temporarily interrupted the treatment, resuming at dose level 10 mg (31 pts) or reducing at 5 mg (49 pts). Main reason for discontinuation/interruption was stomatitis G2-G3. RR and PFS evaluated according to dose intensity, 10 mg vs 5 mg, were respectively 25.9% vs 30% p=0.779, 38 wks (27-44) vs 40 wks (31-48) P=0.614

CONCLUSIONS: efficacy in terms of RR and PFS of the combination EVE/EXE is not related to dose intensity (10 mg vs 5 mg), the discontinuation of the treatment is high with the starting dose of 10 mg, the toxicity is consistent with previous phase II-III studies although we collected some different toxicities.
Title: Abstract Withdrawn
Title: Abstract Withdrawn
**Title:** A phase I study of romidepsin in combination with nab-paclitaxel in patients with metastatic HER-2 negative inflammatory breast cancer (IBC)


**Body:** Inflammatory breast carcinoma (IBC) is the most aggressive form of breast cancer. The hallmark of IBC is regional extension into dermal lymphatics as tumor emboli causing breast edema and erythema. Pathologic characteristics of IBC include high grade, negative hormone receptor status and overexpression of HER2 and E-cadherin. The latter is the most attractive therapeutic target in IBC. In preclinical studies the histone deacetylase inhibitor, romidepsin targeted E-cadherin, affecting tumor emboli and increasing taxane sensitivity.

**Rationale:** In vitro studies show that histone deacetylase inhibitors (HDACi) with taxanes provide synergy to enhance cell death. HDACi alter expression of AIRH1, a regulator of autophagy, typically silenced in breast cancer. In vitro treatment with HDACi induces expression of AIRH1, resulting in enhanced cell death with taxanes. In vitro studies of IBC have demonstrated the utility of HDACi and romidepsin in IBC cell lines. SAHA and romidespin, HDACis, inhibited self-renewal of IBC tumor spheroids from IBC cell lines. This trial combines romidepsin with a taxane proven in metastatic breast cancer to explore whether the combination will be effective in IBC.

**Design:** This is a phase I trial to assess the safety of romidepsin plus nab-paclitaxel in patients with recurrent or metastatic IBC. The maximum tolerated dose (MTD) of romidepsin + weekly nab-paclitaxel was determined to define the dose for the phase II trial. Secondary objectives included describing the adverse event profile and assessing the overall response rate (ORR) and Clinical Benefit Rate (CBR). This study employed a 3+3 design. DLTs included febrile neutropenia or non-hematologic grade 3 or 4 toxicities. Patients were treated with nab-paclitaxel 100 mg/m² iv with romidepsin, 7 mg/m² iv (1st cohort) and 10 mg/m² iv (2nd cohort), on days 1, 8, 15 of a 28 day cycle.

**Results:** Nine patients were treated. The median age was 52. Three patients were treated in the first cohort. Two patients showed progressive disease (PD). One patient has had stable disease (SD) over 10 cycles and continues treatment. DLT was not reached at 7 mg. Toxicities related to romidepsin included neutropenia, anemia and fatigue. Six patients were treated in the 2nd cohort. Grade 3 hypophosphatemia, a DLT, was reached. One patient had complete response (CR). One patient had SD; four patients had PD. Toxicities related to romidepsin were anemia, neutropenia, GI upset, edema, hyperglycemia, fatigue, hypophosphatemia, pruritis, dry mouth, and increased lab values. The overall response rate (ORR) was 33% (3/9). The table below shows results.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Response</th>
<th># Prior Therapies</th>
<th>Metastatic Sites</th>
<th># Cycles on study</th>
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<tbody>
<tr>
<td>1</td>
<td>PD</td>
<td>2</td>
<td>pleura, nodes</td>
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<tr>
<td>1</td>
<td>PD</td>
<td>0</td>
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<td>3</td>
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<td>1</td>
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<tr>
<td>2</td>
<td>PD</td>
<td>0</td>
<td>liver, nodes, bone</td>
<td>2</td>
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**Conclusions:** This phase I trial shows that romidepsin and nab-paclitaxel are well-tolerated in patients with advanced IBC. The
MTD and recommended dose of romidepsin is 10 mg/m2 with nab-paclitaxel 100 mg/m2 days 1, 8, 15 of a 28 day cycle. A phase II trial is planned in recurrent HER negative IBC patients.
Title: Poziotinib, an oral, irreversible pan-HER inhibitor, demonstrates promising clinical activity in metastatic HER2 positive breast cancer patients


Body: Background:
Poziotinib is a novel, oral, irreversible pan-HER inhibitor that has been studied in two completed Phase 1 clinical trials in patients with advanced solid tumors (HM-PHI-101; HM-PHI-102) and is currently being studied in several Phase 2 clinical trials. Preclinical studies have shown poziotinib to be more potent in vitro than other EGFR- and HER2-directed tyrosine kinase inhibitors. This study collates the clinical experience of poziotinib in patients with advanced HER2 positive breast cancer from the two completed Phase 1 trials.

Results:
The maximum tolerated dose (MTD) and safety of poziotinib were evaluated in HM-PHI-101 (once daily, Day 1-14 q3 weeks) and in HM-PHI-102 (continuous dosing). The Dose Limiting Toxicity (DLT) was diarrhea in both studies. Anti-diarrheal medicine was allowed, but prophylactic anti-diarrheal therapy was not used. The most frequently reported Grade 3 AE was diarrhea (40%). The MTD for intermittent dosing of poziotinib was 24 mg and the MTD for continuous dosing was 16 mg. The Dose Limiting Toxicity (DLT) was diarrhea in both studies. Anti-diarrheal medicine was allowed, but prophylactic anti-diarrheal therapy was not used. In total, 10 patients with HER2 positive metastatic breast cancer were treated in the two trials (median age 56.5, range 30-69). These patients were heavily pretreated (median number of previous treatment regimens 5, range 3-9), and all patients had failed treatment with both trastuzumab and lapatinib. The Overall Response Rate (ORR) in these breast cancer patients was 60% and Clinical Benefit Rate (CBR) was 80%. The median duration of response was 32.5 weeks (range 18 - 56 weeks). Two patients in the early dose cohorts of 1 or 2mg had progressive disease. The median progression free survival (PFS) was 28 weeks (6, 6, 12, 17, 25, 31, 37, 49, 57, and 98 weeks).

Conclusions:
Poziotinib showed very promising clinical activity in Phase 1 patients with metastatic HER2 positive breast cancer, who had been heavily pretreated and failed two prior HER2-directed therapies. The ORR in this patient population was 60% and the CBR was 80% in these two early dose finding studies. The AEs observed in these studies was consistent with other pan-HER and EGFR inhibitors. While the majority of DLT cases involves diarrhea, toxicity of other adverse events was relatively tolerable. An intermittent dosing schedule seemed appropriate for poziotinib. Multiple Phase 2 studies with poziotinib are ongoing in patients with breast cancer and other solid tumors.
Title: Activity of HP-based therapies as third and later lines for the treatment of HER2-positive metastatic breast cancer: A retrospective study from a single institution


Body: Background: Dual anti-HER2 blockade with trastuzumab and pertuzumab (HP) plus chemotherapy is an effective therapy (Rx) in the 1st-line setting for HER2-positive metastatic breast cancer (MBC). Our single arm phase II study included patients (pts) treated with HP plus paclitaxel in the 2nd-line setting with progression-free survival (PFS) benefit. Recently, we reported results from a retrospective study of pts treated at our institution, suggesting a longer PFS for those who received HP-based Rxs when compared to any other anti-HER2 based Rxs in the 2nd-line setting. To further assess the activity of this combination in later Rx lines, we conducted a retrospective analysis of pts with HER2-positive MBC who had progressive disease after 2nd-line and were treated with HP-based Rxs in the 3rd and later lines at MSKCC. Historically, the median (med) PFS in this setting with trastuzumab-based Rx is about 3-4 months.

Methods: Pts diagnosed with HER2-positive MBC and treated with HP-based Rxs at MSKCC between 1-1-2011 and 03-30-2015 and who progressed on 2nd-line Rx were identified through an institutional database. Primary endpoint was PFS in 3rd and later treatment lines.

Results: 70 pts who received any HP-based Rx in the 3rd or later lines of treatment were eligible. The med number of prior anti-HER2 Rx was 3. The baseline characteristics and Rxs are summarized in Table 1. The med PFS for the entire cohort was 5.7 months (95% CI, 4.8-6.5).

Conclusions: In this retrospective analysis involving heavily pretreated patients, HP-based Rx appears to be an active regimen and compares favorably to historical data. This supports the NCCN endorsement of HP-based Rx in later lines if HP has not been delivered previously.

Baseline Characteristics and treatments (n=70)

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Title: A pilot study of pertuzumab, trastuzumab and eribulin for patients with advanced HER2 positive breast cancer

Ishihara M, Tamaru S, Oda H, Yamashita Y, Tono Y, Mizuno T and Katayama N. Mie University Hospital, Tsu, Mie, Japan.

Body: [Introduction] The triple therapy of pertuzumab, trastuzumab and taxanes (docetaxel or paclitaxel) is coming into widespread use, because of the beneficial effects on HER2 positive breast cancer. However, we don't have enough information about the efficacy and safety of other agents with trastuzumab and pertuzumab (TP). We studied triple therapy of pertuzumab, trastuzumab and eribulin (PTE) for advanced HER2 positive breast cancer to assess the efficacy, safety and QOL prospectively (UMIN000012018).

[Patients and methods] Responses were assessed by RECIST criteria v1.1. Adverse events (AEs) were graded according to CTCAE v4.0. Patients with advanced HER2 positive breast cancer were treated with pertuzumab (840 mg loading then 420 mg, day 1), trastuzumab (8 mg/kg loading then 6 mg/kg, day 1), and eribulin (1.4 mg/m2, day 1 and 8) every 3 weeks. Dose reduction was allowed when patients developed febrile neutropenia, grade 3-5 non-hematologic toxicity or skipped day 8 eribulin administration because of neutrophil count <1000/mm3. QOL was assessed using FACT-B at baseline and 3 months after initial treatment.

[Results] Ten patients were enrolled. Median age of patients was 60 years-old (35-75). Median number of prior chemoregimen for metastatic disease was 3 (0-5). Two patients had a history of docetaxel allergy. Median number of PTE cycle was 6 (3-12). Eight patients reduced eribulin doses 1.4 mg/m2 to 1.1 mg/m2 because of AEs (2 patients), skipped day 8 eribulin (4 patients), or physician's choice (2 patients). One complete response, 1 partial response and 5 stable disease were achieved at 3 months. Two patients (1 CR and 1 SD) stopped eribulin and received TP as maintenance therapy. At 3 months, all 3 patients with progressive disease developed brain metastasis. Two patients had extracranial progressive lesions, but 1 patient had partial response for extracranial disease.

The common treatment-related AEs were leukopenia, neutropenia, lymphopenia diarrhea, hypokalemia and stomatitis. Grade 3 AEs were leukopenia (7 patients), neutropenia (8 patients), lymphopenia (2 patients), febrile neutropenia (1 patient), hypokalemia (1 patient) and peripheral neuropathy (1 patient). Grade 4/5 AEs were not observed.

Nine patients could be assessed QOL. FACT-B TOI, FACT-G and FACT-B total score had a tendency to be improved at 3 months.

[Conclusion] The PTE therapy showed appropriate clinical effect for extracranial lesions and maintained QOL of patients with advanced HER2 positive breast cancer. It may be a choice for patients who have taxane-resistant diseases or a history of taxane allergy.

Many patients needed to reduce eribulin dosage. When the PTE therapy is referred to advanced HER2 positive breast cancer patients as a palliative chemotherapy, eribulin (1.1mg/m2) might be a reasonable dosage.
Successful targeting HER2 in heavily pretreated HER2-negative metastatic breast cancer patients presenting with elevated serum levels of the HER2 extracellular domain and/or HER2 overexpressing circulating tumor cells


Background: A considerable proportion of patients (pts) with HER2-negative (HER2-) metastatic breast cancer (MBC) present with elevated serum levels of the soluble HER2 extracellular domain (sHER2) and/or HER2-overexpressing circulating tumor cells (CTCs) during their further clinical course. These "occult" HER2-positive (HER2+) pts may well be candidates for HER2-targeted therapy (Tx) albeit normally not subjected to such treatment. This observational study was undertaken to gain more insights into the feasibility of HER2-directed Tx in occult HER2+ MBC pts in the clinical routine. Methods: A total of 30 pts with heavily pretreated HER2- MBC (ER+, n = 26) showing sHER2 values > 15 ng/mL (n = 8), HER2+ CTCs (n = 7), or both (n = 15) were included. Pts had failed 2-16 prior systemic treatments (median: 7) and did not qualify for recruitment onto a controlled clinical trial. sHER was measured by a chemiluminescence assay (Siemens HealthCare, Eschborn, Germany), CTCs were enumerated and checked for HER2 expression by using the FDA-cleared CellSearch™ technology (Veridex, Raritan, NJ). All pts received anti-HER2 Tx with trastuzumab (H: n = 18), lapatinib (L: n = 4), H+L (n = 2), or H+pertuzumab (H+P: n = 6). HER2-targeted Tx was given alone (n = 4), or in combination with endocrine agents (n = 7), cytotoxics (n = 17), or other targeted drugs (n = 2). Responses were scored according to RECIST 1.1, OS was calculated from the start of HER2-directed Ctx until death from any reason or loss to follow-up by using Kaplan-Meier statistics. Results: Anti-HER2 Tx was generally well tolerated. Median treatment duration was 16.1 wks (range 1.0-72.9 wks). In 2 pts with L and 1 pt with H+L, Tx was prematurely stopped due to toxicity (diarrhea, fatigue). 2 pts were too early to evaluate (TE). 11 PR, 12 SD, and 5 PD accounted for an objective response rate (ORR) of 36.7% and a clinical benefit rate (CBR) of 76.7%. Median OS was 62.9 wks. In 25 pts, 9 with PR, 12 with SD, and 4 with PD, results of serial sHER2 measurements at baseline and after 3 wks of Tx were available. Most pts with PD showed increasing sHER2 levels. In the majority of pts with PR or SD, sHER2 decreased by more than 20% from baseline. However, 2 pts with PR following L-based Tx showed increasing sHER2 values. In 19 pts, 8 with PR, 7 with SD, and 4 with PD, repeated CTC counts at week 6 from baseline were available. All pts with PD showed increasing CTCs. All pts with SD and PR presented with decreasing CTC counts, most of them normalizing within 6 wks. Conclusions: Our findings indicate that anti-HER2 Tx may be a valid option in pts with heavily pretreated HER2- MBC with pathological sHER2 values and/or HER2+ CTCs in the clinical routine. Thus, determination of both sHER2 and HER2 expression on CTCs appears to be reasonable in tissue HER2-negative MBC pts. Compared to sHER2, serial CTC measurements may be the more accurate predictor of response to anti-HER2 treatment, particularly in pts receiving L as part of their Tx. Results of ongoing randomized trials in this setting are eagerly awaited.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-13-23

Title: Next-generation sequencing (NGS), array comparative genomic hybridization (aCGH) and patient-derived tumor xenograft (PDX) for precision medicine in advanced breast cancer: A single-center prospective study


Body:

Background
Genomic-based approaches in advanced breast cancer (ABC) were recently demonstrated as feasible in the clinical practice, but only a limited number of patients were actually treated with targeted therapies matching genomic alterations, with low antitumor activity. We conducted a pilot study to evaluate whether precision medicine using NGS and aCGH could be implemented prospectively at a single center in ABC patients. In addition, we examined whether PDX could be derived from ABC and thus could help inform therapeutic decision.

Methods
ABC patients accessible to tumor biopsy were prospectively enrolled at the Institut Paoli-Calmettes in the BC-BIO study (ClinicalTrials.gov, NCT01521676). Tumor tissue from locally recurrent or metastatic disease was immediately frozen after dedicated biopsy. Genomic profiling included high-resolution 4x180K aCGH (Agilent Technologies, Massy, France) and DNA sequencing, using a library of 365 cancer candidate genes (HaloPlex target enrichment kit, Agilent technologies, Santa Clara, CA, USA) and MiSeq analyzer (Illumina, San Diego, CA, USA) with 2x150-bp, paired-end at about 300x coverage. In a subset of patients, fresh tumor was implanted orthotopically in humanized cleared fat pads of NSG mice for establishing xenotransplants.

Results
A total of 34 ABC patients were included, with the following characteristics: median age 54 years (35-77); molecular subtypes: 11 triple-negative (32%), 12 luminal non-HER2 (35%), 4 luminal HER2 (12%), 3 HER2 non-luminal (9%), and 4 unknown (12%); 33 with previous chemotherapy (97%); 22 with previous endocrine treatment (35%); 7 with previous anti-HER2 (21%). Tumor biopsies were obtained from liver (15), skin (6), peritoneum (4), breast (3), node (3), lung (1), pleura (1), and ascitis (1), with a median tumor cellularity of 70% (range 10-90%). aCGH and NGS were available from 34 and 33 patients, respectively. An actionable target was found in 28 patients (82%), corresponding to 66 targets, including 37 mutations (8 in PIK3CA, 7 TP53, 4 ESR1, 2 AKT1, 2 BRCA2, 2 HER2), 22 amplifications (7 for CCND1, 2 CCNE1, 2 FGFR1, 2 IGF1R) and 7 homozygous deletions (3 for PTEN, 2 CDKN2A/B, 1 BRCA2, 1 STK11). A targeted therapeutic proposal was possible, either in a clinical trial (N=18, 52%) or using already registered drugs (N=17, 50%). Ten patients actually received a targeted treatment, 1 of them experienced objective response and 1 showed stable disease for more than 6 months. Of 26 patients subjected to mouse implantation, 10 had successful xenografting (6 triple-negative, 2 HER2, 1 luminal non-HER2, 1 subtype non-attributed), with a median time to reach 10 mm of 148 days. These PDX will be used as models to understand the patient's therapeutic response.

Conclusion
Precision medicine using high-throughput DNA sequencing and aCGH can be implemented at a single center in the context of clinical practice and may allow direct therapeutic proposal in 1/3 of patients, but antitumor activity was minimal. PDX may be obtained in a significant fraction of patients, especially in triple-negative and HER2 subtypes, and could phenotypically complement genomic data.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-13-24

Title: Impact of genomic medicine on clinical decision making in patients with advanced breast cancer at two academic medical centers

Kruse ML L, Santa-Maria CA A, Raska P, Swoboda A, Jain S, Sohal D, Moore H, Budd GT, Abraham J and Montero AJ J. Cleveland Clinic, Cleveland, OH and Northwestern University, Chicago, IL.

Body: Background: A deeper molecular understanding of cancer biology has led to the development of therapies targeting driver mutations. Genomic profiling of tumors is commercially available and has become integrated into many clinical practices as part of a paradigm shift towards personalized care of cancer patients. The current impact of genomic profiling on clinical decision making for patients with advanced breast cancer is not well described.

Methods: Patients with metastatic breast cancer (mBC) who had tumors submitted for commercial genomic analysis from 2013-2015 were identified consecutively at two large academic cancer centers with genomic basket trials open for the majority of the collection period. Demographics, tumor pathology, clinical, and treatment histories were extracted through medical chart review as per an IRB approved protocol. Data from genomic analysis reports was extracted including number and type of mutations, FDA approved therapies and clinical trials available. Genomic analysis was determined to have impacted clinical decision making if the next line of therapy was influenced either by accrual to clinical trial, or a decision to prescribe an FDA-approved therapy. The most frequent somatic mutations and their relative frequencies were determined.

Results: A total of 82 patients with mBC who had undergone commercially available genomic testing were identified. The median age was 49 (range: 29-70). 42 patients (51%) had ER-positive HER2-negative disease, 33 (40%) had ER-negative HER2-negative disease, 4 (5%) had ER-negative HER2-positive disease and 3 (4%) had ER-positive HER2-positive disease. The median number of lines of therapy received prior to genomic profiling was 2 (range 0-15). Genomic analysis reports suggested that 61 (74%) of these patients had at least one FDA approved medication available for at least one somatic mutation, and 79 (96%) had at least one clinical trial available (39 (46%) in the same state, 11 (13%) in the same institution). Genomic testing influenced management in 8 patients (10%), with 6 patients (7%) experiencing a change in next line of therapy attributable to genomic information. In 74 patients (90%), genomic testing results did not affect clinical decision-making. The most frequently observed somatic mutations included: TP53, PI3KCA, MYC, CCDN1, FGF, ZNF703, GATA3, ARID1A, MCL1, PTEN, MYST3, and BRCA1.

Conclusions: Genomic testing did not affect management in the vast majority of mBC patients treated at two major academic cancer centers. Furthermore, the most identified mutated genes found were not targetable. The real world clinical utility of genomic analysis remains limited in breast cancer but may influence clinical decision making in a minority of patients.
**Title:** Abemaciclib, an inhibitor of CDK4 and CDK6, combined with endocrine and HER2-targeted therapies for women with metastatic breast cancer

Goetz MP P, Beeram M, Beck T, Conlin AK K, Dees EC C, Dickler MN N, Helsten TL L, Conkling PR R, Edenfield WJ J, Richards DA A, Turner PK K, Cal N, Chan EM M, Pant S, Becerra CH H, Kalinsky K, Puhalla SL L, Rexer BN N, Burris HA A and Tolaney SM M. Mayo Clinic, Rochester, MN; The START Center for Cancer Care, San Antonio, TX; Highlands Oncology Group, Rogers, AR; Providence Cancer Center Oncology and Hematology Care Clinic, Portland, OR; University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC; Memorial Sloan-Kettering Cancer Center, NY, NY; University of California San Diego Moores Cancer Center, La Jolla, CA; Virginia Oncology Associates, Norfolk, VA; Clinical Research Unit, Institute for Translational Oncology Research, Greenville Hospital System, Greenville, SC; Texas Oncology-Tyler Cancer Center, Tyler, TX; Eli Lilly and Company, Indianapolis, IN; University of Oklahoma Health Sciences Center, Peggy and Charles Stephenson Oklahoma Cancer Center, Oklahoma City, Ok; Texas Oncology-Baylor Sammons Cancer Center, Dallas, TX; Columbia University Medical Center, NY, NY; Magee-Womens Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA; Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN; Sarah Cannon Research Institute (SCRI), Nashville, TN and Dana-Farber Cancer Institute, Boston, MA.

**Body:**

**Background:** Abemaciclib, a small molecule inhibitor of CDK4 and CDK6, induces G1 cell cycle arrest in Rb-proficient human cancers.\(^1\) The clinical safety profile of abemaciclib enables continuous oral dosing to achieve sustained target inhibition, resulting in single-agent antitumor activity against multiple human cancers. The drug also reaches relevant concentrations in the central nervous system and, in patients taking the drug orally, can be detected in the cerebrospinal fluid.\(^2\) For women with previously treated hormone receptor positive (HR+) metastatic breast cancer (MBC), abemaciclib as a single agent achieved a six-month clinical benefit rate of 61.1% and an objective response rate of 33.3%.\(^3\) Clinical trials investigating abemaciclib combined with fulvestrant\(^4\) or aromatase inhibitors\(^5\) have led to randomized Phase 3 studies for women with HR+ breast cancer.\(^6,7\)

**Methods:** This Phase 1b study (NCT02057133) with multiple cohorts evaluates safety and tolerability of abemaciclib combined with endocrine or HER2-targeted therapies for MBC. Secondary objectives include pharmacokinetics (PK) and antitumor activity of abemaciclib when given in combination with other therapies. Cohorts were opened to enrollment sequentially. Patients with HR+ HER2 negative MBC received abemaciclib orally every 12 hours (Q12H) in combination with the following standard therapies daily until progression: letrozole (Part A), anastrozole (Part B), tamoxifen (Part C), exemestane (Part D), or exemestane plus everolimus (Part E). Patients with HER2 positive MBC received abemaciclib orally Q12H in combination with trastuzumab every 21 days until progression (Part F). Adverse events (AEs) were graded by NCI CTCAE v4.0 and tumor response was assessed radiographically using RECIST v1.1.

**Results:** Abemaciclib has been combined with multiple targeted therapies for the treatment of women with MBC. We previously reported safety and early efficacy results for the combinations of abemaciclib with letrozole, anastrozole, tamoxifen, exemestane, and exemestane plus everolimus.\(^5\) Due to limited follow-up at that time, the efficacy results were not mature. Safety, PK, and efficacy results with approximately 6 months of additional follow-up will be reported across all parts of the study. The most common treatment-emergent AEs include effects on the gastrointestinal and hematopoietic systems. Consistent with previously reported results for both single-agent abemaciclib and the combination of abemaciclib with fulvestrant, tumor responses have been observed among women receiving abemaciclib in combination with targeted therapies for MBC.

**Conclusions:** This study for women with MBC demonstrates the potential for abemaciclib to be combined with therapies targeting specific signaling pathways.

Efficacy, safety, and treatment decision-making in the AVANTI German observational study of first-line bevacizumab (BEV)-containing therapy for locally advanced, recurrent, or metastatic breast cancer (aBC)

Müller V, Jakob A, Aktas B, Grafe A, Fett W, März W, Bruch H, Pott D, Klare P, Boller E, Kiewitz C and Schneeweiss A. Universitätsklinikum Hamburg-Eppendorf, Klinik und Poliklinik für Gynäkologie, Hamburg, Germany; Ortenau-Klinikum, Offenburg, Germany; Universitätsklinikum Essen, Essen, Germany; MVZ Nordhausen gGmbH, Praxis Dr Grafe/Brustzentrum der Frauenklinik, Südharz-Klinikum Nordhausen gGmbH, Nordhausen, Germany; Onkologische Praxis, Wuppertal, Germany; Paracelsus-Klinik Osnabrück, Osnabrück, Germany; Schwerpunktpraxis Bonn, Bonn, Germany; Schwerpunktpraxis Hämatologie und Onkologie, Bottrop, Germany; Brustzentrum Berlin, Berlin, Germany; iOMEDICO Clinical Research Organisation, Freiburg, Germany; Roche Pharma AG, Grenzach-Wyhlen, Germany and Universitäts-Klinikum Heidelberg, Nationales Centrum für Tumoreraffektionen, Heidelberg, Germany.

Background: In Europe, BEV is approved as first-line therapy for metastatic breast cancer in combination with either paclitaxel (PAC) or capecitabine (CAP).

Methods: The ongoing multicenter non-interventional AVANTI study aims to determine the safety and efficacy of first-line BEV–PAC or BEV–CAP in the context of routine oncology practice in Germany and to assess selection criteria that influence therapy choice. Eligible patients (pts) have previously untreated aBC and no contraindications for BEV. Chemotherapy schedule, diagnostics, and frequency of follow-up visits are at the physician's discretion. Data are collected for 1 year after the start of BEV, with 6-monthly follow-up for 1.5 years after the end of documented observation or BEV discontinuation, whichever occurs first.

Results: Between Oct 2009 and Feb 2015, 2168 pts treated at 331 German centers received BEV–PAC (N=1774) or BEV–CAP (N=394). The most common reasons driving treatment choice were efficacy (66% BEV–PAC, 60% BEV–CAP), guidelines (55% BEV–PAC, 50% BEV–CAP), and tolerability (40% BEV–PAC, 45% BEV–CAP). Compared with pts receiving BEV–PAC, the BEV–CAP subgroup included relatively fewer pts with ≥3 metastatic sites, visceral metastases, and stage IV disease at diagnosis, and relatively more pts with triple-negative aBC (TNBC) and prior (neo)adjuvant chemotherapy. At the time of data cut-off for this interim analysis (Mar 1, 2015), median duration of observation was 10.8 months (range <0.1–47.5). BEV was typically continued for longer than chemotherapy (median 5.9 months [95% CI 5.6–6.3] vs 4.6 months [95% CI 4.4–4.9], respectively). Among pts with hormone receptor-positive disease, only 9% received concurrent endocrine therapy with BEV. The most common reason for stopping treatment was disease progression (483 of 1529 [32%] who had stopped BEV–PAC; 157/345 [46%] who had stopped BEV–CAP). At data cut-off, 1245 pts (57%) had experienced a PFS event. Median PFS was 10.1 months (95% CI 9.6–10.7) overall, 10.7 months (95% CI 10.1–11.3) for BEV–PAC, and 8.1 months (95% CI 6.6–9.0) for BEV–CAP. Median PFS in clinically important subgroups was: TNBC 7.1 months (95% CI 6.2–8.0); ≥3 metastatic sites 9.7 months (8.7–11.2); anthracycline- and/or taxane-pretreated 9.2 months (8.5–9.9); ≥65 years old 9.9 months (9.1–10.7). Safety was consistent with the well-established safety profiles of the two regimens. Grade ≥3 adverse events occurred in 17% of pts (16% BEV–PAC, 18% BEV–CAP). There were no new safety signals.

Conclusions: Interim results of this large non-interventional study indicate that first-line BEV-containing regimens represent an active and well-tolerated therapy option for aBC. Data collection in non-inferiority studies based on routine clinical practice typically differs from that in prospective clinical trials. Nevertheless, these results from AVANTI suggest that the efficacy and tolerability of BEV–PAC and BEV–CAP seen in the E2100, RIBBON-1, and TURANDOT trials can be replicated in routine oncology practice. Further analyses focusing on the incidence, management, and potential risk factors for elevation of blood pressure are ongoing.
Body: Background: Growing evidence suggests that concomitant inhibition of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin pathway could enhance and extend the clinical benefit of endocrine therapies in hormone receptor-positive (HR+) metastatic breast cancer (mBC). In this Phase Ib study (NCT02058381), alpelisib (a p110α-selective inhibitor) or buparlisib (a pan-PI3K inhibitor) was combined with tamoxifen and goserelin acetate in premenopausal women with mBC, a more prevalent patient population in Asian vs Western countries.

Methods: Premenopausal women with HR+/human epidermal growth factor receptor 2-negative (HER2–) locally advanced or mBC and no prior endocrine therapy for metastatic disease were recruited in Taiwan, Republic of Korea, and Thailand. Patients (pts) received tamoxifen (20 mg once daily [QD]) and goserelin (3.6 mg Q28D) with either alpelisib (350 mg QD; Group 1) or buparlisib (100 mg QD; Group 2) on a continuous dosing schedule in 28-day cycles. The primary objective was to define the recommended Phase II dose (RP2D) for each combination, based on dose-limiting toxicities (DLTs) observed during Cycle 1, using a dose de-escalation design. Secondary objectives included pharmacokinetics, safety and tolerability (per Common Terminology Criteria for Adverse Events v4.03), efficacy (per Response Evaluation Criteria In Solid Tumors v1.1), and impact on quality of life.

Results: As of February 2, 2015, 12 pts, all Asian, have been treated in the first cohort. In Group 1, 6 pts with a median age of 43 were treated with alpelisib (350 mg starting dose), and no DLTs were observed in Cycle 1. In Group 2, 6 pts with a median age of 47 were treated with buparlisib (100 mg starting dose), and 1 DLT of Grade (G) 3 alanine aminotransferase/aspartate aminotransferase elevation was observed. In Group 1, significant toxicities included hypokalemia (G3: 1 pt), anemia (G3: 1 pt), leukopenia (G3: 1 pt), and infections (G3: 1 pt; G1/2: 1 pt); no G4 toxicities were reported. In Group 2, significant toxicities included liver toxicity (G4: 1 pt; G3: 1 pt; G1/2: 2 pts), psychiatric disorders (G4: 1 pt; G3: 1 pt; G1/2: 1 pt), rash (G3: 1 pt; G1/2: 2 pts), hypertension (G3: 1 pt; G1/2: 1 pt), and hyperglycemia (G3: 1 pt). No pts in Group 1, and 5/6 pts in Group 2, have discontinued treatment due to adverse events (AEs). Median treatment duration was 110 days in Group 1 and 71 days in Group 2.

Conclusions: The combination of alpelisib (350 mg) with tamoxifen and goserelin resulted in a manageable toxicity profile. Meanwhile, the same combination with full-dose buparlisib (100 mg) was less well tolerated; despite the appearance of only one DLT during Cycle 1, the majority of pts subsequently stopped treatment due to AEs. An expansion phase is ongoing, and results will be integrated with safety, tolerability, and efficacy results for the first 15 pts enrolled in each group. PIK3CA status at baseline will also be assessed.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-13-28

Title: Identification of patients (pts) at high risk for early death with advanced or metastatic breast cancer (MBC), not receiving salvage treatment after 1st-line (1stL) therapy with trastuzumab (T) – Results of a prospective national non-interventional study (NIS) in Germany

Jackisch C, Schoenegg W, Reichert D, Welslau M, Selbach J, Harich H-D, Tesch H, Keitel S and Hinke A. Sana-Klinikum Offenbach GmbH, Offenbach, Germany; Gynäkologische Praxis, Berlin, Germany; Gemeinschaftspraxis für Hämatologie und Onkologie, Westerstede, Germany; Hämato-Onkologische Schwerpunktpraxis am Klinikum Aschaffenburg, Aschaffenburg, Germany; Schwerpunktpraxis und Tagesklinik im Medizinischen Zentrum Duisburg Nord, Duisburg, Germany; Hämatologisch-Onkologische Schwerpunktpraxis, Hof, Germany; Hämatologisch-Onkologische Gemeinschaftspraxis am Bethanien-Krankenhaus, Frankfurt, Germany; Roche Pharma AG, Grenzach-Wyhlen, Germany and WiSP Research Institute, Langenfeld, Germany.

Body: Background: The advent of T as targeted agent has markedly improved survival in pts with HER2-positive (HER2+) MBC. Standard of care is the permanent inhibition of the HER2neu pathway. It would be beneficial to identify patients with an early unfavorable course of the disease upfront, who might benefit from continuous anti-HER2 treatment, since more effective HER2-targeted agents are available.

Patients and methods: We recruited 1843 pts with HER2+ MBC from 2000-2010 in a national prospective NIS in Germany. 1042 pts received T as part of their 1st-L combination therapy either with chemotherapy (CT) and/or endocrine therapy (ET) for MBC. At that time, treatment beyond progression with T was the only available treatment option as 2nd-L anti-HER2 treatment. HER2-positivity (IHC3+ or IHC2+ and FISH+) was assessed according to standard procedures. Detailed information on study treatment and course of disease was collected for at least 1 y (year). Thereafter, long-term outcome data were retrieved up to 11 y.

Results: 153 pts were selected as, presumably, "high risk" cohort (C1). C1 was defined by either early death reported without any formal detection of progressive disease (PD) (C1a; n = 94), or death occurring within 3 months (mth) after PD, without any salvage therapy given (C1b; n = 59). A second cohort was defined by at least one documented salvage treatment after PD under 1st-L T therapy (C2; n = 365). In C2, 2nd-, 3rd-, 4th-, 5th- and 6th-L therapy were reported in 70%, 46%, 27%, 15%, and 7% of pts. Median overall survival (OS) in C1 vs. C2 was 14.4 (95% confidence interval: 12.0 - 18.2) vs. 45.0 mth (41.2 - 53.4). 3 y-survival was 11% in C1, compared to 65% in C2. Median time to progression in C2 was 11.8 mth (10.9 - 13.0). A correlation with treatment intensity could be ruled out as, on the contrary, less pts in C1 received ET ± T only (11% vs. 17%) and taxane treatment was more frequent in the high risk population (59% vs. 45%).

Compared to C2 pts, C1 pts are characterized by an older median age (63.4 vs. 58.4 y) and higher number of elderly pts, defined as > 70 y (26 vs. 11%; p<0.0001). In addition, negative estrogen receptor status (ER-) was more frequent in C1 than in C2 (41 vs. 28%, p=0.0038), as were hepatic lesions (50 vs. 41%, p=0.070), the number of metastatic sites (p=0.015), and a poorer ECOG status (p<0.0001). No major differences between the two cohorts were detected with respect to tumor grading, stage IV at presentation, adjuvant therapy, relapse-free interval as well as metastatic lesions confined to the bones and lungs.

Conclusions: Based on our findings, pts displaying the characteristics age > 70 y, ER-, high metastatic load, and poor ECOG status are less likely to receive 2nd-L therapy due to the high risk for early PD or death. About 30% of pts with HER2+ tumors go off treatment with each step of further-line therapy. This implicates that treatment including a permanent blockade of the HER2neu pathway implementing the available new compounds in this field should be considered even in the subset of pts at high risk for relapse.
Trastuzumab emtansine improves overall survival versus capecitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer: Final results from the phase 3 EMILIA study

Diéras V, Miles D, Verma S, Pegram M, Welslau M, Baselga J, Krop I, Blackwell K, Kang B, Xu J, Green M and Gianni L. Institut Curie, Paris, France; Mount Vernon Cancer Center, Northwood, United Kingdom; Sunnybrook Odette Cancer Center, Toronto, ON, Canada; Stanford Cancer Institute, Palo Alto, CA; Medical Office Hematology, Aschaffenburg, Bavaria, Germany; Memorial Sloan Kettering Cancer Center, NY, NY; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Genentech, Inc, South San Francisco, CA and San Raffaele Hospital, Milan, Italy.

Body: Introduction

T-DM1 is indicated for the treatment of advanced HER2-positive MBC in patients who previously received trastuzumab and a taxane (separately or in combination) based on data from the phase 3 EMILIA study (BO21977/TDM4370g; NCT00829166). In the primary PFS and second interim OS analyses, respectively, T-DM1 significantly improved PFS (median 9.6 vs 6.4 months; HR=0.65; 95% CI, 0.55–0.77; p<0.0001) and OS (median 30.9 vs 25.1 months; HR=0.68; 95% CI, 0.55–0.85; p<0.0006) compared with capecitabine (X) plus lapatinib (L). T-DM1 treatment was associated with fewer grade ≥3 AEs (41% vs 57%) vs XL. Here we present the final OS analysis from EMILIA.

Methods

EMILIA was a randomized, open-label study of patients with centrally confirmed HER2-positive (IHC3+ and/or FISH amplification ratio ≥2.0), unresectable, locally advanced or MBC, previously treated with trastuzumab and a taxane. Patients were randomized 1:1 to T-DM1 (3.6 mg/kg IV every 3 weeks) or X (1000 mg/m² PO twice daily, days 1–14 every 3 weeks) plus L (1250 mg PO daily). The final OS analysis was to be conducted following 632 events, and these results are descriptive only. Since the OS efficacy boundary (HR<0.71, p=0.0025) was crossed in the second interim analysis, a protocol amendment allowed crossover from XL to T-DM1.

Results

From Feb 2009 to Oct 2011, 991 patients were randomized to T-DM1 (n=495) or XL (n=496). Patient disposition by the data cutoff (31 Dec 2014) is shown in Table 1. OS was longer with T-DM1 vs XL (median OS 29.9 vs 25.9 months; HR=0.75; 95% CI, 0.64–0.88; p=0.0003). In a sensitivity analysis, which censored crossover patients at the time of switching from XL to T-DM1, the HR was 0.69 (95% CI, 0.59–0.82; p<0.0001). The overall safety profile was similar to previous analyses (Table 2). More grade ≥3 thrombocytopenia occurred with T-DM1 vs XL (14.3% vs 0.4%). Cardiac dysfunction occurred in 2.7% of T-DM1 patients vs 3.5% of XL patients.

Table 1. Patient disposition.

<table>
<thead>
<tr>
<th></th>
<th>T-DM1 (n=495)</th>
<th>XL (n=496)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median treatment duration, months</td>
<td>7.6</td>
<td>X: 5.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L: 5.5</td>
</tr>
<tr>
<td>Median duration of follow-up, months</td>
<td>47.8</td>
<td>41.9</td>
</tr>
<tr>
<td>Discontinued study, n (%)</td>
<td>364 (74)</td>
<td>404 (82)</td>
</tr>
<tr>
<td>Crossover, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per protocol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not applicable</td>
<td>136 (27)</td>
</tr>
<tr>
<td>Non-protocol therapy&lt;sup&gt;b&lt;/sup&gt;</td>
<td>X: 252 (54)</td>
<td>X: 53 (11)</td>
</tr>
<tr>
<td></td>
<td>L: 224 (48)</td>
<td>L: 74 (15)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Median duration of follow-up among per-protocol crossover patients was 24.1 months.
By investigator choice after study treatment discontinuation; X or L could be given in combination with each other or other agents after progression.

Table 2. Safety summary in patients who received ≥1 dose of study treatment.

<table>
<thead>
<tr>
<th>n (%)</th>
<th>T-DM1 (n=490)</th>
<th>XL (n=488)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade ≥3 AEs</td>
<td>233 (47.6)</td>
<td>291 (59.6)</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>91 (18.6)</td>
<td>99 (20.3)</td>
</tr>
<tr>
<td>AEs leading to dose reduction</td>
<td>91 (18.6)</td>
<td>X: 205 (42.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L: 98 (20.1)</td>
</tr>
</tbody>
</table>

Conclusions
This final analysis of EMILIA shows an OS benefit of T-DM1 compared with XL. While median drug exposure was longer with T-DM1 than XL, T-DM1 was associated with fewer grade ≥3 AEs and AEs leading to dose reduction compared with XL. These final OS results confirm that T-DM1 treatment improved survival, even in the presence of treatment crossover, and reaffirm T-DM1 as the standard of care in patients with previously treated HER2-positive MBC.
Title: Neutralization of BCL2/BCL-XL enhances the cytotoxicity of T-DM1 in vivo

Zoeller JJ J, Bronson RT T, Sampath D, Leverage JS J and Brugge JS S. Harvard Medical School, Boston, MA; Genentech, San Francisco, CA and AbbVie, Chicago, IL.

Body: One of the most recent advances in the treatment of HER2+ breast cancer is the development of the antibody-drug conjugate, T-DM1, composed of trastuzumab (T) linked to the cytotoxic maytansinoid (DM1). T-DM1 has proven clinical benefits for patients with advanced and/or metastatic breast cancer who have progressed on prior HER2-targeted therapies. However, T-DM1 resistance ultimately occurs and represents a major obstacle in the effective treatment of this disease. We previously identified BCL2 upregulation as a critical component and biomarker of the adaptive response to inhibition of PI3K/mTOR or HER2, and thus examined whether BCL2/BCL-XL combinatorial strategies could improve the initial efficacy of T-DM1. Here, we demonstrate that combined inhibition of BCL2/BCL-XL proteins plus T-DM1 significantly enhances the cytotoxicity of T-DM1 in vivo.

The effectiveness of T-DM1 plus BCL2/BCL-XL inhibition was evaluated in two patient-derived xenograft (PDX) models of advanced HER2+ER- resistant disease (PDX8 and PDX12). Animals were randomized into one of four treatment arms: T-DM1, ABT-737, T-DM1 + ABT-737 or vehicle controls. Our initial results after a 14d treatment period indicate that combined treatment with T-DM1 and ABT-737, the dual BCL2/BCL-XL inhibitor, confers an exceptional tumor cell cytotoxic advantage characterized by widespread elimination of the tumor cells.

To evaluate whether ABT-263, the clinically relevant BCL2/BCL-XL inhibitor, mimics ABT-737, we randomized animals into one of four treatment arms: T-DM1 (administered weekly), ABT-263 (administered daily), T-DM1 + ABT-263 or vehicle controls. To minimize thrombocytopenia that is induced by ABT-263, we included a fifth treatment arm that received pulsed treatment of ABT-263 + T-DM1. Notably, unlike continuous treatment, pulsed administration of ABT-263 reduced weight loss to vehicle levels and allowed recovery of platelet counts. Evaluation of pathological responses by H&E staining indicated that T-DM1 + ABT-263 mimics T-DM1 + ABT-737. To better distinguish tumor cells from stromal elements, we used epithelial membrane antigen immunostaining to specifically visualize tumor cells and Trichrome stain to visualize stromal content and scored the tissue sections blindly. ABT-263 had no observable effect. T-DM1 induced a 38.75% and 20% average reduction in tumor cell content in the two PDX models, whereas the combined treatment caused a 74% and 54% average reduction after the 14d treatment period. The loss of tumor cell content was associated with an increased stromal reaction at the tumor bed. T-DM1 treated tumors contained 27.5% and 47.5% average stromal content, whereas combination treated tumors contained 86% and 85.6% average stromal content. Importantly, T-DM1 + pulsed ABT-263 treatment elicited a similar response as continuous treatment in the PDX8 model, but was not as effective in PDX12.

The dramatic improvement in tumor regression observed in these preclinical studies, together with the safety benefits of modified dosing of ABT-263, provides substantial rationale for the clinical investigation of this combination therapy. Furthermore, thorough investigation of treatments that combine anti-apoptotic drugs with tumor-targeted chemotherapeutics could have broad implications in other cancer types.
What are the real-world treatment patterns and medical costs in patients with metastatic breast cancer treated with ado-trastuzumab emtansine?


Body: Background: Ado-trastuzumab emtansine (T-DM1) was approved by the FDA (02/2013) for the treatment of HER2+ metastatic breast cancer (mBC). This study assessed the real-world treatment (tx) patterns and medical costs in patients (pts) receiving T-DM1 or other targeted therapy [TT] or chemotherapy [CHT] for the tx of HER2+ mBC in the US.

Methods: Adult women with mBC initiated on T-DM1 (index date) covered by their health plan ≥365 days before and ≥30 days after the index date were selected in a large US commercial claims database (Q2 2009–Q2 2014). Pts were observed from the index date to the end of health plan enrollment (study period). Patient characteristics at T-DM1 initiation were reported and tx patterns, including T-DM1 tx duration, discontinuation (no T-DM1 claim for ≥60 days) and switch to a new TT or CHT (among pts who discontinued T-DM1), were analyzed using Kaplan Meier (KM) analyses.

In addition, T-DM1 pts were exactly matched to pts treated with another TT or CHT with similar profiles (same line of therapy and metastatic sites) on a 1:1 ratio. Tx change, defined as the initiation of or a switch to a new TT or CHT, and medical costs, measured up to 6 months after index date, were compared between pts receiving T-DM1 vs. other TT or CHT using multivariate Cox and GLM regression models, respectively.

Results: A total of 240 T-DM1 pts were selected. Mean age was 54 years and pts had on average 2.9 distinct metastatic sites. Most prevalent sites were bone/bone marrow (69.6%), liver (47.1%), and lung/pleura (40.4%). Median time from mBC diagnosis to index date was 25.0 months. Pts were observed for a median of 5.6 months after index date. 8.3% of pts were initiated on T-DM1 in 1st line therapy, 30.4% in 2nd line, 15.4% in 3rd line, 17.5% in 4th line, and 28.3% in later lines. 9.2% of pts were initiated on T-DM1 concomitantly with hormonal therapy. Pts received a mean of 6.2 doses (median: 5.0) of T-DM1 over the study period. KM median T-DM1 tx duration estimate was 7.4 months. KM rates of T-DM1 discontinuation and switch at 6 months were 18.6% and 23.1%, respectively.

Among the matched sample (n=228 in each cohort), T-DM1 pts had a lower risk of tx change (hazard ratio (HR) [95% CI]: 0.61 [0.40; 0.94]) compared to pts treated with other TT or CHT. Among T-DM1 pts, those receiving T-DM1 in 1st or 2nd lines of therapy had a lower risk of tx change than those receiving T-DM1 in later lines (HR [95% CI]: 0.34 [0.14; 0.80]). Once adjusted for potential confounding factors, T-DM1 pts had lower medical costs (adjusted, $1,630 per pt per month [pppm]) compared to pts treated with other TT or CHT (unadjusted, $5,075 vs. $6,204; p<.05). The medical cost difference was mainly driven by the outpatient cost difference (adjusted, $1,002 pppm; p<.05). Incremental cost associated with adverse events accounted for 25% (adjusted, $255 pppm) of the outpatient cost difference.

Conclusions: In this real-world study of mBC pts treated with T-DM1 shortly after its approval, most pts were initiated on T-DM1 in 2nd and later lines of therapy. When compared to pts with similar profiles initiated on other TT or CHT, T-DM1 pts had a lower risk of tx change and lower medical costs.
Title: A chart review of patient characteristics, treatment patterns and response in metastatic breast cancer patients treated with ado-trastuzumab emtansine in first-line therapy and beyond


Background: Ado-trastuzumab emtansine (T-DM1) approved for HER2+ unresectable locally advanced metastatic breast cancer (mBC) has been shown to significantly improve progression-free and overall survival in patients (pts) previously treated with trastuzumab and a taxane. However, little is known about real-world patterns and outcomes of T-DM1.

Method: Pt-level data was collected from 90 US oncologists using an online chart extraction tool. Oncologists randomly selected eligible adult mBC pts started on T-DM1 on or after February 22nd 2013. Pts demographics, clinical information, and T-DM1 patterns and responses were described. Among pts whose T-DM1 response was assessed, univariate logistic regression models were used to assess the association between each factor and the probability of achieving complete response (CR).

Results: Among the 303 pts, median follow-up after T-DM1 initiation was 5.1 months; 58.4% started T-DM1 in the 2nd half of 2014. Median age was 58 years, most pts were Caucasian (62.0%), median number of metastatic sites was 2, and 65.3% of pts had a mBC diagnosis (dx) within 1 year of the BC dx. Most common metastatic sites were liver (51.5%), lung/pleura (42.6%), and bone/bone marrow (41.6%). Median time from mBC dx to T-DM1 initiation was 7.1 months. 34.0% of pts started T-DM1 in 1st line for mBC, 55.4% in 2nd line, and 10.6% in later lines after mBC dx; most common prior treatments were trastuzumab, pertuzumab, and taxane. Best response achieved while on T-DM1 was CR in 17.5% of pts, partial response (PR) in 46.2%, stable disease in 11.2%, recurrence/progression in 3.6%, and the response was unknown in 21.5% of pts. CR/PR was achieved within a median of 5 months of T-DM1 initiation. At the end of follow-up, 80.2% were still on T-DM1, 3.3% had switched, 12.9% had discontinued without switching, and 3.6% were deceased. 44.9% of pts discontinued/switched after CR/PR and 32.7% after progression. When physicians were surveyed about their practice, 30.0% reported intention to interrupt T-DM1 after CR and 7.8% after an a priori determined number of cycles. Among pts whose response on T-DM1 was assessed (78.5%), race (Asian), initiation of T-DM1 shortly after mBC dx or in 1st line after mBC, ≤2 metastatic lesions, single metastatic site, and estrogen (ER)+/progesterone (PR)+ at T-DM1 initiation were found to be significant predictors of CR, while pts who progressed between mBC dx and T-DM1 were less likely to achieve CR.

Table 1. Factors Associated with CR

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio and 95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian (vs non-Asian)</td>
<td>2.9 (1.2 - 7.4)</td>
</tr>
<tr>
<td>T-DM1 ≤ 1 year after mBC dx (vs &gt; 1)</td>
<td>3.2 (1.4 - 7.6)</td>
</tr>
<tr>
<td>T-DM1 in 1st line after mBC dx (vs later lines)</td>
<td>3.8 (2.0 - 7.2)</td>
</tr>
<tr>
<td>≤2 metastases (vs&gt;2)</td>
<td>3.8 (1.9 - 7.6)</td>
</tr>
<tr>
<td>1 metastatic site (vs &gt;1)</td>
<td>4.4 (2.3 - 8.4)</td>
</tr>
<tr>
<td>ER+/PR+ (vs other status)</td>
<td>4.9 (2.5 - 9.7)</td>
</tr>
<tr>
<td>Progression between mBC dx and T-DM1 (vs no prog)</td>
<td>0.2 (0.1 - 0.4)</td>
</tr>
</tbody>
</table>

Conclusions: Most pts started T-DM1 as 1st or 2nd line therapy for mBC and were still treated with T-DM1 at the end of follow-up. CR/PR, assessed by treating oncologists, was achieved in >50% of pts within 5 months of T-DM1 initiation.
Title: Genomic parameters affecting the outcome of patients with advanced breast cancer treated with trastuzumab


Body: Background-Aim: There is an unmet need for de-selecting HER2-positive patients with advanced breast cancer (ABC), since only some of those patients benefit from the addition of anti-HER2 agents to chemotherapy. The aim of this study was to investigate candidate biomarkers, including MYC and MET, in parallel with an extended array of biomarkers previously associated with trastuzumab (T) resistance.

Patients and Methods: Two hundred and twenty-nine ABC patients treated with T and chemotherapy over a period of 13 years were included in the study. Paraffin tumors were retrospectively centrally assessed with immunohistochemistry (IHC) for breast cancer subtypes; fluorescence in situ hybridization (FISH) for HER2, TOP2A and centromere (CEN) 17, MYC and CEN8, MET and CEN7; qPCR for MYC and MET copy number (CN); and, for PI3K activation (PIK3CA mutations, PTEN and phospho-mTOR IHC). Patterns of CEN CN aberrations corresponding to chromosome "polysomy" were also evaluated, with cut-offs based on normal tissue. Time to progression (TTP) and survival were evaluated from the initiation of T as first-line treatment.

Results: Median follow-up was 70 months. Of the 229 patients treated with T as HER2-positive, central analysis identified 90 cases being HER2-negative, as per current guidelines (39.3% of the total cohort). HER2-positive patients showed a trend for survival benefit over HER2-negative patients (median 50.7 vs. 38.1 months, respectively, p=0.118). HER2-positive tumors were subtyped as Luminal-HER2 (n=77) and HER2-enriched (n=53); 156 patients presented with ABC and 65 with disease initially diagnosed at stage IV (de novo ABC). MET and MYC CN gains (≥2.5 copies) were found in 40 (25%) and 15 (9%) cases with qPCR, while MET and MYC amplification with FISH was present in 4 (2.5%) and 31 (18%) cases, respectively. Concordance between FISH and qPCR was low for MYC (kappa value 0.46) and absent for MET. Polysomy was collectively observed in 70 cases, in 54 of them (32% of all tumors) concerning any 1 of the 3 examined chromosomes. This condition, called restricted polysomy, interacted with ABC presentation, conferring decreased survival to patients with ABC (HR=2.32, 95% CI 1.43-3.76, Wald's p=0.001) but not to those with de novo ABC (interaction p=0.077). MYC CN gain was the only marker significantly associated with increased risk for progression (HR=3.22, 95% CI 1.66-6.24, p<0.001) and death (HR=5.45, 95% CI 2.89-10.28, p<0.001) at univariate analysis. Adjustment of all tested markers with standard clinicopathological parameters revealed that along with poor patient performance status that was associated with poor prognosis, MYC CN gain was an independent adverse prognosticator for both TTP and survival (all p-values <0.001). The HER2-enriched subtype was independently associated with T benefit for TTP (p=0.001) and survival (p=0.051). The interaction between restricted polysomy and disease presentation was also independently significant for survival (p=0.041).

Conclusions: MYC CN gain is a strong unfavorable prognosticator in T-treated ABC patients. Distinguishing between HER2-positive subtypes seems important for identifying T benefit in ABC. Chromosomal polysomy may distinctly affect T benefit in patients with pre-treated and de novo ABC.
Title: Neoadjuvant chemotherapy with docetaxel, carboplatin and weekly trastuzumab (TCH) is active in HER2-positive early breast cancer: Results after a median follow-up of over 4 years


Body: Background:
HER2-positive breast cancer is known to carry an adverse prognosis compared to HER2-negative disease, which may however be compensated by the use of HER2-targeted agents. Therefore, most patients with HER2-positive disease larger than 5 mm receive chemotherapy and trastuzumab. Data from adjuvant trials have shown that the combination of docetaxel, carboplatin and weekly trastuzumab (TCH) is well tolerated and as effective as anthracycline containing regimes. Previous investigations on neoadjuvant treatment with TCH showed pCR-rates in the range of 40%, however, survival data have not yet been presented. Here we present 4-year follow-up data for a cohort of 51 patients treated with neoadjuvant TCH.

Methods:
We treated 51 patients with operable HER2-positive breast cancer with a neoadjuvant schedule of docetaxel (75 mg/m2) and carboplatin (AUC 6) q3w and trastuzumab (2(4)mg/kg) q1w. Lymph node involvement was verified by SLNB or core-cut-biopsy. Patients were diagnosed at a mean age of 55 years, 68.6% had ER positive tumors, 39.2% presented with grade 3 disease and 49% of patients were node-positive. Patients were monitored every two cycles by ultrasound. After 6 cycles of chemotherapy all patients had surgery. Axillary dissection was performed in case of positive lymph node status prior to TCH. After surgery trastuzumab was continued q3w up to one year.

Results:
In 50 patients TCH could be administered as planned without dose reductions or delays. One patient suffered from an allergic reaction on taxane after the second cycle, resulting in replacement by gemcitabine. Side effects were mild, no grade III/IV toxicities occurred and no case of cardiomyopathia was observed. 21 (41.18%) patients achieved a pCR, 18 (72.0%) patients converted from cN+ to ypN0. Outcome data at a median follow-up of 51.6 months are as follows.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n=51)</th>
<th>pCR (n=21)</th>
<th>N+ (n=25)</th>
<th>cN+ → ypN0 (n=18)</th>
<th>ER positive (n=35)</th>
<th>G3 (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS (n/%)</td>
<td>42/82.35</td>
<td>17/80.95</td>
<td>17/68.0</td>
<td>16/88.89</td>
<td>31/88.57</td>
<td>16/80.0</td>
</tr>
<tr>
<td>DDFS (n/%)</td>
<td>46/90.2</td>
<td>19/90.48</td>
<td>20/80.0</td>
<td>17/94.44</td>
<td>33/94.29</td>
<td>18/90.0</td>
</tr>
<tr>
<td>OS (n/%)</td>
<td>48/94.18</td>
<td>21/100.0</td>
<td>22/88.0</td>
<td>18/100.0</td>
<td>34/97.14</td>
<td>19/95.0</td>
</tr>
</tbody>
</table>

Conclusion:
Outcome following neoadjuvant TCH as observed in our analysis compares well to outcome data observed in adjuvant trastuzumab trials such as HERA (4-year follow-up; DFS 78.6% and OS 89.3%) or BCIRG006 (36-month follow-up; DFS 82% and OS 91% in the TCH-arm). Particularly among patients with ER positive disease and those experiencing axillary conversion we observed an excellent outcome. Importantly, TCH was well tolerated in our cohort. Therefore our data support the use of TCH as neoadjuvant therapy regimen for patients with HER-positive breast cancer. They also strongly encourage the use of docetaxel and carboplatin as chemotherapy backbone in studies investigating the dual blockade with trastuzumab and pertuzumab in the neoadjuvant setting.
Title: Outcome with use of 12 weeks of adjuvant or neoadjuvant trastuzumab in a resource constrained setting


Body: Background: Adjuvant trastuzumab has improved overall survival in women with HER2 receptor positive breast cancer. However, only a small fraction (4%) of eligible patients in resource constrained settings have access to this drug. A patient assistance program of 12 weeks of adjuvant or neoadjuvant trastuzumab was thus started for those who did not have any access to trastuzumab due to financial constraints. We undertook a retrospective analysis of outcomes in women who were enrolled between January 2011 to December 2012 in this patient assistance program.

Methods: Patients received four cycles of anthracycline based chemotherapy (AC/CAF/EC/CEF) and 12 doses of weekly paclitaxel (80mg/m2) with trastuzumab (4mg/kg loading followed by 2mg/kg) in the neoadjuvant or adjuvant setting in either sequence (anthracycline followed by taxane trastuzumab or taxane trastuzumab followed by anthracycline). Patients received adjuvant hormonal therapy depending on the hormone receptor status. The primary endpoint of this analysis was disease free survival (DFS).

Results: A total of 103 patients with HER2 receptor positive breast cancer were analysed. The median age was 46 (24-65) years, 50% were premenopausal, 60.7% had stage III disease (86.8% had node positive disease) and 37% patients had ER and or PR positive disease. Forty patients (38.8%) had breast conserving surgery while the rest had modified radical mastectomy. At a median follow-up of 34 (7-46) months the 3-year DFS and overall survival was 77.2% and 82.7% respectively. Among patients who developed recurrence one had only local recurrence, 4 had both local and distant recurrence and 11 had distant metastasis alone. Of the 15 patients who developed distant metastasis 7 had brain involvement. Symptomatic cardiac dysfunction developed in four patients, two of whom died while in the other 2 ejection fraction recovered. The results are summarised in the table.

<p>| Patient Characteristic and outcome with 12 weeks of adjuvant or neoadjuvant Trastuzumab |
|-------------------------------------|-----------------|----------------------|-------------|------------------------|-----------------|-----------------------|</p>
<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Node Positive (%)</th>
<th>Hormone Positive (%)</th>
<th>DFS at 3 years</th>
<th>OS at 3 years</th>
<th>Brain Mets (%)</th>
<th>Grade 3/4 Cardiac Toxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>86.8</td>
<td>37</td>
<td>77.2</td>
<td>82.7</td>
<td>6.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Conclusions: These results suggest that 12 weeks of neoadjuvant or adjuvant trastuzumab is an acceptable alternative in patients who lack access to full 1 year of trastuzumab.
Title: Efficacy of trastuzumab re-therapy in the clinical routine of HER2-positive breast cancer patients who relapsed after completed anti-HER2 (neo)adjuvant therapy – 5th interim analysis of the national non-interventional study (NIS) ML21589

Hanker L, Hitschold T, Grafe A, Förster F, Schröder J, Janssen J, Reichert D, Hielscher C, Keitel S and Hesse T. Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Klinik für Frauenheilkunde und Geburtshilfe, Lübeck, Germany; Klinikum Worms, Brustzentrum, Worms, Germany; MVZ Nordhausen, Nordhausen, Germany; Schwerpunktpraxis für Gynäkologische Onkologie und Palliativmedizin, Chemnitz, Germany; Onkologische Schwerpunktpraxis, Muelheim an der Ruhr, Germany; Gemeinschaftspraxis für Hämatologie und Onkologie, Westerstede, Germany; Gynäkologie Zentrum Stralsund, Stralsund, Germany; Roche Pharma AG, Grenzach-Wyhlen, Germany and Diakoniekrankenhaus, Klinik für Frauenheilkunde, Rotenburg, Germany.

Body: Background
HER2 overexpression occurs in approx. 20% of breast tumors and is associated with increased aggressiveness and mortality. Anti-HER2 re-therapy with trastuzumab (Herceptin®, T) is an established therapeutic option for the treatment of recurrent/metastatic HER2-positive breast cancer (MBC). Patients (pts) receiving T re-therapy appear to benefit from the retreatment after a relapse-free (neo)adjuvant anti-HER2 therapy.

Methods
This NIS is conducted in Germany to gain additional knowledge about efficacy of 1st-line T retreatment in the clinical routine. 122 sites enrolled a total of 239 pts with locally recurrent and/or MBC who relapsed after (neo)adjuvant treatment with T in their medical history. 230 pts met the eligibility criteria. The current analysis presents data collected between 10/2008-04/2015. Tumor progression was clinically assessed by the investigator.

Results
Median observation period for the total population (n=230) was 41.7 months (m) (range 0.4-94.5).

At start of T retreatment, 20.0% (n=46) of the pts presented with local recurrence only, 79.6% (n=183) presented with distant metastases ± local recurrence and one patient (0.4%) presented with elevated tumor markers. 27.4% (n=63) of the pts developed exclusively non-visceral metastases and 52.2% (n=120) were diagnosed with visceral (± non-visceral) metastases. Median duration of T re-therapy in the first line setting was 9.0 m (95% confidence interval (CI): 7.6-10.1). In 69.6% (n=160) of the cases, T was added to chemotherapy (CT). 15.2% of the pts (n=35) were treated with T+CT + endocrine therapy (ET).

A median progression free survival (PFS) of 10.1 m was observed among all eligible patients (n = 230) (95% CI: 8.5-12.0). The evaluation by risk groups revealed a PFS of 23.7 m (95% CI: 13.3-NE*) for patients with local recurrence only, 11.8 m (95% CI: 8.3-20.1) for patients with non-visceral metastases and 7.6 m (95% CI: 6.2-9.9) for patients with visceral metastases. *Not evaluable

Median PFS according to treatment regimen was 8.3 m for pts who were treated with T+CT (n=125, 54.3%), 17.7 m in pts who received T+CT+ET (n=35, 15.2%) and 11.2 m in pts treated with T+ET (n=38, 16.5%). Pts who received T monotherapy (n=32, 13.9%) had a median PFS of 13.4 m.

Of 230 pts, 121 deaths were documented (52.6%) within the observation period. The median overall survival (OS) was 29.6 m for all pts (95% CI: 27.3-36.8). Median OS was statistically reliable for pts with visceral metastases only: 19.4 m (95% CI: 16.9-27.3). The 2-year survival rate was 62.2% for all pts, 81.9% for pts with local recurrence only, 80.7% for pts with non-visceral metastases and 45.6% for pts with visceral metastases.

Conclusions
The survival observed for pts with HER2-positive MBC receiving T re-therapy in the clinical routine is in line with the results of recently published data. In terms of the 2-year survival rate, 81.9% of the pts with local recurrence were still alive and thus show the most favourable prognosis. T re-therapy provides an efficient treatment option regardless of given as combination-therapy together with CT and/or ET or as monotherapy.
Title: Cardiac safety of trastuzumab without an anthracycline in patients with HER2-positive early stage breast cancer: A single center experience


Body: Background: Trastuzumab (H) improves disease-free survival and overall survival in HER2+ early breast cancer (EBC) but is associated with risk of treatment-induced cardiotoxicity especially when administered after an anthracycline. We performed a single center retrospective study to assess the cardiac safety of adjuvant trastuzumab therapy without anthracyclines in a real-world clinical setting.

Methods: Patients (pts) with HER2+ early breast cancer (EBC) who received H without anthracycline-based chemotherapy between January 2010 and June 2014 were studied. Patients enrolled in a clinical trial were excluded. Tumor characteristics, chemotherapy regimen, cardiovascular risk factors, left ventricular ejection fraction (LVEF), and treatment interruption data were collected. A cardiac event (C.E.) was defined as New York Heart Association class III or IV heart failure with LVEF decline of > 10% to < 55% or possible/probable cardiac death, as previously defined by the NSABP B-31 trial.

Results: In total, 174 pts with HER2+ EBC treated with H-based therapy without anthracyclines were identified. Median age was 59 years (range, 32 to 85 years), 72 (41%) had hypertension, 55 (32%) had hyperlipidemia, 29 (16%) had diabetes, and 5 (3%) had coronary artery disease. At baseline, all pts had a LVEF > 50% (median, 66%; range, 50% to 81%). Two (1.1%) pts developed a C.E. Both pts had risks factors for C.E. (1 - age > 60 years, hypertension, and prior history of anthracycline exposure; 1- age > 60 years, hypertension, hyperlipidemia, and baseline LVEF of 50-55%). After discontinuation of H, both patients had recovery of LVEF to > 50% and resolution of heart failure symptoms. Twelve (6.9%) pts developed asymptomatic LVEF decline of > 10% points to < 55% during H therapy. Of the 14 patients who developed cardiotoxicity, H was prematurely interrupted or discontinued in 8 patients.

Conclusion: In our single center experience of patients being treated off study, the incidence of C.E.s and asymptomatic LVEF decline during H therapy without an anthracycline was 1.1% and 6.9%, respectively. These appeared higher than events reported in clinical trials possibly due to the inclusion of an older group of women with a higher prevalence of cardiovascular risk factors. Overall, the incidence of symptomatic heart failure is low for H without an anthracycline even in this older group.
Title: Pertuzumab overcomes chemotherapy/trastuzumab resistance in ER+/Her2+ tumors classified as luminal functional subtype by the 80-gene BluePrint assay in the prospective neo-adjuvant breast registry symphony trial (NBRST)

Peter B, Pat W, Paul B, Jennifer B, Pellicane JV V, Murray MK K, Dul CL L, Mislowsky AM M, Nash CH H, Richards PD D, Lee LL L, Stork-Sloots L, de Snoo F, Untch S, Gittleman M, Akbari S and Rotkis MC C. Dallas Surgical Group, Dallas, TX, Netherlands; Nashville Breast Center, Nashville, TN; Breast & Melanoma Specialists of Charleston, Charleston, SC; The Breast Place, Charleston, SC; Virginia Breast Center, Bon Secours Cancer Institute, Richmond, VA; Akron General Hospital, Akron, OH; St. John Hospital & Medical Center, Detroit, MI; Coastal Carolina Breast Center, Murrells Inlet, SC; Northeast Georgia Medical Center, Gainesville, GA; Blue Ridge Cancer Care, Roanoke, VA; Comprehensive Cancer Center, Palm Springs, CA; Agenda Inc, Irvine, CA; Breast Care Specialists, Allentown, PA; Virginia Hospital Center, Arlington, VA and Northern Indiana Cancer Research Consortium, South Bend, IN.

Body: Background
The prospective Neo-adjuvant Breast Registry Symphony Trial (NBRST) enrolled over 1000 US patients between June 2011 and December 2014. The aim of NBRST study is to compare chemosensitivity as defined by pathological Complete Response (pCR) using the 80-gene BluePrint functional subtype profile vs. conventional IHC/FISH subtyping. Treatment was at the discretion of the physician utilizing standard NCCN regimens. Pertuzumab, a monoclonal antibody, inhibits the dimerization of HER2 with other HER receptors. Pertuzumab received US FDA approval for the neo-adjuvant treatment of HER2-positive breast cancer in September 2013. Essentially all patients with HER2 positive cancers were treated with chemotherapy + trastuzumab and after this date pertuzumab was added, creating 2 distinct groups of Her2 treated patients.

The aim of the current analysis is to compare the pCR rate of trastuzumab (H) vs trastuzumab and pertuzumab (H + P) by conventional and BluePrint functional subtype.

Methods
The current analysis includes women from the NBRST study, with histologically proven breast cancer, who received neo-adjuvant chemotherapy plus H or H + P and who provided written informed consent. Pathological assessment of Her2 was done according to ASCO CAP guidelines at the time of diagnosis. BluePrint (BP) classifies patients into Luminal, HER2 or Basal-type. pCR is defined as T0/isN0. All pCRs were verified with a de-identified copy of the surgical pathology report. Fisher’s exact test was used to compare pCR rates within different subgroups.

Results
252 IHC/FISH Her2+ patients received H (166) or H + P (86). The median age was 53 (range 23-81). 8% was stage I, 68% stage II and 24% stage III. 65% were ER positive.

BP classified 55% of patients as HER2, 32% as Luminal, and 14% as Basal-type.

The pCR rates and p-values within different subgroups of clinical Her2+ patients are provided in the table below.

<table>
<thead>
<tr>
<th>(n)</th>
<th>H (pCR rate)</th>
<th>H + P (pCR rate)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=252)</td>
<td>40%</td>
<td>59%</td>
<td>0.005</td>
</tr>
<tr>
<td>IHC/FISH Her2+/ER+ (163)</td>
<td>30%</td>
<td>57%</td>
<td>0.001</td>
</tr>
<tr>
<td>IHC/FISH Her2+/ER- (89)</td>
<td>69%</td>
<td>63%</td>
<td>0.82</td>
</tr>
<tr>
<td>BP HER2 (138)</td>
<td>57%</td>
<td>78%</td>
<td>0.01</td>
</tr>
<tr>
<td>BP Luminal (80)</td>
<td>4%</td>
<td>38%</td>
<td>0.0002</td>
</tr>
<tr>
<td>BP Basal (34)</td>
<td>47%</td>
<td>38%</td>
<td>0.69</td>
</tr>
</tbody>
</table>
Conclusions
Addition of pertuzumab to trastuzumab significantly increased response rate in ER+/Her2+, BP HER2 and BP Luminal patients but not in ER-negative and BP Basal patients. Pertuzumab overcame resistance to NCT/trastuzumab in a substantial proportion of the IHC/FISH Her2+/BP Luminal subgroup; indicated by a significantly increased pCR rate.
Title: Efficacy and cardiac safety in neoadjuvant treatment of Her2 positive breast cancer with concomitant nonpegylated liposomal doxorubicin, docetaxel and dual blockade with trastuzumab and pertuzumab: A retrospective analysis


Body: Background: Approval to pertuzumab as part of a complete treatment regimen for patients with early stage breast cancer (EBC) before surgery (neoadjuvant setting) was granted by the FDA in September 2013. Since then, the relevance of neoadjuvant treatment in Her2 overexpressing breast cancer has increased considerably. This for instance has been emphasized by the results of the Neosphere Study, in which dual blockade of Her2 was combined with docetaxel as chemotherapy backbone and resulted in favorable pCR rates. But it is likely, that anthracyclines could play an important role in enhancing the effectiveness of the above mentioned treatment. However, there is only little data about the cardiac safety of this combination. The use of liposomal doxorubicin might be a valuable alternative with low cardiotoxicity, as it has been shown in comparable publications without the use of pertuzumab. Therefore we report pCR-rate and cardiac safety of a single arm, retrospective, multicenter analysis of neoadjuvant treatment for Her2 positive EBC with liposomal doxorubicin, docetaxel, trastuzumab and pertuzumab.

Methods: In this study 42 women with Her2 positive EBC were investigated in 4 oncological departments in Austria. 41 patients were treated with liposomal doxorubicin (50 mg/m²), docetaxel (75 mg/m²) concurrent with trastuzumab and pertuzumab in standard dosage for 6 cycles as neoadjuvant therapy. One patient refused to receive a chemotherapy but agreed to be treated with combined antibody therapy alone. All patients were free of cardiovascular disease and had a left ventricular ejection fraction (LVEF) of ≥50%. Cardiac function was measured by LVEF and was examined at regular intervals (cycles 0-3, cycle 6, FU). Clinical response was evaluated by diagnostic breast imaging after cycles 3 and 6. All patients underwent surgery after neoadjuvant chemotherapy. The absence of any residual invasive cancer in the breast and axilla was defined as pathological complete response (pCR). Median follow up was 1.3 years.

Results: Median age of the patients was 49 years. After 6 cycles of treatment the pCR rate was 76.2%. In this cohort a negative estrogen-and/or progesteron receptor was predictive for pCR (p<0.001). These patients achieved pCR in 95.2%. The antibody only treatment in one case also resulted in a pCR. No patient progressed during treatment. Only one of the patients (2,4%) suffered symptomatic heart failure after surgery. The patient initially presented with an LVEF of 16%.

Conclusions: In this multicenter analysis we observed a considerably high rate of pCR in HER2-positive EBC treated with liposomal doxorubicin, docetaxel, trastuzumab and pertuzumab. Especially the group of hormone receptor negative patients showed a remarkable response rate. The addition of liposomal doxorubicin entails a very favorable cardiotoxicity profile. This regimen is a safe treatment option in patients with HER-2 positive breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-14-12

Title: Interim results from the first open-label, multicenter, phase IIIb study investigating the combination of pertuzumab with subcutaneous trastuzumab and a taxane in patients with HER2-positive metastatic breast cancer (SAPPHIRE)

Woodward N, De Boer RH H, Redfern A, White M, Young J, Truman M and Beith J. University of Queensland and Mater Hospital, Cancer Services, Brisbane, QLD, Australia; Royal Melbourne Hospital, Parkville, VIC, Australia; Royal Perth Hospital, Perth, WA, Australia; Monash Cancer Centre, Moorabbin, VIC, Australia; Roche Products Pty. Limited, Dee Why, NSW, Australia and Chris O'Brien Lifehouse, Camperdown, NSW, Australia.

Body: Background: Intravenous (IV) trastuzumab has proven clinical benefits in patients (pts) with human epidermal growth factor receptor 2 (HER2)-positive breast cancer (BC). The use of pertuzumab, which targets HER2 through an independent epitope to that of trastuzumab, in combination with IV trastuzumab and docetaxel has shown improved efficacy with acceptable toxicity in metastatic (m) BC. Subcutaneous (SC) and IV trastuzumab formulations have shown comparable efficacy. This study aimed to assess the safety, tolerability, and efficacy of combining IV pertuzumab with SC trastuzumab and a taxane, as 1st-line therapy in pts with HER2+ mBC, a combination for which results have not previously been reported. Here we present demographics and interim safety data.

Methods: This is an open-label, multicentre, phase IIIb study. The primary objective was the safety and tolerability of IV pertuzumab with SC trastuzumab and investigator's choice of taxane. Pts aged ≥18 years with confirmed HER2-positive [IHC3+ or ISH+] mBC with at least one measurable lesion and/or non-measurable disease according to RECIST version 1.1 and ECOG performance status (PS) 0-2 were included. Pts received IV pertuzumab every 3 weeks (loading dose=840 mg; subsequent doses=420mg) combined with SC trastuzumab at 600mg/5mL every 3 weeks and the investigator's choice of taxane (docetaxel, paclitaxel, or nab-paclitaxel). Treatment continued until disease progression, unacceptable toxicity, or consent was withdrawn, whichever occurred first. The incidence and severity of adverse events (AEs), serious (S) AEs and AEs leading to premature discontinuation of study treatment were analyzed.

Results: The planned 50 pts have been recruited from 12 centres; mean age 53 (SD-12.0) years; the majority white (84%), ECOG PS 0 (n=33) and PS 1 (n=15). 98% were females; 61% post-menopausal. Taxanes of choice were nab-paclitaxel (n=36), docetaxel (n=13) and paclitaxel (n=1). Any grade AEs (n=627) were reported in 100% pts; majority grade 1-2, the most common being diarrhoea, fatigue, peripheral neuropathy, alopecia, nausea, rash, headache and vomiting. Grade 3+ AEs (n=54) were reported in 52% pts, most commonly neutropenia (10%), febrile neutropenia (8%) and diarrhea (6%). SAEs (n=36) were reported in 48% pts; most commonly pyrexia (14%), febrile neutropenia (8%), neutropenia (4%), pulmonary embolism (4%) and cellulitis (4%). Five AEs of suspected cardiac disorders were reported in 4 pts (atrial fibrillation, cardiomyopathy, myocardial ischemia, palpitations and ejection fraction decreased). AEs leading to study drug discontinuation (n=3) were reported in 3 pts (LVEF decreased, syncope and blister). AEs leading to chemotherapy discontinuation (n=14) were reported in 20% pts.

Title: Pertuzumab (P) use in first-line HER2-positive metastatic breast cancer (mBC) in US community oncology practices: Treatment patterns and outcomes


Body: **Background**: Pertuzumab was FDA-approved in 6/2012 for use in first-line in combination with trastuzumab (H) and docetaxel for patients (pts) with HER2-positive mBC. This retrospective study investigated the clinical characteristics, treatment patterns, safety, and outcomes for pts with HER2-positive mBC who received a P-containing regimen in first-line in US community oncology practices.

**Methods**: This study utilized iKnowMed electronic health records, Claims Data Warehouse, and Social Security Death Index. Pts with HER2-positive mBC, who received a P-containing regimen between 6/2012 and 6/2014 and were followed through 12/2014, had ≥2 visits within the McKesson Specialty Health/US Oncology Network, and were not on clinical trials during the study period, were eligible.

**Results**: Of the 322 pts who received a P-containing regimen in the first-line setting, 25% were ≥65 years of age, 63% were post-menopausal, 61% had hormone receptor-positive mBC, 84% had a performance status of 0 or 1, and 76% had a Charlson Comorbidities Index of 0. Twenty-one percent of pts had 1 site of metastasis noted, 32% had 2 sites, and 47% had 3 or more sites. Pts with de novo mBC made up 40% of this cohort. Of the pts with recurrent mBC, over 60% received H in the early-stage BC setting. In the first-line mBC setting, 93% of the 322 pts received H+P+taxane, and 7% received H+P with other chemotherapy agent(s). Common adverse events reported included: fatigue (49%), diarrhea (44%), nausea (33%), peripheral neuropathy (33%), neutropenia (24%), and rash (22%). Further analyses including outcomes of these 322 pts will be presented.

**Conclusions**: First-line P was given in combination with H and chemotherapy agent(s) (93% taxane). No new safety signals were observed. More details on the clinical characteristics, specific treatment patterns, and safety will be presented, along with the progression-free survival of these pts receiving first-line P-containing therapy in a real-world setting.
Incidence and management of diarrhea in patients with HER2-positive breast cancer treated with pertuzumab

Swain SM M, Schneeweiss A, Gianni L, Stein A, McNally V, Heeson S, Portera C, Yoo B, Cortes JC and Baselga J. Washington Cancer Institute; National Center for Tumor Diseases, University Hospital Heidelberg; San Raffaele Hospital; Genentech, Inc; Roche Products Ltd; Vall D’Hebron University Hospital and Memorial Sloan Kettering Cancer Center.

BACKGROUND: Pertuzumab (P) in combination with trastuzumab (T) and docetaxel (D) is the approved first line SOC in patients with HER2 positive metastatic breast cancer and is approved neoadjuvantly in patients with HER2 positive stage Ib-IIlc breast cancer. Because of its role in heterodimerization with EGFR, P may cause adverse events associated with EGFR antagonists. Diarrhea is the most commonly reported AE due to P. Increased usage of P has generated clinical questions regarding the incidence and management of diarrhea. Here we report safety analyses of diarrhea from three P containing studies.

METHODS: The safety population evaluated in this exploratory analysis included 804 patients from CLEOPATRA, 416 patients from NeoSphere, and 223 from TRYPHAENA. Diarrhea incidence, severity (NCI-CTCAE v3.0), and management in P containing arms were analyzed.

RESULTS: The incidence and management of diarrhea in MBC (Table 1), EBC (Table 2):

CLEOPATRA

<table>
<thead>
<tr>
<th></th>
<th>P+T+D n=408</th>
<th>Pla+T+D n=396</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Grades</td>
<td>279 (68)</td>
<td>193 (49)</td>
</tr>
<tr>
<td>≥ Grade 3</td>
<td>38 (9)</td>
<td>20 (5)</td>
</tr>
<tr>
<td>Median time to 1st event (days) all grades / Interquartile Range (IQR)</td>
<td>8 (4,44)</td>
<td>23 (6,82)</td>
</tr>
<tr>
<td>Discontinuation of any study drug</td>
<td>8 (0.2)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Treatment n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidiarrheal treatment</td>
<td>164 (40)</td>
<td>77 (19)</td>
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NeoSphere X4 followed by adjuvant FECx3

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<tr>
<th>Neoadjuvant tx followed by T up to 1 year</th>
<th>T+D n=107</th>
<th>P+T+D n=108</th>
<th>P+T n=94</th>
<th>P+D x3→P+T+D x3 n=72</th>
<th>FEC+P+T x3→P+T+D x3 n=72</th>
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<tbody>
<tr>
<td>All Grades</td>
<td>41 (38)</td>
<td>55 (51)</td>
<td>46 (43)</td>
<td>53 (56)</td>
<td>46 (64)</td>
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<tr>
<td>≥ Grade 3</td>
<td>4 (4)</td>
<td>7 (7)</td>
<td>3 (3)</td>
<td>5 (5)</td>
<td>3 (4)</td>
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<td>Median time to 1st event (days) all grades / Interquartile Range (IQR)</td>
<td>7 (4,24)</td>
<td>8 (3,26)</td>
<td>19 (4,117)</td>
<td>6 (3,21)</td>
<td>9 (4,30)</td>
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<tr>
<td>Discontinuation of any study drug</td>
<td>0</td>
<td>0</td>
<td>0</td>
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TRYPHAENA

<table>
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<tr>
<th>TCH+P x6 n=76</th>
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<tr>
<td>FEC x3→P+T+D x3 n=75</td>
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<th>Neoadjuvant tx followed by T up to 1 year</th>
<th>T+D n=76</th>
<th>P+T+D n=75</th>
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<td>All Grades</td>
<td>47 (63)</td>
<td>55 (72)</td>
<td>55 (72)</td>
<td>55 (72)</td>
</tr>
<tr>
<td>≥ Grade 3</td>
<td>4 (5)</td>
<td>9 (12)</td>
<td>9 (12)</td>
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<td>69 (64,82)</td>
<td>6 (3,21)</td>
<td>69 (64,82)</td>
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<td>Discontinuation of any study drug</td>
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</table>
pertuzumab(P)+trastuzumab(T)+docetaxel(D) FEC= 5FU, epirubicin, cyclophosphamide TCH= docetaxel,(T)carboplatin(C), trastuzumab(H)

The overall incidence of diarrhea events is greatest in the first cycle containing P: in CLEOPATRA (P+T+D) 43%, in NeoSphere (P+T+D) 34%, (P+T) 21%, and in TRYPHAENA (FEC+P+T→P+T+D) 40%, (FEC→P+T+D c4) 46%, and (TCH+P) 55%. Of patients experiencing diarrhea, the median number of events (all grades) for patients receiving P+T+D in CLEOPATRA and NeoSphere was 2 and 1 respectively, and 2 for patients receiving TCH+P in TRYPHAENA.

CONCLUSIONS:
Diarrhea was common in all P containing arms but events were mostly low grade and occurred more often with the first cycle. Events and management were similar in the EBC and MBC setting. Approximately half of patients required antidiarrheal treatment. However, rates of study drug discontinuation due to diarrhea were low. Studies of treatment associated diarrhea management are planned.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-14-15

Title: Pre-treatment stromal tumour-infiltrating lymphocytes (S-TILs) are correlated with complete response (CR) to chemotherapy (Chemo) plus trastuzumab (T) in HER2-positive (H+) metastatic breast cancer (MBC)


Body: Background. We have previously reported that ChemoT produces durable (>5 years) CR in a minority of pts with H+MBC, prompting a search for predictive markers. Extensive lymphocytic infiltration of cancers is correlated with high levels of immune gene signatures. International consensus guidelines on TILs define "lymphocyte-predominant BC" at a threshold of S-TILs of 50-60% versus tumour cells. High levels of S-TILs has been correlated with improved outcome in HER2+ early stage BC pts treated with ChemoT. We investigated the degree of S-TIL infiltration in metastatic biopsies from pts with HER2+MBC prior to ChemoT, and attempted to determine whether S-TILs predicted CR in HER2+MBC.

Methods. We searched a database of all pts with HER2+ MBC treated at our institution with anti-HER2 therapy over 15yrs to identify pts who achieved CR according to RECIST 1.0 criteria, which lasted for at least 6 months. We matched them with an equal number of pts from the database who were treated during the same period, but who had progressive (POD) or stable disease (SD) as best response to T. Pts must have at least one pre-treatment tumour sample available for S-TILs assessment, and adequate clinical and follow-up information. S-TILs (mononuclear cells including lymphocytes and plasma cells) contained within the boundaries of invasive tumour were identified on a representative haematoxylin and eosin stained slide and scored as a percentage of the stromal area alone, according to the International TILs Working Group 2014 methodology [Salgado R, 2015]. S-TILs were assessed specifically for this study by a senior pathologist who scored the samples and who was blinded to pts response and clinical details.

Results. Out of 246 MBC pts registered in the HER2+ database we identified 31 CR pts with at least one available pre-treatment metastatic sample. A cohort of 31 matching POD-SD pts was randomly obtained from the same database. In 8 cases (7 CR / 1 POD-SD) S-TILs could not be assessed due to inadequate material, or for other technical reasons. The final study sample is 54 pts (24 CR / 30 POD-SD). Pts characteristics are as follows: median age (range): CR 55 (29-78) / POD-SD 56 (26-89), hormone receptor (HR) pos: CR 12 (50%) / POD-SD 18 (60%), De Novo MBC at diagnosis: CR 13 (54%) / POD-SD 8 (27%) [p<0.05]. All pts received chemotherapy with T (+ lapatinib in 3 pts as part of a clinical trial), and continued on T until POD. Pre-treatment S-TILs >50% were statistically significantly more frequent in CR (50% of pts) than POD/SD (20%) [chi-square p=0.02]. No statistically significant difference in the HR status was observed between the two groups (CR vs POD-SD) or between the high and low S-TILs pts.

Conclusions. S-TILs >50% in the pre-treatment tumour biopsy of HER2+MBC were significantly correlated with subsequent CR to ChemoT, supporting the hypothesis that the immunological effects of T may play a role in determining response. Speculatively, S-TILs might identify pts with a higher likelihood of benefit from T. Further study of the potential role of S-TILs as predictors of T benefit are required.
Title: T-cell clonality increases after neoadjuvant treatment with trastuzumab and pertuzumab


Body: Background: Baseline TIL density and T-cell clonality predict pathological complete response (pCR) to neoadjuvant (NA) trastuzumab (TRAS). Preclinical data suggests that a major mechanism of TRAS is enhancement of antibody-dependent cytoxicity (ADCC) and T-cell mediated tumor kill and that the combination of TRAS with pertuzumab (PERT) may enhance ADCC. This study quantifies both TIL density and T-cell clonality in both pre and post-NA samples from patients receiving NA TRAS +/- PERT.

Methods: Sequential subjects treated with NA docetaxel, carboplatin and TRAS +/- PERT were identified and pre-treatment (n=39) and post-treatment specimens (n=44, 36 pairs of pre and post-treatment; 19 with pCR) were sequenced using the ImmunoSEQ platform (Adaptive Bioscience, Seattle, WA). T-cell density and dominant T-cell clonality were measured by TCR sequencing and then associated with pCR using a two-tailed Mann-Whitney U test comparing patients with pCR versus those without. Paired values were compared using the sign test to evaluate change in pre and post-treatment T-cell density and clonality. TIL density was performed using standard IHC methodology.

Results: There was a trend towards increased T-cell clonality after treatment (p=0.08). T-cell clonality post-treatment was significantly increased compared to pre-treatment in those samples where post-treatment tumor was available (p=0.01). There was no difference in T-cell clonality between pre- and post-treatment in normal tissue from pts achieving a pCR (p=1.00). There was no difference in post-treatment T cell clonality when treated with TRAS vs. TRAS + PERT (p=0.19). No differences in the % of pretreatment TILs or T-cell clonality were seen in those who obtained a pCR vs. those who did not (p=0.39 and p=0.85). In addition, no differences in T-cell clonality were seen based on ER status in either pre-treatment or post-treatment samples (p=0.40/0.48)

Conclusions: After treatment with TRAS +/- PERT, there was an increase in T-cell clonality suggesting an enhanced immune response as a mechanism of action for HER2 targeting antibodies. This analysis is currently limited by a small sample size and high rate of pCR. We plan to include additional paired samples which may help to better demonstrate the increased immune response that occurs with HER-2 targeting antibodies.
Title: Prognostic value of tumor-infiltrating lymphocytes in residual tumors after neoadjuvant chemotherapy concomitant with trastuzumab for HER2-positive breast cancer

Kurozumi S, Inoue K, Matsumoto H, Hayashi Y, Tozuka K, Kubo K, Komatsu K, Takai K, Nagai SE E, Oba H, Horiguchi J, Takeyoshi I and Kurosumi M. Division of Breast Surgery, Saitama Cancer Center, Saitama, Japan; Division of Breast Oncology, Saitama Cancer Center, Saitama, Japan; Saitama Cancer Center, Saitama, Japan and Gunma University Graduate School of Medicine, Gunma, Japan.

Body: Background:
Neoadjuvant chemotherapy (NAC) with taxanes, followed by fluorouracil, epirubicin, and cyclophosphamide (FEC), with concurrent trastuzumab is known to achieve a high pCR rate of more than 60% for HER2-positive breast cancer (BC) as well as good prognoses in those obtaining pCR. On the other hand, the prognostic significance of tumor-infiltrating lymphocytes (TILs) has recently been described in triple-negative BC. However, the prognostic and predictive values of TILs in HER2-positive BC remain unclear. In the present study, we examined the grades of TILs in pre-treatment cancer tissues and residual tumors after NAC with trastuzumab, and also investigated its predictive utility for pCR and prognostic power for HER2-positive BC.

Patients and Methods:
A total of 128 Japanese women with HER2-positive BC received either paclitaxel or docetaxel followed by FEC, with concomitant trastuzumab. The proportional grades of stromal (Str)-TILs in pre-treatment biopsy specimens and residual tumors after NAC with trastuzumab were determined as follows: low grade (0-10%), intermediate grade (10-40%), and high grade (40-90%), using the criteria of the International Working Group for TILs in BC. Analysis 1: The relationship between the grades of Str-TILs in pre-treatment tumors and pCR rates was investigated. Relapse-free survival (RFS) and cancer-specific survival (CSS) were analyzed for a correlation with pre-treatment Str-TILs. Analysis 2: Alterations in the grade of Str-TILs were examined in the residual tumors of non-pCR patients, and RFS and CSS were analyzed for a correlation with residual Str-TILs.

Results:
pCR was achieved in 83 out of the 128 patients (pCR rate, 64.8%) who received NAC with trastuzumab, and RFS was significantly better in the pCR group than in the non-pCR group (p = 0.0071). Analysis 1: The patient distribution of the Str-TILs grade in pre-treatment tumors was as follows: high: 24 (18.8%); intermediate: 38 (29.7%); and low: 66 (51.6%). pCR rates correlated with the Str-TILs grade in pre-treatment tumors: 83.3% in the high group, 71.1% in the intermediate group, and 54.5% in the low group (p = 0.026); however, the Str-TILs grade in pre-treatment tumors did not correlate with survival. Analysis 2: In 45 non-pCR patients, the distribution of the Str-TILs grade in residual tumors was as follows: high: 9 (20.0%); intermediate: 8 (17.8%); and low: 28 (62.2%), respectively. In non-pCR patients, the rate of a high Str-TILs grade was greater in residual tumors than in pre-treatment tumors (residual, 20.0%, pre-treatment, 8.9%). RFS was significantly better with a high grade than with a low grade of residual Str-TILs (p = 0.033).

Conclusions:
The status of TILs in pre-treatment tumors predicted responses to NAC concomitant with trastuzumab in HER2-positive BC. The grade of TILs was higher in residual tumors than in pre-treatment tumors, and, among non-pCR patients, the prognosis of patients with a high residual-TILs grade was better prognosis than that of patients with a low residual-TILs grade. We speculate that an examination of TILs in residual tumors after NAC with trastuzumab may be necessary for selecting patients with a good prognosis from non-pCR patients.
Title: Phase I study of single-agent pyrotinib, a novel irreversible pan-ErbB receptor tyrosine kinase inhibitor, in patients with ErbB2+ metastatic breast cancer

Xu B, Ma F, Chen S, Li Q, Yang F, Zhang Y, Chen X, Zhong D and Zhang G.  Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China;  Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China and  Jiangsu Hengrui Medicine Co., LTD, Shanghai, China.

Body: Pyrotinib is a novel small molecule irreversible pan-ErbB receptor tyrosine kinase inhibitor. This phase I study was designed to evaluate the safety, pharmacokinetics and antitumor activity of pyrotinib in Chinese patients with ErbB2+ metastatic breast cancer (n=38). The majority of patients were previously treated with multiple lines (≤5) of anti-tumor regimens including trastuzumab. The dose-escalation study was conducted at 80, 160, 240, 320, 400 and 480 mg levels in patients receiving oral pyrotinib with food once daily on a 28-day cycle. Dose-limiting toxicity (DLT) was grade 3 diarrhea, which occurred in two out of two patients treated with 480mg pyrotinib during the dose-escalation. Therefore, the maximum tolerated dose (MTD) of pyrotinib was determined to be 400 mg per day. In general, pyrotinib was safe and well tolerated when administered to patients. The majority of adverse events which occurred in the study were grade I and II. The pharmacokinetic results showed linear characteristics along the range of doses tested, and supports a once daily dosing regimen. The overall objective response rate (ORR) was 52.8% and disease control rate (DCR) was 80.6% in 36 patients (19 PR and 10 SD), with the highest ORR of 55.6% (5/9) and 87.5% (7/8) observed in 320 mg and 400 mg cohorts, respectively. The median duration of response was 32.3 weeks. In addition, 3 (8.3%) subjects achieved stable disease for at least 24 weeks. Kaplan-Meier median progression-free survival (PFS) was 35.3 weeks (95%CI: 23.6–39.9 weeks) among all subjects treated with 80-400 mg, and the median PFS for 320 mg and 400 mg cohorts was 39.9 weeks (95%CI: 31.1–47.4 weeks). Based on tolerability, safety, PK and efficacy data of 6 dose-cohorts, 320 mg and 400mg of pyrotinib once daily could be considered a recommended regimen for Phase II studies. The promising antitumor activity of pyrotinib observed in patients with ErbB2+ metastatic breast cancer, who had previous treatment with anthracyclines, taxanes, and trastuzumab, warrants its further study (ClinicalTrials.gov number, NCT01937689).
ONT-380 in the treatment of HER2+ breast cancer central nervous system (CNS) metastases (mets)

Murthy RK K, Hamilton E, Borges VF F, Moulder S, Aucoin N, Welch S, Chaves J, Falkson CI I, Walker L and Ferrario C. University of Texas MD Anderson Cancer Center, Houston, TX; Sarah Cannon Research Institute, Nashville, TN; University of Colorado Cancer Center, Aurora, CO; hopsital de la Cité-de-la-Sante, Laval, QC, Canada; London Regional Cancer Program, London Health Sciences Centre, London, ON, Canada; Northwest Medical Specialties PLLC, Tacoma, WA; University of Alabama Comprehensive Cancer Center, Birmingham, AL; Oncothyreon Inc, Seattle, WA; Segal Cancer Centre -Jewish General Hospital, Montreal, QC, Canada and Tennessee Oncology PLLC, Nashville, TN.

Body: Background: The risk of CNS involvement in patients with HER2+ metastatic breast cancer (MBC) is high. The natural history of HER2+ CNS metastases (mets) is different from other breast cancer subtypes; there is a longer time from metastatic diagnosis to CNS relapse, greater control of extracranial disease at the time of CNS relapse, and longer median overall survival from the time of CNS relapse. Local treatments, i.e., surgery and/or radiation, remain the mainstay of treatment for HER2+ CNS disease as standard systemic therapy options have limited efficacy. ONT-380, a potent HER2 selective tyrosine kinase inhibitor with minimal EGFR-like side effects, has been associated with increased survival compared to lapatinib or neratinib in an animal model of HER2+ CNS disease. Here, we describe 24 pts from two studies with response-evaluable CNS mets treated with ONT-380 in combination with other systemic therapies.

Methods: Pts with asymptomatic untreated or post-treatment progressive CNS mets were enrolled in ongoing phase 1b studies of ONT-380 + ado-trastuzumab emtansine (T-DM1) or ONT-380 ± trastuzumab (T) ±capecitabine (C). All pts received treatment in 21 day cycles including ONT-380 300 mg PO BID and approved doses of either T-DM1, T or C alone, or T+C. Eligibility criteria for all pts included prior treatment with T and a taxane, and for pts receiving T and/or C, prior T-DM1. Prior lapatinib was allowed. Assessments included safety, systemic tumor response per RECIST 1.1, and CNS tumor response by MRI every 2 cycles per modified RECIST 1.1.

Results: 24 pts (10 with untreated CNS mets and 14 with progressive CNS mets after local therapy) received ONT-380 plus T-DM1 (n =14), C (n=1), T (n = 5) or T+C (n = 4) for 1-8 cycles. Of these 24 pts, 14 are evaluable for response in the CNS (at least one follow-up MRI): 7 pts have not yet been rescanned, and 3 are non-evaluable. In the 14 response-evaluable pts, best CNS response has been: 1 CNS CR (T-DM1), 4 CNS PR (T-DM1 n = 2; T+C n = 1; C n=1) and 9 CNS SD (T-DM1 n = 5; T n = 3; T+C n=1). Pts with CR or PR (prior lapatinib n=2; prior pertuzumab n=2; prior T-DM1 n=2) all had > 50% decrease in CNS target lesions. One CNS non-evaluable pt (T) had a 15% increase in their target lesion and underwent surgical resection; pathology, however, revealed no viable tumor with only necrotic tissue present. Two additional CNS non-evaluable pts (T-DM1 n=1; C+T n=1) were taken off study due to systemic PD after treatment was held for AEs (Gr 3 AST/ALT elevation [n=1]; Gr 4 edema in thalamic lesion [n=1]). No other ≥ Gr 3 ONT-380 related events were reported. Systemic disease control was also seen, with a best response in 17 evaluable pts of 6 PR, 9 SD, and 2 PD. Conclusions: This case series demonstrates early clinical signs of activity of ONT-380 against HER2+ CNS mets in combination with other systemic agents. Further study of the CNS activity of ONT-380 is ongoing and further studies are being planned. Updated results will be reported. Clinical trial information: NCT01983501, NCT02025192.
Title: A phase 1b study of ONT 380, an oral HER2-specific inhibitor, combined with ado trastuzumab emtansine (T-DM1), in HER2+ metastatic breast cancer (MBC)

Ferrario C, Hamilton E, Aucoin N, Falkson CI, Khan Q, Krop IE, Welch S, Bedard PL, Conlin A, Chaves J, Vo A, Walker L and Borges V. Segal Cancer Centre - Jewish General Hospital, Montreal, QC, Canada; Sarah Cannon Research Institute, Nashville, TN; Hopital de la Cite-de-la Sante, Laval, QC, Canada; University of Alabama Comprehensive Cancer Center, Birmingham, AL; University of Kansas Medical Center, Westwood, KS; Dana-Farber Cancer Institute, Boston, MA; London Regional Cancer Program, London Health Sciences Centre, London, ON, Canada; Princess Margaret Cancer Center – University Health Network, Toronto, ON, Canada; Providence Oncology and Hematology Care Clinic, Eastside, Portland, OR; Northwest Medical Specialties PLLC, Tacoma, WA; Oncothyreon Inc, Seattle, WA; University of Colorado Cancer Center, Aurora, CO and Tennessee Oncology PLLC, Nashville, TN.

Body: Background: ONT 380 is a potent HER2 selective tyrosine kinase inhibitor with minimal EGFR-like side effects. Preclinical studies demonstrate synergism with trastuzumab (T) and chemotherapy, as well as activity in models of HER2+ CNS disease. Based on the potential for increased clinical activity of dual HER2 blockade in the context of a targeted cytotoxic agent, we evaluated the safety, tolerability, and anti-tumor activity of ONT-380 in combination with T-DM1 in patients (pts) with MBC with and without CNS metastases (mets).

Methods: 3+3 dose escalation with MTD expansion cohorts in pts with/without CNS mets evaluating ONT-380 (300 or 350 mg PO BID) combined with T-DM1 3.6 mg/kg IV q 21 days. Prior treatment with T and taxane was required; prior lapatinib and asymptomatic brain mets (treated or untreated) were allowed. Assessments included safety, PK, and systemic (RECIST 1.1) and CNS (modified RECIST) tumor response, with brain MRI at baseline and q 6 wks in pts with CNS mets at baseline. DLT was defined as the occurrence of protocol-specified events during the 1st treatment cycle.

Results: As of 01 June 2015, 51 pts have been enrolled and have received between 1 and 22 cycles, with safety data available for 43 pts (n=36 at 300 mg BID; n=7 at 350 mg BID). Pts had a median of 2 prior treatments for MBC, including T (n=43); pertuzumab (n=15) and lapatinib (n=7). The MTD for ONT-380 was 300 mg BID with 5/36 pts (14%) with DLT (Gr 3 AST/ALT [n=4]; Gr 2 vomiting/Gr1 diarrhea [n=1]) vs. 3/7 pts (43%) with DLT at 350 mg BID (Gr 3 vomiting [n=1]; Gr 3 hypersensitivity [n=1]; Gr 3 fatigue [n=1]). Overall, the majority of AEs have been Gr 1 or 2. The most common AEs, regardless of relationship, were nausea, fatigue, diarrhea, vomiting, thrombocytopenia, AST/ALT elevation, headache, decreased appetite, epistaxis, constipation, and hypokalemia. Gr 3 AST/ALT elevation has occurred in 7/43 pts (16%), with no events meeting Hy's law, and has been reversible with dose interruption and reduction except in 2 pts found to have progressive liver mets. No Gr 3 diarrhea has occurred at any dose. ONT-380 PK was dose proportional, and no drug-drug interaction was observed with T-DM1. In 33 of 43 pts with data available from at least one follow-up scan to evaluate response, best systemic response regardless of dose was 11 PR, 16 SD, and 6 PD, with clinical benefit rate (CBR= CR, PR, or SD >6 mos) of 19/33 (58%). The most common reason for treatment discontinuation was PD, with 3 pts coming off study for AEs (n=1 each of Gr 3 hypersensitivity, Gr 2 vomiting, Gr 3 AST/ALT). Eight pts to date have been evaluable for CNS response (untreated or progressive CNS mets with ≥1 follow-up MRI), with best CNS response of 1 CNS CR, 2 CNS PR, and 5 CNS SD, with a CNS CBR of 5/8 (63%). All pts with CNS response are still active.

Conclusion: Treatment with ONT-380 300 mg BID and T-DM1 3.6 mg/kg has been tolerable. Early evidence of anti-tumor activity has been seen, including clinical benefit in patients with CNS mets. Updated results will be presented.
Title: A phase I trial of ganetespib (heat shock protein 90 inhibitor) in combination with paclitaxel and trastuzumab in patients with human epidermal growth factor receptor-2 positive (HER2+) metastatic breast cancer (MBC)


Body: Introduction: Targeted therapies in HER2+ MBC significantly improve outcomes but efficacy is limited by therapeutic resistance. HSP90 is a molecular chaperone involved in the stability and function of multiple signaling onco-proteins. HER2 is an acutely sensitive HSP90 client and HSP90 inhibition can overcome trastuzumab resistance. Our group reported objective responses with 17-AAG plus trastuzumab in HER2+ MBC. Ganetespib, a synthetic, second generation HSP90 inhibitor has increased potency and tolerability compared with earlier agents. We reported anti-tumor activity in metastatic HER2+ and triple negative breast cancer with single agent ganetespib. Preclinically, HSP90 inhibition has synergistic anti-tumor activity with taxanes and trastuzumab. This study will define the MTD and RP2D of ganetespib plus paclitaxel and trastuzumab in HER2+ MBC.

Methods: In this 3+3 phase I dose escalation study, patients with trastuzumab-resistant HER2+ MBC receive weekly trastuzumab and paclitaxel (80mg/m$^2$) with ganetespib on day 1, 8, 15 of a 28 day cycle. HR+ positive patients are required to have at least one prior line of endocrine therapy. DLT of ganetespib monotherapy is diarrhea and therefore patients receive prophylactic anti-motility agents. Based on prior experience with ganetespib plus docetaxel in NSCLC, only 3 dose levels of ganetespib were explored: 100mg/m$^2$, 150mg/m$^2$ and a 3rd cohort of 125mg/m$^2$, if needed. Secondary endpoints include evaluation of effects of ganetespib on the pharmacokinetics (PK) of paclitaxel and preliminary efficacy assessment.

Results: The dosing cohorts (100 mg/m$^2$ (n=3) and 150 mg/m$^2$ (n=6)) have been completed without any DLTs. Median age was 46 years (range 29-65), median prior lines of chemotherapy and anti-HER2 therapy were 3 (range 2-6) and 3 (range 2-4) respectively, including prior pertuzumab in 9/9 and T-DM1 in 8/9 patients. There were no grade 3/4 adverse events (AEs) related to ganetespib. Most common AEs related to ganetespib were diarrhea, fatigue, anemia and rash. Paclitaxel PK data available from 6/9 patients are not appreciably different from those reported in literature. Overall response rate was 25% (2/8 had PR in 150 mg/m$^2$ cohort; 1 patient was not evaluable), SD in 63% (5/8), and clinical benefit rate (CR+PR+SD>24 weeks) was 50% (4/8). 3 patients remain on study.

Conclusion: The RP2D of ganetespib is 150mg/m$^2$ in combination with paclitaxel and trastuzumab. The combination was safe and well tolerated. Updated PFS and PK data will be presented. Despite prior taxanes, pertuzumab and T-DM1, clinical activity of this triplet regimen in this heavily pre-treated cohort is very promising and together with our prior experience with 17-AAG plus trastuzumab and single agent ganetespib warrants further study in HER2+ MBC. A phase 2 trial is being planned in trastuzumab-refractory HER2+ MBC who have progressed on prior pertuzumab and T-DM1. Additionally, the protocol is amended to assess the safety of ganetespib in combination with paclitaxel, trastuzumab and pertuzumab in the first-line setting.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-14-22

Title: A single-center, open-label phase 1b study of entinostat, and lapatinib alone, and in combination with and trastuzumab in patients with HER2+ metastatic breast cancer after progression on trastuzumab


Body: Background: Our in vitro and in vivo preclinical data showed that entinostat enhances the efficacy of lapatinib in HER2 positive (HER2+) breast cancer cells via FOXO3-mediated Bim1 expression, which resulted in enhanced apoptosis in HER2 targeted therapy (lapatinib and trastuzumab)-resistant breast cancer (IBC and non-IBC) cells [Lee et al.]. Based on these findings, we conducted a phase 1b trial of entinostat to determine the maximal tolerated dose (MTD) in combination with lapatinib alone and in combination with lapatinib and trastuzumab for metastatic HER2+ breast cancer patients (pts), who progressed on trastuzumab.

Method: This was a single-center, open-label phase 1b study to evaluate the dose limiting toxicity (DLT) and determine MTD. 3+3 dose escalation schedule was used for Cohorts 1 and 2. Pts received lapatinib and entinostat (Cohort 1) or entinostat, lapatinib, and trastuzumab (Cohort 2). Initial dose of lapatinib 1250mg in Cohort 1 and 1000mg for Cohort 2 to match standard dose in combination with trastuzumab dose. In Cohort 1, entinostat was given PO on day 1 and 15 every 28 days cycle at dose levels 10 mg (level 0), 12 mg (level 1), or 15 mg (level 2). The dose levels for Cohort 2 were 12 mg (co-level 0) or 15 mg (co-level 1) on day 1 and 15 every 28 days cycle. While lapatinib and entinostat were given 28 days cycle due to entinostat dosing, the dosing of trastuzumab followed approved schedule every 21 days starting at 8mg/kg loading followed by 6mg/kg q 3 wks in Cohort 2 and 3. After the MTD of entinostat in cohort 2 was determined at 12mg, an expansion cohort of 10 pts (cohort 3) was conducted.

Results: Median age was 52 (26-69 yrs). Median number of prior trastuzumab-based regimens was 2 (1-6), 8 pts had lapatinib containing treatment prior to the trial, including 5 pts who had clinical benefit. 16 had ER+ and 13 ER negative, and 9 had IBC. Clinical efficacy and toxicity of treatment is summarized in table 1. Out of 14 pts who had clinical benefit (CR, PR, SD), 6 had IBC. Three pts are still on therapy (1CR, 1PR, 1SD).

Table 1. Clinical Efficacy, Toxicity of combination

<table>
<thead>
<tr>
<th>Receptor Status</th>
<th>Response</th>
<th>Grade 3 toxicity</th>
<th>Grade 4 toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+/ER- (N=8)</td>
<td>CR (N=1; 8M), SD (N=4;1,2,4M)</td>
<td>Lapatinib dose reduction: 3 pts Rash (2) Abdominal pain + dyspnea (1)</td>
<td>Entinostat dose reduction: 2pts Neutropenia (1 at 12mg, 1 at 15mg)</td>
</tr>
<tr>
<td>HER2+/ER+ (N=7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cohort 2/3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+/ER- (N=8)</td>
<td>CR (N=2; 3,6M), PR (N=2;4,5M) SD (N=5;1,2,4,6M)</td>
<td>Lapatinib dose reduction: 2 pts Diarrhea (N=1 at 12mg N=1 at 10mg) Entinostat dose reduction: 5 pts Neutropenia (N=2 at 12 mg) Leukopenia (N=1 at 12mg) Anemia (N=1 at 12mg)</td>
<td>Entinostat dose reduction: 2pts Hypokalemia (N=1 at 12mg) Thrombocytopenia (N=1 at 15mg)</td>
</tr>
<tr>
<td>HER2+/ER+ (N=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CR: complete response, PR: partial response, SD: stable disease, N=number of pts, M=months

Conclusion: MTD was reached at 12mg q 2wkly entinostat, lapatinib 1000 mg daily and trastuzumab 8 mg/kg followed by 6mg/kg q 3 wks. This combination was safe and had promising clinical efficacy in patients with trastuzumab-resistant metastatic HER2+ breast cancer including IBC, warranting further study.
A phase I/II study of the combination of lapatinib and oral vinorelbine in HER2 positive metastatic breast cancer

Chen TW-W Wei-Wu, Yeh D-C, Chao T-Y, Lin C-H, Chow LW-C Wing-Cheong, Hsieh Y-Y, Huang S-M, Cheng A-L, Huang C-S and Lu Y-S. National Taiwan University Hospital, Taipei, Taiwan; Taichung Veterans General Hospital, Taichung, Taiwan; Division of Hematology and Oncology, Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan; National Taiwan University Hospital, Taipei, Taiwan; Comprehensive Centre for Breast Diseases, UNIMED Medical Institute, Wan Chai, Hong Kong and National Taiwan University Hospital, Taipei, Taiwan.

Body: Background The combination of lapatinib and oral vinorelbine for HER2 positive metastatic breast cancer (MBC) is appealing due to the convenience of oral combinations, but the overlapping toxicities may be of concern. A phase I/II study was designed to understand the tolerability and efficacy of this combination treatment.

Methods Female MBC patients (pts) with HER2 positive, but regardless of ER status, were eligible. In the phase I part, pts refractory to HER2-targeted treatment or chemotherapy were eligible. In the phase II part, only pts who had not exposed to HER2-targeted treatment in the metastatic setting were eligible. Hormonal treatments were not allowed during the study for ER positive pts. Lapatinib was given once daily and oral vinorelbine was given on days 1 and 8 of a 21-day cycle. A 3+3 standard dose escalation rule was applied in phase I part (Table 1). The maximum tolerated dose (MTD) was the highest dose level with less than 33% of pts experiencing dose limiting toxicity (DLT) and will used as starting dose for phase II part. The primary endpoint of the phase II part was progression-free survival (PFS). Tumor response was assessed according to RECIST 1.1. Pt number estimation in the phase II part was based on Simon 2 stage design.

Results From 2009 Jun to 2013 Feb, a total of 46 pts were enrolled in phase I (n=15) and II (n=31) parts. The median age was 52.8 (range 34.3-84.0) and the median follow-up time was 39.2 months (range 6.1-62.2). Twenty-eight (60.9%) pts were ER positive. In the phase I part, 2 pts in dose level III had DLTs (Grade 3 neutropenia and grade 3 diarrhea (n=1), prolonged neutropenia delaying next cycle treatment (n=1)). Other common ≥40% grade 1/2 adverse events (AE) in the first-cycle include diarrhea (80.0%), skin rash (66.7%), fatigue (60.0%), and vomiting (40.0%). The MTD was determined at lapatinib 1000 mg plus oral vinorelbine 50 mg/m2. In the phase II part, 11 pts had intra-patient dose escalation of vinorelbine from 50 to 60 mg/m2 after first-cycle if no major toxicities were noted. Grade 3/4 AEs in the first-cycle include neutropenia (12.9%), diarrhea (3.2%) and infection (3.2%). Grade 1/2 AEs were similar to the phase I results. Persistent treatment (in 8 cycles) increased the rate of grade 3/4 neutropenia (22.6%) and ALT/AST elevation (6.5%).The median PFS was 5.6 months (95% CI 5.2-5.9); 6 (19.4%) pts had PR, and the clinical benefit rate (CBR) was 38.7%. A higher dose (level III vs. level II) of vinorelbine was not associated with a better CBR (p=0.71), PFS (p=0.73), or OS (p=0.11). Among pts who had disease progression records, 13.2% (5/38) had brain metastasis progression. Long-term disease control (under treatment for more than 2 years) were achieved in 13.0% (6/46, 2 ER positive, 4 ER negative) pts.

Conclusion The combination of lapatinib 1000 mg and oral vinorelbine 60 mg/m2 was general tolerable with manageable toxicities. Clinical efficacy was demonstrated with long-term responders observed.

Table 1

<table>
<thead>
<tr>
<th>Vinorelbine D1 &amp; D8/21-day cycle (mg/m2)</th>
<th>Lapatinib Daily/21-day cycle (mg)</th>
<th>No. of pt recruited</th>
<th>No. of pt with grade 3/4 toxicities in first-cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 0 30</td>
<td>1000</td>
<td>3 (15(I)+31(II))</td>
<td>0</td>
</tr>
<tr>
<td>Level I 40</td>
<td>1000</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Level II 50</td>
<td>1000</td>
<td>6+20</td>
<td>6(II)</td>
</tr>
<tr>
<td>Level III 60</td>
<td>1000</td>
<td>3+11</td>
<td>2(I)</td>
</tr>
<tr>
<td>Level IV 60</td>
<td>1250</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Body: Background: Systemic treatment of central nervous system (CNS) metastases remains a challenge partially due to poor drug penetration. Lapatinib and capecitabine are drugs with modest efficacy in treatment of brain metastases from HER2-positive (+) breast cancer (BC) and were shown to cross the blood-tumor barrier in clinical craniotomy specimens (Lin N et al., CCR 2009, Morikawa A et al., Neuro Oncol 2015). However, intratumoral drug concentrations observed were sub-optimal and heterogeneous. Administration of shorter-duration, high dose tyrosine kinase inhibitor is proposed as a way to improve efficacy and tolerability based on Norton-Simon modeling and drug exposure in the CNS (Traina T et al., JCO 2008, Grommes C et al., Neuro Oncol 2011, Chien AJ et al., J Clin Oncol 2014). In this study, we examined optimization of high dose lapatinib administration with or without capecitabine to inform the design of a phase I trial for BC patients with HER2+ CNS metastases.

Methods: Mice bearing BT-474 BC xenograft tumors were treated with various lapatinib doses and schedules. A standard continuous daily dose (100mg/kg) was compared to various intermittent dosing schedules (at 100mg/kg, 400mg/kg, and 800mg/kg). In addition, high dose lapatinib (800mg/kg) was administered with capecitabine either concurrently or in tandem. Xenografts were treated when tumors reached 100mm$^3$. Tumor volumes were evaluated for antitumor efficacy, and mice weights were measured for toxicity. Significance testing for between-group comparisons was conducted using a mixed effect model for repeated measures.

Results: Intermittent schedules of lapatinib at 100mg/kg given as 3 days on/11 days off (3/11), 5 days on/9 days off (5/9), and 7 days on/7 days off (7/7) had a similar efficacy in tumor control: percent change in tumor volume of 225% (7/7), 222% (5/9), and 223% (3/11) (NS). Therefore, the 3 days on (with 4 days off or 11 days off) schedule was subsequently chosen to evaluate for tolerability and antitumor efficacy of higher lapatinib dose. The 3 days on/4 days off (3/4) group at 800mg/kg demonstrated the highest tumor reduction (-69%) compared to the daily continuous dosing group (-18%) (p=0.04), but a trend toward higher toxicity was observed (p=0.12). Evaluation of concurrent vs. tandem administration of capecitabine with lapatinib at 800mg/kg given in 3 days on/11 days off was conducted. The concurrent treatment was discontinued early due to high toxicity. However, tandem administration of capecitabine with high dose lapatinib was tolerable without a significant difference in weight changes (p=0.62).

Conclusions: The intermittent schedule allows delivery of high dose lapatinib, which has better anti-tumor activity than standard continuous dosing. If given intermittently, high dose lapatinib is tolerable, even with capecitabine if given in tandem/sequence. Based on the result of these experiments, a phase I trial of high dose lapatinib using 3 days on/11 days off schedule in tandem with capecitabine is currently proposed for treatment of HER2-positive BC patient with CNS metastases.
Title: Single agent and combined targeting of PI3K, mTOR, HER2 and ER signaling in a panel of HER2+/ER+ versus HER2+/ER- breast cancer cell lines


Body: Background: Altered PI3K/mTOR signaling, through activating mutations in PIK3CA and/or PTEN loss, has been implicated in resistance to both hormonal and HER2-directed therapeutics for breast cancer. Recent data from the BOLERO-1 phase III clinical trials demonstrate an improvement in progression free survival (PFS) of 7.2 months in patients with HER2+/estrogen receptor negative (HER2+/ER-) advanced breast cancer when treated with trastuzumab and paclitaxel plus the mTORC1 inhibitor, everolimus (Afinitor®). However, improvement in PFS was not observed in patients with HER2+/ER+ positive disease. These data suggest that hormone receptor levels may influence the response of HER2-amplified cells to both HER2 and PI3K/mTOR directed therapies in the absence of hormonally directed therapies. In this study, we investigated the role of ER in HER2+ breast cancer by screening a large panel of HER2+/ER- and HER2+/ER+ breast cancer cell lines for responses to single agent and combined treatment with PI3K, mTOR, HER2 and ER-directed therapeutics.

Materials and Methods: The anti-proliferative activity of BKM120 (pan-PI3K inhibitor), BYL719 (p110α-specific) and everolimus were assessed as single agents or in combination with trastuzumab and/or tamoxifen in a panel of six HER2+/ER+ versus six HER2+/ER- breast cancer cell lines in two-dimensional culture. Drug response IC50s were generated from actual cell counts as measured by Z2-particle counting. Biomarker analyses were conducted using baseline mRNA microarray (Agilent) and reverse phase protein array (RPPA) profiling of each of the cell lines to determine associations with response or resistance data.

Results: RPPA analysis confirmed the presence of higher levels of ER protein in the cell lines designated as ER+ and significantly higher levels of PTEN protein were detected in those cell lines. Interestingly, each of the PI3K/mTOR pathway inhibitors tended to have increased single agent activity in the ER+ relative to the ER- lines. Combined activity with trastuzumab and either BKM120 or BYL719 was observed in 6 of the 12 cell lines tested and occurred independent of ER status. Similarly, combined activity of everolimus and trastuzumab was also observed in both HER2+/ER+ and HER2+/ER- cell lines. In 3/6 HER2+/ER+ cell lines the addition of tamoxifen provided no benefit to the combination of trastuzumab and PI3K/mTOR pathway inhibitor, whereas in two cell lines mild antagonism was observed with the triple combination. Finally, one cell line did show significant potentiation from the addition of endocrine therapy on top of HER2/PI3K/mTOR targeting.

Discussion: These data confirm levels of estrogen receptor are likely playing a role in response to single agent PI3K/mTOR pathway inhibition and highlight the potential utility of combining endocrine therapy with HER2/PI3K/mTOR-directed therapeutics in a sub-group of HER2-amplified breast cancers. Further in depth biomarker analyses may reveal additional molecular alterations responsible for this differential sensitivity to the double and triple combinations and is underway.
Phase I expansion of S-222611, a reversible inhibitor of EGFR and HER2, in advanced solid tumors, including HER2-positive breast cancer patients with brain metastases

Baird RD D, Arkenau H-T, Deva S, Cresti N, Garcia-Corbacho J, Hogarth L, Frenkel E, Kawaguchi K, Arimura A, Donaldson K, Posner J, Sarker D, Jodrell D, Plummer R, Spicer J and Italiano A. University of Cambridge, Cambridge, United Kingdom; Sarah Cannon Research, London, UK, London, United Kingdom; King's College London, Guy's Hospital, London, UK, London, United Kingdom; Northern Centre for Cancer Care, Newcastle Upon Tyne, UK, Newcastle, United Kingdom; University of Texas Southwestern Medical Center, Dallas, TX, USA, Dallas, TX; Shionogi & Co. Ltd., Osaka, Japan, Osaka, Japan and Institut Bergonie, Bordeaux, France, Bordeaux, France.

**Body:** BACKGROUND

S-222611 is an oral, reversible ErbB tyrosine kinase inhibitor of EGFR and HER2 with potent pre-clinical activity. MTD was not reached during the dose-escalation phase, (maximum dose 1600 mg QD). PK and efficacy data supported a daily dose of 800 mg. An expansion cohort of patients has been treated to further explore safety and efficacy.

**METHODS**

Subjects with advanced solid tumors expressing EGFR and/or overexpressing HER2 were enrolled. S-222611 800 mg daily was administered until disease progression or unacceptable toxicity.

**RESULTS**

76 patients were included in this phase 1 expansion cohort with a variety of tumor types. Dose reduction was required because of adverse events in 15 patients; the most frequent of which being diarrhea and elevated bilirubin. Two patients discontinued treatment due to drug-related adverse events. Of the 25 patients with HER2-positive metastatic breast cancer (MBC), 4 partial responses were observed, and prolonged stable disease (≥ 6 months) was observed in 3 additional patients. These 25 patients had received prior HER2-directed therapy as shown in Table 1.

Table 1. Prior therapies received by patients with HER2-positive MBC

<table>
<thead>
<tr>
<th>Prior therapy</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>22 (88)</td>
</tr>
<tr>
<td>T-DM1</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>16 (64)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>23 (92)</td>
</tr>
</tbody>
</table>

Six of these patients had brain metastases, in whom 1 intracranial response and 2 prolonged stable disease (≥ 6 months) were observed (Table 2).

Table 2. HER2-positive MBC patients with brain metastases - best overall response to S-222611

<table>
<thead>
<tr>
<th>Pts #</th>
<th>HER2 IHC</th>
<th>Brain metastases</th>
<th>Best overall response (RECIST 1.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>3+</td>
<td>Target lesion</td>
<td>PR</td>
</tr>
<tr>
<td>Patient 2</td>
<td>3+</td>
<td>Target lesion</td>
<td>SD (≥12 M)</td>
</tr>
<tr>
<td>Patient 3</td>
<td>3+</td>
<td>Target lesion</td>
<td>SD (6.0 M)</td>
</tr>
<tr>
<td>Patient 4</td>
<td>3+</td>
<td>Non-target lesion</td>
<td>SD (4.7 M)</td>
</tr>
<tr>
<td>Patient 5</td>
<td>3+</td>
<td>Non-target lesion</td>
<td>SD (3.3 M)</td>
</tr>
<tr>
<td>Patient 6</td>
<td>3+</td>
<td>Non-target lesion</td>
<td>NE</td>
</tr>
</tbody>
</table>
The patient showing intracranial response was previously treated with lapatinib and capecitabine after diagnosis of BM.

CONCLUSIONS

S-222611 was well tolerated at a dose of 800 mg once daily. Anti-tumour activity, including shrinkage of brain metastases, was evident in a heavily pre-treated population of patients with HER2-positive breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P4-14-27

Title: Effect of docetaxel duration on clinical outcomes: Results from the phase III trial CLEOPATRA

Miles D, Fung A, Yoo B, Knott A, Heeson S, Portera C and Swain S. Mount Vernon Cancer Center, Northwood, United Kingdom; Genentech, Inc., South San Francisco, CA; Roche Products Ltd., Welwyn Garden City, United Kingdom and Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC.

Body: Introduction:
In the CLEOPATRA study, pertuzumab (P) plus trastuzumab (T) and docetaxel (D) significantly improved median progression-free survival (PFS) and overall survival (OS) compared with placebo (Pla) plus T and D in pts with HER2-positive metastatic breast cancer. Study treatment was given every 3 weeks until disease progression (PD) or unacceptable toxicity. D starting dose was 75 mg/m2 and could be escalated or reduced per protocol. A minimum of 6 cycles of D was recommended. If D was discontinued, patients could continue P+T or Pla+T. To evaluate the potential association between D duration and clinical outcomes, we conducted post hoc exploratory analyses of the CLEOPATRA study.

Methods:
As of 11 Feb 2014 data cutoff, the safety population analyzed included 804 pts (396 Pla+T+D; 408 P+T+D) who received at least one dose of any study medication. Exposure and clinical outcomes of study treatment groups are presented by dichotomized subgroup of pts who received <6 cycles of D and those who received more than 6 cycles. Median PFS and OS were estimated using Kaplan-Meier methods. Cox regression analyses were used to estimate hazard ratios (HRs).

Results:
The median number of D cycles received was 8 for both arms. Forty-one % of pts received ≤6 (14% <6; 27% exactly 6) and 59% received >6 cycles.

Docetaxel Duration and Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
<th>D ≤6 cycles (n=329, 41%)</th>
<th>D &gt;6 cycles (n=475, 59%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pla+T+D (n=159)a</td>
<td>P+T+D (n=170)a</td>
</tr>
<tr>
<td></td>
<td>Pla+T+D (n=237)</td>
<td>P+T+D (n=238)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Exposure</th>
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</tr>
</thead>
<tbody>
<tr>
<td>#D Cycle, median (range)</td>
<td>6 (1,6)</td>
</tr>
<tr>
<td># Study Treatment Cycle, median (range)</td>
<td>7 (1,78)</td>
</tr>
<tr>
<td>Duration of Study Treatment in month, median (range)</td>
<td>6 (1,54)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Outcomes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS in month, median (range)</td>
<td>8.2 (6.2,9.0)</td>
</tr>
<tr>
<td>95% CI</td>
<td>HR=0.59 (0.44,0.79)</td>
</tr>
<tr>
<td>OS in month, median (range)</td>
<td>29.8 (22.2,39.2)</td>
</tr>
<tr>
<td>95% CI</td>
<td>HR=0.67 (0.45,0.90)</td>
</tr>
</tbody>
</table>

N/A=not available  aTwo pts in each group had missing D cycle
<table>
<thead>
<tr>
<th>Reason (n,%)</th>
<th>90 (57)</th>
<th>118 (69)</th>
<th>180 (76)</th>
<th>196 (82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE/intercurrent illness</td>
<td>34 (38)</td>
<td>27 (23)</td>
<td>60 (33)</td>
<td>72 (37)</td>
</tr>
<tr>
<td>Patient reason&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 (3)</td>
<td>5 (4)</td>
<td>5 (3)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Standard practice</td>
<td>36 (40)</td>
<td>59 (50)</td>
<td>65 (36)</td>
<td>41 (21)</td>
</tr>
<tr>
<td>Adequate therapy</td>
<td>12 (13)</td>
<td>22 (19)</td>
<td>36 (20)</td>
<td>59 (30)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>9 (5)</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Missing</td>
<td>4 (4)</td>
<td>4 (3)</td>
<td>5 (3)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Discontinuation of all study treatment (T+D+Pla or P), n</td>
<td>159</td>
<td>170</td>
<td>237</td>
<td>238</td>
</tr>
<tr>
<td>Reason (n,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE/intercurrent illness</td>
<td>15 (9)</td>
<td>22 (13)</td>
<td>25 (11)</td>
<td>32 (13)</td>
</tr>
<tr>
<td>Death</td>
<td>10 (6)</td>
<td>5 (3)</td>
<td>4 (2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Disease progression</td>
<td>111 (70)</td>
<td>110 (65)</td>
<td>182 (77)</td>
<td>154 (65)</td>
</tr>
<tr>
<td>Patient reason&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 (11)</td>
<td>16 (9)</td>
<td>12 (5)</td>
<td>14 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (4)</td>
<td>16 (9)</td>
<td>12 (5)</td>
<td>35 (15)</td>
</tr>
<tr>
<td>Missing</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

<sup>b</sup> Included failure to return, refused treatment, withdrawal, protocol violation

Conclusions:
Consistent with the overall study results, addition of P to T+D showed significant improvement in clinical outcomes regardless of whether ≤6 or >6 cycles of D were received. In the poster, three subgroup (<6, 6 and >6 D cycles) analyses and time-dependent Cox regression analysis to capture the dynamic variations in D exposure will be presented.
Title: XMT-1522 induces tumor regressions in pre-clinical models representing HER2-positive and HER2 low-expressing breast cancer


Body: XMT-1522 is an anti-HER2 antibody-drug conjugate (ADC) comprised of a novel anti-HER2 antibody (HT-19) and the Dolaflexin ADC platform, which allows for conjugation of 12-15 proprietary auristatin drug payload molecules per antibody without aggregation or detrimental impact on pharmacokinetics. The HT-19 antibody binds to a novel HER2 extracellular domain epitope and does not compete for HER2 binding with trastuzumab or pertuzumab. In vitro, XMT-1522 has sub-nanomolar potency in cell lines expressing as few as 25,000 HER2 antigens per cell, and is ∼100X more potent than ado-trastuzumab emtansine (T-DM1) across a panel of 25 cell lines representing a range of tumor indications and HER2 expression levels. In the BT-474 HER2-positive breast cancer model, treatment with the HT-19 antibody alone at a single 5 mg/kg dose is inactive, while XMT-1522 ADC at a single 2 mg/kg or 5 mg/kg dose induces durable complete tumor regression, indicating that the primary mechanism of XMT-1522 is cytotoxic payload delivery, not inhibition of HER2 signaling. T-DM1 at a single 5 mg/kg dose in the same model is inactive, consistent with the significant improvement in potency of XMT-1522 compared to T-DM1. Biodistribution studies using a HER2-targeted Dolaflexin ADC in the BT-474 model demonstrate accumulation of intact ADC and released active drug payload in tumor at levels significantly higher than normal tissues. In a HER2-positive patient-derived xenograft (PDX) model, XMT-1522 induces durable complete tumor regression after a single 1 mg/kg dose, while T-DM1 at a 10 mg/kg dose achieves tumor growth delay without regression. In a HER2 1+ PDX model without HER2 gene amplification, a single 3 mg/kg dose of XMT-1522 achieves partial tumor regression where a 10 mg/kg dose of T-DM1 is inactive. Combination of XMT-1522 with trastuzumab does not block the ability of the ADC to bind HER2 or efficiently internalize, and in vivo the combination of low dose XMT-1522 with full dose trastuzumab and pertuzumab is synergistic in a HER2-driven model. The exposure achieved with XMT-1522 at well-tolerated doses in cynomolgus monkey is several fold higher than the exposure needed in mice to achieve complete tumor regressions across models representing a range of HER2 expression and tumor indications. Based on these data, XMT-1522 will soon enter clinical testing in breast cancer patients with both HER2-positive tumors and HER2 low-expressing (IHC 1+ and 2+/FISH-) tumors.
Title: One-third of HER2 positive patients have 80-gene luminal subtype that is resistant to chemo-trastuzumab but sensitive to chemo-trastuzumab-pertuzumab: Critical implications for the adjuvant setting from the NBRST phase 4 neoadjuvant study

Beitsch P, Whitworth P, Baron P, Beatty J, Pellicane JV V, Murray MK K, Dul C, Mislowsky AM M, Nash CH H, Richards PD D, Lee LA A, Stork-Sloots L, de Snoo F, Untch S, Gittleman M, Akbari S and Rotkis MC C. Dallas Surgical Group, Dallas, TX; Nashville Breast Center; Breast & Melanoma Specialists of Charleston; The Breast Place, Charleston; Virginia Breast Center, Bon Secours Cancer Institute; Akron General Hospital; St. John Region; Coastal Carolina Breast Center; Northeast Georgia Medical Center; Blue Ridge Cancer Care; Comprehensive Cancer Center; Agendia Inc; Breast Care Specialists; Virginia Hospital Center and Northern Indiana Cancer Research Consortium.

Body: Background
The phase 4 Neo-adjuvant Breast Registry Symphony Trial (NBRST) enrolled over 1,000 US patients between June 2011 and December 2014. The aim of NBRST study is to compare chemo-sensitivity as defined by pathological Complete Response (pCR) using the 80-gene BluePrint (BP) functional subtype profile vs. conventional IHC/FISH subtyping. Treatment was at the discretion of the physician utilizing standard NCCN regimens. Pertuzumab, a monoclonal antibody, inhibits the dimerization of HER2 with other HER receptors. Pertuzumab received US FDA approval for the neo-adjuvant treatment of HER2-positive breast cancer in September 2013. Essentially all patients with HER2 positive cancers were treated with chemotherapy + trastuzumab and after this date pertuzumab was added, creating 2 distinct groups of Her2-treated patients.

The aim of the current analysis is to compare the pCR rate of chemo-trastuzumab (c-t) vs chemo-trastuzumab plus pertuzumab (c-t-p) by conventional and 80-gene BP functional subtype. 80-gene BP functional subtype was derived by supervised cluster analysis for concordant mRNA and protein expression.

Methods
The current analysis includes women from the NBRST study, with histologically proven breast cancer, who received neo-adjuvant treatment, had 80-gene subtyping and provided written informed consent. Pathological assessment of HER2 was performed according to ASCO CAP guidelines at the time of diagnosis. 80-gene BluePrint (BP) classifies patients into Luminal, HER2 or Basal-type. pCR is defined as T0/isN0. All pCRs were verified with a de-identified copy of the surgical pathology report. Fisher's exact test was used to compare pCR rates within different subgroups.

Results
286 IHC/FISH HER2+ patients received c-t (175) or c-t-p (111). Of these 80-gene BP subtype classified 53% as HER2-type, 33% as Luminal-type and 14% as Basal-type. 64% were ER positive.

The pCR rates and p-values within different subgroups of clinical HER2+ patients are provided in the table below.

<table>
<thead>
<tr>
<th></th>
<th>c-t</th>
<th>c-t-p</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=286)</td>
<td>41%</td>
<td>57%</td>
<td>0.01</td>
</tr>
<tr>
<td>BP HER2 (153)</td>
<td>58%</td>
<td>73%</td>
<td>0.06</td>
</tr>
<tr>
<td>BP Luminal (93)</td>
<td>6%</td>
<td>39%</td>
<td>0.0002</td>
</tr>
<tr>
<td>BP Basal (40)</td>
<td>45%</td>
<td>45%</td>
<td>1.0</td>
</tr>
<tr>
<td>IHC/FISH HER2+/ER+ (183)</td>
<td>31%</td>
<td>53%</td>
<td>0.003</td>
</tr>
<tr>
<td>IHC/FISH HER2+/ER- (103)</td>
<td>59%</td>
<td>64%</td>
<td>0.68</td>
</tr>
</tbody>
</table>
Conclusions
One-third of ASCO/CAP Her2+ patients had 80-gene BP Luminal subtype and demonstrated resistance to c-t (pCR 6%). Addition of Pertuzumab overcame resistance in this group (pCR 39%). This finding in the neoadjuvant setting suggests a substantial potential benefit in the adjuvant setting and thus an urgent need to consider treatment in at-risk patients as well as confirmatory tissue analysis from independently reported trials.
Title: Whole exome sequencing of pre-treatment biopsies from the neoALTTO trial to identify DNA aberrations associated with response to HER2-targeted therapies

Pusztai L, Shi W, Jiang T, Nuciforo P, Holmes E, Harbeck N, Sotiriou C, Rimm D, Hatzis C, de la Peña L, Armour A, Piccart-Gebhart M and Baselga J. Yale University, New Haven, CT; Vall d’Hebron Institute of Oncology, Barcelona, Spain; Frontier Science (Scotland) Ltd; University of Munich; Jules Bordet Institute; Memorial Sloan Kettering Cancer Center; SOLTI Clinical Trial Group and Novartis.

Body: Background: We examined if alterations in nucleic acid variants, genes, pathways, and overall mutational load and clonal entropy are associated with pathologic complete response (pCR) and survival after neoadjuvant anti-HER2 therapies in the NeoALTTO trial.

Methods: Whole exome sequencing was performed of 203 baseline biopsies with outcome information. The mean nucleotide coverage was 150x with >90% of target bases showing > 30x coverage in > 99% of samples. Somatic mutations were called by MuTect and indels by Strelka, using pooled reference normal DNA. Significantly mutated genes (FDR<10%) were identified by MutSigCV. Mutations in 714 canonical biological pathways were assessed and mutational load and genome clonal entropy (MATH) were calculated. Association with pCR and survival were evaluated by logistic regression adjusted for ER status and Cox-proportional hazards regression.

Results: Only 12 genes had mutation rates significantly above background and among these only PI3KCA was associated with lower pCR rate (OR=0.42, p=0.019). Genes with somatic mutations in more than 10 patients were also assessed, but none were associated with pCR or survival. Clonal entropy or adjusted mutation load also did not correlate with response. Mutations in 33 pathways showed significant association with response in the entire cohort. In the trastuzumab arm, 23 of the 33 pathways showed an association with response but none was independent of PIK3CA mutation. We constructed “PIK3CA-gene network” that included all unique genes (n=439) from these 23 pathways. Of the 66 patients in the trastuzumab arm, 50 carried at least one mutation in one of the 439 genes and among these only 2 achieved pCR (4%) compared to 9 of 16 pCR (56%) among the wild type (OR=0.035; p < 0.001). The same genes/mutations had little impact on pCR in the lapatinib arm (pCR 20%). In the lapatinib arm, mutations in 3 pathways conferred higher probability of pCR. The “Regulation of RhoA activity” pathway, had the most significant association with pCR in the entire cohort (OR=3.77, p=0.0009) and in the lapatinib (pCR 67% vs 17%, OR=14.8, p=0.008) and lapatinib + trastuzumab (OR=3.0, p=0.06) arms, but not in the trastuzumab arm (OR=1.4, p=0.7). Event free and overall survival were also significantly higher in patients who had mutations in this pathway. Twenty seven of the 48 genes in this pathway had mutations affecting 33 patients but different genes were affected in different individuals.

Conclusions: There are no high frequency recurrent single mutations associated with response to HER2-targeted therapies, other than PIK3CA. We identified several biological pathways, including RhoA activity, and a network of PIK3CA associated genes that are significantly associated with response when affected by mutations, however, different genes are mutated in different individuals.
**Body:** Background: Up to 26% of patients with early-stage HER2+ breast cancer develop recurrences within 5 to 8 years, despite receiving trastuzumab-based adjuvant therapy. Neratinib (Puma Biotechnology Inc), an irreversible pan-HER tyrosine kinase inhibitor, is currently being investigated in a large phase 3 randomized, placebo-controlled, double-blind trial (ExteNET) to determine if it can further reduce the risk of recurrence in women with early stage HER2+ breast cancer after trastuzumab-based adjuvant therapy (clinicaltrials.gov NCT00878709). The positive findings from the primary efficacy analysis of this study were reported recently [Chan et al. ASCO 2015]. We report an updated analysis of efficacy from ExteNET after 3 years of follow-up.

**Methods:** Women with HER2+ breast cancer were randomly assigned to oral neratinib 240 mg/day or matching placebo for 1 year, stratified by nodal status, hormone-receptor status and prior trastuzumab regimen. The study was initiated in April 2009. In October 2011, recruitment was stopped and follow-up was limited to 2 years. In January 2014, follow-up was restored to 5 years. During years 1 and 2, physical examinations were performed at 0, 1, 3, 6, 9 and 12 months (year 1) and then every 4 months (year 2). Mammograms were performed annually. After completing 2 years, patients were asked to re-consent to an additional 3 years of follow-up. During years 3 to 5, disease-free survival (DFS) and survival events were identified from medical records. The primary study endpoint is invasive DFS (iDFS). Secondary efficacy endpoints are DFS including ductal carcinoma in situ (DFS-DCIS), time to distant recurrence (TDR), distant disease-free survival (DDFS), cumulative incidence of central nervous system (CNS) recurrences and overall survival. The primary efficacy analysis was performed after 2 years of follow-up in July 2014. A further descriptive analysis of DFS, to investigate the durability of treatment effect, will be performed after 3 years of follow-up. Data for overall survival will remain blinded until 248 events have occurred. Hazard ratios (HR) with 95% confidence intervals (CI) were estimated using stratified Cox proportional-hazards models.

**Results:** The intention-to-treat population included 2840 patients (neratinib, N=1420; placebo, N=1420). Two-year rates for iDFS were 93.9% with neratinib and 91.6% with placebo (HR 0.67, 95% CI 0.50–0.91; P=0.009). Two-year rates for DFS-DCIS were 93.9% with neratinib and 91.0% with placebo (HR 0.63, 95% CI 0.46–0.84; P=0.002). Two-year data for other secondary endpoints were supportive of the primary analysis (TDR: HR 0.71, 95% CI 0.50–1.00; P=0.05; DDFS: HR 0.75, 95% CI 0.53–1.04; P=0.09). Overall survival data are not yet mature.

**Conclusions:** After 2 years, there was a significant iDFS benefit with a 1-year course of neratinib versus placebo in women with early-stage HER2+ breast cancer after trastuzumab-based adjuvant therapy. Data for the 3-year analysis are expected to be available in October/November 2015 and will be presented at the meeting.
Body: Background: In HER2+ early breast cancer (eBC) pCR rates after standard neoadjuvant chemo- + anti-HER2 therapy differ according to hormone-receptor (HR) status. Molecular analysis reveals HER2+/HR+ BC as a distinct entity within HER2+ BC. The ADAPT HER2+/HR+ phase II trial aims to identify early responders to endocrine + anti-HER2 therapy.

Methods: The trial completed recruitment in January 2015 (n=376). Patients (pts.) were randomized to 12 weeks of neoadjuvant therapy: A:T-DM1 (3.6 mg/kg q3w) vs. B:T-DM1 with endocrine therapy (ET) (pre-: tamoxifen; postmenopausal: aromatase inhibitor) vs C:trastuzumab q3w+ET. After surgery, standard chemotherapy at investigators’ discretion and completion of 1y trastuzumab were recommended. Trial tests pCR (yPN0 and ypT0/is) in after T-DM1 or T-DM1+ET compared to T+ET. Biomarkers are measured at baseline and after 3 weeks.

Results: Pre-planned interim analysis (n=130) aimed to identify an early-response biomarker (e.g. Ki-67 drop) and to validate trial assumptions. Median age was 49 years; 55% were pre-menopausal; 40% had cT1 tumors, 51% cT2; 68% had cN0, 27% cN1; 75% had G3. Median baseline Ki67 was 30%. In all arms, more than 95% received all 4 therapy cycles. 16 SAEs were reported in 13 pts (A:7; B:6; C:3); all CTC grade 1 (1), 2 (11) or 3 (4); all pts completely recovered without sequelae. Overall pCR rate was 30.8%: T-DM1: 40.5%, T-DM1+ET: 45.8%, T+ET: 6.7%. The difference between either arm T-DM1 arm vs. T+ET was significant (p<0.001). Exploratory analysis suggests benefit of adding ET to T DM1 in pre- (pCR: 27.3% for T-DM1 single agent vs. 45.5% with ET) but not in postmenopausal pts (pCR: 60% vs. 46.2%). Substantial early therapy response did not permit Ki-67 quantification in the 3-week biopsy in 43.1% due to low cellularity (<500 tumor cells). PIK3CA mutation analysis (n=114) revealed a mutation rate of 15.8% (n=18). Overall pCR rate was 35.4% (n=96) for wildtype and 17.6% (n=18) for tumors with PIK3CA mutation. Ongoing biomarker analyses include further mutation analysis and intrinsic subtypes in the total trial collective.

Conclusions: The WSG-ADAPT HER2+/HR+ phase II trial is internationally the first large prospective randomized phase II trial specifically conducted within this distinct subtype. Interim analysis demonstrated for the first time clinically meaningful pCR rates (>40%) after short therapy (12 weeks) of T-DM1 ± ET without systemic chemotherapy in HER2+/HR+ eBC. Final efficacy and safety data will be presented at the meeting together with results of the correlative science program.
Title: Ten year follow-up of the BCIRG-006 trial comparing doxorubicin plus cyclophosphamide followed by docetaxel (AC®T) with doxorubicin plus cyclophosphamide followed by docetaxel and trastuzumab (AC®TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2+ early breast cancer patients

Slamon DJ, Eiermann W, Robert NJ, Giermek J, Martin M, Jasiowka M, Mackey JR, Chan A, Liu M-C, Pinter T, Valero V, Falkson C, Fornander T, Shiftan TA, Bensfia S, Hitier S, Xu N, Bée-Munteanu V, Drevot P, Press MF, and Crown J. UCLA, Los Angeles, CA; GBG, Munchen, Germany; USO, The Woodlands, TX; Maria Sklodowska-Curie Centre, Warsaw, Poland; GEICAM, Madrid, Spain; Maria Sklodowska-Curie Memorial Cancer Institute, Krakow, Poland; Cross Cancer Institute, Edmonton, Canada; Breast Cancer Research Centre - WA & Curtin University, Perth, Australia; Sun Yat-Sen Cancer Center, Taipei, Taiwan; Petz Aladar Megyei Oktato Korhaz Onkoradiologica, Gyor, Hungary; The University of Texas MD Anderson Cancer Centre, Houston, TX; University of Alabama, Birmingham, AL; Karolinska University Hospital, Stockholm, Sweden; Sharp Memorial Hospital, San Diego, CA; Sanofi, Cambridge; Sanofi, Chilly-Mazarin, France; Genentech, South San Francisco, CA; TRIO, Paris, France; USC/Norris Comprehensive Cancer Center, Los Angeles, CA and ICORG, Dublin, Ireland.

Body: Background: The intent of the BCIRG-006 study was to assess the relative benefit and safety of two trastuzumab-based regimens compared to a then standard (non-trastuzumab) regimen in the adjuvant treatment of early HER2+ breast cancer. We present the final evaluation of the long-term results from this trial that was designed to determine how to maximize both efficacy and safety.

Material and Methods: Between April, 2001 and March, 2004, we randomized 3222 HER2+ breast cancer patients with axillary lymph node-positive or high risk node-negative disease, to either standard AC (60/600 mg/m2 q3wk x 4) followed by T (100 mg/m2 q3wk x 4) (AC-T) or two trastuzumab-containing regimens. The trastuzumab-based regimens were AC followed by T with trastuzumab (H) x 1 year (AC-TH), or TCarbo (75 mg/m2 / AUC6 q3wk x 6) with H x 1 year (TCH). Patients were prospectively stratified by number of positive nodes (0, 1-3 vs 4+) and hormone receptor status. Patients with ER and/or PR positive (HR+) disease received hormone-directed therapy for 5 yrs post chemotherapy. The primary endpoint was disease-free survival (DFS). Secondary endpoints included overall survival (OS) and safety, with extensive cardiac evaluation (symptomatic events and asymptomatic LVEF declines).

Results: Baseline characteristics of all arms of the study population were well balanced. Of the 3222 patients (1073 in AC-T, 1074 in AC-TH and 1075 in TCH) initially randomized, we have efficacy and safety data with a median follow-up of 10.3 years. Of the original 3222 patients, 511 deaths occurred prior to study end while 1817 patients reached the 10 years follow-up point. During the study period, a total of 508 patients were lost to follow-up, 162 withdrew consent prior to 10 years and an additional 224 patients had missing data prior to 10 years. We will present the DFS benefit of the trastuzumab containing arms (AC-TH and TCH) compared to AC-T on this study population. We will also present cumulative data on the incidence of protocol-defined, symptomatic or significant asymptomatic cardiac events in each of the study arms and compare them to the 3- and 5-year study follow-up results.

Discussion: The BCIRG-006 10-year follow-up final results will contain an assessment of the relative long-term benefit and safety of adding trastuzumab to the adjuvant treatment of HER2+ early breast cancer, as well as the efficacy/safety of anthracycline versus non-anthracycline based regimens when combined with adjuvant trastuzumab.
2015 San Antonio Breast Cancer Symposium

Publication Number: S5-05

**Title:** Trastuzumab emtansine improves overall survival versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer: Final overall survival results from the phase 3 TH3RESA study

Wildiers H, Kim S-B, Gonzalez-Martin A, LoRusso PM M, Ferrero J-M, Yu R, Smitt M and Krop I. University Hospitals Leuven, Leuven, Belgium; Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; Centro Oncológico MD Anderson International España, Madrid, Spain; Yale Cancer Center, Yale University Medical Center, New Haven, CT; Centre Antoine Lacassagne, Nice, France; Genentech, Inc, South San Francisco, CA and Dana-Farber Cancer Institute, Boston, MA.

WITHELD PENDING PRESS CONFERENCE
Title: BRCA mutations, therapy response and prognosis in the neoadjuvant GeparQuinto study

Fasching PA A, Loibl S, Eidtmann H, Tesch H, Untch M, Hilfrich J, Schem C, Rezai M, Gerber B, Costa SD, Blohmer JU U, Fehm TN N, Huober J, Liedtke C, Müller V, Nekljudova V, Weber K, Rack B, Rübner M, Wang L, Ingle JN N, Weinshilboum RM M, von Minckwitz G and Couch F. University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany; GBG Forschungs GmbH, Neu Isenburg, Germany; Christian-Albrechts-University, Kiel, Germany; CHOP Onkologie Bethanien, Frankfurt, Germany; Eilenriede-Klinik, Hannover, Germany; Breast Center of Düsseldorf, Lülsenkranckenhau, Düsseldorf, Germany; University of Rostock, Rostock, Germany; Magdeburg University Hospital, Magdeburg, Germany; University of Ulm, Ulm, Germany; Heinrich-Heine-University, Düsseldorf, Germany; Charite, Berlin, Germany; Lübeck University Hospital, Lübeck, Germany; Hamburg University Hospital, Hamburg, Germany; Ludwig Maximilian University, Munich, Germany; Division of Clinical Pharmacology, Mayo Clinic College of Medicine, Mayo Medical School–Mayo Foundation, Rochester, MN; Mayo Clinic Rochester, Rochester, MN and Division of Experimental Pathology, Mayo Clinic, Rochester, MN.

Body: Background: Mutations in BRCA1 and BRCA2 influence the molecular pathogenesis of breast cancer. However, little is known about the association between mutations, response to therapy, and prognosis. We therefore analysed these associations in triple negative breast cancer (TNBC) patients in the neoadjuvant GeparQuinto Study.

Methods: The GeparQuinto study recruited 1956 patients with HER2 negative disease including a prespecified group of 671 TNBC patients with untreated breast cancer. Patients were randomized to receive four cycles EC (90/600 mg/m2; q3w) followed by four cycles docetaxel (100 mg/m2; q3w), with or without bevacizumab (15 mg/kg). Here we present the analysis for both randomization arms combined. Sufficient blood was available of 482 TNBC patients. BRCA1 and BRCA2 genotyping was successful in 469 patients with available germline DNA and was conducted by SureSelect custom capture and sequencing on HiSeq 2500. Mutation status was correlated with pathological complete response rates (pCR) and disease free survival (DFS).

Results: A total of 74 (15.8%) mutations in BRCA1 (n=61) and BRCA2 (n=13) were detected after initial sequencing. A pCR (ypT0/ypN0) was observed in 50% (n=37) of mutation carriers but only 31.1% (n=123) of patients without mutations (p=0.002). Similar results were observed for pCR (pT0is/pN0) (52.7% vs. 36.5%; p=0.010). pCR (ypT0/ypN0) was predictive of disease free survival (DFS) in all patients (Hazard Ratio, HR=0.23; 95%CI: 0.15-0.37; p<0.001) and in patients without BRCA mutations pCR (ypT0/ypN0) (HR= 0.20; 95%CI: 0.11-0.34; p<0.001). However in mutation carriers this effect was reduced (HR=0.48; 95%CI: 0.18 1.27; p=0.129).

Conclusion: TNBC Patients with BRCA mutations showed a higher frequency of pCR than patients without BRCA mutations after treatment with epirubicin, cyclophosphamide and docetaxel (+/ bevacizumab), suggesting that BRCA mutations may influence pCR in response to treatment regimens that do not include platin chemotherapy. pCR as a surrogate marker for DFS was also confirmed in patients without BRCA mutations. In addition, the effect of pCR on prognosis seemed to be smaller among the mutation carriers, although the number of mutation carriers was too low to test for differences between mutation carriers and wildtype patients.

The project has partly been funded within NIH-NIGMS U19 GM61388 and the Breast Cancer Research Foundation.
Preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1–positive, estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028

Rugo HS S, Delord J-P, Im S-A, Ott PA A, Piha-Paul SA A, Bedard PL L, Sachdev J, Le Tourneau C, van Brummelen E, Varga A, Saraf S, Pietrangelo D, Karantza V and Tan A.  UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Institut Universitaire du Cancer, Toulouse, France; Seoul National University Hospital, Seoul, Republic of Korea; Dana-Farber Cancer Institute, Boston, MA; The University of Texas MD Anderson Cancer Center, Houston, TX; Princess Margaret Cancer Centre, Toronto, ON, Canada; Honor Health, Scottsdale, AZ; Institut Curie, Paris, France; Netherlands Cancer Institute, Amsterdam, Netherlands; Gustave Roussy, Villejuif, France; Merck & Co., Inc., Kenilworth, NJ and Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Body: Background: The programmed cell death 1 (PD-1) pathway is used by tumors to evade immune surveillance. Pembrolizumab is a humanized anti–PD-1 monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Pembrolizumab has shown robust antitumor activity against several advanced malignancies, including triple-negative breast cancer. We assessed the safety and efficacy of pembrolizumab in patients with PD-L1–positive, ER+/HER2-negative advanced breast cancer.

Methods: KEYNOTE-028 (ClinicalTrials.gov, NCT02054806) is an ongoing multicohort, open-label phase 1b study evaluating the safety and efficacy of pembrolizumab in patients with PD-L1–positive advanced solid tumors. Key eligibility criteria for this cohort include ER+ and HER2-negative tumor status defined by institutional standards, locally advanced or metastatic disease, ECOG performance status of 0 or 1, failure of or inability to receive standard therapy, and PD-L1 expression in stroma or in ≥1% of tumor cells as assessed at a central laboratory using a prototype immunohistochemistry assay with the 22C3 antibody (Merck). Pembrolizumab was administered at a dose of 10 mg/kg every 2 weeks for up to 24 months or until confirmed progression or intolerable toxicity. Response is based on RECIST v1.1 as assessed by investigator review every 8 weeks for the first 6 months and every 12 weeks thereafter. Primary efficacy end point is overall response rate (ORR).

Results: Of the 248 patients with ER+/HER2-negative breast cancer who had evaluable tumor samples screened for PD-L1 expression, 48 (19%) had PD-L1–positive tumors. Of these, 25 patients were enrolled. Median age was 53 years (range, 36-79), and 44% of patients had an ECOG performance status of 1. Patients were heavily pretreated, with 76% having received ≥3 prior lines of therapy for advanced disease, including 48.0% who received ≥5 prior lines. Analyses of ORR, duration of response, and adverse events are ongoing and will be completed by September 4, 2015.

Conclusion: Data from this KEYNOTE-028 cohort will provide information on the antitumor activity and safety of pembrolizumab in patients with heavily pretreated, PD-L1–positive, ER+/HER2-negative advanced breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: S5-08

Title: Late side-effects of breast cancer radiotherapy: Second cancer incidence and non-breast-cancer mortality among 40,000 women in 75 trials

Taylor C, Correa C, Anderson S, Duane F, Ewertz M, Jagsi R, Pierce L, Swain S, Whelan T, Wang Z, Wang Y, Peto R and McGale P. NDPH Clinical Trial Service Unit, Oxford University, Oxford, United Kingdom; Cancer Centre and Research Institute University of Southern Florida, Tampa, FL; University of Pittsburgh, Pittsburgh, PA; Odense University Hospital, Odense, Denmark; University of Michigan, MI; Washington Cancer Institute MedStar Washington Hospital Center, WA and McMaster University and Juravinski Cancer Centre, Ontario, Canada.

Body: Introduction
Breast cancer radiotherapy cures many women, but, as with other therapies, can cause late side-effects.

Methods
We undertook meta-analyses of individual patient data from the trials of breast cancer radiotherapy, relating various characteristics of the regimens tested to cause-specific mortality rate ratios (RRs) and second cancer incidence RRs. Doses to cardiac structures were calculated for trials with some heart disease death(s), lung doses were calculated for megavoltage trials with some lung cancer(s) in the second decade after radiotherapy, and oesophagus doses were calculated for megavoltage trials with some oesophageal cancer(s). Trial radiotherapy regimens were reconstructed for a woman with typical anatomy using virtual simulation and 3-dimensional CT planning (and, for a few regimens, manual planning).

Results
Information was available on 40,781 women in 75 evenly randomised comparisons of radiotherapy versus not. Median follow-up was 9.7 years and 20,345 died, 6064 without recurrence. Smoking information for included women was unavailable.

Mean normal tissue radiation doses for irradiated women were: heart 6.3 Gy (range <1-18), ipsilateral lung 17.2 Gy (range 5.8-27.2) and oesophagus 10.5 Gy (range <1-27.3).

Allocation to radiotherapy increased non-breast-cancer mortality (RR=1.15, 95% CI 1.09–1.22, 2p<0.0001), due mainly to heart disease (RR=1.30, 1.15–1.46, 2p<0.0001). The heart disease death rate was strongly related to the estimated heart radiation dose, and increased approximately linearly by 4.1% per Gy (95% CI 2.4–6.2, 2p<0.00001).

Second cancer incidence was increased (RR=1.23, 1.12–1.36, 2p<0.0001). The site with most events was the contralateral breast (RR=1.20, 1.08–1.33, 2p=0.0006 with 881 versus 673 cases). Excluding the first decade, lung cancer incidence was increased (RR=2.10, 1.48–2.98, 2p=0.00003 with 94 versus 40 cases after the first decade). There were also excesses of oesophageal cancer (RR=2.42, 1.19–4.92, 2p=0.01), mainly in trials where the internal mammary chain and supraclavicular fossa were irradiated, and leukaemia (RR=1.71, 1.05–2.79, 2p=0.03).

Conclusions
Since these trials, normal tissue doses from breast cancer radiotherapy have at least halved so the excess relative risks will be at least halved. Background disease rates have also changed, so the absolute risks will be different for women today. Modelling the effects of such changes suggests that for women who have smoked throughout adult life and will continue smoking, even modern radiotherapy may cause an absolute lung cancer risk of a few per cent, making this the main late side-effect in smokers. However, for non-smokers (and ex-smokers) with healthy hearts who would, under current guidelines, be offered radiotherapy, the expected reduction in breast cancer mortality greatly outweighs any increase in other mortality.
Title: PIK3CA status in circulating tumor DNA (ctDNA) predicts efficacy of buparlisib (BUP) plus fulvestrant (FULV) in postmenopausal women with endocrine-resistant HR+/HER2– advanced breast cancer (BC): First results from the randomized, phase III BELLE-2 trial

Baselga J, Im S-A, Iwata H, Clemons M, Ito Y, Awada A, Chia S, Jagiello-Gruszfeld A, Pistilli B, Tseng L-M, Hurvitz S, Masuda N, Cortés J, De Laurentiis M, Arteaga CL L, Jiang Z, Jonat W, Hachemi S, Le Mouhaër S, Di Tomaso E, Urban P, Massacesi C and Campone M. Memorial Sloan Kettering Cancer Center, New York, NY; Seoul National University College of Medicine, Seoul, Republic of Korea; Aichi Cancer Center, Nagoya, Japan; Ottawa Hospital Research Institute, Ottawa, Canada; Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokyo, Japan; Institut Jules Bordet, Brussels, Belgium; BC Cancer Agency, Vancouver, Canada; Maria Skłodowska-Curie Memorial Cancer Centre and Institute of Oncology (MCMCC), Warsaw, Poland; Ospedale di Macerata, Macerata, Italy; Taipei Veterans General Hospital, Taipei, Taiwan; UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA; National Hospital Organization Osaka National Hospital, Osaka, Japan; Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Istituto Nazionale Tumori Fondazione G. Pascale, Naples, Italy; Vanderbilt-Ingram Cancer Center, Nashville, TN; Beijing 307 Hospital of PLA, Beijing, China; University Hospital Schleswig-Holstein, Kiel, Germany; Novartis Pharma S.A.S., Paris, France; Novartis Institutes for BioMedical Research, Cambridge, MA; Novartis Pharma AG, Basel, Switzerland and Institut de Cancérologie de l'Ouest–René Gauducheau Centre de Recherche en Cancérologie, Nantes, France.

WITHELD PENDING PRESS CONFERENCE
Title: Occurrence of natural ESR1 mutations during acquisition of endocrine resistance in breast cancers and widely used ER+ cell lines


Body: Background: There is great interest in the mutational landscape of metastatic breast cancer (BC) for personalised medicine. Knowledge about how this landscape differs in recurrent lesions from primary BC as a result of metastatic selection or the impact of treatment will extend our knowledge of mechanisms of endocrine resistance and determine the manner in which diagnostics and treatment are integrated. A small number of limited series have reported an increased presence of ESR1 mutations in metastatic lesions after endocrine treatments including aromatase inhibitors (AIs) which are the most common agents for ER+ postmenopausal BC.

Aim: To determine the change in mutational profile of 16 genes affected by driver mutations in patients with metastatic BC at the time of progression after an AI.

Methods: We conducted targeted sequencing with a custom AmpliSeq panel in 48 matched pairs of FFPE archival blocks of pre-treatment diagnostic and recurrent lesions. 24 of these were metastatic, 24 local recurrences. The genes were AKT1, BRAF, CDH1, ERBB2, ESR1, GATA3, KIT, KRAS, MAP2K4, MAP3K1, PIK3CA, PIK3R1, PTEN, RUNX, SF3B1 and TP53. Only mutations with high depth (>250x) were retained. Germline mutations were identified by 1000 Genomes Project data. C:G>T:A transitions with low frequency (<10%) were removed as artefacts from formalin cross-linking.

Results: Median coverage was 782-fold. Good quality data was available on 42 pairs. At least one mutation was found in 34 pairs. Three cases had lost IHC expression of ER in the recurrence. A total of 115 mutations in coding regions or splice sites were identified, the largest number in PIK3CA (in 20 samples), CHD1 (18), MAP3K1 (12), TP53 (12) and PTEN (9). In 47 instances the mutation was private to one or other sample. There was no difference in the number of mutations between the presentation and recurrent lesions. Six ESR1 mutations were found, all private to 5 recurrent lesions (all IHC ER+). Four of these mutations have been previously reported (2x E380Q, 2x D538G), one was novel (D484G) and also in the ligand-binding domain. One had an additional previously reported mutation (H524L). Four of these lesions were metastatic but one in a local recurrence suggesting that the metastatic process is more likely, but not essential in selecting the emergence of ESR1 mutations. HER2 mutations were identified in 3 patients; in two cases private in the presentation tumour while in the other case the mutation in the recurrent lesion (L755S) differed from that in the primary. L755S has been reported to be sensitive to neratinib in experimental systems. CDH1 (e-cadherin) mutations were numerically higher than reported in other series. The mutations were across the gene and private in 4 pretreatment and 2 recurrent lesions (6 had identical mutations). They were not associated with lobular phenotype.

Conclusions: These data confirm that recurrences after AI but not primary ER+ tumours often contain ESR1 mutations that could influence clinical decision making. The high number of CDH1 mutations at diagnosis may be at least in part because of the selection of recurrent ER+ cases. The data stress the individuality of mutational profiles in recurrent BC and the need for individual interpretation of the data.
2015 San Antonio Breast Cancer Symposium

Publication Number: S6-03

Title: Anastrozole versus tamoxifen for the prevention of loco-regional and contralateral breast cancer in postmenopausal women with locally excised ductal carcinoma in-situ (IBIS-II DCIS)

Cuzick J, Forbes JF, Sestak I, Howell A, Bonanni B, Bundred N, Levy C, von Minckwitz G, Eiermann W, Neven P, Stierer M, Holcombe C, Coleman RE E, Jones LJ J and Ellis I. University of Newcastle, Calvary Mater Hospital, Australia New Zealand Breast Cancer Trials Group Newcastle, Newcastle, Australia; Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University London, London, United Kingdom; Genesis Breast Cancer Prevention Centre, Manchester, United Kingdom; Instituto Europeo di Oncologia, Milan, Italy; South Manchester University Hospital, Manchester, United Kingdom; Centre François Baclesse, Caen, France; German Breast Group, Neu-Ilsenburg, Germany; Interdisciplinary Oncology Center Mnchen, Munich, Germany; UZ Gasthuisberg Ziekenhuis, Leuven, Belgium; Vienna International Health Centre, Vienna, Austria; Royal Liverpool University Hospital, Liverpool, United Kingdom; Weston Park Hospital, Sheffield, United Kingdom; Barts Cancer Institute, John Vane Science Centre, London, United Kingdom and University of Nottingham, Molecular Medical Sciences, Nottingham, United Kingdom.

WITHELD PENDING PRESS CONFERENCE
Title: Patient-reported outcome (PRO) results, NRG Oncology/NSABP B-35: A clinical trial of anastrozole (A) vs tamoxifen (tam) in postmenopausal patients with DCIS undergoing lumpectomy plus radiotherapy

Ganz PA A, Cecchini RS S, Julian TB B, Margolese RG G, Costantino JP P, Vallow LA A, Albain KS S, Whitworth PW W, Cianfrocca ME E, Brufsky A, Gross HM M, Soori GS S, Hopkins JO O, Fehrenbacher L, Sturtz K, Wozniak TF F, Seay TE E, Mamounas EP P and Wolmark N. NSABP/NRG Oncology; UCLA Schools of Medicine and Public Health and Jonsson Comprehensive Cancer Center; University of Pittsburgh; Allegheny Cancer Center at Allegheny General Hospital; Jewish General Hospital, McGill University; NRG Oncology Statistics and Data Management Center (SDMC); Mayo Clinic; Loyola University Chicago Cardinal Benardin Cancer Center; Nashville Breast Center; Fox Chase and Northwestern (ECOG); Magee-Women's Hospital, University of Pittsburgh; Dayton NCORP; Missouri Valley Cancer Consortium; Novant Health; Kaiser Permanente Northern California; Colorado Cancer Research Program; Christiana Care CCOP; Atlanta Regional CCOP and UF Health Cancer Center at Orlando Health.

WITHELD PENDING PRESS CONFERENCE
Title: A phase II trial of neoadjuvant palbociclib, a cyclin-dependent kinase (CDK) 4/6 inhibitor, in combination with anastrozole for clinical stage 2 or 3 estrogen receptor positive HER2 negative (ER+HER2-) breast cancer (BC)


Body: Background

ER+ BC is associated with activated CDK4/6. The CDK4/6 inhibitor palbociclib (P) markedly improves time to progression in advanced ER+HER2- BC. We conducted a neoadjuvant phase II trial to determine the activity of P in primary breast cancer as a prelude to adjuvant studies.

Methods

To assess molecular changes induced by anastrozole (A) or P+A, patients (pts) were treated initially with A alone (1mg PO daily) for 28 days in cycle 0 (C0) before the addition of P (125mg PO daily on D1-21 each cycle) on C1D1. P+A was administered for 4 28-day cycles followed by C5 with A alone for 2-4 weeks (wks) before surgery. P was added in C5 for 10-12 days immediately prior to surgery in the last 20 pts enrolled to assess molecular changes induced by A, either alone or in combination with P immediately prior to surgery, in resected tumor. Goserelin was added in premenopausal pts.

Research tumor biopsies were obtained at baseline, C1D1, and C1D15. Central Ki67 analysis was performed at all timepoints, those with Ki67 >10% at C1D15 went off study treatment.

The primary endpoint was complete cell cycle arrest (CCA), defined as Ki67 <2.7%, at C1D15. Patient stratification was based on PIK3CA mutation status with an initial focus on PIK3CA wild type (WT) disease. Pts with PIK3CA mutant (Mut) tumors enrolled to a separate cohort. A sample size of 33 pts in the PIK3CA WT cohort was chosen based on the Fleming's single-stage phase II design to test the hypothesis that P+A leads to > 50% improvement over A in CCA rate on C1D15 biopsy (44% with A alone based on historical data, vs 66% with P+A, power = 0.8, alpha=0.05). The primary endpoint is met if >20 pts achieved CCA in this cohort.

Correlative endpoints included assessment of markers of proliferation, apoptosis, senescence, Rb, gene expression microarray, intrinsic subtype, and next generation sequencing of 83-gene panels, which will be reported at the meeting.

Results

Between 4/23/2013 and 4/24/2015, 50 pts (33 PIK3CA WT, 11 PIK3CA Mut, 2 pending, 4 tissue quantity or quality not sufficient for sequencing (QNS)) were enrolled to the study. Median age was 57.5 (range: 34.1–79.6) years. Four pts, all with WT PIK3CA, went off study due to Ki67 >10% on C1D15 biopsy, 26 pts completed treatment and surgery, 1 refused surgery, 3 withdrew study treatment in C1, and 16 continued to receive study drug (2 in C0, 3 in C1, 4 in C2, 5 in C3, 1 in C4, and 1 in C5). Among the 40 pts currently evaluable for the primary endpoint (C1D15 Ki67), CCA occurred in 34 (85%) pts, including 9 of 9 (100%) PIK3CA Mut, 22 of 28 (78.5%) WT, and 3 of 3 QNS pts. Preliminary analysis of available data indicated a significantly lower Ki67 value after 2 wks of P+A (C1D15) compared to that on A alone (C1D1) (p=0.034, n=18).

Conclusion

This study met the primary endpoint demonstrating that P+A is a highly effective anti-proliferative combination. The sequential biopsy design clearly demonstrated that P+A increased cell cycle control over A alone. P+A was effective regardless of PIK3CA mutation status and these results support the evaluation of this combination in the adjuvant setting for ER+HER2- BC.
van Ramshorst MS S, van der Heijden-van der Loo M, Dackus GMHE MHE, Linn SC C and Sonke GS S. Netherlands Cancer Institute, Amsterdam, Netherlands and Comprehensive Cancer Organisation, Utrecht, Netherlands.

Body:
2015 San Antonio Breast Cancer Symposium

Publication Number: S6-07

Title: Moved to S6-07

Gluz O, Nitz U, Liedtke C, Christgen M, Sotlar K, Grischke EM M, Forstbauer H, Braun M, Warm M, Hackmann J, Uleer C, Aktas B, Schumacher C, Bangemann N, Lindner C, Kuemmel S, Clemens M, Potenberg J, Staib P, Kohls A, Pelz E, Kates RE E, Wuerstlein R, Kreipe HH H and Harbeck N. Westdeutsche Studiengruppe GmbH, Moenchengladbach, Germany; Ev. Hospital Bethesda, Breast Center Niederrhein, Moenchengladbach, Germany; University Clinics Schleswig-Holstein/Campus Luebeck, Women's Clinic; Medical School Hannover, Institute of Pathology; University of Munich (LMU), Institut of Pathology; University Clinics Tuebingen, Women's Clinic; Practice Network Troisdorf; Rotkreuz Clinics Munich; Clinics of Cologne - Hospital Holweide; Marien-Hospital Witten; Gynecologic Oncologic Practice Hildesheim; University Clinics Essen, Women's Clinic; St. Elisabeth Hospital Cologne; Charité Berlin, Clinic of Gynecology; Agaplesion Diakonie Clinic; Clinics Essen-Mitte, Breast Center; Mutterhaus der Borromäerinnen Trier; Ev. Waldkrankenhaus; St. Antonius Hospital, Clinics of Hematology and Oncology; Ev. Hospital Ludwigsfelde; Pathology Viersen; Palleos Healthcare Services, Statitistics and Breast Center, University of Munich and CCCLMU.

Body:
Title: HERMIONE: A phase 2, randomized, open label trial comparing MM-302 plus trastuzumab with chemotherapy of physician's choice plus trastuzumab, in anthracycline naive HER2-positive, locally advanced/metastatic breast cancer patients previously treated with pertuzumab and ado-trastuzumab emtansine (T-DM1)

Miller K, Cortes J, Hurvitz SA A, Krop IE E, Tripathy D, Verma S, Riahi K, Reynolds JG G, Wickham T, Molnar I and Yardley DA A. Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; University of California Los Angeles, Los Angeles, CA; Dana-Farber Cancer Institute, Boston, MA; MD Anderson Cancer Center, Houston, TX; Sunnybrook Odette Cancer Centre, Toronto, Canada; Merrimack Pharmaceuticals, Inc, Cambridge, MA and Sarah Cannon Research Institute, and Tennessee Oncology, PLLC, Nashville, TN.

Body: Background: Although HER2-targeted therapies such as pertuzumab and T-DM1 have improved patient outcomes, treatment resistance typically occurs. MM-302 is a HER2-targeted liposomal doxorubicin in development by Merrimack Pharmaceuticals. In a Phase 1 study, patients with HER2-positive metastatic breast cancer (MBC) were treated with MM-302 alone and in combination with trastuzumab with or without cyclophosphamide. MM-302 had an acceptable safety profile and promising efficacy was observed in patients not previously exposed to an anthracycline.

Trial design: HERMIONE (NCT02213744) is a randomized Phase 2, two-arm, open-label trial designed to evaluate if MM-302 can address an unmet medical need in patients with anthracycline naïve, trastuzumab-, pertuzumab- and T-DM1-pretreated HER2-positive locally advanced breast cancer (LABC)/MBC. Patients are randomized 1:1 to receive MM-302 (30mg/m2, Q3W) plus trastuzumab (6mg/kg, Q3W) or chemotherapy of physician's choice (vinorelbine, capecitabine, or gemcitabine) plus trastuzumab (6mg/kg, Q3W).

Eligibility criteria: Centrally confirmed HER2-positive LABC/MBC, no prior anthracycline exposure, prior trastuzumab in any setting, prior pertuzumab and T-DM1 in the LABC/MBC setting, unlimited prior lines of therapy, ECOG 0-1 and LVEF ≥50%. CNS metastases are permitted if stable and without symptoms or steroids for 4 weeks.

Specific aims: The primary endpoint is independently assessed progression free survival (PFS). Secondary endpoints include investigator assessed PFS, overall survival, response rate, safety and patient related outcomes.

Statistics: 250 patients will be enrolled to observe 191 PFS events for 90% power to detect a Hazard Ratio of 0.625. The MM-302 arm will be compared to the control arm on the primary endpoint of PFS using a stratified log-rank test at one-sided 0.025 level.

Accrual status: First patient in was December 2014 and enrollment is expected to be complete in late 2016. Sites are open in the US, Canada and Western Europe and are currently enrolling patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-02

Title: A phase I-II trial of dasatinib in combination with trastuzumab (T) and paclitaxel in the first line treatment of HER2 positive metastatic breast cancer (MBC) patients: GEICAM/2010-04

Ocaña A, Ruiz Borrego M, Gil-Martín M, Antolín S, Guerrero Á, Vidal Boixader L, Martín M, Trigo Pérez JM, Rojo F, Jerez Y, Atienza M, Pernas S, Hernando A, Carrasco E, Benito S, Caballero R and Pandiella A. Complejo Hospitalario Universitario de Albacete, Albacete, Spain; Hospital Universitario Virgen del Rocio, Sevilla, Spain; Instituto Catalán de Oncología de L’Hospitalat, L’Hospitalet de Llobregat, Barcelona, Spain; Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; Instituto Valenciano de Oncología, Valencia, Spain; Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain; Hospital Clínico Universitario Virgen de la Victoria, Málaga, Spain; Fundación Jiménez Díaz, Madrid, Spain; Hospital General Universitario Gregorio Marañón, Madrid, Spain; GEICAM Spanish Breast Cancer Group, Madrid, Spain and Centro de Investigación del Cáncer, CSIC-Universidad de Salamanca, Salamanca, Spain.

Body: Background: Resistance to targeted-therapies is a current problem in our clinical practice. In HER2 overexpressing tumors, resistance to trastuzumab-based therapies is widely observed. Expression of SRC has been pre-clinically linked to trastuzumab resistance and the addition of the multi-tyrosine kinase inhibitor dasatinib to trastuzumab increases its antitumor activity (Seoane S, et al. J Natl Cancer Inst. 2010). On previous studies, Dasatinib showed a good toxicity profile, with low grade 3-4 toxicity rates (E. Mayer, et al. J Clin Oncol 2009). We have designed a phase I-II trial combining dasatinib with standard trastuzumab/paclitaxel. (ClinicalTrials.gov Identifier: NCT01306942). In this abstract we report the description of the phase II part of the trial which is ongoing.

Trial Design: Eligible patients must be HER2+ (evaluated by central laboratory and assessed by immunohistochemistry and fluorescent in-situ hybridization) MBC and candidates for trastuzumab + chemotherapy as first line treatment. Taxanes and trastuzumab administered in the adjuvant setting are permitted if given >12 months before the inclusion. Patients with CNS involvement are eligible if treated and clinically stable without medication. Treatment consists of trastuzumab 2 mg/kg weekly (following a loading dose of 4 mg/kg in cycle 1), weekly paclitaxel (80 mg/m², 3 weeks on-one week off) and dasatinib 100 mg once daily (based on the recommended phase II dose from the phase I part, Gil-Martín M, et al. European Breast Cancer Conference 2014, P-041) in a 28-days cycle. Patients are treated until radiologic or symptomatic progression or unacceptable toxicity.

The primary objective for this phase II is efficacy, measured by Objective Response Rate (ORR according to RECIST 1.1). Secondary objectives are: Safety (evaluated using NCI-CTCAE v 4.03), Clinical Benefit Rate, Time to Progression, Progression Free Survival and Response Duration. Pharmacodynamic biomarkers include pAKT, pS6, pSRC, pErk1/2 in tumor tissue samples, mononuclear cells in blood samples and in skin tissue. An additional exploratory objective is to evaluate the correlation between the early presence of lymphocytosis and efficacy. The study will be considered positive if ORR increases 25% from a 50% ORR observed in previous studies with paclitaxel + trastuzumab. We need to include 28 evaluable patients to demonstrate this hypothesis (with an alpha error of 0.05 and a statistical power of 80%), assuming a 10% drop-out rate. Twenty one patients have already been included. Recruitment is expected to finish by the end of 2015.
Title: Phase III clinical trial to evaluate patient's preference for subcutaneous (SC) versus intravenous (IV) trastuzumab administration in patients with HER2 positive, advanced breast cancer (ABC) under IV trastuzumab treatment for at least 4 months and without disease progression. ChangHER-SC study (GEICAM/2012-07)

Ciruelos E, González E, Lluch A, Garrigós L, Quiroga V, Antón A, Pascual T, Montaño Á, Angulo MdM, Cámara MdC, Amigo Y, Carrasco E and Casas A. Hospital Universitario 12 de Octubre, Madrid, Spain; Hospital Universitario Virgen de las Nieves, Granada, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Hospital del Mar, Barcelona, Spain; Institut Català d’Oncologia Badalona-HU Germans Trias i Pujol, Badalona, Barcelona, Spain; Hospital Universitario Miguel Servet, Zaragoza, Spain; Hospital Universitario Virgen del Rocío, Sevilla, Spain and GEICAM, Spanish Breast Cancer Group, Madrid, Spain.

Body: Background: Patients with HER2-positive, ABC, usually receive anti-HER2 treatment for several months or even years. Trastuzumab IV is administered weekly or 3-weekly, mandating patients to visit the hospital on a regular basis to receive infusions that are prepared in the hospital's pharmacy and administered to patients over 30-90 minutes. These mean some inconveniences for the patients and increase treatment costs. Subcutaneous administration could improve convenience of trastuzumab treatment. This study was designed to evaluate patient's preference for IV or SC trastuzumab.

Trial Design: Phase III, open label, multicenter study, running in 27 Spanish sites. HER2 positive, ABC patients, receiving IV trastuzumab (in monotherapy or in any combination), without evidence of disease progression for at least 4 months, and a life expectancy of at least 3 months, are eligible. Patients are randomized to receive SC trastuzumab, either from a vial or from a single injection device (SID), at a fixed dose of 600 mg, every 3 weeks, for 4 cycles. Before starting SC administration, patients receive an additional IV cycle. Patients in arm A receive 2 SC cycles using the vial and then two cycles more with the SID. In arm B patients receive the opposite sequence. After these four cycles, patients continue with SC or IV trastuzumab, depending on their decision, until disease progression. The primary objective is to evaluate patient's preference for IV or SC trastuzumab and, secondary objectives are, to evaluate patient's preference for the SC administration using the vial or the SID, the Health Care Professional satisfaction, the associated costs of the different administration options (with a Time and Motion pharmaco-economic study) and the safety of the different treatment administrations. Assuming a 65% rate of patients preferring SC trastuzumab and with a precision ±7.5%, a total of 195 patients have to be recruited to allow for a 20% of expected drop-out rate. Recruitment started in September 2013 and 158 patients have been included so far. Analysis of the primary endpoint will take place once the last patient has completed the 4 cycles of the study treatment. (ClinTrials.gov reference NCT01875367).
2015 San Antonio Breast Cancer Symposium

Publication Number: OT3-01-04

Title: An open-label, single-arm, phase II study of pertuzumab with high-dose trastuzumab for the treatment of central nervous system progression post-radiotherapy in patients with HER2-positive metastatic breast cancer (PATRICIA)

Lin NU U, Pegram MD D, Lai C, Lacasia A, Stein A, Yoo B and Perez EA A. Dana-Farber Cancer Institute, Boston, MA; Stanford Cancer Institute, Stanford, CA; Genentech Inc., South San Francisco, CA and Mayo Clinic, Jacksonville, FL.

Body: Background: Central nervous system (CNS) metastases are observed in up to half of patients with HER2-positive metastatic breast cancer (MBC), with incidence likely to continue to rise due to longer survival through improved systemic treatments. While radiotherapy-based approaches can be effective, there are potential short- and long-term toxicities, and patients frequently progress. CNS response to existing systemic therapies has been generally poor, and there is a high unmet need with no approved treatment for CNS metastases in HER2-positive MBC. Combination of the HER2-targeted monoclonal antibodies trastuzumab and pertuzumab provides a more comprehensive blockade of HER2 than either antibody alone, and data from the phase III CLEOPATRA trial suggest that adding pertuzumab to trastuzumab and docetaxel may delay onset of CNS disease. Trastuzumab concentrations in the CNS are increased under conditions of an impaired blood–brain barrier (BBB) and subtherapeutic levels in the CNS may be related to insufficient dosing rather than inability to cross the BBB. The PATRICIA trial is evaluating the addition of pertuzumab with high-dose trastuzumab to a patient's current systemic treatment for HER2-positive MBC patients with CNS progression post-radiotherapy and stable systemic disease.

Study design: In this US-based, phase II, open-label, single-arm study, patients will receive intravenous pertuzumab (840 mg loading dose followed by 420 mg every 3 weeks) in combination with intravenous high-dose trastuzumab (6 mg/kg weekly) in addition to their current systemic therapy (except for ado-trastuzumab emtansine or lapatinib) until disease progression or unacceptable toxicity.

Eligibility criteria: Patients aged ≥18 years with confirmed HER2-positive MBC with new and/or progressive CNS lesions >60 days after whole-brain radiotherapy or stereotactic radiosurgery for CNS metastases, performance status 0–1, and stable systemic disease will be eligible. Patients must have a baseline left ventricular ejection fraction (LVEF) ≥50%, no significant history of cardiac disease or current use of anthracyclines, life expectancy >12 weeks, and not be pregnant or lactating.

Aims: The primary efficacy endpoint will be objective response rate (ORR) in the CNS, assessed by the investigator using RANO–BM criteria. Secondary endpoints will include duration of CNS response, progression-free survival (CNS and/or non-CNS), overall survival, and safety. Pharmacokinetic and patient-reported outcomes will also be evaluated. LVEF will be assessed throughout treatment and follow-up. An interim analysis will be performed when 15 patients have completed 2 cycles, and the study will be stopped if no clinical benefit (complete response, partial response, or stable disease in the CNS) is seen or if two or more patients have congestive heart failure events related to trastuzumab or pertuzumab.

Statistical methods: The recruitment target is 40 patients; with 35 evaluable, the 95% confidence interval around an estimated ORR of 20% will be 8.4–36.9%. The trial opens for accrual in Q3 2015.
Title: PANACEA (IBCSG 45-13/BIG 4-13): A phase Ib/II trial evaluating the efficacy of pembrolizumab and trastuzumab in patients with trastuzumab-resistant, HER2-positive, metastatic breast cancer


Body: Background
Preclinical and correlative clinical data suggest that HER2-positive breast cancer could be amenable to immunotherapeutic approaches. We hypothesize that immune evasion contributes to tumor growth and progression in HER2-positive tumors and anti-PD1 restores T cell cytotoxicity reverting trastuzumab resistance.

Eligibility criteria
Patients with HER2-positive, PD-L1 expressing, unresectable loco-regional or metastatic breast cancer with disease progression during treatment with trastuzumab or subsequent anti-HER2 therapy of not more than 3 lines. HER2 overexpression and PD-L1 expression confirmed in central laboratories based on tissue obtained within 1 year prior to study entry.

Objective
The primary objectives of this phase Ib/II study are to determine the recommended dose (RP2D) of pembrolizumab in combination with standard dose trastuzumab, and to evaluate the efficacy and safety profile of the drug combination in patients with PD-L1 positive, HER2 overexpressing unresectable loco-regional or metastatic breast cancer.

Trial design
The phase Ib portion of the trial will use a standard 3+3 design to determine the RP2D of standard-dose trastuzumab with three pembrolizumab dose levels: 2 mg/kg, 10 mg/kg, or a fall-back dose of 1 mg/kg. A Simon optimal two-stage design will be used in the phase II portion to assess the primary outcome of objective response. Pembrolizumab at the RP2D will be given with trastuzumab (6 mg/kg i.v. every 3 weeks) until disease progression or lack of tolerability. The null hypothesis that the true objective response rate (ORR) is 7% will be tested against a one-sided alternative rate of 22%. In the first stage, 17 patients will be enrolled. If there are zero or one responses, enrollment will stop. Otherwise, 23 additional patients will be accrued. The null hypothesis will be rejected if 6 or more objective responses are observed in 40 evaluable patients. This design has a type-I error of 0.05 and 85% power. If the null hypothesis is true, the probability is 0.66 that enrollment will stop at the end of the first stage. Two concurrent analyses of the data will take place at the end of the first stage. One analysis will assess ORR using the criteria described above. The second will be a detailed review of safety data.

Statistical methods
Primary and secondary endpoints will be based on patients enrolled in the phase II trial. ORR will be presented with a two-sided 90% confidence interval calculated using the method of Atkinson and Brown. The distributions of time-to-event secondary endpoints, such as duration of response and time to progression, will be summarized using the method of Kaplan-Meier.

Present accrual and target accrual
As of 3 June 2015 11 patients have been screened for HER2 and PD-L1 positivity, and 3 successfully enrolled, completing the first dose cohort. Target enrollment of this phase Ib/II trial is 6-46 evaluable patients. Discussions are ongoing to include a PD-L1 negative cohort.

Contact information
Conducted by the International Breast Cancer Study Group and the Breast International Group in collaboration with Merck Sharp & Dohme Corp.
Title: Phase I/II trial of ruxolitinib in combination with trastuzumab in metastatic HER2 positive breast cancer

Kalinsky K, Chi D-C, Lee S, Bhardwaj A, Makower D, Cigler T, Crew KD D, Hershman DL L, Califano A, Silva J and Maurer M. New York Presbyterian - Columbia University Medical Center; Mount Sinai Medical Center; Montefiore Medical Center and New York Presbyterian - Weill Cornell Medical Center.

Body: Background:
Integrated analysis of whole genome RNAi screening with computationally reverse engineered interactome models identified IL6/JAK/STAT as a master regulator pathway essential for growth of ErbB2/HER2 positive breast cancer. Ruxolitinib (R), FDA-approved treatment for myelofibrosis, inhibits JAK1 and JAK2. The combination of R plus Trastuzumab (T) is synergistic in tumor growth inhibition in mouse xenografts of HER2 amplified breast cancer cell lines. These data provide a strong rationale for studying the efficacy of combination R and T in a clinical trial.

Trial Design:
A multi-center, open-label, phase I/II (P1/2) trial of R plus T in HER2+ metastatic breast cancer (MBC) who have progressed on T-based therapy. P1 will be an adaptive design with 10 patients, using the time-to-event continual reassessment method. The recommended P2 dose (RP2D) will be used in a non-randomized, open-label P2 trial with 30 evaluable patients (pts). Given the anticipated limited overlapping toxicities, approximately 36 pts (range: 32-40) are expected for the P1/2. The duration of a treatment cycle will be 21 days. R will be taken orally twice a day continuously. The P1 dosing range will be 10-25 mg BID (dose level 0: 20 mg BID). T will be administered on Day 1 of each cycle at standard dosing. Objective Response Rate (ORR) will be assessed by imaging every 9 weeks. Blood samples will be obtained for biomarker analysis, pre-treatment, on-treatment on C2D1, and then at progression. Pre-treatment biopsies from archival tissue or new biopsy, on treatment biopsy on C2D1, and upon progression of disease will be discussed with pts with accessible disease.

Main Eligibility Criteria:
1. HER2 positive MBC
2. Progression on HER2-directed therapy in metastatic setting, including Pertuzumab and T-DM1
3. Measurable or non-measurable disease
4. LVEF great than 50%
5. No history of prior JAK2 inhibitor
6. No HIV-positive or active infection
7. No concurrent medications that are potent CYP3A4 inhibitor or inducer

Specific Aims:
1. Primary: P1: MTD of combined R + T. P2: Progression Free Survival (PFS)
2. Secondary: a) Clinical: ORR, clinical benefit rate (CBR), and tolerability. Pts will be stratified by hormone receptor (HR) status to explore differences in efficacy between HR+ and HR-.
   b) Explore potential predictive tumor and blood-based predictive biomarkers at baseline, on treatment, and progression: (tumor: pSTAT3 expression); serum: IL-6, IL-8, C-reactive protein; circulating tumor cell pSTAT3 expression; and tumor gene expression.

Statistical Methods:
Assuming a historical PFS of 8 weeks with single-agent agent HER2-targeted therapy in HER2+ MBC after progressing on T-based therapy, we predict that pts receiving the combination of R plus T will have a PFS of at least 13 weeks. With a 2-sided alpha of 0.05, we have 80% power to detect a difference with 30 pts.

Target Accrual:
Sample Size: 32-40 pts; projected over 2 years at 4 sites: Columbia, Einstein, Mount Sinai, and Cornell. Trial accruing since Fall 2014.
Title: Phase II study of trastuzumab and pertuzumab alone and in combination with hormonal therapy or chemotherapy with eribulin in women aged ≥60 with HER2/neu overexpressed locally advanced and/or metastatic breast cancer

Body: Her2 overexpression is both a predictive and prognostic marker with tumors overexpressing Her2 having an aggressive natural history, but also responding to targeted therapy. The standard of care for Her2 positive metastatic cancer is docetaxel paired with combined antibody therapy of pertuzumab (P) and trastuzumab (T). Older patients are known to have more difficulty tolerating traditional cytotoxic chemotherapy. Neoadjuvant studies have shown a proportion of patients have pathologic complete responses (pCR) with dual Her2 targeted therapy without chemotherapy. The NEOSPHERE trial demonstrated at 17% pCR after 3 cycles of T+P. The Translational Breast Cancer Research Consortium has shown 12-28% pCR with the combination of estrogen deprivation, trastuzumab, and lapatinib (TBCRC 006 and 023). We have designed a phase II study of T+P alone and then in combination with hormonal or chemotherapy after progression in women age ≥60 with Her2 overexpressed locally advanced or metastatic breast cancer (BC). As a primary endpoint, this study seeks to evaluate the overall response rate (ORR) of dual Her2 targeted therapy with T+P without chemotherapy in older patients with locally advanced or metastatic Her2 positive BC (cohort 1). At progression, depending on tumor characteristics and disease status, chemotherapy with eribulin or hormone therapy with anastrozole plus fulvestrant will be added (cohort 2 – A and B). ORR for cohorts 1, 2A and 2B will be determined. Secondary end points will evaluate clinical benefit, progression free survival, overall survival, tolerability, safety, and quality of life. Translational studies involving circulating tumor cells identified through OncoCEE – Biocept system and glycoprotein 88 expression will be performed. Eligibility includes patients' age ≥60 with locally advanced or metastatic Her2 positive BC treated with 0-3 lines of chemotherapy. Patients must have an ejection fraction >50% and meet set hematologic and metabolic lab criteria. Her2 status is per ASCO/ACP guidelines. Excluded patients include patients with active brain metastasis, second malignancies, anticancer treatment <3 weeks prior to the start of therapy. Patients must have not received pertuzumab, eribulin, anastrozole, or fulvestrant in the metastatic setting. A true ORR of 40% will be considered active. The study was designed assuming 25% of patients initially respond to T+P and 75% progress to cohort 2. With a type I error rate of 0.05 and power of 0.90, 40 patients will need to enroll in order to have 30 patients in cohort 2 (15 per arm). Data will be analyzed after eight patients are enrolled. If there are no responders in cohort 1 and 2, the accrual will be stopped and declared inefficient. After 15 patients are enrolled, if no more than 3 of the 15 respond, the therapy will be considered not promising and halted. Currently there are two patients enrolled at the University of Maryland. We are in negotiations to expand to additional sites. Questions can be directed to prosenblatt@umm.edu.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** OT3-01-08

**Title:** NRG oncology/RTOG 1119: Phase II randomized study of whole brain radiotherapy with concurrent lapatinib in patients with brain met from HER2-positive breast cancer — A collaborative study of RTOG and KROG (NCT01622868)

White JR R, Moughan J, Kim IA, Peereboom DM M, De Los Santos JF F, Sperduto PW W and Mehta MP P. The Ohio State University, Columbus, OH; NRG Oncology Statistics and Data Management Center; Seoul National University Bundang Hospital; Cleveland Clinic Foundation; University of Alabama at Birmingham Cancer Center; Metro-Minnesota CCOP and University of Maryland.

**Body:**

**Background:** The addition of trastuzumab to cytotoxic chemotherapy has improved outcomes for patients with HER2+ breast cancer. Increased survival coupled with limited blood-brain barrier (BBB) penetration of trastuzumab may contribute to the increased incidence of brain metastases (mets) in these patients. Half of these patients die of intracranial disease progression rather than extracranial systemic disease. Therefore, strategies to improve survival must include increased CNS disease control. Lapatinib crosses the BBB & demonstrates modest activity against intracranial mets. Based upon preclinical data & phase I study results, it's hypothesized that lapatinib plus WBRT can improve the intracranial disease control compared to whole brain radiotherapy (WBRT) alone.

**Trial design:** RTOG 1119 is a randomized phase II trial that will evaluate if there is a sufficient enough signal in improved 12-week complete response (CR) rate following WBRT with the addition of lapatinib versus WBRT alone in patients with brain met from HER2+ breast cancer to warrant a future phase III trial.

**Eligibility criteria:** Eligibility includes HER2+ breast cancer with at least one measurable, unirradiated parenchymal brain met (≥10 mm if solitary, & > 5 mm if multiple on enhanced MRI). The two populations targeted for accrual include patients with 1) newly diagnosed, multiple brain mets or 2) progressive brain mets after stereotactic radiosurgery (SRS) or surgical resection of 1-3 mets. Patients are stratified by breast-specific graded prognostic assessment; use of non-CNS penetrating HER2 targeted therapy; & prior SRS or surgical resection. Non-CNS penetrating HER2 targeted therapy is permitted throughout the study, but patients not on trastuzumab, pertuzumab or any other breast cancer therapy at study entry are not permitted to begin this therapy while on protocol treatment, but may begin it 24 hours after its completion. Prior lapatinib is allowed, last dose > 21 days prior to study entry.

**Specific aims:** Primary objective is to determine if there is an increase in CR rate in the brain at 12 weeks post WBRT as determined by MRI scan of the brain, with the addition of lapatinib to WBRT compared to WBRT alone. Secondary objectives includes: CR rate at 4 weeks on MRI post WBRT, objective response rate on MRI at 4 & 12 weeks, evaluation of lesion specific MRI response rates; CNS progression-free survival rate, overall survival rate, & adverse event rates.

**Statistical methods:** The randomization of experimental & control arms is set as 1:1. With 114 eligible subjects there will be 86% power to detect a 15% absolute increase in CR rate at a significance level of 0.10, using a 1-sided Z-test for 2 proportions. Targeted accrual is 143 accounting for up to a 5% ineligibility rate, 15% patients not evaluable for the primary endpoint due to death, patient withdrawal, or other reasons.


**Contact Information:**


Supported by NCI U10 grants CA21661, CA180868, CA180822, CA37422 & UG1CA189867.
Title: Phase II study of gemcitabine, trastuzumab, and pertuzumab in the treatment of metastatic HER2-positive breast cancer after prior trastuzumab/pertuzumab- or pertuzumab-based therapy

Iyengar NM M, Argolo D, Smyth L, Chen MF F, Hudis CA A and Dang CT T. Memorial Sloan Kettering Cancer Center, NY, NY.

Body: Background: Despite significant therapeutic advances in the treatment of breast cancers that overexpress human epidermal growth factor receptor-2 (HER2), the large majority of patients (pts) with metastatic disease will experience progression. The development of new HER2 directed therapies has led to improvements in progression free and overall survival; however optimal therapeutic sequencing remains under study. The combination of taxanes with trastuzumab (H) and pertuzumab (P) is active in the first line setting; however the efficacy of continuing dual anti-HER2 therapy with HP after initial progression is unknown.

Trial design: This is a single arm phase II trial of gemcitabine (G) with HP for pts with metastatic HER2-positive breast cancer who have had prior HP-based treatment. G is administered at 1200 mg/m$^2$ on days 1 and 8 of an every 3 week cycle. H is given 8 mg/kg load → 6 mg/kg every 3 weeks or 4 mg/kg load → 2 mg/kg every week. P is given 840 mg load → 420 mg every 3 weeks. All agents will be given intravenously. This trial is currently enrolling pts.

Eligibility criteria: Eligible pts are adults with stage IV HER2-positive (3+ IHC or FISH $\geq$ 2.0 of primary or metastatic site) breast cancer. Pts may have received prior treatment with HP- or P-based therapy in the (neo)adjuvant, unresectable, locally advanced, or metastatic setting. In the metastatic setting, $\leq$ 3 prior chemotherapies are permitted. Prior G is allowed only if it was not combined with P. Bone-only non-measurable disease is permitted. Adequate organ and bone marrow function, left ventricular ejection fraction $> 50\%$, and ECOG status $\leq 1$ are required. Pts with previously treated brain metastases stable for $\geq 2$ months may be enrolled.

Specific aims: The primary objective is to determine the progression free survival (PFS) at 3 months. Secondary objectives include overall survival, safety and tolerability. An exploratory endpoint is to compare PFS determined by RECIST criteria versus 18-F FDG-PET response criteria.

Statistical methods: Based on historical data, the anticipated median PFS in this setting is 3 months. The study will be considered positive if the 3-month PFS is $\geq 70\%$. A Simon optimal 2-stage design will be used. If 12/21 pts in stage 1 are alive and progression free at 3 months, stage 2 will accrue for a total of 45 pts. If at least 27/45 pts are alive and progression free, the trial will be deemed a success. This design assumes a 10% type I and type II error.

Present accrual and target accrual: 3 out of a planned 45 pts have been enrolled.

Contact information: For more information, please visit clinicaltrials.gov (NCT02252887).
Title: A prospective, open-label, single-arm, multi-center, phase II exploratory study to evaluate the efficacy and safety of poziotinib (NOV120101) in patients with HER2-positive metastatic breast cancer who have received at least two prior HER2-directed regimens

Park Y-H, Jung KH, Sohn JH, Lee KS, Lee KH, Kim J-H, Kim J-Y, Jung J, Han H, Park W-Y and Im S-A. Samsung Medical Center, Seoul, Korea; Asan Medical Center, Seoul, Korea; Yonsei Medical Center, Seoul, Korea; National Cancer Center, Goyang, Korea; Chungbuk National University Hospital, Cheongju, Korea; Seoul National University Bundang Hospital, Soengnam, Korea; National OncoVenture, Goyang, Korea; Hanmi Pharmaceutical Co., Ltd., Seoul, Korea and Seoul National University Hospital, Seoul, Korea.

Body: Background: Poziotinib is a novel, oral, irreversible pan-HER inhibitor that has shown promising clinical activity in Phase 1 studies of patients (pts) with advance HER2 positive breast cancer who have failed at least 2 prior lines of HER2-directed therapy. A Phase 2 study of poziotinib was initiated in Korea in March 2015 in pts with HER2+ metastatic breast cancer. This phase 2 study is designed to seek accelerated approval for poziotinib for the treatment of metastatic breast cancer in Korea. Trial Design: Prospective Phase 2, open-label, single-arm, multi-center study in pts with recurrent, Stage IV breast cancer with HER2-overexpression who had received at least 2 prior HER2-directed regimens

Eligibility Criteria: Histologically confirmed breast cancer patients, at least 19 years of age, with confirmed HER2 positive evaluable tumors (per RECIST, 1.1) who have adequate hematologic, renal, and hepatic function and have failed at least two HER2-directed regimens that included a taxane-containing anticancer chemotherapy, with a life expectancy of at least 12 weeks.

Specific Aims: The Primary Efficacy Endpoint of the study was Progression-Free Survival (PFS). The Secondary Efficacy Endpoints included: PFS rate at 12 weeks post-dose; Objective Response Rate (ORR) including Complete Response (CR) and Partial Response (PR) rates; Disease Control Rate (DCR) including CR, PR, and Stable Disease (SD); Duration of Disease Control; Overall Survival (OS); Time to Progression (TTP); Time to Objective Response and Duration of Objective Response. The Exploratory Endpoints included: Population Pharmacokinetic (PK) Profile and Exploratory Genomic and Biomarker Analyses.

Statistical Methods: In the randomized, multicenter, 2-arm, open-label study of trastuzumab emtansine (TH3RESA18), the median PFS was shown to be 3.3 months in subjects with optimal treatment per Investigator’s Choice. This ongoing study with poziotinib expects a median PFS of 4.5 months based on data from a previous Phase 1 study of poziotinib (NOV120101). Based on the following assumptions, a 5% one-sided significance level, and 80% power, and 2 months of accrual and 12 months of follow-up, 66 subjects will be required. Accounting for a 10% drop-out rate, a total of 74 subjects will be recruited into this ongoing Phase 2 study.

Present Accrual and Target Accrual: 17 patients enrolled as of May 20, 2015 with a total target enrollment of 74 patients

Contact information:
ClinicalTrials.gov Identifier: NCT02418689.
**Title:** Thrombokinetic studies of ado-trastuzumab emtansine (T-DM1)

Gadi VK K, Butler BS S, Corson J and Slichter SJ J. Fred Hutchinson Cancer Research Center, Seattle, WA; University of Washington, Seattle, WA and Bloodworks Northwest, Seattle, WA.

**Body:**

**Background:** Patients treated with Ado-Trastuzumab-Emtansine (T-DM1) are at risk of thrombocytopenia with the reported rate of 13% in metastatic patients of >3 toxicity. A recent report supports that off-target uptake of T-DM1 into megakaryocyte precursors may result in thrombocytopenia. It is unclear if this is the only mechanism to explain the thrombocytopenia since many patients have lower grade effects on platelets and often report mild mucosal bleeding. In this study, we will test whether platelet half-life in circulation or platelet function are also affected by treatment with T-DM1.

**Trial design:** We initiated a single institution Phase 1 study of T-DM1 in metastatic or unresectable breast cancer in any line of therapy. The general method is to collect autologous platelets, radiolabel the platelets ex vivo, and reinfuse them into patients to measure recovery (twice, at day 4 and again on day 7-9 days). The first 21-day cycle on protocol is to measure baseline platelet recovery (no T-DM1) followed by two cycles where platelet recovery is measured after T-DM1 infusion. Only, three radiolabeled platelet infusions occur on trial (2 with drug) after which disease response is assessed and patients can remain on drug at the discretion of the primary treating oncologist. In addition, patients will have platelet aggregation studies and bleeding times measured. Adult patients with Her2+ breast cancer are eligible for treatment provided they have adequate bone marrow (ANC > 1500 cells/mm3, Platelet >100,000/mm3, hemoglobin >9 g/dL), cardiac (Ejection fraction > 50%), kidney and liver function at baseline and have an ECOG performance score better than 2. Patients’ prior treatment with chemotherapy or trastuzumab, if any, must have a >21 day washout period. Prior T-DM1 treatment is not permitted nor is a personal history of known platelet disorder (ITP, von Willebrand’s disease). Patients with brain metastases are permitted to participate provided the disease is treated and is stable for >30 days.

**Statistical methods:** The primary goal is to measure platelet T1/2 following T-DM1 exposure and to compare this to baseline recovery. The proposed sample size of 20 participants is selected to insure that at least 15 patients will have complete data for all time points and study procedures. The first test performed will compare pre-therapy platelet lifespan to platelet lifespan on T-DM1 (cycles 1 and 2). Using platelet lifespan from normal controls to approximate pre-therapy values (mean 10.7 days, standard deviation 2.0), for 15 patients a two-sided paired t-test with alpha=0.05 would have 82% power to detect a 15% (1.605 days) decrease in platelet lifespan, assuming a correlation of 0.5 between paired values. The actual analysis will fit a linear mixed effects model, using a two-sided Wald test to compare pre-therapy to the two post-therapy values.

**Accrual:** The study activated in January 2015. One patient has undergone all study procedures. 3 Additional patients have been screened and 2 of these patients chose not to participate citing that the study had too many procedures and one was discovered to have had prior T-DM1 treatment. The investigators are in the process of opening the study to Her2 positive non-breast cancer patients to accelerate accrual.
Title: Phase II trial of lapatinib and everolimus for HER2 positive metastatic breast cancer

Barr JA A, Sharma P, Fabian CJ J, Yeh H, Baccaray S, Springer M and Khan QJ J. University of Kansas Cancer Center, Westwood, KS.

Body: Background:
Although the treatment of HER2 positive metastatic breast cancer (MBC) has improved with anti-HER2 agents and chemotherapy, most patients will eventually develop resistance to these agents. Preclinical studies have shown that mTOR inhibition may reverse trastuzumab resistance. We hypothesize that combining mTOR inhibitor everolimus with lapatinib will be an effective strategy for patients who have progressed on prior anti-HER2 therapies.

Trial Design:
We are conducting an open-label phase II pilot study of the combination of everolimus and lapatinib for pts with HER-2 positive MBC. Eligible pts must have histologically documented locally advanced (inoperable) or metastatic HER-2 positive breast cancer that have progressed on at least one HER-2 based regimen in the metastatic or locally advanced setting. Pts with disease progression during or within 12 mos of the completion of adjuvant trastuzumab are eligible. Pts with untreated asymptomatic brain metastases are allowed. Pts with symptomatic brain metastases are allowed to enroll after they have completed radiation and are off steroids. Eligible pts are started on everolimus 5 mg PO daily and lapatinib 1250 mg PO daily without interruption. Among subjects progressing on lapatinib, lapatinib is continued and everolimus initiated. Pts will continue to receive treatment until there is evidence of progressive disease (PD), unacceptable toxicity, or withdrawal of consent. Pts will have radiological evaluation every 8 weeks with CT, bone scan, and MRI brain (for pts with known brain metastasis at baseline).

Specific Aims:
Primary objective is to assess the effectiveness of the combination of RAD-001 and lapatinib as measured by the six-month Overall Response Rate in women with MBC who have progressed on trastuzumab and/or lapatinib based therapies. Secondary objectives are six-month PFS, safety and tolerability of the combination, six-month objective CNS response rate, six-month clinical benefit rate of systemic disease, and six-month clinical benefit rate in CNS.

Statistical methods:
The response rate of lapatinib monotherapy in heavily pre-treated patients is estimated to be 7% (Blackwell 2009). For an expected ORR of 17%, a sample size of 45 subjects will provide 79% power to detect the difference at 0.10 Type I error rate according to 1-sided exact binomial test.

Present accrual and target accrual:
The trial has accrued 20 patients with a target accrual of 45 patients.
Title: MetaPHER: A phase IIIb multicenter, open-label, single-arm safety study of subcutaneous trastuzumab, in combination with pertuzumab and docetaxel in patients with HER2-positive advanced breast cancer


Body: Background: In patients (pts) with HER2-positive metastatic breast cancer (BC), the phase III CLEOPATRA study (NCT00567190) demonstrated significant improvement in progression-free (PFS) and overall survival (OS) from first-line treatment with intravenous (IV) pertuzumab (PERJETA® [P IV]) plus trastuzumab (Herceptin® [H IV]) plus docetaxel (D IV). HannaH (NCT00950300), a phase III trial in pts with HER2-positive early BC demonstrated that fixed-dose trastuzumab subcutaneous (Herceptin® [H SC]) is non-inferior to weight-based H IV infusion in the co-primary endpoints of serum trough concentration and pathologic complete response, which was supported by the long-term efficacy endpoints of event-free survival (EFS) and OS. The safety profile of H SC was consistent with the known safety profile of H IV. H SC is comprised of 600 mg trastuzumab and recombinant human hyaluronidase (rHuPH20) as an excipient, allowing a significantly reduced administration time compared with H IV (5 mins versus 30–90 mins, respectively). The MetaPHER study is designed to investigate the safety and efficacy of H SC in combination with P IV and D IV in pts with HER2-positive advanced BC.

Trial design: MetaPHER is a phase IIIb multicenter, open-label, single-arm study, with eligible pts receiving H SC 600 mg/5 mL q3w, P IV (840 mg loading dose, then 420 mg at each subsequent cycle q3w), and D IV (at least 6 cycles at 75 mg/m² q3w with possible escalation to 100 mg/m² q3w; after Cycle 6, continuation of docetaxel is at the investigator’s discretion). Study treatment is administered until disease progression, unacceptable toxicity, withdrawal of consent, death, or predefined study end, whichever occurs first. Pts will receive post-treatment follow-up for safety and survival.

Eligibility: Women aged ≥18 years with metastatic or locally recurrent HER2-positive BC, ECOG performance status 0 or 1, and a left ventricular ejection fraction ≥50% are eligible. Exclusion criteria include disease-free interval of <6 months from completion of adjuvant or neoadjuvant non-hormonal treatment to disease recurrence; previous systemic non-hormonal therapy for metastatic or locally recurrent BC; history of persistent grade ≥2 hematological toxicity; current grade ≥3 peripheral neuropathy; or clinically significant cardiovascular disease.

Aims: The primary objective is to evaluate the safety and tolerability of H SC in combination with P IV and D IV in pts with HER2-positive advanced BC. Secondary endpoints include PFS, OS, objective response rate and incidence of anti-trastuzumab and anti-rHuPH20 antibody formation.

Statistical methods: Safety and efficacy results will be summarized descriptively to include all enrolled pts who received at least one dose of any study drug; the study is not designed for formal hypothesis testing. A sample size of 400 pts provides reasonable precision for the estimation of grade ≥3 AEs and cardiac AEs.

Accrual: MetaPHER is enrolling, FPI was May 2015, and target enrollment is 400 pts; clinicaltrials.gov ID: NCT02402712.

Contact information: For more information or to refer a patient, email global.rochegenentechtrials@roche.com or call 1-888-662-6728 (USA only).
Title: Randomized phase II study of Hangeshashinto (TJ-14) for chemotherapy induced oral mucositis in patients with breast cancer (Hangesha-B study)


Body: Background: Oral mucositis is a common complication of systemic chemotherapy for cancer, and is associated with higher risk of infection, pain, chemotherapy dose reduction. Severe mucositis impairs oral function and seriously affects nutrition and quality of life of the patients. Hangeshashinto (TJ-14) is a traditional Japanese herbal (Kampo) medicine reduces the level of prostaglandin E2 and affects the cyclooxygenase activity, and alleviates chemotherapy induced oral mucositis. We conducted a randomized phase II trial to investigate whether Hangeshashinto (TJ-14) prevents or controls chemotherapy induced oral mucositis.

Patients and Methods: Patients who develop moderate to severe chemotherapy induced oral mucositis (WHO grade>1) during any cycle of chemotherapy are randomly assigned to receive either Hangeshashinto (TJ-14) (n=25) or placebo (n=25). Patients receive the administration of Hangeshashinto (TJ-14) or placebo for 3 weeks at the beginning of the next course of chemotherapy. The patients are advised to dissolve 2.5g of Hangeshashinto (TJ-14) or placebo in 50ml drinking water, and divide it into twice or three times in an oral cavity. Patients rinse their oral cavity with it three times daily. The signs of oral mucositis is assessed by the investigator during the screening cycle. The CTCAE v4.0 grading is used to assess the severity of oral mucositis. The primary endpoint is duration time of oral mucositis, and secondary endpoints include incidence of oral mucositis, incidence of diarrhea, blood levels of CRP, The change of body weight, and blood levels of albumin.

Accrual: This study began in June 2015. The expected end of accrual of 50 patients will be the last quarter 2017.
Title: ROSCO: A randomised phase III, stratified CEP17/TOP2A biomarker trial of neo-adjuvant 5-flourouracil, epirubicin and cyclophosphamide vs docetaxel and cyclophosphamide chemotherapy

Rea DW W, Haywood L, Francis AM M, Bowden SJ J, Brookes CM M, MacKenzie M, Cameron D, Stein R, Earl HM M, Thomas J, Abraham J, Stanley A, Starczynski J, McGoldrick T, Treharne-Jones P, Billingham L and Bartlett JM M. University of Birmingham, Birmingham, United Kingdom; Western General Hospital, Edinburgh, United Kingdom; University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; Independent Cancer Patient Voices, London, United Kingdom; University of Edinburgh, Edinburgh, United Kingdom; University of Cambridge, Cambridge, United Kingdom; Addenbrooke's Hospital, Cambridge, Cambridge, United Kingdom; Heart of England NHS Foundation Trust, Birmingham, United Kingdom; Ontario Institute for Cancer Research, Toronto, Canada, ON, Canada; Sandwell and West Birmingham NHS Trust, Birmingham, United Kingdom; University College London, London, United Kingdom and Aberdeen Royal Infirmary, Aberdeen, United Kingdom.

Body: Background: Patients with high risk early breast cancer undergoing chemotherapy are frequently treated with both anthracycline and taxane-based chemotherapy exposing patients to multiple toxicities. Molecular predictors of response to specific chemotherapy agents are emerging. Abnormal duplication of the centromeric region of chromosome 17 (CEP17) and either Topoisomerase 2A (TOP2A) gene amplification or deletion, have been identified in a recent meta-analysis as potent markers of anthracycline sensitivity (Bartlett et al JCO 2015). The ROSCO study has been designed to prospectively test the utility of these markers to select anthracycline or taxane based chemotherapy in the neoadjuvant setting.

Key entry criteria: Confirmed invasive breast cancer; centrally confirmed CEP17 duplication and TOP2A status; primary tumour>2cms or documented axillary node metastasis Exclusion criteria include breast cancer with good risk features i.e. Grade 1/2 ER PR rich (Q score 7/8), HER2 negative tumours.

Study treatment: Patients will undergo central testing for CEP-17 and TOP2A and be randomised to 4 cycles of FEC100 (5-Flourouracil 500mg/m2 Epirubicin 100mg/m2 cyclophosphamide 600mg/m2) or 4 cycles of TC (Docetaxel 75mg/m2 Cyclophosphamide 600 mg/m2). Following chemotherapy patients will undergo surgical resection, (Institutional standard). Patients with residual invasive cancer will receive 4 cycles of the alternative chemotherapy to that received in the neoadjuvant setting. HER2 positive patients will receive concurrent trastuzumab and continue standard adjuvant trastuzumab. Patients with biopsy proven axillary node metastases will undergo combined blue dye and radiisotope tracer guided sentinel lymph node biopsy (SLNB) and axillary lymph node clearance as a single procedure during breast surgery. Adjuvant endocrine and radiation therapy will be Institutional standard

Endpoints: The primary endpoint is pathological complete response (no invasive disease in breast or axilla (pCR)). Secondary endpoints include: clinical and radiological response; rate of breast conservation; patient reported outcomes; safety, tolerability and long term outcomes. In patients with proven nodal involvement the false negative rate of a negative post treatment SLNB compared to axillary node clearance will be reported.

Sample size and stratification: 1050 patients will be randomised in a 1:1 ratio stratified by nodal status, ER, HER2, and biomarker status (CEP-17 amplified or TOP2A amplified/deleted) vs normal (CEP-17 normal TOP2A normal).

Analysis: The primary analysis will assess the interaction between the treatment effect and CEP17/TOP2A status to determine if a differential treatment effect exists between CEP17/TOP2A Normal and CEP17/TOP2A Abnormal patients. pCR will be analysed using a logistic regression model including co-variates for treatment, CEP17/TOP2A status and an interaction term of the two effects Sample size is based on the ability to detect an absolute improvement in pCR in the biomarker abnormal group from 21% in the TC treated group to 30% in the FEC treated group. With 90% power at 10% significance level.

Contact Information: the ROSCO Trial Office ROSCO@trials.bham.ac.uk.
Title: An observational study of dose dense chemotherapy with lipegfilgrastim support in early breast cancer

Lyons T, Mallet V, Collins D, Malone E, Milewski M, Egan K, Hennessy B, Grogan L, Breathnach O and Morris P. Cancer Clinical Trials and Research Unit, Beaumont Hospital, Dublin, Ireland.

Body: Background: The combination of the anthracycline, doxorubicin with cyclophosphamide (AC) is a widely used chemotherapy regimen in early stage breast cancer. Studies have shown that dose dense chemotherapy (incorporating AC) improved both disease-free survival and overall survival compared to once every 3 week treatment with daily subcutaneous G-CSF support. An important advance in the use of dose dense chemotherapy regimens was the development of pegylated forms of G-CSF, which offered the convenience of a single subcutaneous injection, rather than multiple daily injections. Lipegfilgrastim is a pegylated long-acting covalent conjugate of filgrastim (G-CSF). In a pivotal randomised phase III study in breast cancer lipegfilgrastim was shown to be non-inferior to pegfilgrastim. Although lipegfilgrastim is licensed in Europe and can facilitate every 2 week (dose dense) scheduling of chemotherapy there are a lack of prospective data about its efficacy in this setting. In this prospective, non-interventional, study we are investigating the incidence of treatment-related neutropaenia following four cycles of dose dense AC with lipegfilgrastim support.

Methods: The primary end point of this prospective, single arm study is to determine the incidence of treatment-related neutropaenia, defined as an absolute neutrophil count (ANC) of <1.0 x 10^9/L, following four cycles of dose dense AC with lipegfilgrastim support. The secondary end points are to (1) determine the incidence of febrile neutropaenia, defined as temperature > 38Â°C and ANC <1.0 x 10^9/L, during 4 cycles of dose dense AC with lipegfilgrastim and (2) examine the incidence of treatment-related neutropaenia during subsequent intravenous chemotherapy post completion of AC. Eligibility criteria include, patients with stage I-III breast cancer, planned treatment with dose dense AC in the adjuvant or neoadjuvant setting, age ≥ 18 years, Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1 and adequate bone marrow function. Patients with prior exposure to chemotherapy and/or G-CSF, who are pregnant, have a cardiac or other concurrent illness, which at the investigator's discretion contraindicates the use of AC will be excluded. We will enrol 40 consecutive patients who are planned to undergo dose dense AC. Based on previous clinical trials, we expect that the incidence of treatment-related neutropaenia will be <12%. However, it is likely that the current study will more closely resemble real-world practice and a higher incidence of treatment-related neutropaenia may be observed. This study has been approved by the Institutional Review Board and 9 patients have been consented to date. (registered with clinicaltrials.gov).
2015 San Antonio Breast Cancer Symposium

**Publication Number:** OT3-02-04

**Title:** Randomised phase II study evaluating, as first-line chemotherapy, single-agent oral vinorelbine administered with two different schedules in patients with hormone receptor positive, HER2-negative advanced breast cancer (TempoBreast-1 trial)

De la Haba J, Cazzaniga M, Freyer G, Costa L, Petru E, Bartsch R, Staroslawska E, de Almeida C, Villanova G and Cardoso F. Hospital Reina Sofia, Cordoba, Spain; Ospedale San Gerardo, Monza, Italy; Centre Hospitalier Lyon Sud, Pierre Benite, France; Hospital Santa Maria, Lisbon, Portugal; Medical University, Graz, Austria; Medical University, Vienna, Austria; Oncology Center, Lublin, Poland; Pierre Fabre Medicament, Boulogne-Billancourt, France; Pierre Fabre Medicament, Boulogne-Billancourt, France and Champalimaud Foundation, Lisbon, Portugal.

**Body:** Background: Single-agent chemotherapy (CT) is the standard treatment option for patients (pts) with hormone receptor (HR) positive disease progressing after previous hormone therapy (HT). Oral CT offers significant advantages over intravenous CT because of its greater convenience, its ease of administration and reduced need for hospitalisation. Metronomic CT could be considered as a multi-targeted therapy for advanced breast cancer, combining effects not only on tumour cells but also on their microenvironment by inhibiting angiogenesis and stimulating anticancer immune response. Metronomic oral vinorelbine (OV) has been evaluated in phase I and II trials setting 50 mg total dose three times per week as the reference dosing. This schedule provides promising efficacy results, combined with a potentially improved safety by reducing the risk of neutropenia. Weekly administration of oral vinorelbine is one of the standard chemotherapy options in the management of advanced breast cancer. This study will provide key clinical data regarding the optimal management of this pt population: HR positive and HER2-negative disease previously treated by a HT.

**Trial design:** In this open-label study, pts are randomised to receive (1 cycle = 3 weeks): OV 50 mg total dose three times weekly on Mondays, Wednesdays and Fridays (metronomic schedule) or OV 60 mg/m²/week (day 1, 8, 15) for the first cycle, then increased to 80 mg/m²/week from the second cycle in the absence of grade 3 or 4 toxicity (weekly schedule). Treatments are continued until disease progression, unacceptable toxicity or pt.’s refusal. Main eligibility criteria include: age ≥ 18 years, documented locally recurrent or metastatic disease previously untreated by CT, HR positive disease previously treated by at least one HT, HER2-negative disease, Karnofsky PS ≥ 70. Primary objective: disease-control rate. Secondary objectives: other efficacy parameters, evaluation of safety profiles of both arms and quality of life assessment. Statistical methods: the one-sample multiple testing procedure for phase II clinical trials described by Fleming is used. This procedure employs the standard single stage test procedure at the last one of 2 pre-specified testings, while both allowing for early termination (should extreme results be seen) and essentially preserving the size and power of the single stage procedure. 160 patients will be enrolled in this randomised phase II study (80 per treatment arm). Randomisation is stratified according to centre, prior taxane, prior everolimus and presence of visceral metastases. Study accrual is planned to start Q3 2015 with a duration of 24 months.
Title: Phase II study of eribulin in combination with gemcitabine for the treatment of patients with locally advanced or metastatic triple negative breast cancer. ERIGE Trial on behalf of the Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)

Musolino A, Caldara A, Montemurro F, Frassoldati A, Cavazzini G, Cavanna L, Todeschini R, Camisa R, Tognetto M and Pinto C. Medical Oncology Unit and Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC), University Hospital of Parma, Parma, Italy; Oncologia Medica, Ospedale Santa Chiara, Trento, Italy; Divisione di Oncologia Medica 1, Fondazione del Piemonte per l’Oncologia, Istituto per la Ricerca e la Cura del Cancro di Candolo, Candoli, Italy; Oncologia Clinica, Azienda Ospedaliero Universitaria di Ferrara, Ferrara, Italy; Oncologia Medica, Azienda Ospedaliera C. Poma, Mantova, Italy; Hospital of Piacenza, Piacenza, Italy and Medical Oncology Unit, Policlinico S.Orsola-Malpighi, Bologna, Italy.

Body: Background: There are no well-established chemotherapy regimens for metastatic triple negative breast cancer. The combination of a microtubule inhibitor (eribulin) with a nucleoside analog (gemcitabine) may synergistically induce tumor cell death, especially in tumors like triple negative breast cancers characterized by high cell proliferation, aggressive tumor behavior, and chemo-resistance.

Trial design: This is an open-label, national multicenter phase 2 study evaluating the combination of eribulin (0.88 mg/m2) plus gemcitabine (1000 mg/m2) on day 1 and 8, q21 as either first- or second-line treatment of locally advanced or metastatic triple negative breast cancer (TNBC). A prospective, molecular correlative study is being carried out on germinal DNA of study population.

Eligibility criteria: Patients must have locally advanced or metastatic breast cancer with estrogen receptor-negative (ER < 1%), progesterone receptor-negative (PR < 1%), and human epidermal growth factor receptor 2-negative (Her2-) (0, 1+) or, e.g. in HER2 2+ cases, fluorescent in situ hybridization (FISH) < ratio of 1.8, status. Previous chemotherapy including an anthracycline and a taxane (unless one or both were clinically contraindicated) is mandatory. Up to one prior chemotherapy regimen for metastatic disease is permitted, with the exception of treatment with eribulin and gemcitabine. Patients must have: Measurable disease; Eastern Cooperative Oncology Group (ECOG) performance status 0-2; adequate bone marrow reserve, liver and renal function.

Specific aims: The main objective of this trial is to evaluate the activity of eribulin plus gemcitabine in terms of overall response rate (ORR; RECIST 1.1). Secondary end-points are: Feasibility, safety (CTC-AE V4.0), progression-free and overall survival. The molecular correlative study aim is to assess the role of germinal DNA polymorphisms and BRCA mutations in predicting efficacy and toxicity with the combination regimen.

Statistical methods: The primary endpoint is the ORR. The study is being carried out according to Optimal Simon’s two stage design. We chose the lower activity (p0) of 0.20 and target activity level (p1) of 0.35. A total of 83 (37 in the first stage, 46 in the second one) assessable patients will be needed to guarantee 90% power under a α-level of 5%. Genotypic correlations with clinical responses and toxicities will be performed using a two-tailed Fisher’s exact test. A logistic regression analysis including patients’ genotypes will be used to identify independent prognostic variables influencing both clinical responses and toxicities. Present accrual and target accrual: A total of 83 eligible patients will be enrolled from multiple institutions. To date (June 09, 2015), the first stage has been closed with 37 patients enrolled. The second stage is now recruiting 46 additional patients.
Body: Background: Poly(ADP-ribose) polymerase (PARP) enzymes are involved in DNA repair and are activated by DNA strand breaks. DNA damage from carboplatin has been associated with activation of PARP. Preclinical data indicate that PARP inhibition potentiates the anti-tumor effect of platinum chemotherapy. BMN 673 (Talazoparib) is an oral, selective PARP inhibitor. The phase I single agent maximum tolerated dose (MTD) of BMN 673 given once daily was 1mg po qd. Myelosuppresion was primary dose-limiting toxicity (DLT), including grade 3-4 thrombocytopenia. Carboplatin with paclitaxel is a current standard treatment for many solid tumors, including ovarian, bladder, upper gastrointestinal, breast and non-small cell lung cancer. Myelosuppression, including thrombocytopenia, is also seen with this combination. This phase 1 study is combining BMN 673 with carboplatin once every 3 weeks and weekly paclitaxel.

Trial Design: Two dosing schedules are being investigated. In both schedules intravenous carboplatin will be administered on day 1 and paclitaxel on days 1, 8, 15 of a 21-day cycle. BMN 673 will be dosed orally once daily for days 1-7 (schedule A) or days 1-3 (schedule B) starting on day 1 of each cycle. After 4-6 cycles of the combination therapy, subjects may continue the combination, change to carboplatin and intermittent BMN 673 without paclitaxel or change to BMN 673 alone with continuous daily dosing. Each schedule will have a 6 subject dose expansion at the MTD. The starting dose level for schedule B will be the MTD from Schedule A. The MTD for each schedule will be considered the recommended phase 2 dose (RP2D). Pharmacokinetic samples will be collected. Planned exploratory correlative studies include RAD51 and gamma-H2AX changes in peripheral blood mononuclear cells and examination of mechanism of secondary resistance by comparing mutation profiles in tumors from biopsy specimens.

Key eligibility criteria include age 18 or older with a measurable or evaluable solid tumor malignancy that is metastatic or unresectable. Subjects must have tumor type for which there is a reasonable expectation of response to carboplatin and paclitaxel or they must have BRCA germline or somatic mutation. Adequate performance status and organ function is required. Stable, treated brain metastases are allowed. No prior carboplatin for metastatic disease is allowed.

Objectives: The primary objectives are to determine the MTD and RP2D of BMN 673 given on the 7 and 3 day schedules in combination with carboplatin and paclitaxel. Secondary objectives include evaluation of the anti-tumor activity, pharmacokinetic parameters, and the safety and tolerability of the combination.

Statistical Plan: A standard 3+3 phase 1 dose escalation design is used. Assuming 3-6 subjects per dose level with two schedules including 6 subject dose expansion cohorts and assuming 6 inevaluable subjects, the maximum sample size is 66.

Study Status: The trial will be activating in summer 2015 at the Cancer Institute of New Jersey and University of Wisconsin. It is anticipated that 2-3 patients will be accrued per month with accrual completed within 28 months. For more information: www.clinicaltrials.gov (NCT02317874).
2015 San Antonio Breast Cancer Symposium

Publication Number: OT3-02-07

Title: Neo-DDRD: A biomarker-driven neoadjuvant feasibility study in breast cancer

McIntosh SA A, Parkes EE E, James CR R, Lioe T, Lowry K, Keating KE E, Khambata-Ford S and Kennedy RD D. Centre for Cancer Research and Cell Biology, Queen's University, Belfast, Belfast, Co Antrim, United Kingdom; Belfast City Hospital, Belfast, Co Antrim, United Kingdom and Almac Diagnostics, Craigavon, Northern Ireland, United Kingdom.

Body: Background
Anthracycline-based chemotherapy reduces the risk of early breast cancer recurrence but to date it has not been possible to predict which patients specifically benefit from this treatment. The Almac DDRD assay, a 44 gene signature, has been developed to detect DNA damage response deficiency (DDRD) within breast and other cancer types. The DDRD assay has been shown to predict benefit from DNA damaging chemotherapy in retrospective analyses in neoadjuvant and adjuvant settings (Mulligan et al, JNCI 2014). This pilot study aims to prospectively evaluate the feasibility and utility of this signature in the neoadjuvant setting in breast cancer. It will inform the design of a phase II randomised study where the DDRD assay will be utilised to guide neoadjuvant chemotherapy selection.

Study design
A single-centre, non-randomised feasibility study of 30 women with a histologically confirmed diagnosis of breast cancer, where neoadjuvant chemotherapy is the treatment modality of choice.

Inclusion criteria:
Age >18 years
Early (T1-2, N0-1), locally advanced or inflammatory breast cancer
Normal left ventricular function, haematological and biochemical parameters
ECOG PS 0-1

Exclusion criteria:
Metastatic disease
Bilateral breast cancer
Pregnancy/breastfeeding
Inability to give informed consent

At diagnosis, patients consented to providing two core needle biopsies in addition to a diagnostic biopsy. Axillary nodal status was determined by axillary ultrasound, fine needle aspiration cytology, and pre-treatment sentinel node biopsy if pre-operative axillary staging was negative.

Chemotherapy was administered as per standard institutional practice: 6 cycles flououruracil, epirubicin and cyclophosphamide (FEC) in node-negative patients; 3 cycles of FEC followed by 3 cycles of docetaxel in node positive patients. Neoadjuvant trastuzumab was given in Her2 positive patients. Imaging (mammography and ultrasound) was repeated after three cycles, and repeat FFPE and snap frozen core biopsies undertaken. At conclusion of treatment, patients underwent surgery as appropriate (mastectomy or breast conservation at the discretion of the operating surgeon), with axillary node clearance for patients who were node positive at diagnosis. Further biopsies (FFPE and fresh frozen) were taken intra-operatively. Plasma samples were obtained at diagnosis, mid-treatment and surgery. Pathological reporting of the surgical specimen was standardised using the residual cancer burden (RCB) reporting system.

Aims
· To assess the feasibility of carrying out the DDRD assay on diagnostic core biopsy specimens
· To evaluate the turn-around time for the assay to assess feasibility of integration into the breast cancer treatment pathway
· To assess the correlation of DDRD assay scores and pathological tumour response
· Exploratory biomarker analysis will be carried out within Queen's University, Belfast, including:
  o correlation of DDRD score with changes in Ki67 protein level after three cycles of chemotherapy
  o exploratory analysis of chemokine expression in peripheral plasma samples, and correlation with pathological response to treatment

Accrual
The study opened in April 2014. Accrual to date is 14 patients.
Title: Scalp cooling alopecia prevention trial (SCALP) for patients with early stage breast cancer

Nangia JR R, Wang T, Rude M, Osborne C, Papish S, Abraham J, Holmes F, Savin M, Paxman R, Hilsenbeck SG G, Osborne CK and Rimawi M. Baylor College of Medicine; US Oncology; Regional Cancer Care Associates; Cleveland Clinic and Paxman Coolers LTD.

Body: Background
Adjuvant chemotherapy treats micro-metastatic disease and decreases the risk of breast cancer recurrence. However, it may be associated with distressing side effects, including alopecia. Women with breast cancer rate chemotherapy-induced alopecia as one of the most severe, troublesome, and distressing side effects of chemotherapy. In many countries, scalp cooling has been introduced to prevent or reduce chemotherapy-induced alopecia. The theory is that scalp cooling causes cutaneous vasoconstriction, which reduces blood flow to the hair follicles during peak plasma concentrations of the chemotherapeutic agents and therefore reduces cellular uptake of these agents. It also results in reduced biochemical activity, which makes hair follicles less susceptible to the damage of the chemotherapy agents. Historically success rates are have been variable, but based on non-randomized studies, scalp cooling appears to be effective in preventing chemotherapy-induced alopecia especially in more recent studies.

Methods
We are conducting a prospective multi-center randomized controlled non-blinded trial to evaluate the safety and efficacy of the Orbis Paxman Hair Loss Prevention System in reducing the incidence of chemotherapy-induced alopecia. Women with stage I-II breast cancer who will receive neoadjuvant or adjuvant anthracycline- or taxane-based chemotherapy, for at least four cycles are eligible. Participants are randomized in a 2:1 ratio to scalp-cooling or no cooling. Scalp-cooling is done using the Orbis Paxman Hair Loss Prevention System prior to, during and after each chemotherapy administration. The primary efficacy endpoints are hair preservation, defined as CTCAE v4 alopecia <2, and device safety. Two hundred and thirty five (235) patients are planned to be enrolled which will provide 85% power to detect a 20% difference in hair preservation, 15% in control group and 35% in scalp-cooling group. Secondary endpoints include: wig/scarf use and quality of life assessed by the EORTC QLQ-30, HADS and BIS. Study participants will be followed for 5 years post-study for time to first recurrence, overall survival, site of first recurrence, and incidence of isolated scalp metastasis.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** OT3-02-09

**Title:** Seraphina – Safety efficacy and patient reported outcomes of advanced breast cancer patients: Therapy management with NAB-paclitaxel in daily routine

Fasching PA A, Wallwiener M, Lux MP P, Mueller V, Schneeweiss A, Tesch H, Brucker SY Y, Haeberle L, Spall T, Belleville E and Lück H-J. University Hospital of Erlangen, Erlangen, Germany; University Hospital of Heidelberg, Heidelberg, Germany; University Hospital of Hamburg-Eppendorf, Hamburg, Germany; National Center for Tumor Diseases, Heidelberg, Germany; Oncology Bethanien, Frankfurt/Main, Germany; University Hospital of Tuebingen, Tuebingen, Germany; ClinSol GmbH&Co KG, Wuerzburg, Germany and GOPH, Hannover, Germany.

**Body:** BACKGROUND

Treatment of patients with advanced breast cancer (ABC) has evolved significantly. Nevertheless, further improvement in ABC treatment is a high medical need. Besides the prolongation of progression free survival (PFS) and overall survival (OS) the major objective of new therapeutic approaches is the enhancement of quality of life (QoL). A recent advance for the treatment of ABC was the development of the cremophor-free albumin-bound paclitaxel, nab-Paclitaxel.

SPECIFIC AIMS/TRIAL DESIGN

The aim of this non-interventional study is the analysis of efficacy and safety data of ABC patients within routine treatment with nab-Paclitaxel. A key focus will be the assessment of patient reported outcomes (PRO), health economic aspects and the influence of breast cancer patient characteristics on prognosis, adverse event frequencies, PRO and therapy decision making. Patients with ABC, who experienced failure of first-line treatment for metastatic disease and for whom standard anthracycline-containing therapy is not indicated, will be followed up under real-life conditions. Sixty sites, equally distributed with regard to their organizational structure (hospital and office based) and medical disciplines (gyneco-oncologists and medical oncologists) will document 1,200 patients. The primary objective is the assessment of PFS under real-life conditions. Secondary objectives include the assessment of overall and breast cancer specific survival, the influence of age on prognosis and QoL, as well as the incidence of (serious) adverse events (AE). PRO including FACT-B, FACT-Taxane, and nab-Paclitaxel treatment specific questions will be collected in a web based application and compared to paper based reporting. Furthermore, biomaterials will be collected to allow translational research projects.

ELIGIBILITY CRITERIA:

Adult women (>18 years) with ABC and treated with nab-Paclitaxel.

STATISTICAL METHODS/TARGET ACCRUAL:

In Germany nab-Paclitaxel is indicated for patients with metastatic breast cancer after failure of a previous therapy in ABC. In this therapeutic setting several studies have shown high efficacy and acceptable toxicity. However, populations within clinical trials are selected and may be different from the general patient population in clinical practice. Therefore this study aims at the capture of PFS, PRO and AE in the general population for which nab-Paclitaxel is used in clinical practice. Nab-Paclitaxel treatment will be documented over a period of up to 6 months, followed by a 30 months progression/ survival follow-up. Target accrual is 1,200 patients. We assume that at most 10% are lost to follow-up before the median survival time is reached. Kaplan-Meier curves will be calculated, especially the median survival time with 95% confidence interval.
Title: Nordic trip, a randomized phase 3 study in early triple negative breast cancer

Loman N, Linderholm B, Joensuu H, Ejlertsen B, Johannsson OT T, Geisler J, Bendahl P-O, Ahlgren J and Lindman H. Skane University Hospital, Lund University, Lund, Sweden; Sahlgrenska Academy and University Hospital, Gothenburg, Sweden; Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland; Rigshospitalet, DBCG Secretariat, Copenhagen, Denmark; Landspitali, University Hospital, Reykjavik, Iceland; Akershus University Hospital, Lørenskog, Norway; Örebro University Hospital, Örebro, Sweden and Akademiska Hospital, Uppsala University, Uppsala, Sweden.

Background: Adjuvant treatment among the heterogeneous group of triple negative breast cancers (TNBC) is still a challenge. Recently published data indicate that platinum salts may have a role in the treatment of TNBC (v Minckwitz 2014), especially in BRCA-associated cases (Tutt 2014). Subgroup analyses from two large adjuvant studies indicate that also oral capecitabine may add a significant value in this subset of BC (Lindman 2010, O'Shaughnessy, 2010).

As a planned collaboration between the nordic countries, we are currently preparing a three-armed randomized phase 3 study of the addition of carboplatin and capecitabine respectively to a chemo backbone consisting of a taxane-antracycline based treatment in both the adjuvant and the neoadjuvant setting.

Objectives: The primary endpoint will be invasive disease-free survival (IDFS). Secondary endpoints will include IDFS in subsets of TNBC, eg according to gene-expression-based subtypes of TNBC (Lehmann 2011), and BRCA-mutation status. In the cohort treated neoadjuvant, biomarkers predicting pathological response will be identified with the purpose to validate them in the adjuvant cohort.

Method: 1800 patients (pts) with early TNBC stage 1 (>10 mm) - 3 will be randomized 1:1:1 to three treatment arms.
A: nab-paclitaxel (nP) followed by epirubicine-cyclophosphamide (EC)
B: nP-capecitabine followed by EC-capecitabine.
C: nP-carboplatin followed by EC
Treatment will be given either in the adjuvant or in the neoadjuvant setting, the primary endpoint will include all randomized pts from both cohorts.

Statistical considerations: We assume a 75% five-year IDFS with standard treatment (A), and that a relative reduction of the event incidence of 30% for each of the two experimental treatments (B and C) would be a clinically relevant finding (HR=0.7). In order to achieve 80% power at a 0.05 significance level for each of the two comparisons A vs. B and A vs. C, 600 patients need to be evaluated in each of the three treatment arms. Stratification factors: pre- vs postoperative treatment, nodal status and treatment site.
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

Smith J, O'Shaughnessy J, Song J and Berrak E. US Oncology, The Woodlands, TX; Baylor Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX and Eisai Inc, Woodcliff Lake, NJ.

Body: Eribulin mesylate, a mitotic inhibitor, is indicated 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 d1 and 8 of a 21d cycle. A modified biweekly dose regimen—which may improve safety profile without compromising efficacy—is also being explored.

This presentation describes an ongoing phase 2, open-label, single-arm, multicenter study of eribulin (1.4 mg/m²) administered intravenously (IV) biweekly (d1 and 15) in 28d cycles for the treatment of pts with human epidermal growth factor receptor (HER)2-negative MBC previously treated with 2–5 chemotherapy regimens.

Approximately 58 female pts (aged ≥18 yrs) will be enrolled to have 55 evaluable pts; accrual has not yet commenced. Pts with ≥1 measurable lesion ≥10 mm in longest diameter (nonlymph node) or ≥15 mm in short-axis diameter (lymph node) with an Eastern Cooperative Oncology Group performance status ≤2 and life expectancy ≥3 months will be included. Exclusion criteria include treatment with chemotherapy, radiation, biological, or targeted therapy within the last 2 weeks (or 5 × half-life); existing anticancer-therapy–related toxicities of grades ≥2 (except alopecia); and prior malignancy other than carcinoma in situ of the cervix or nonmelanoma skin cancer (unless prior malignancy was treated >5 yrs ago with no evidence of recurrence). Pts will receive treatment as long as clinical benefit is demonstrated or until intercurrent illness, unacceptable toxicity, or disease progression.

The study consists of 3 phases: a screening phase, treatment phase (estimated median treatment duration 5 months), and posttreatment survival follow-up phase. The study is divided into 2 stages (Simon 2-stage design): stage 1 (n=15 evaluable pts who have completed 3 cycles of treatment) allows for an interim analysis of efficacy results to end the trial early in the case of low anticancer activity (<1 responder [objective response rate; ORR] and <8 responders [disease control rate; DCR]). Otherwise, the study will proceed to Stage 2 (40 evaluable pts). Final analysis will take place after all ongoing pts complete ≥5 cycles of treatment or discontinue from treatment and ≥75% pts experience disease progression or death.

The primary objective is to evaluate efficacy in terms of ORR and DCR. A modified Simon 2-Stage Design will be used in hypothesis testing for these endpoints as well as the stage 1 interim futility analysis. Secondary objectives include evaluation of progression-free survival (PFS), overall survival (OS), safety, and tolerability. PFS and OS will be analyzed using Kaplan–Meier product-limit estimates. Median PFS, OS, and cumulative probability of PFS and OS at 3, 6, and 12 months will be calculated with 2-sided 95% confidence intervals, if estimable. The final analysis thresholds for success are defined as ≥7 responders by ORR or ≥32 responders by DCR out of 55 evaluable pts.
Title: OPTIMA (optimal personalised treatment of early breast cancer using multi-parameter analysis), a prospective trial to validate the predictive utility and cost-effectiveness of gene expression test-directed chemotherapy decisions

Stein RC, Marshall A, Hall PS, Bartlett JMS, Rooshenas L, Campbell A, Cameron DA, Rea D, Macpherson I, Earl HM, Poole CJ, Francis A, Morgan A, Harmer V, Pinder SE, Stallard N, Donovan J, Huime C, McCabe C, Hughes-Davies L, Makris A and Dunn JA. National Institute for Health Research University College London Hospitals Biomedical Research Centre, London, United Kingdom; University of Warwick, Coventry, United Kingdom; University of Edinburgh, Edinburgh, United Kingdom; Ontario Institute for Cancer Research, Toronto, ON, Canada; University of Bristol, Bristol, United Kingdom; Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, United Kingdom; Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom; University of Cambridge, Cambridge, United Kingdom; University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; Independent Cancer Patients' Voice, London, United Kingdom; Imperial College Healthcare Trust, London, United Kingdom; King's College London, London, United Kingdom; Academic Unit of Health Economics, Leeds Institute of Health Sciences, University of Leeds, Leeds, United Kingdom; University of Alberta, Edmonton, AB, Canada; Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom and Mount Vernon Cancer Centre, East and North Hertfordshire NHS Trust, Middlesex, United Kingdom.

Body: Background: Multi-parameter gene expression assays (MPAs) are widely used to estimate individual patient residual risk and to guide chemotherapy use in hormone-sensitive HER2-negative node-negative early breast cancer. These uses of MPAs have not yet been prospectively validated. OPTIMA aims to validate the use of MPA testing to predict 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 preliminary phase (OPTIMA prelim) evaluated the performance of MPAs to identify a suitable test(s) to be used in the main efficacy trial and assessed the feasibility and acceptability of a large UK trial. Eligible patients are men or women aged 40 years or older who have surgically resected early stage breast cancer, which is ER-positive and HER2-negative and who have either 1-9 involved axillary lymph nodes or tumors of at least 30mm diameter. Randomization is to standard management (chemotherapy followed by endocrine therapy) or to MPA-directed treatment. Those with a tumor categorized as "high-risk" by the test will be assigned to standard management whilst those at "low-risk" will be treated with endocrine therapy alone. OPTIMA prelim used Oncotype DX as the primary discriminator; the main trial will use Prosigna (PAM50). The co-primary outcomes are (1) Invasive Disease Free Survival (IDFS) and (2) cost-effectiveness of test-directed therapy compared to standard practice. Secondary outcomes include IDFS in "low-risk" patients, distant disease free survival, breast cancer specific survival, overall survival and quality of life. 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 over 4 years 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. It confirmed the acceptability of randomization to patients with a 47% acceptance rate, and to clinicians and hence the feasibility of a large prospective trial of test-directed treatment running in 100-plus UK sites. It showed that investment into research on test-directed therapy, especially with Prosigna, should be of substantial value to the NHS.

Conclusion: OPTIMA, as one of two large scale prospective trials validating the use of test-guided chemotherapy in node-positive hormone-sensitive early breast cancer will have a global impact on patient treatment. Recruitment into the main efficacy trial will commence in October 2015.

Funding: Project funded by the UK 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.
Randomized phase 3 study of a novel, long-acting G-CSF (SPI-2012) versus pegfilgrastim in the management of chemotherapy-induced neutropenia in early-stage breast cancer patients receiving docetaxel and cyclophosphamide (TC) (ADVANCE study)

Schwartzberg L, Vacirca JL, Hager SJ, Adoo CS, Ibrahim EN, Bhat G, Choi MR, Allen LF, Tedesco KL and Agajanian R. The West Clinic, Memphis, TN; North Shore Hematology/Oncology Associates, East Setauket, NY; California Cancer Associates for Research and Excellence, Fresno, CA; Arizona Center for Cancer Care, Glendale, AZ; Beaver Medical Group, Highland, CA; Spectrum Pharmaceuticals, Irvine, CA; New York Oncology Hematology (US Oncology/Mckesson Specialty Health), Albany, NY and The Oncology Institute of Hope and Innovation, Downey, CA.

Background: SPI-2012 is a distinct biologic that uses the innovative, proprietary long-acting protein/peptide discovery technology (LAPSCOVERY™) to enhance the activity of G-CSF. SPI-2012 consists of a novel, modified recombinant human G-CSF conjugated to the Fc fragment of IgG4 via a polyethylene glycol linker to produce a new, more potent, longer-acting G-CSF with a potentially unique distribution to areas rich in Fc receptors. The primary endpoint of this Phase 3 study is to compare the efficacy of a single dose of SPI-2012 with pegfilgrastim in patients with early-stage breast cancer (ESBC) receiving TC chemotherapy, as measured by the Duration of Severe Neutropenia (DSN) in Cycle 1. Key secondary objectives include the comparison of SPI-2012 with pegfilgrastim during Cycle 1 in: Time to Absolute Neutrophil Count (ANC) Recovery; Depth of ANC Nadir and Incidence of Febrile Neutropenia. Safety and pharmacokinetics will also be assessed.

Trial Design: This is a randomized, open-label, active-controlled, multicenter study comparing the efficacy and safety of SPI-2012 to pegfilgrastim. Patients (n=506) will be randomized in a 1:1 ratio to receive either SPI-2012 (equivalent to 3.6 mg G-CSF) or pegfilgrastim (equivalent to 6.0 mg G-CSF) once per chemotherapy cycle (up to 4 cycles), approximately 24 hrs after chemotherapy.

Eligibility Criteria: This study will enroll histologically confirmed ESBC patients who are eligible to receive adjuvant or neoadjuvant TC chemotherapy and at least 18 years of age, with adequate hematologic, renal and hepatic function. Patients will be excluded if they have active concurrent malignancy or life-threatening disease; a known sensitivity or previous reaction to E. coli derived products or any of the products to be administered during study participation; concurrent adjuvant cancer therapy; locally recurrent/metastatic or contralateral breast cancer; previous exposure to filgrastim, pegfilgrastim, or other G-CSF products in clinical development prior to the administration of study drug; bone marrow or hematopoietic stem cell transplant or radiation therapy prior to enrollment or are pregnant or breast-feeding.

Statistical Methods: The goal of the study is to demonstrate non-inferiority of SPI-2012 to pegfilgrastim. For the Primary Efficacy Analysis, the mean DSN in Cycle 1 will be compared between the SPI-2012 and pegfilgrastim treatment arms. A 2-sided 95% confidence interval (CI) of the difference between the mean DSN of the SPI-2012 arm and the mean DSN of the pegfilgrastim arm will be calculated using bootstrap resampling with treatment as the only stratification factor. For the Secondary Efficacy Analyses, the results will each be summarized by treatment arm and Cycle. The two-sided 95% CI for the difference between the treatment arms will be calculated.

Target Accrual: Approximately 506 pts.
**Title:** A phase 1 study in patients with metastatic breast cancer to evaluate the feasibility of magnetic resonance imaging with ferrumoxytol as a potential biomarker for response to treatment with nanoliposomal irinotecan (nal-IRI, MM-398)


**Body:**

**Background:** Nal-IRI (MM-398, nanoliposomal irinotecan) is designed for extended circulation relative to free irinotecan and to exploit leaky tumor vasculature for enhanced drug delivery to tumors. Tumor deposition of nal-IRI and subsequent conversion to SN-38 in both neoplastic cells and tumor associated macrophages (TAM) may positively correlate with response to therapy. In phase I studies of nal-IRI, activity has been shown in metastatic breast cancer (MBC), pancreatic and colorectal cancer. Ferumoxytol (FMX) is an iron-oxide superparamagnetic nanoparticle that has been used off-label for its MRI contrast properties. FMX has long-circulating pharmacokinetics and is taken up by TAMs with similar distribution patterns to nal-IRI in preclinical models. A single site pilot study established the feasibility of performing quantitative FMX MRI. Thirteen patients with advanced cancer (3 with ER/PR+ MBC) were imaged with FMX MRI and treated with nal-IRI. Median tumor lesion FMX uptake in the pilot study was 32.6 and 34.5 µg/mL at 1 h and 24 h, respectively. Lesions with FMX uptake above the median were associated with greater reductions in tumor size following treatment with nal-IRI as determined by CT lesion measurements. The relationship between FMX levels in tumor lesions and nal-IRI activity may serve as a potential biomarker for nal-IRI deposition and response in solid tumors. This study has been expanded to include additional MBC patients to further evaluate the technical feasibility of FMX MRI at multiple study sites, and to evaluate activity of nal-IRI in patients with MBC.

**Trial Design:** Three cohorts of 10 patients with MBC in the following categories will be enrolled: ER and/or PR positive/HER2-negative, triple negative (TNBC) and MBC with brain metastases. An imaging phase will be followed by a treatment phase. The imaging phase consists of a baseline MRI scan, FMX infusion, and follow-up MRI scans at 1-4 and 24 h after infusion. The treatment phase begins 1-6 days after imaging and consists of nal-IRI 80 mg/m² q2w. A pretreatment biopsy is required for correlative studies.

**Study Objectives:** The primary objective of this multisite expansion is to investigate the feasibility of FMX quantitation in tumor lesions at multiple lesion sites in breast cancer. The secondary objective is to characterize the efficacy of nal-IRI in patients with metastatic breast cancer.

**Eligibility Criteria:** The key inclusion criteria include patients with MBC, ECOG 0 or 1 with adequate bone marrow reserve and no prior topoisoomerase 1 inhibitor or anti-VEGF treatment. ER and/or PR positive/HER2-negative and TNBC patients must have had 1-3 prior lines of chemotherapy in the metastatic setting and have at least 2 measurable lesions. Patients with brain metastasis must be neurologically stable and have new or progressive brain metastases after prior radiation therapy with at least one lesion measuring ≥ 1 cm in longest diameter on gadolinium-enhanced MRI.

**Status:** This trial is currently recruiting patients.
Body: PURPOSE: Increasing Black patients’ participation in cancer clinical trials is particularly important because of the population's lower survival rate. Accrual to clinical trials remains low among the general population (1-3%), with recruitment of Blacks the lowest of all groups at 0.5-1.5%. INSPIRE-BrC aims to increase trial participation rates among Black breast cancer patients and examine the relationship between the intervention and attitudes/beliefs on the decision to participate. METHODS: The study was approved by the Institutional Review Board at all sites and opened in March 16, 2014. Black breast cancer patients at five MedStar sites will view a 15 minute, culturally-tailored video about clinical trials, which targets six cultural and attitudinal barriers to participation. A pre-test/post-test method is used to determine the impact of the video on three variables -- likely participation in therapeutic clinical trials; attitudes toward therapeutic clinical trials (assessed based on the 6 barriers); and actual trial enrollment. Participants are followed for six months after participating in the intervention to assess clinical trial enrollment status. ELIGIBILITY CRITERIA: The study includes patients with invasive breast cancer, who are Black or African American and have never participated in a therapeutic clinical trial. Participants can be male or female and must be 18 years or older. EXPECTED FINDINGS: We hypothesize that the intervention will increase clinical trial enrollment compared to our 2012 clinical trial enrollment baseline rate of 6% (22/384) for Black breast cancer patients in five MedStar hospitals. The primary outcome measure is the proportion of Black breast cancer patients who agree to participate in a therapeutic clinical trial among those who sign consent to INSPIRE-BrC. Study findings have the potential to increase patient recruitment as a promising tool for rapid dissemination of a theory-driven, evidence-based model to enhance clinical trial accrual among Black cancer patients. STATISTICAL METHODS: Bivariate associations between enrollment status and patient-level characteristics will be examined by t-tests for continuous variables and chi-square tests for categorical variables. Paired t-tests and McNemar's test for paired proportions will be used to measure the changes in attitudes and beliefs, measured based on pre-post test. Multiple logistic regression models will be used to predict enrollment status including many factors identified in the literature as predictors, which are, but not limited to age, gender, SES, insurance status, and stage. ACCRUAL: As of June 2, 2015, the total accrual across all five sites is 177 patients.

INSPIRE enrollments as of June 2, 2015

| MedStar Franklin Square Medical Center | 10 |
| MedStar Georgetown University Hospital | 16 |
| MedStar Harbor Hospital | 12 |
| MedStar Union Memorial Hospital | 19 |
| MedStar Washington Hospital Center | 120 |
| Total | 177 |

The accrual goal is 200 patients. We anticipate meeting this goal in July 2015, and analyzing the data in January 2016.

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Assessment of cancer Concerns at the End of treatment (ACE): What do survivors need and want?


Body: Discussion:
There are over 14 million cancer survivors in the United States today and this population will continue to grow. Not only are survivors at risk for recurrence, but increasingly complex treatments place them at risk for a range of long-term and late effects. Provision of survivorship care plans (SCPs) at an end of treatment (EOT) visit that includes referrals to appropriate resources may be assumed to improve the care provided to survivors. There are, however, few relevant studies examining the outcomes of providing patients with referrals to resources identified through the completion of patient reported outcomes (PROs) reported in the literature. Current research has focused on documenting the frequency with which SCPs are provided and the perceived usefulness of these documents. Such data are a necessary but an insufficient step in the creation of practice guidelines in part guided by PROs, or standards of care that support the provision of SCPs. This study will provide a comprehensive description of breast cancer (BC) survivors' symptom burden and other concerns, as well as health behaviors as they enter the survivorship phase of care. These items will be evaluated by data generated by a pre visit PROs questionnaire completed through the patient portal. Significant symptoms and concerns will be addressed during a clinical encounter at the EOT visit. It will also describe referrals triggered by these data.

Trial Design:
Quasi-experimental single-group design with historical controls. Our study describes patient-reported symptom burden, desire for assistance, quality of life (QoL) and health behaviors of BC survivors who have completed initial treatment using a web-based platform to collect PRO data. As well, it estimates differences in referral and uptake between participants and historical controls.

Eligibility Criteria:
Survivors must understand English, have internet access and a working email address, and be age 18 years or older within 1 year of completing initial treatment (chemotherapy, radiotherapy, and/or surgery). Participants may still be receiving hormonal or targeted therapy.

Specific Aims:
1) Describe physical and psychosocial PROs of BC survivors after completion of treatment so as to define targets and develop metrics for future intervention; 1A) examine diagnosis and treatment variables that moderate PROs; 2) determine provider satisfaction with a web based patient questionnaire that includes a summary of significant patient concerns; 3) estimate the impact of providing the summary of patient concerns on utilization of/referral to available services relative to historic controls.

Statistical Methods:
Parameter estimates with confidence intervals for concerns and desire for help. Bivariate associations between unmet needs and quality of life. Non-parametric comparison of referral patterns between ACE participants and historical controls.

Present Accrual and Target:
To date 107 BC survivors have consented to complete a self-report survey questionnaire prior to their scheduled EOT visit, 61 completed the survey, 28 pending a visit. Target 250.
2015 San Antonio Breast Cancer Symposium

Publication Number: OT3-03-03

Title: A prospective study of glycoprotein 88 (GP-88) blood test in healthy women undergoing screening for breast cancer (BC) with mammography (MM)

Tkaczuk KHR HR, Campassi C, Kesmodel S, Bellavance E, Rosenblatt P, Nichols E, Feigenberg SJ J, Coughlin P, Drogula C, Urban B, Galandak J, Dromi S, Kuo L, Yue B, Hicks D and Serrero G. University of Maryland, Baltimore, MD; A&G Pharma, Columbia, MD and University of Maryland Baltimore Washington Medical Center, Glen Burnie, MD.

Body: Background: Population based BC screening with XRAY mammography (MM) has been widely accepted as standard of care for women aged 40+ with average risk of developing BC. Sensitivity and specificity of MM is dependent on breast tissue density and up to ~20% of BC are undetected by MM. The development of a dependable, low cost blood-based BC screening test to increase the sensitivity and specificity of currently existing BC screening methods is needed.

Rationale: GP88 is expressed & secreted by BC cells & is not expressed by normal mammary epithelial cells, 2 retrospective randomized multi-site trials (a training study & a validation study of 300 cases each) demonstrated that elevated GP88 expression in estrogen positive (ER+) invasive BC was statistically correlated with a 4-fold increase in the risk of 5-yr BC recurrence. GP88 was an independent predictor of BC recurrence in multivariate analysis of other factors such as PR expression, tumor size, grade, lymph node status & stage. The quantitative GP88 EIA was developed to determine the amount of GP88 in biological fluids. The blood based EIA assay is highly specific for GP88 & both sensitive & linear over a wide dynamic range, i.e. detection of GP88 concentrations from 0.1 to 20ng/ml. A baseline GP-88 level of 28.4 ± 5 ng/ml was established by us for healthy volunteers (HV). In BC pts a statistically significant increase of serum GP88 was observed in early stage pts (40.7 ± 16 ng/ml; p=0.007). Stratification of BC pts according to their clinical outcomes shows that pts having no evidence of disease (NED) have serum GP88 levels within the range of HV. These data suggest that pts with breast tumors express & secrete high levels of GP88.

Objectives: 1. To determine prospectively GP-88 blood levels in HV at average risk of developing BC screened by MM & in women with recently biopsy-confirmed BC. 2. To establish the statistical distribution of GP88 serum levels in subjects by baseline BIRAD classification (1-6). 3. To determine if the initial GP88 level is predictive of change in BIRADS classification from baseline to 12-mos follow-up. 4. To determine if baseline GP88 level is predictive of the appearance of BC at 12 mos follow-up in HV who were cancer-free at study entry.

Inclusion Criteria: Female, aged >=40 yrs old, presenting for screening or diagnostic MM or diagnostic workup and/or biopsy due to abnormal MM <= 10 wks before study entry.

Study procedures: Serum levels of GP88 in subjects with average BC risk factors will be measured prospectively at baseline; 3-6 mos & 6-12 mos & correlated with BIRADS reading of the screening MM, BIRADS 1-6; GP88 serum level will be correlated with pathologic results of breast biopsies performed on subjects with suspicious BIRADS (4 & 5) MM & final pathologically confirmed diagnosis of breast cancer as BIRADS 6.

Study Progress: The study is ongoing; currently we have 308 subjects enrolled, the total number of subjects will be up to 725 & screened up to 1400. Study is UM IRB approved & is conducted at the University of Maryland Medical Center (UMMC) and UM Baltimore Washington Medical Center (BWMC). Funding is provided by Maryland Industry Partnership Grant (MIPS)& Avon Grant No. 02-2013-018.
NRG-BR002: A phase IIIR/III trial of standard of care therapy with or without stereotactic body radiotherapy (SBRT) &/or surgical ablation for newly oligometastatic breast cancer

Chmura SJ J, Winter KA A, Salama JK K, Woodward WA A, Borges VF F, Al-Hallaq H, Matuszak M, Jaskowiak NT T, Milano MT T, Bandos H and White JR R. University of Chicago; NRG Oncology Statistics and Data Management Center; Duke University Medical Center; M.D. Anderson Cancer; University of Colorado; University of Michigan; University of Rochester; University of Pittsburgh and Ohio State University.

Body: Background:
The current standard of care for metastatic breast cancer patients (pts) is to deliver palliative chemotherapy, biologic &/or hormonal therapy when appropriate, with radiation &/or surgery reserved for the management of symptomatic or non-responsive (mets). For selected pts with limited metastatic (met) disease, mets-directed ablative therapy, with either surgical resection or high dose conformal radiotherapy (in addition to standard systemic therapies) to lung, liver, CNS adrenal, & multiple organs has been shown to result in long-term disease control numerically superior to systemic therapy alone. NRG-BR002 is a randomized Phase II trial to evaluate stereotactic body radiotherapy (SBRT) &/or surgical resection (SR) of all met sites in newly oligometastatic breast cancer in addition to standard systemic therapy.

Trial Design & Eligibility
For the Ph IIIR, eligible breast cancer pts who have received up to 6 months of first line systemic therapy without progression will be randomized to receive either standard systemic therapy with mets directed therapy as needed (control arm) versus ablative therapy of all met sites with either body SBRT &/or surgical resection (SR) (per the treating physician discretion) to determine if there is an improvement median progression free survival (PFS). If this aim is met the trial continues as a Ph III to evaluate if SBRT/SR improves 5 year overall survival. Secondary aims include local control in the met site, new distant met rate, & technical quality. The primary translational endpoint tests whether < 5 CTCs (per 7.5ml of blood) is an independent prognostic (outcome) marker for improved PFS & OS in oligometastatic breast cancer.

Women with pathologically confirmed met breast cancer to < /= 2 sites, with up to 6 months of standard first line systemic therapy & the primary site disease is controlled. CNS mets are ineligible. ER/PR & HER-2 neu are required on either the primary or met site. Site radiation credentialing & a facility questionnaire are required. Randomization is to standard systemic therapy with local radiotherapy/ surgery for palliation when necessary vs ablative therapy of all mets with SBRT &/or SR.

Statistics:
For the Ph IIIR portion to detect a signal for improved median PFS from 10.5 months to 19 months with 95% power & accounting for ineligible/lost pts, 146 pts will be required. For the Ph III, an additional 246, for a total of 402 pts will be required to definitively determine if ablative therapy improves 5-year overall survival from 28% to 42.5% (HR=0.67), with 85% power & a 1-sided type I error of 0.025. For the translational research, the number of pts accrued in the Ph IIIR & Ph III portions will provide sufficient power ≥ 91% to detect whether < 5 CTC's is a prognostic marker for improved PFS & OS.

Present Accrual & Target Accrual
NRG BR002 activated 12/24/2014 with a target accrual of 146 pts for the Ph II component & 256 additional for the Ph III.

Contact Information:

Support:
Supported by NRG Oncology grants U10CA180868 & U10CA180822 from the National Cancer Institute (NCI).
Title: Real-time imaging of lymph node metastasis in response to systemic ezrin inhibitor treatment in breast cancer

Ghaffari A, Hoskin V, Mullins G, Greer P, Kiefer F, Madarnas Y, SenGupta S and Elliott B. Queen's University, Kingston, ON, Canada; Queen's University, Kingston, ON, Canada; Cancer Research Institute, Queen's University, Kingston, ON, Canada and Max-Plank Institute for Molecular Biomedicine, Muster, Germany.

Body: Lymph node (LN) metastasis is a key driver of recurrence and survival in breast cancer (BC) patients. However, the mechanisms of metastatic dissemination of tumour cells from LNs to distant sites and their predictors of response to systemic therapy remain poorly understood, mainly due to a lack of non-invasive in vivo imaging models. We have recently described ezrin, a pro-metastatic crosslinker protein, as a regulator of tumour lymphangiogenesis and metastasis in BC (Breast Cancer Res. 2014; 16(5): 438). Furthermore, we demonstrated significant association of high ezrin expression with lymphovascular invasion in a cohort (n=63) of premenopausal patients with invasive BC (p =0.024). These findings prompted us to examine the role of ezrin in migration and invasion of metastatic tumour cells in LNs and their response to ezrin-targeted therapy. Using a locally accrued LN positive patient cohort (n=94), we demonstrated a significant association between high ezrin levels and reduced recurrence-free survival (univariate Log-rank test, p=0.033), suggesting that ezrin is a potential predictor of relapse in LN positive BC. To address the mechanistic role of ezrin in LN metastasis, we developed a novel intravital imaging model using a lymphatic reporter transgenic mouse (B6-prox1-mOrange2-pA-BAC) to examine the response of tumour-draining LN to anti-ezrin systemic therapy in real time. Next, we tested the effects of a small molecule ezrin inhibitor (NSC668394) in vitro and observed significant suppression of ezrin activation (p-T567) and cancer cell invasive phenotype. Intravital imaging of inguinal LN metastases, derived from subcutaneously implanted breast adenocarcinoma E0771-LMV (lung metastatic variant) cells, demonstrated significant reduction in mobility and invasiveness (Mann Whitney, p<0.0001) of metastatic cells following systemic treatment with NSC668394 (0.5 mg/kg at 24h and 8h prior to imaging). Interestingly, LN metastases engagement by host T cell (CD3+) was notably increased, whereas T cell mobility was not affected by ezrin inhibition. Our findings present a novel non-invasive imaging model to study the LN metastasis response to anti-cancer therapy in real time, and provide new insight into the role of ezrin as a potential anti-metastatic target in BC.

(Supported by CRS, CIHR, CBCF, BCAK, Queen's SRC).
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-01-02

**Title:** Multimodality molecular imaging with dynamic 18F-fluorodeoxyglucose positron emission tomography (FDG PET) and MRI to evaluate response and resistance to neoadjuvant chemotherapy (NAC)


**Body:** Background: Using quantitative FDG PET to measure glucose metabolism and perfusion, and dynamic contrast-enhanced (DCE) MRI to measure perfusion, we previously identified a metabolic signature for breast cancer resistant to NAC. This imaging signature is (1) persistent or increased tumor perfusion despite treatment, (2) an altered pattern of glucose kinetics in response to therapy, and (3) pre-therapy mismatch between tumor metabolism (MRFDG) and glucose delivery (K1) (high ratio of MRFDG/K1). These patterns predict poor response, early relapse and death independent of established prognostic factors, including pathologic response. Identification of factors associated with resistance or response to therapy is the translational goal of "Quantitative Dynamic PET and MRI in Breast Cancer Therapy," part of the Seattle Breast SPORE (1P50CA138293).

Methods: Patients (Pts) undergoing NAC for histologically confirmed breast cancer (stage II-III) were approached for this trial (CCIRB# 7587). FDG PET and DCE-MRI were obtained pre-therapy, 2-12 weeks after start of NAC (mid-therapy) and after completion of NAC. Breast biopsies were obtained pre-therapy and post-NAC. FDG PET included a dynamic scan with kinetic analysis. PET measures included SUVmax, MRFDG, K1, Ki, and Patlak. 3T DCE-MRI measurements included semi-quantitative vascular parameters of peak enhancement (PE), signal enhancement ratio (SER), washout fraction, functional tumor volume, and apparent diffusion coefficient (ADC) from diffusion-weighted MRI (DWI). Breast biopsies were assayed by immunohistochemistry and gene expression profiling. NAC was per physician's choice with most pts receiving weekly paclitaxel (with trastuzumab if HER2+) followed by doxorubicin/cyclophosphamide.

Results: 32 pts have completed the study. Pathologic complete response (pCR), defined as absence of invasive cancer in the breast, was observed in 9 (28%); near pCR defined as only microscopic residual invasive cancer in 3 (9%) more pts. Mid-therapy decline in SUVmax and K1 was associated with near pCR; (p-value 0.06, 0.04, respectively). Pre-therapy PET measures of MRFDG and K1 were not predictive of pCR. On MRI, pre-therapy PE (p=0.009), SER (p=0.01), washout fraction (p=0.02), ADC (p=0.08, trend) and mid-therapy change in volume (p=0.05) were each predictive of pCR. Gene profiling of pre-therapy biopsies showed correlation between high MRFDG/K1 ratio in basal and luminal B tumors.

Conclusions: Assessment of serial changes in tumor metabolism and perfusion by FDG PET and DCE-MRI is feasible in the clinic. Mid-therapy decline in metabolism and glucose delivery was predictive of pCR; consistent with prior retrospective series. Baseline DCE-MRI and DWI measures show promise to predict response, and associations of mid-therapy change in MR functional tumor volume with pCR agree with findings of another multisite clinical trial (ISPY). These imaging parameters may serve as useful biomarkers to inform future neoadjuvant trials. Integration of imaging data with gene expression profiling revealed that the pattern of metabolism in luminal B tumors was closer to that of the basal subtype compared to other ER-positive tumors.
Title: Stratifying triple-negative breast cancer prognosis using 18F-FDG-PET/CT imaging

Yue Y, Cui X, Bose S, Audeh W, Zhang X and Fraass B. Cedars-Sinai Medical Center, Los Angeles, CA.

Body: Introduction: Triple-negative breast cancer (TNBC) is a highly diverse group of cancers, and may benefit from molecular-targeted therapies. This study aims to stratify prognosis of TNBC patients using pre-treatment 18F-FDG PET/CT, alone and with correlation to immunohistochemistry biomarkers.

Method: 200 consecutive TNBC breast cancer patients treated between 2008 and 2012 who received lumpectomy or mastectomy as primary treatment were retrieved. Among the full cohort, 79 patients had pre-treatment 18F FDG PET/CT scans. Immunostaining status (percentage and intensity) of basal biomarkers (EGFR, CK5/6), Ki-67, P53, and other clinicopathological variables (age, tumor size, pathological T/N stage, nuclear grade, and lymph node metastasis) were obtained. Three PET image features were evaluated: maximum uptake values (SUVmax), mean uptake (SUVmean) and target volume (SUVvol) defined by SUV>2.5. The relationships among tumor metabolic activities and clinicopathological factors were evaluated. All variables were analyzed versus disease-free survival (DFS) using univariate and multivariate Cox analysis, Kaplan-Meier curves and log-rank tests. The optimal cutoff points of variables were estimated using time-dependent survival receiver operating characteristic (ROC) analysis.

Results: All PET features significantly correlated with proliferation marker Ki-67 (all p<0.010). SUVmax stratified the prognosis of TNBC patients with optimal cutoff derived by ROC analysis (≤3.5 vs >3.5, AUC=0.654, p=0.006). Basal biomarkers EGFR and CK5/6 and image features SUVmax, SUVmean, SUVvol were significant associated with DFS in univariate Cox analysis, whereas SUVmax (p=0.001) and EGFR (p=0.001) were also significant in multivariate Cox analysis. To integrate prognosis of biological and imaging markers, patients were first stratified by EGFR into low (≤15%) and high (>15%) risk groups. Further, SUVmax was used as a variable to stratify the two EGFR groups. In the high EGFR group, patients with high FDG uptake (SUVmax>3.5) had worse survival outcome (median DFS=7.6 months) than those patients with low FDG uptake (SUVmax≤3.5, median DFS=11.6 months). In the low EGFR group, high SUVmax also indicated worse survival outcome (17.2 months) than low SUVmax (22.8 months). The risk stratification with integrative EGFR and PET was statistically significant with log-rank p<<0.001.

Multivariate Cox analysis for disease-free survival

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathology, T stage, ≤ 3 vs &gt;3</td>
<td>2.337(0.428-7.384)</td>
<td>0.148</td>
</tr>
<tr>
<td>EGFR, ≤15% vs &gt; 15%</td>
<td>9.109(1.997-41.55)</td>
<td>0.004</td>
</tr>
<tr>
<td>CK5/6, ≤ 50% vs &gt; 50%</td>
<td>1.471(0.598-3.614)</td>
<td>0.401</td>
</tr>
<tr>
<td>SUVmax, ≤3.5 vs &gt; 3.5</td>
<td>3.883(1.13-13.32)</td>
<td>0.031</td>
</tr>
</tbody>
</table>

TNBC patient risk groups stratified by EGFR and SUVmax (with the median values of variables)

<table>
<thead>
<tr>
<th>Risk groups (EGFR&gt;15, SUVmax&gt;3.5)</th>
<th>patient#</th>
<th>DFS months</th>
<th>EGFR %</th>
<th>SUVmax</th>
<th>SUVmean</th>
<th>SUVvol</th>
<th>Ki-67%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (-, -)</td>
<td>12</td>
<td>22.8</td>
<td>5</td>
<td>2.0</td>
<td>0.6</td>
<td>0.2</td>
<td>34</td>
</tr>
<tr>
<td>2 (-, +)</td>
<td>15</td>
<td>17.2</td>
<td>5</td>
<td>8.9</td>
<td>4.3</td>
<td>7.2</td>
<td>67</td>
</tr>
<tr>
<td>3 (+, -)</td>
<td>13</td>
<td>11.6</td>
<td>50</td>
<td>2.7</td>
<td>2.6</td>
<td>0.9</td>
<td>35</td>
</tr>
<tr>
<td>4 (+, +)</td>
<td>37</td>
<td>7.6</td>
<td>60</td>
<td>11.3</td>
<td>5.2</td>
<td>10.9</td>
<td>60</td>
</tr>
</tbody>
</table>
Conclusions: Pre-treatment 18F-FDG PET/CT imaging has significant prognostic value for predicting survival outcome of TNBC patients. Integrated with basal-biomarker EGFR, PET imaging can further stratify patient risks in the pre-treatment stage, and help select appropriate treatment strategies for individual patients.
Title: uPAR PET imaging in breast cancer: First-in-humans studies using 64Cu-DOTA-AE105 and 68Ga-NOTA-AE105


Body: Objective
The urokinase-type plasminogen activator receptor (uPAR) is a well-established prognostic biomarker in many cancer types including breast cancer (BC). Numerous studies using immunohistochemically evaluation of uPAR expression in tissue samples from BC patients have shown that not only is uPAR consistently overexpressed, but also carries strong prognostic value and is associates with overall survival. Accordingly, uPAR is an obvious target for identifying BC and for phenotyping aggressiveness in BC. Using whole body Positron Emission Tomography (PET) imaging rather than tissue specimens circumvents possible sampling error and allows for staging. Our objective was therefore to perform first-in-humans studies of uPAR-specific PET imaging in BC using either 64Cu-DOTA-AE105 or 68Ga-NOTA-AE105

Methods
Six patients with BC and scheduled for surgery were included. Prior to operation, patients were either PET/CT scanned 1, 3 and 24 h after injection of the uPAR PET ligand 64Cu-DOTA-AE105 (n=3; half life of 64Cu: 13 h) or PET/CT scanned 10 min, 1 h or 2 h after injection of 68Ga-NOTA-AE105 (n=3; half life of 68Ga: 1 h). PET Images were visually analyzed for visible tumor uptake of 64Cu-DOTA-AE105 or 68Ga-NOTA-AE105 and Standardized Uptake Values (SUV) were obtained by manually drawing volumes of interest (VOIs) around the primary tumor as well as identified metastases. Results are given as SUVmax. Tumor-to-background ratios relative to liver, kidney, blood and muscle were also calculated. Surgical tumor specimens were obtained from all patients during subsequent surgery. In addition to routine pathological examination, tissue was analyzed for ex vivo uPAR expression as target validation.

Results
Both primary tumors and metastases were visually detectable. For 64Cu-DOTA-AE105 SUVmax values were 2.9–4.0, and 2.9-4.0 after 1 and 3 h, respectively. Tumor-to-background ratios after 1 h were 0.91 (tumor-liver), 1.65 (tumor-kidney), 0.96 (tumor-blood) and 8.9 (tumor-muscle), respectively. Tumor-to-background ratios after 3 h were 0.50 (tumor-liver), 0.96 (tumor-kidney), 4.2 (tumor-blood) and 11.4 (tumor-muscle), respectively. Ex vivo analysis by immunohistochemistry confirmed uPAR expression in all primary cancer lesions. For 68Ga-NOTA-AE105, SUVmax was 5.0, 3.8 and 4.2 after 10 min, 1 h and 3 h, respectively (first patient analyzed). Tumor-to-background ratios after 10 min were 2.8 (tumor-liver), 0.4 (tumor-kidney), 1.6 (tumor-blood) and 8.4 (tumor-muscle), respectively. Tumor-to-background ratios after 1 h were 3.2 (tumor-liver), 0.6 (tumor-kidney), 1.7 (tumor-blood) and 7.1 (tumor-muscle), respectively.

Conclusion
This is the first study in humans using PET imaging of uPAR in BC. Both primary tumors and metastases were clearly visible with robust PET tracer uptake and a high and sufficient contrast between tumors and background. Our data supports continuation into phase II clinical studies using uPAR PET for staging and risk stratification, which potentially may be used for selection of treatment strategy in BC.
Title: High contrast visualization of breast cancer lesions with $^{11}$Cvorozole PET

Cohen J, Pareto D, Bernstein C, Farelly P, Fisher P, Franceschi D, Jhanwar Y, Rizk C, Scherl W, Shroyer K, Vallabhajosula S and Biegon A. Stony Brook University, NY; Vall d'Hebron Hospital, Barcelona, Spain and Weill Cornell Medical Center, NY.

Body: More than a half of breast tumors are known to overexpress estrogen synthase (aromatase, Cyp19A gene product); and aromatase inhibitors are the mainstay of current hormonal adjuvant therapy in breast cancer. Vorozole is a potent aromatase inhibitor which was labeled with carbon11 and recently used to image aromatase with PET in healthy men and women. Here we describe the first case of breast cancer to be imaged with this tracer. A 68 year old woman recently diagnosed with stage 4 invasive lobular carcinoma was given 7.3 mCi $^{11}$Cvorozole intravenously. Forty minutes after injection, she was positioned in the prone position in a high resolution PET/CT (Siemens) scanner; with both breasts in the field of view. PET emission data were collected over a 50 minute period. The PET images revealed a large area of very high intensity in the left breast; corresponding in location and size to the diagnostic mammography; and multiple smaller regions with high intensity in the sternum and thoracic spine. The dynamic study included 5 frames of 10 min. duration each. Regions of interest (ROIs) were drawn with PMOD over the first frame and the corresponding time-activity curves were obtained. ROIs were placed in 2 locations of the carcinoma, in the breast adjacent to the tumor and in the contralateral breast. The ratio of tracer uptake in the tumor to the uptake in the same location in the contralateral breast ranged from 4.8 to 7.2 in the first frame (40-50 min). Both absolute uptake and ratio of tumor to contralateral breast decreased over time between 50 and 90 minutes post injection; suggesting a short (10-20min) acquisition may be sufficient. The $^{11}$Cvorozole PET image compares favorably with other imaging studies performed on the same patient, including FDG and MRI; supporting further investigation and optimization of this tracer in breast cancer.
Title: \(^{18}\)F-radiolabeled PARP-1 inhibitor uptake as a marker of PARP-1 activity in breast cancer


Body: Objectives: The nuclear enzyme PARP-1 plays a central role in sensing DNA damage and facilitating repair. Tumors with BRCA1/2 mutations are highly dependent on PARP-1 as an alternative mechanism for DNA repair, and PARP inhibitors generate synthetic lethality in tumors with BRCA mutations, resulting in cell cycle arrest and apoptosis. Zhou et al. recently synthesized an \(^{18}\)F-labeled PARP-1 inhibitor (\(^{18}\)F-FluorThanatrace) for PET, and demonstrated high specific tracer uptake in a xenograft model of breast cancer (Zhou, Bioorg Med Chem, 22:1700, 2014). The current study seeks to quantify the relationship between \(^{18}\)F-FluorThanatrace binding (both in vitro and on PET imaging of human tumor xenografts) and the level of constitutively active PARP-1, using multiple human breast cancer cell lines, including a BRCA1 defective line.

Methods: BRCA1 defective HCC1937, triple negative MDA-MB-231, and luminal A MCF-7 human breast cancer lines were assessed for constitutive PARP-1 activity via a chemiluminescent ELISA assay for PAR and by Western blot. The same cell lines were incubated with \(^{18}\)F-FluorThanatrace over various time increments, and tracer uptake was assayed via a gamma counter. Specificity of tracer binding was verified via co-incubation with competitive inhibitor Olaparib, and specific tracer uptake was calculated as the difference between uptake with and without Olaparib. Specific tracer uptake was compared to levels of constitutive PARP-1 activity in all cell lines. In addition, HCC1937 and MDA-MB-231 xenograft tumor models were imaged via \(^{18}\)F-FluorThanatrace-PET/CT, and PET uptake was correlated with PARP-1 activity.

Results: BRCA1-defective HCC1937 had higher constitutive PARP-1 activity than cell lines with intact BRCA1. In vitro levels of \(^{18}\)F-FluorThanatrace uptake correlated with constitutive PARP-1 activity across cell lines. In addition, \(^{18}\)F-FluorThanatrace measured by PET in xenograft breast cancer tumor models correlated with constitutive PARP-1 activity.

Conclusions: Tumor uptake of \(^{18}\)F-FluorThanatrace, both in vitro and on PET imaging of xenograft tumor models, quantitatively reflects differences in PARP-1 activity across different breast cancer cell lines, including BRCA1 defective. This motivates further studies of \(^{18}\)F-FluorThanatrace as an in vivo measure of PARP-1 activity and possibly as a predictive marker for PARP-1 therapy in patients, including those with BRCA1/2 mutations.
Title: Radiotherapy may induce enhanced uptake on $^{18}$F-fluoroestradiol PET scans

Venema CM M, van der Veen SJ J, Glaudemans AWJM WJM, Schröder CP P, de Vries EFJ FJ and Hospers GAP AP. University Medical Center Groningen, Groningen, Netherlands.

Body: Introduction: Whole body imaging of $^{18}$F-fluoroestradiol (FES) uptake combined with positron emission tomography (PET) has been applied for diagnosis and prediction of therapy response in estrogen receptor (ER) positive breast cancer patients. A maximal standardized uptake value (SUVmax) of 1.5 has previously been defined as the optimal threshold for FES uptake to differentiate between ER-positive and negative tumor lesions. FES uptake in healthy tissue differs per anatomic site and can be influenced by extrinsic factors. Previous FES PET studies have suggested that radiotherapy (RTx) may cause heterogeneous enhanced uptake in lungs. The cause of this uptake has not been elucidated yet. This is an exploratory study to evaluate whether patients with RTx prior to FES PET show enhanced FES uptake in tissues in the irradiation field.

Methods: Our FES PET database was screened between 2009 and June 2015 for patients who had RTx $\leq$ 6 months before FES PET, independent of location of RTx. Irradiation fields were reconstructed and fused with the FES PET scan. The main outcome was the presence of enhanced FES uptake, defined as visually increased FES uptake above background with a SUVmax $>$1.5 in the irradiation field in the absence of an oncologic substrate on the concordant (contrast enhanced) CT scan.

Results: A total of 133 patients were identified in the database; 29 were eligible for the study. Mean age at the first FES PET scan was 57 (SD 9) years. 28/29 patients had breast cancer, 2/28 had a concurrent other malignancy. Seventeen patients (59%) showed enhanced uptake in the RTx field. 15/17 patients were irradiated in the thoracic area, and 2/17 at the hip. Enhanced uptake was mostly located at the dorsomedial side of the lungs, or subcutaneously after thoracic- or hip RTx, respectively. Patients with normal FES uptake in the radiation field were irradiated in the pelvic/lumbal spine area (n=5), hip (n=3) or thorax (n=4). Time range between RTx and FES PET did not differ between patients with and without enhanced FES uptake (mean 50.9 days [range 0-156] vs. mean 56.8 days [range 0-178]). Systemic treatment at the time of FES PET did not differ between the patients with and without enhanced uptake. Radiation dosage in gray (Gy) was related to enhanced FES uptake, after RTx in the thoracic area or at the hip.

Conclusion: RTx can induce enhanced uptake in the irradiation field on the FES PET scan in part of the patients. Irradiation-induced FES uptake most frequently occurs in lung tissue. Therefore, physicians should keep the possibility of radiation-induced aspecific enhanced tracer uptake in mind, when interpreting FES PET scan of patients that have recently received RTx.
Title: Abstract Withdrawn

Body:
**Title:** Prospective study to evaluate changes in bone quality in premenopausal women with breast cancer undergoing adjuvant chemotherapy


**Body:**

**Background:** Chemotherapy-induced menopause results in rapid bone loss. The rate of bone loss is several-fold higher than those who have natural menopause, which results in a higher risk for bone fractures. High-resolution peripheral quantitative computed tomography (HR-pQCT) is a precise, low radiation and non-invasive technology that evaluates bone microstructure and microarchitectural changes. HR-pQCT scans, unlike dual-energy x-ray absorptiometry scans (DXA), are able to distinguish between cortical and trabecular bone, measure volumetric rather than areal bone density, and quantify whole bone strength using geometric and microarchitectural measurements.

**Methods:** Premenopausal women with stage I-III BC undergoing adjuvant chemotherapy received DXA and HR-pQCT scans at baseline, 6 and 12 months. The distal tibia and radius were evaluated by HR-pQCT. Baseline scans were completed before the third cycle of chemotherapy. Subjects were excluded if they had an endocrine disorder, bone fracture in the past year, bisphosphonate therapy in the last 12 months or a T-score <-2.0 at any site. Paired t-tests were used to observe the change over time in bone microarchitecture and volumetric density, in particular, cortical bone thickness and trabecular bone density. Multiple linear regression was used to evaluate the association between baseline factors and change in cortical bone thickness and trabecular bone density.

**Results:** A total of 21 subjects were consented, 18 enrolled and 13 were considered evaluable. Baseline characteristics include mean age 45.6; 38% White, 19% African American, 29% Hispanic and 14% Asian; mean body mass index (BMI) was 28.6 kg/m2 . At 12 months from baseline a significant decrease in cortical thickness was observed (change= 0.03 mm, p=0.02). There were no significant changes in trabecular bone density at 6 or 12 months. In a linear regression younger age was associated with a worsening in trabecular density from baseline at both 6 and 12 months (p=0.04 and p<0.01). There were no associations between race, ethnicity, BMI, or chemotherapy regimen with changes in trabecular bone density or cortical thickness.

**Conclusions:** We found that premenopausal women undergoing adjuvant chemotherapy experienced a significant decrease in cortical bone thickness after 12 months at the distal tibia. The decrease was highest in younger women. Future studies will evaluate the association between early changes in bone microarchitecture and subsequent osteoporosis.

<table>
<thead>
<tr>
<th>QCT scan measures</th>
<th>Change from BL at 6 mos</th>
<th>Change from BL at 12 mos</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Cortical thickness (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L or R radius</td>
<td>0.01</td>
<td>0.03</td>
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<tr>
<td>L or R tibia</td>
<td>0.01</td>
<td>0.03</td>
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<tr>
<td>Trabecular bone density (g/cm3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L or R radius</td>
<td>0.56</td>
<td>2.32</td>
</tr>
<tr>
<td>L or R tibia</td>
<td>0.71</td>
<td>4.09</td>
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</tbody>
</table>
Title: Correlation of apparent diffusion coefficient with standardized uptake value in invasive lobular carcinoma of breast using in hybrid 18F-FDG PET/MR

Choi JE, Kang SH, Lee SJ and Kong EJ. Yeungnam University College of Medicine, Daegu, Korea and Department of Nuclear Medicine, Daegu, Korea.

Body: Purpose: To compare the apparent diffusion coefficient (ADC) with maximum standardized uptake values (SUV) derived from combined 18F-FDG PET/MRI in invasive lobular carcinoma (ILC) patients.

Methods: From 2012 Aug to 2015 Feb, 53 women with histologically proven ILC (mean age, 50.3 ± 7.7 y-o) underwent hybrid 18F-FDG PET/MR (Siemens Biograph mMR) scan for preoperative assessment. During PET acquisition, simultaneous diffusion-weighted imaging (DWI, b values: 0, 400, 800 s/mm²) was performed using breast coil. ILC over than 2 Cm in size were analyzed. Regions of interest (ROI) were drawn covering the entire ILC on the attenuation-corrected PET-image and the monoexponential ADC-map on PET/MR workstation. All the patients were received surgical treatment within 3 weeks after scan. Spearman rank correlation coefficient (ρ) was calculated to examine the correlation between SUVmax and ADC value.

Results: Total 25 ILC (5.4±1.3 cm; 2.1-6.3 cm) of 22 women were enrolled. 24 ILC showed ER (+)/PR (+) and all ILC showed HER2 (-). 15 patients were confirmed metastatic axillary lymph nodes. The mean of SUVmax was 2.14 ± 2.2 (0.91-11.9) and mean of ADC was 1.02 x10-3 (0.82 -1.31 x10-3) mm²/s. Higher histologic grade was correlated with higher SUV (ρ = -0.6, p=0.001). And SUV showed inverse correlation with ADC (ρ = -0.48, p=0.016).

Conclusion: The present data show inverse correlation between increased glucose-metabolism and cellularity in ILC patients. 18F-FDG PET and DWI thus may offer complementary information for the evaluation of treatment response in ILC.
Title: Diagnosis of pathological complete response by vacuum-assisted minimal invasive biopsy after neoadjuvant chemotherapy in breast cancer - Results from a prospective pilot study

Heil J. Universitäts-Frauenklinik, Heidelberg, Baden-Württemberg, Germany.

Body: Purpose:
To explore the ability of vacuum-assisted-biopsy (VAB) to diagnose pathological complete response (pCR) or residual tumor in breast cancer patients after neoadjuvant chemotherapy (NACT).

Patients and methods: 50 patients (22 with clinical / imaging complete response 28 with clinical / imaging residual tumor) were included in this review-board approved prospective pilot study between 08/14 and 02/15. Vacuum-assisted-biopsy (VAB) was performed after NACT and before surgery. Negative predictive values (NPV) and false-negative-rates (FNR) to predict a pCR in surgical specimen (=diagnose pCR through VAB) were the main outcome measures.

Results:
The cohort (n=50) consisted of 15 (30%) triple negative (TNBC), 13 (26%) HER2 positive (HER2+) and 22 (44%) hormone receptor positive / HER2 negative (HR+/HER2-) cancers. pCR in surgical specimen was diagnosed in 23 (46%) cases of the whole cohort. The NPV of the VAB diagnosis of pCR was 94.4% (95% CI: [0.84; 1.00]). The FNR was 3.7% (95% CI: [0; 0.12]) and specificity 73.9% (95% CI: [0.54; 0.94]). Taking only those biopsies into account that pathology confirmed to be representative of the (former) tumor region, improved the outcome of VAB to a specificity of 100% while the NPV (94.4% (95% CI: [0.84; 1.00])) and the FNR (4.8% (95% CI: [0.00; 0.15])) stayed the same.

Conclusion:
Overall accuracy of VAB diagnosis of pCR questions the necessity of surgical intervention to diagnose a pCR. A confirmative, multi-center, intra-individually-controlled clinical trial including patients with clinical complete, near complete or partial response to NACT to validate the above mentioned results is warranted.
The precise diagnosis of breast lesions represents a significant problem in women under the age of 50, especially given the high prevalence of confounding factors such as dense breast. No new approaches have been developed to augment standard of care in the more precise detection of breast cancer. The combination of breast imaging with a robust protein signature that would detect biochemical cues of breast cancer offers a potentially attractive approach to detection regardless of the quality of the radiographic evidence. We have recently tested a protein signature (KARIFY BREAST™) composed of immune-regulatory cytokines, growth factors and tumor-associated autoantibodies (TAAbs). Here, we confirm the hypothesis that this protein signature, combined with standard of care can increase the precision of the diagnosis of breast cancer in women under the age of 50. We have tested this method in a prospective study of 351 women at 8 centers across the US in a randomized and blinded manner. Presented is both data from the initial blood draw and results of the six-month follow up blood draw. The achievement of 93% sensitivity and greater than 80 percent specificity was demonstrated.

Methods: Provista-001 enrolled 351 patients from 9 sites across the US and will follow patients for 6 months prior to first blood draw under IRB approval. Upon enrollment, patients were randomized to either training or validation groups. Clinical truth was set at equal to or greater than 80% sensitivity/specificity. Serum protein biomarkers and autoantibodies identified in prior proteomic screens were measured prior to biopsy. Individual biomarker (25 serum protein biomarkers (SPB) and TAAbs) concentrations were measured, together with specific patient data were evaluated using various logistic regression models. Additionally, 200 patients were used as a training set to develop and refine new models, which were then validated in the remaining 151 subjects. Clinical findings were compared to biopsy (largely BIRADS 4) or were followed for 6 months and re-assessed (BIRADS 3). The novel algorithm utilizing patient data, SPBs and TAAb concentrations and regression models were able to distinguish benign from breast cancer lesions in a statistically significant manner. Importantly, the SPBs alone were unable to adequately distinguish benign lesions, consistent with prior work. However, the addition of TAAbs markedly increased both the sensitivity (93%) and specificity (80.3%) of the assay in this group of women. The use of the algorithm in conjunction with imaging detected more lesions than imaging alone.

Our findings suggest that when used in combination, the protein signature developed here and breast imaging provides a more precise detection methodology than either alone. This is particularly important in women under the age of 50 where detection is difficult. The follow-up data at six months (BIRADS 3) have yielded additional data in this understudied group of women. Such as the apparent lack of effect of breast density on early detection when using the algorithm.
Incidental radiographic findings at the time of breast cancer diagnosis

Brothers JM M, Kidwell KM M, Brown RK K and Henry NL. University of Michigan, Ann Arbor, MI.

Background: Perioperative staging imaging to evaluate for distant metastases is frequently performed in patients with newly diagnosed breast cancer, despite clinical guidelines recommending against their routine use in stage I and II disease. In addition, recent technological advances in imaging have led to increased sensitivity for findings, many of which are unrelated to breast cancer. We assessed whether the presence of incidental findings on staging imaging is associated with a higher risk of breast cancer recurrence.

Patients and Methods: A retrospective review of staging imaging for distant metastases was performed in 340 patients with stage II or III invasive breast cancer diagnosed in 2008-2009 at a large academic medical center. Data related to patient demographics, pathology, treatment, and recurrence were abstracted from the electronic medical record. Kaplan Meier curves and Cox proportional hazards models were used to assess the association between the presence of incidental findings and time to disease recurrence.

Results: A total of 169 of 340 patients (49.7%) underwent staging evaluation for distant metastases (CT chest, CT abdomen/pelvis, bone scan, and/or PET-CT). Of these, 146 (86.4%) had at least one suspicious or indeterminate radiographic finding. To clarify these findings, 73 (43.2%) patients underwent follow-up imaging or procedures. Nineteen patients were diagnosed with metastatic disease, 18 of whom were initially thought to have stage III disease and one was thought to have stage II disease. In the 127 patients without definite evidence of metastatic disease who underwent staging imaging, 32 were diagnosed with disease recurrence. With median follow-up of 4.9 years, the presence of indeterminate or suspicious findings at diagnosis was not associated with a significant difference in time to disease recurrence, adjusted for stage, hormone receptor status, and HER2 status (HR 1.44, 95% CI 0.51-6.03, p=0.55).

Time to Disease Recurrence by Imaging Modality

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th># With Indeterminate or Suspicious Finding(s)</th>
<th># Without Indeterminate or Suspicious Finding(s)</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients without mets at diagnosis</td>
<td>127</td>
<td>23</td>
<td>1.44</td>
<td>0.51-6.03</td>
<td>0.55</td>
</tr>
<tr>
<td>CT Chest</td>
<td>83</td>
<td>52</td>
<td>1.89</td>
<td>0.80-4.46</td>
<td>0.15</td>
</tr>
<tr>
<td>CT Abdomen/Pelvis</td>
<td>91</td>
<td>45</td>
<td>1.38</td>
<td>0.63-3.04</td>
<td>0.43</td>
</tr>
<tr>
<td>Bone Scan</td>
<td>35</td>
<td>123</td>
<td>1.04</td>
<td>0.44-2.46</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Time to Disease Recurrence by Type of Radiographic Abnormality

<table>
<thead>
<tr>
<th>Type of Abnormal Finding</th>
<th># With Indeterminate or Suspicious Finding(s)</th>
<th># Without Indeterminate or Suspicious Finding(s)</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary nodules</td>
<td>70</td>
<td>66</td>
<td>1.65</td>
<td>0.76-3.58</td>
<td>0.20</td>
</tr>
<tr>
<td>Liver lesions</td>
<td>46</td>
<td>91</td>
<td>1.72</td>
<td>0.83-3.60</td>
<td>0.15</td>
</tr>
<tr>
<td>Borderline or enlarged lymph nodes</td>
<td>20</td>
<td>119</td>
<td>0.57</td>
<td>0.17-1.96</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Conclusions: Staging imaging for distant metastases frequently reveals indeterminate findings, whose presence was not associated with a significant risk of disease recurrence in this analysis. Due to low yield for the diagnosis of metastases, staging imaging should not routinely be performed in stage II breast cancer patients.
Title: Plasma autoantibodies associated with basal-like breast cancers


Body: Basal-like breast cancer (BLBC) is a rare aggressive subtype that is less likely to be detected through mammographic screening. Identification of circulating markers associated with BLBC could have promise in detecting and managing this deadly disease.

Methods: Using samples from the Polish Breast Cancer study, a high-quality population-based case-control study of breast cancer, we screened 10,000 antigens on protein arrays using 45 BLBC patients and 45 controls, and identified 748 promising plasma autoantibodies (AAbs) associated with BLBC. ELISA assays of promising markers were performed on a total of 145 BLBC cases and 145 age-matched controls. Sensitivities at 98% specificity were calculated and a BLBC classifier was constructed.

Results: We identified a 13-AAbs (CTAG1B, CTAG2, TP53, RNF216, PPMLN1, PIP4K2C, ZBTB16, TAS2R8, WBP2NL, DOK2, PSRC1, MN1, TRIM21) that distinguished BLBC from controls with 33% sensitivity and 98% specificity. We also discovered a strong association of TP53 Ab with its protein expression (p=0.009) in BLBC patients. In addition, MN1 and TP53 AAbs were associated with worse survival (MN1 Ab marker HR=2.25 95%CI= 1.03-4.91 p=0.04; TP53, HR=2.02, 95%CI 1.06-3.85, p=0.03). We found limited evidence that Ab levels differed by demographic characteristics.

Conclusions: These AAbs warrant further investigation in clinical studies to determine their value for further understanding the biology of BLBC and possible detection. Currently, they are also being tested in a large national blind validation trial using a well characterized independent sample set.
Title: Prediction of response to neoadjuvant chemotherapy in ER+ breast cancer: Magee equation recurrence versus oncotype DX® recurrence score


Body: Background and Aim: The Magee equation recurrence score (MS) based on tumor pathological characteristics can be used to estimate the actual Oncotype DX® recurrence score (RS). This study’s aim was to test the correlation of MS with RS and their usefulness in predicting tumor response to neoadjuvant chemotherapy (NCT) in patients with estrogen receptor (ER) positive, HER2 negative invasive breast carcinoma (BC). Methods: Pathological data required for MS calculation such as Nottingham Score, H-scores for ER and PR, HER2, Ki 67 and tumor size were obtained via pre-therapy slide review or pathology/electronic medical record. Actual RS was measured (at Genomic Health) on core biopsies from 60 patients who received NCT. Pre-therapy tumor size was measured using imaging. Percentage tumor volume reduction (%TR) was measured. Substantial %TR was defined as at least 50% reduction in tumor size (>50%TR). MS was calculated using Magee equation (http://path.upmc.edu/onlineTools/ptvr.html). MS and RS were categorized by score <18 (referred as MS18 and RS18, respectively) and ≥31 (referred as MS31 and RS31, respectively). Correlation between MS, RS and >50%TR were determined by using the Spearman’s correlation coefficient. The mean levels of pathological parameters and score levels which had >50%TR or <50%TR were compared by ANOVA at a 2-sided α level of 0.05. The impact of the scoring systems for >50%TR by the scoring systems was assessed by calculating the area under (AUC) the receiver operating characteristic curve (ROC); AUC values between 0.7–0.8 represent considerable discrimination. Results: The mean patient age was 52 ±13 years. The mean pre-NCT tumor size was 48 ±36 mm. >50%TR was observed in 27 (45%) patients, but there was no pathological complete response. Low level of ER H-score (<100) was significantly associated with >50%TR (p=0.01). The Spearman’s correlation coefficient (rs) for the MS and RS was 0.522 (p<0.001) with an average coefficient of determination (r²) of 0.298. Neither, MS nor RS correlated with >50%TR (p=0.08, p=0.2, respectively). The AUC values were calculated from the MS, RS, MS18, RS18, MS31 and RS31 (AUC=0.625, 0.602, 0.589, 0.572, 0.525 and 0.522, respectively). Conclusions: MS and Oncotype DX® RS have a good correlation in this small ER+ neoadjuvant cohort. However, neither MS nor RS correlated with %TR. Lower ER expression as a single variable is an excellent predictor of response to NCT and may be used alone in making therapy decisions in small number of cases. For moderate to strongly ER+ cases, the decision between primary surgery versus neoadjuvant systemic therapy may be difficult, but MS may be used in lieu of actual Oncotype DX® RS when it is not available.
Body: Introduction
Mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-AKT pathways are two major hyper-activated cascades in triple-negative breast cancer (TNBC) that critically regulate cancer progression by enhancing cell survival, proliferation, metastasis, EMT, cancer stem cell regulate, and transformation. While many therapeutic agents targeting kinases in these pathways are being developed, the development of predictor of response for such agents is critical to successfully translate them into the clinic. Genomic analysis (amplification, deletion of mutation) is one of the prediction methods. However, these technologies do not always reflect the intrinsic functionalities/activities of the kinase molecules. Therefore, we hypothesized that kinase activity predicts the response to the targeted therapy in TNBC.

Materials and methods
Seventeen TNBC cell lines were used in this study. To analyze cell growth inhibition, cells were incubated for 72 h with various concentrations of trametinib or wortmannin, then processed for sulforhodamine B (SRB) staining assay. To measure MEK or PI3K enzymatic activity, TNBC cell lines were lysed and immunoprecipitated with magnetic beads conjugated with MEK antibody or with PI3K p110α antibody. Kinase reaction buffer including respective substrate and ATP was added to the immunoprecipitates and incubated for 120 minutes at 37 °C. Resultant ADP was quantified by HPLC and determined MEK and PI3K activities. Protein mass of MEK, PI3K, phospho-MEK and phospho-PI3K were determined by Western Blot analysis. Total protein amount was measured by A280. Lactate dehydrogenase (LDH) activity was measured by N-assay L LDH Nittobo. Total protein and LDH were used to normalize MEK and PI3K activities for the further analysis.

Results
Seventeen TNBC cell lines were classified into 4 groups depending on pattern of inhibition to two inhibitors as follows; Wortmannin (PI3K inhibitor) sensitive group (W, 2/17), Trametinib (MEK inhibitor) sensitive group (T, 2/17), Both sensitive group (S, 5/17) and Resistant group (R, 8/17). We found that ratio of PI3K activity and MEK activity showed good agreement to the cell classification (PPV [Wortmannin]: 67 %, PPV [Trametinib]: 33 %, NPV: = 100 %). The other parameters; enzymatic activity of MEK or PI3K, protein mass of MEK, PI3K, phospho-MEK, or phospho-PI3K, ratios of the protein mass, and the phospho-protein did not show statistically significant agreement to the classification. Mutational status and enzymatic activities or cell classification had no correlation. Additionally, MEK activity correlated to downstream phospho-ERK expression level (R = 0.7309).

Conclusion
Our results show that relative activity of two relevant kinases in the signaling cascade could predict the cell lines that will not respond to molecular targeting agents against corresponding cascades. Our concept should be warranted in the clinical study with statistically sufficient number of patients.
Title: Effect of multidisciplinary case conferences on physician decision making: Breast diagnostic rounds

Foster T, Bouchard-Fortier A, Olivotto I and Quan ML. University of Calgary, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada and University of Calgary, Calgary, AB, Canada.

Body: Background: Participation in multidisciplinary case conferences (MCCs) continues to be highly variable, in spite of proven benefits. One contention is the lack of perceived utility on patient management.

Purpose: To evaluate the utility of MCCs on physician decision making in benign and malignant breast disease management.

Methods: At the Foothills Medical Centre, diagnostic breast MCCs occur biweekly. Patients with interesting or challenging diagnostic or management issues were discussed by attending surgeons, radiologists, pathologists, nurse coordinators and oncologists. Prior to case discussion, the presenting physician was asked to specify his/her management plan. Their response was recorded and compared to the management plan consensus after the MCC discussion. For each case a clinical summary and question was provided by the presenting physician, followed by a review of diagnostic images and/or pathology. After group discussion, a management consensus was achieved and documented. A management change was defined as a difference compared to the pre-MCC plan or if there was no definite management plan prior to MCC.

Results: From November 5th, 2014 to May 6th, 2015, 52 patients were discussed in 11 MCCs (1 to 8 patients per MCC). No MCCs were cancelled due to insufficient patients. Of these, 23 (44%) had a change in the management plan compared to the pre-MCC intent including 7 cases where there was no clear plan prior to MCC. Among the 23 cases with a management change, 12 (52%) were due to new or clarified information from radiology review, 6 (26%) were due to new or clarified details from pathology review and 5 (22%) changes occurred from both radiology and pathology review. All cases presented resulted in a consensus management recommendation.

Conclusion: The MCCs had a substantial impact on physician decision making. Nearly half of cases presented resulted in a change in clinical recommendation, the majority of which were based on new/clarified diagnostic imaging or pathology information. Presentation of cases at MCCs should be encouraged given their clinical impact on patient care, especially for challenging diagnostic or management issues.
Title: Therapeutic targeting of Rac GTPases in ER+ and HER2+ breast cancer

Hampsch RA A, Shee K and Miller TW W. Dartmouth College, Hanover, NH.

As crucial regulators of cell motility, survival, and proliferation, Rac GTPases (Rac1/2/3) have been implicated in cancer. We previously found that P-REX1, a guanine nucleotide exchange factor that activates Rac GTPases, forms a PI3K-driven positive feedback loop to promote activation of Rac enzymes, receptor tyrosine kinases, PI3K/AKT/mTOR, and MEK/ERK in ER+ breast cancer cells. Importantly, inhibition of Rac GTPases with a small molecule (EHT1864) simultaneously suppressed activation of both the PI3K/AKT/mTOR and MEK/ERK pathways. While co-targeting of the PI3K/AKT/mTOR and MEK/ERK pathways with drug combinations has anti-tumor activity in preclinical models and is being tested in ongoing clinical trials, targeting Rac as a common upstream signaling node may be a more efficient means of simultaneously targeting these two oncogenic pathways.

In breast cancer cell lines, treatment with EHT1864 decreased activation of AKT, mTOR, p70S6K, and S6 in a dose-dependent manner. Pulldown of activated Rac from cell lysates revealed that GTP-bound Rac1 and/or Rac3 bind MEK1/2, ERK1/2, p70S6K, S6, Raptor, Rictor, and mTOR. Temporal analysis indicated that EHT1864 inhibits phosphorylation of p70S6K (an mTORC1 substrate) before AKT is inhibited, suggesting that Rac may directly activate p70S6K and/or mTORC1. Mining of sensitivity data from ~700 cell lines to a panel of 138 drugs (COSMIC/GDSC database) revealed that the growth-suppressive effects of EHT1864 correlate most strongly with growth-suppressive patterns induced by a p70S6K inhibitor, supporting the notion that Rac directly activates p70S6K. Additionally, EHT1864 treatment dose-dependently decreased phosphorylation of ERK1/2 and MEK1/2, and induced apoptosis in breast cancer cell lines. In a panel of 16 breast cancer lines, cells with activating mutations in PIK3CA (encodes the p110-alpha subunit of PI3K) exhibit increased EHT1864 sensitivity. Pharmacokinetic analysis of EHT1864 (100 mg/kg, i.p.) in plasma in NSG mice revealed that drug was present at >50 uM for 1 h after administration. In mice bearing s.c. ER+/HER2+ BT-474 breast cancer xenografts, EHT1864 (100 mg/kg BID) significantly slowed tumor growth compared to vehicle control. Thus, therapeutically targeting Rac could be an effective means of reducing cancer cell proliferation and survival by simultaneously suppressing both the PI3K/AKT/mTOR and MEK/ERK signaling pathways in ER+ and HER2+ breast cancers.
Title: The dynamic duo: A breast cancer-targeting nanoparticle loaded with a cytotoxic peptide as a treatment for metastatic disease

Vishnubhotla P, Khaled AR, Khaled AS S, Perez JM, Bassiouni R, Flores O and Nierenberg D. Orlando VA Medical Center, Orlando, FL; University of Central Florida, Orlando, FL and Cedars-Sinai Medical Center, Los Angeles, CA.

Body: Metastatic breast cancer is a uniformly fatal disease with a 5-year survival rate of 15 percent. To date there are no effective approaches for targeted therapy. To develop a treatment for metastatic cancer, our group discovered a novel cytotoxic peptide, CT20p, and developed a nanotechnology-based platform to deliver and concentrate CT20p in breast tumors. CT20p was derived from Bax, a member of the Bcl-2 family. Unlike the parent protein, CT20p does not cause apoptosis and its cytotoxicity is independent of caspases and Bcl-2 overexpression. Rather, the intracellular target of CT20p is a protein called chaperonin-containing T-complex (CCT), which is required for the folding of actin and tubulin into their native forms. Inhibition of CCT activity by CT20p, indicated by decreased F-actin and tubulin, impaired the polymerization of microfilaments and microtubules, causing loss of cell migration and adhesion that promoted breast cancer cell death. In contrast, normal, non-transformed cells were resistant to the cytotoxicity of CT20p. On its own, CT20p is not membrane-permeable. To deliver the peptide to cells, we used nanoparticles formed with a novel aliphatic hyperbranched polyester polymer (HBPE-NPs). The surface of HBPE-NPs retains carboxylic acid groups for labeling of targeting ligands to enable accumulation in tumors. To concentrate on breast cancer, we functionalized the HBPE-NPs with either folate (FOL) or glutamate (GLU), which target the folate receptor (FR) or the metabotropic glutamate receptor (GRM-1) respectively. FR and GRM-2 are essential metabolic components that are highly expressed in solid tumors like breast cancer. In vitro targeting studies using triple negative breast cancer cell (TNBC) lines established that folate FOL or GLU-HBPE-NPs loaded with fluorescent dyes were readily up taken at high efficiency by TNBC cells. HBPE-NPs also contain unique hydrophobic cavities especially suited for encapsulating CT20p. We found that once the CT20p-HBPE-NPs were taken up by cancer cells, the peptide was released inside cells under acidic conditions (e.g. endosomes) and directly interacted with its intracellular target, CCT. Studies using primary cells derived from human breast tumors confirmed the targeted uptake of HBPE-NPs as well as demonstrated the cancer-specific cytotoxicity of CT20p. We treated a murine TNBC xenograft model with nanomolar amounts of FOL-CT20p-HBPE-NPs and achieved 100% regression of established tumors as well as prevented tumor growth. These studies indicated that CT20p is a potent and specific anti-cancer agent due to its inhibition of CCT, an essential molecular complex highly expressed in cancer cells, and that the peptide can be efficiently delivered to tumor sites using HBPE-NPs decorated with ligands to receptors, such FR or GRM-1, found on tumor cells.
Title: Designing a novel platinum chemotherapeutic (IO-125) for treatment of breast cancer

Roy M, Sengupta A, Sarkar A, Mylavarapu S, Modi S, Gupta N, B H, Hossain S, Ansari A, Pandey M, Yadav Y and Sengupta S. Invictus Oncology Pvt Ltd, New Delhi, Delhi, India; India Innovation Research Center, New Delhi, Delhi, India and Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

Body: Triple-negative breast cancer (TNBC) is an aggressive form of cancer occurring in 15-20% of breast cancer patients, with most patients relapsing on currently approved therapy. Recent studies have shown activity of platinum chemotherapy in this class of patients. IO-125 is a novel platinum (II) chemotherapeutic agent with an unique coordination environment. In this study, we investigated the anti-tumor activity of IO-125 in pre-clinical models of TNBC.

The coordination environment in IO-125 facilitates supramolecular assembly and releases diaminocyclohexane (DACH)-platinum in a sustained pH-dependent manner. In vitro cell viability studies using an array of breast cancer cell lines shows IO-125 exerts increased potency compared to carboplatin or oxaliplatin. The maximum tolerated (platinum-equivalent) dose (MTD) of IO-125 in mice was 8-fold higher than the MTD (platinum-equivalent) dose of oxaliplatin. The biodistribution and pharmacokinetic profile of IO-125 in plasma and tumor revealed preferential tumor accumulation, significantly increased area-under-the-curve (AUC), a reduction in clearance (CL) and a longer terminal half-life (42 hours) in comparison to oxaliplatin (18 hours). In addition, DNA-Pt adduct formation in tumors was significantly higher for IO-125. When administered at their respective MTDs, IO-125 led to sustained regression of the tumor in a 4T1 syngeneic breast cancer model. Based on these observations, we conclude that IO-125 may emerge as a novel therapeutic against triple negative breast cancer.
Identification of compounds from natural sources with selective activity against triple-negative breast cancer molecular subtypes

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Triple negative breast cancers (TNBCs) lack expression of the estrogen and progesterone receptors (ER/PR) and do not have amplified HER2. While targeted therapies for ER+/PR+ and HER2-amplified breast cancers have greatly improved patient survival, there are no targeted therapies for TNBCs and no effective therapies to treat metastatic disease. There is a need to identify new therapeutic agents and molecular targets for treating TNBCs, but efforts have been limited by a lack of understanding of the subtypes of these heterogeneous diseases. However, gene expression profiling of TNBC patients recently identified 6 molecular subtypes of TNBC and representative cell lines, providing the first opportunity to identify subtype-specific leads for TNBC.

We performed high-content screening to evaluate novel libraries of extracts from Texas plants and diverse fungal cultures for antiproliferative and/or cytotoxic activity in a panel of cell lines modeling five different TNBC molecular subtypes. The aim was to identify extracts with selective activity in a single cell line. We hypothesized that extracts found to have selective activity in one of these cell lines may target a protein or cellular process critical to the growth of that subtype. We identified 11 extracts with selective activity against cell lines representing four different TNBC molecular subtypes. From a fungal culture we identified a new compound called maximiscin, which was found to have selective cytotoxic efficacy against the MDA-MB-468 cell line of the basal-like 1 subtype. From a plant extract we isolated deguelin, which had selective activity in the MDA-MB-453 cell line, a model of the luminal androgen receptor (LAR) subtype.

The molecular mechanisms of action of each compound were investigated in cell line models. Initial cell cycle studies using flow cytometry showed that maximiscin caused an accumulation of cells in G1 after 18h of treatment. Protein microarray studies indicated that maximiscin increased levels of phospho-p53, which was consistent with the observed G1 accumulation. Based on these findings, we hypothesized that maximiscin induces DNA damage and investigated the effects of maximiscin on the phosphorylation of several DNA damage response proteins. Maximiscin increased phosphorylation of Chk1, Chk2, p53 and H2A.X as soon as 2h after treatment, indicating an accumulation of DNA damage.

Previous studies have shown that LAR TNBC cells are particularly sensitive to PI3K inhibitors in vitro compared to other TNBC subtypes. The effects of deguelin on PI3K-Akt-mTORC1 signaling were evaluated in both MDA-MB-453 and MDA-MB-231 cells. Phosphorylation of both ribosomal protein S6 and 4E-BP1 were dramatically reduced in MDA-MB-453 cells 2h after deguelin treatment. Interestingly this was not observed in MDA-MB-231 cells, suggesting inhibition of mTORC1 signaling may be involved in the selective activity of deguelin in MDA-MB-453 cells. Preliminary studies suggest deguelin may also decrease androgen receptor abundance in MDA-MB-453 cells, indicating multiple molecular mechanisms may be involved in its selective effects. These results demonstrate that compounds with selective activity against TNBC subtypes can be identified from nature.
2015 San Antonio Breast Cancer Symposium

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Title: Genomic analysis and efficacy of entinostat in basal-like and HER2-overexpressing models

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Body: Background. The histone deacetylase inhibitor Entinostat has been under development for patients with metastatic estrogen receptor-positive breast cancer, and its use in Basal-like and HER2-overexpressing breast cancer has been limited. Here we provide results of a study using cell-lines and genetically engineered mouse (GEM) models of breast cancer to examine the effects of Entinostat and combination therapies on multiple in vitro and in vivo models.

Methods. Breast cancer cell lines SKBR3, BT474 and MCF-7 were treated with or without Entinostat at their IC50 doses and their gene expression profiles determined. Two breast cancer GEM models (MMTV-neu and C3-Tag) were used to test the efficacy of Entinostat alone, or in combination with cyclophosphamide, or a murine version of an anti-mouse PD1 immune checkpoint inhibitor.

Results: Entinostat was an effective in vitro drug with IC50 ranging from 700 to 1500 nM. Supervised analysis of gene expression data coming from treated cell lines showed induction of multiple major histocompatibility complex (MHC) class I/II genes on 6p21. In addition, several MHC genes were also upregulated in C3-Tag treated by Entinostat; because of these findings, the GEM models are currently being treated with the combination of Entinostat and the anti-mouse PD-1 inhibitor. In vivo, GEM tumors treated only with Entinostat were observed to have high objective response rates of 88% and 33% in MMTV-Neu and C3-Tag, respectively. The combination with cyclophosphamide provided significant improvements in the C3-Tag mice over either agent alone, with 11 of 17 mice achieving a CR or PR, and an extension of overall survival (98 days vs 33 days); Entinostat (42d, p=0.002) and Cytoxan (47d, p=0.001).

Conclusions. Entinostat showed anti-tumor activity in vitro and in vivo, and was synergistic with cyclophosphamide in the Basal-like C3-Tag mouse model. The upregulation of MHC genes induced by Entinostat at RNA level suggested the interaction with immune response, which is currently under study and will be presented.
Title: MEDI3039, a novel highly potent tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) receptor agonist, induces apoptotic cell death in breast cancer cells

Greer YE E, Tice D and Lipkowitz S. National Cancer Institute, Bethesda, MD and MedImmune, LLC, Gaithersburg, MD.

Body: TRAIL receptor agonists are attractive anti-tumor agents because of their capability to induce apoptosis in cancer cells by activating death receptors 4 and 5 (DR4 and DR5) with little toxicity against normal cells. We previously reported that GST-TRAIL efficiently induced cell death in breast cancer cells, particularly mesenchymal triple negative breast cancer (TNBC) – so called basal B breast cancer cells (Rahman et al., Adv. Cancer Res. 2009). Recently, a newly developed multivalent TRAIL receptor agonist designed to activate DR5, has been shown to be a TRAIL super-agonist with significantly enhanced potency in multiple cancer cell lines (Swers et al., Mol Cancer Ther. 2013). We hypothesized that MEDI3039, developed from this TRAIL super-agonist, is a potential new therapeutic agent to be used in human breast cancer treatment.

As model systems, we used 19 breast cancer cell lines that can be categorized into 4 different groups: ER+, HER2 amplified, TNBC basal A and TNBC basal B. MEDI3039- or GST-TRAIL-induced cell death was analyzed by an MTS assay in 96 well format after 72h of treatment. MEDI3039- or GST-TRAIL-induced caspase activation was measured by Caspase-glo 3/7 assay. Z-VAD-FMK was used as a pan-caspase inhibitor. To verify the receptor for MEDI3039, siRNA against DR4 and DR5 were transfected to cells and tested in MTS assay and Western blotting.

MEDI3039 induced cell death in MDA-MB231 (TNBC basal B), and the IC50 was 4.71pM. By contrast, GST-TRAIL induced cell death in this cell line with an IC50 of 624 pM (a 132 fold difference). MEDI3039 and GST-TRAIL induced cell death was completely inhibited by Z-VAD-FMK, indicating that cell death was the result of caspase-mediated apoptotic pathway. Knockdown of DR5, but not DR4, inhibited MEDI3039-induced cell death, demonstrating that MEDI3039-mediated apoptosis requires DR5. MEDI3039 induced cell death in multiple breast cancer cell lines, but the sensitivity varied between cell lines from the four different subtypes. TNBC basal B group was the most sensitive (avg IC50= 1.4 pM), TNBC basal A group was next most sensitive (avg IC50 = 203 pM), HER2 amplified group was less sensitive (avg IC50 = 314 pM), and ER+ group was the least sensitive to MEDI3039 (avg IC50= 403 pM). This was similar to what was observed with GST-TRAIL. Importantly, MEDI3039 was at least 2 orders of magnitude more potent compared with GST-TRAIL in most cell lines tested. Drug combination experiments indicated that MEDI3039 has synergistic effect with multiple drugs, including cisplatin, MK1775. Animal breast cancer xenograft experiments are planned to test the efficacy of MEDI3039 in vivo. Further work to identify biomarker(s) that correlate with MEDI3039 sensitivity, and effective combinations that enhance the toxicity of MEDI3039 especially in the resistant breast cancer subtypes are ongoing. In conclusion, MEDI3039 is a potent TRAIL receptor agonist in breast cancer cells and has potential as a cancer drug in breast cancer patients, especially those with TNBC basal B.
Title: Molecular characterization of CDK 4/6 inhibitors (palbociclib, abemaciclib, and ribociclib) in hormone receptor-positive (HR+) breast cancer cell lines

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Body: Background: Palbociclib (Ab) in combination with letrozole or fulvestrant (ful) has been shown to significantly improve disease-free survival in patients with HR+ breast cancer, in first and second line metastatic settings. Although Ab and two other CDK4/6 inhibitors [Abemaciclib (Ab) and Ribociclib (Ri)] are evaluated in multiple clinical trials, predictive biomarkers for these inhibitors have not been identified. To elucidate their molecular mechanisms in endocrine-responsive and -resistant cancers and identify potential predictive biomarkers, a series of preclinical experiments were conducted.

Methods: All three inhibitors, each as a single agent and combined with AI or ful, were used to treat cell lines: MCF-7aro (ER+/aromatase+/endocrine responsive), Let-R (letrozole-resistant), LTEDaro (long-term estrogen deprived line of MCF-7aro), and Rad-R (Everolimus-resistant). Molecular pathways were investigated through reverse phase protein array analysis (RPPA). Western blot analyses were performed to validate key results from the RPPA.

Results and Discussion: Ab, Pa, and Ri inhibit proliferation of MCF-7aro with IC50 values: 24nM, 77nM, and 234nM, respectively. These inhibitors and AIs inhibit androgen (converted to estrogen by the expressed aromatase)-dependent proliferation of MCF-7aro in a synergistic manner. Our protein expression analyses confirm why ER+ breast cancer is most sensitive to treatment with CDK4/6 inhibitors. In MCF-7aro, 17β-estradiol up-regulates expression of RB, cyclin D, and FoxM1. The expression of these proteins can be suppressed by ful/AI + CDK4/6 inhibitors in a synergistic manner. Moreover, ful has been found to induce the expression of p21, an inhibitor of cyclin D, possibly by the elimination of ER-antagonized p53 activation. Since levels of pRB (S807/S811) are most sensitive to treatment with CDK4/6 inhibitors + ful, we hypothesize that pRB could be a predictive biomarker for this therapy.

Ab, Pa, and Ri inhibit proliferation of Let-R (IC50 values: 170nM, 530nM, and 5730nM, respectively). Notably, our analysis has revealed that ful + CDK4/6 inhibitors effectively reduce the expression of Aurora-A, Plk1, and CHK1. Since these proteins play important roles in endocrine resistance, our molecular studies may explain why these drugs improve disease-free survival in cancer progression after one endocrine therapy. Also, Rad-R (IC50 values: 57nM, 136nM, and 1140nM, respectively) results suggest the potential utility of CDK4/6 inhibitors against Everolimus resistance.

Unexpectedly, LTEDaro was found to be significantly more sensitive to CDK4/6 inhibitors as a single agent. Ab, Pa, and Ri inhibit proliferation of LTEDaro with IC50 values: 6.3nM, 10.9nM, and 63nM, respectively. If LTEDaro is considered to be a model of late stage endocrine resistance, our results suggest CDK4/6 inhibitors could be valuable for heavily treated patients.

Conclusion: Preclinical studies using our endocrine-responsive and –resistant cell lines on three CDK4/6 inhibitors have produced valuable information about molecular characteristics associated with their treatments. These results will be verified in tumor biopsies of patients receiving CDK 4/6 inhibitors.
Title: Eribulin impairs the transport of TGF-β type I receptor leading to inhibition of downstream non-canonical TGF-β signaling necessary for cancer metastasis and survival

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Body: Microtubule targeting agents (MTAs) continue to be some of the most valuable drugs used in the treatment of breast cancers. While decades of research have shown that these drugs cause mitotic arrest in cells by suppressing the dynamic instability of microtubules, recent evidence demonstrates that the ability of MTAs to disrupt microtubule-dependent transport of key signaling components, including proteins and microRNAs, in interphase cells likely contributes to their anticancer actions. TGF-β receptors are known to undergo constant cycling from the plasma membrane to intracellular portions of the cell, a process which is microtubule dependent. This microtubule-dependent trafficking has been shown to regulate the nuclear translocation of the TGF-β type I receptor, TGFβR1. Nuclear translocation of TGFβR1 activates the expression of genes including Snail and MMP2, which facilitate the invasiveness, motility and metastasis of cancer cells. We tested the hypothesis that a 2 h treatment of breast cancer cells with eribulin or 4 other clinically relevant MTAs, would differentially disrupt interphase microtubules and alter the internalization and trafficking of TGFβR1 to the nucleus; thereby impacting downstream signaling events. Cells were serum starved for 12 hours and then treated for 2 h with concentrations of MTAs that caused comparable disruption of the interphase microtubule network; 100 nM was used for the destabilizers, eribulin and vinorelbine and 1 µM was used for the stabilizers, paclitaxel, docetaxel and ixabepilone. Following the 2 h treatment, cells were stimulated with 10 ng/mL TGF-β1 for 30 min. The results show that there are distinct differences between the effects of microtubule stabilizers and destabilizers on TGFβR1 trafficking. Eribulin and vinorelbine decreased the nuclear localization of TGFβR1 in a panel of breast cancer cell lines with initial studies suggesting that eribulin impairs this trafficking to a greater extent. In contrast, the microtubule stabilizers, particularly ixabepilone, increased TGFβR1 localization in the nucleus. Additionally, TGFβR1 was extensively localized along stabilizer-induced microtubule bundles. Overall, our work suggests that eribulin is the most effective MTA at inhibiting TGFβR1 nuclear accumulation and subsequent phosphorylation of Smads 2 and 3. The downstream signaling effects of these MTAs on TGFβR1 induced transcription of Snail and MMP2 are also being investigated. Eribulin induced inhibition of non-canonical TGF-β signaling is consistent with previous studies that show that a 7 day treatment with eribulin reversed TGF-β-mediated EMT in breast cancer cells.1,2 Our data suggest that inhibition of the nuclear transport of TGFβR1 could be a potential mechanism for the eribulin-mediated EMT reversal. These studies begin to shed light into the diverse mechanisms of MTAs and lay the groundwork to identify patient populations that might respond optimally to different MTAs.


This work is funded by Eisai Inc.
Title: Eribulin affects E-cadherin localization consistent with a reversal of the epithelial-to-mesenchymal transition

Body: Microtubule targeting agents (MTAs) are some of the most important compounds used to treat breast cancer. While both microtubule stabilizers and destabilizers have utility against breast cancers, not all patients respond and there are differences among the agents. Eribulin is a microtubule depolymerizer used to treat patients with locally advanced or metastatic breast cancer who have previously received at least two chemotherapeutic regimens, including an anthracycline and a taxane. Our goal was to compare the effects of eribulin to four other clinically relevant MTAs on signaling processes important for cancer cell metastasis that are known to be regulated by the cytoskeleton. One process is the appropriate localization of E-cadherin, which plays a critical role in maintaining an epithelial phenotype by facilitating the ability of cells to form cell-cell contacts. These contacts help prevent cell motility and epithelial to mesenchymal transition (EMT) by sequestering several proteins involved in EMT at the plasma membrane, including β-catenin. E-cadherin is absent from the cell membrane in cells with a mesenchymal phenotype. We hypothesized that eribulin could affect the internalization of E-cadherin and promote its retention at the cell periphery and that it might do so differently than other MTAs because eribulin has been shown to reverse EMT in breast cancer cells and in xenograft models. Mesenchymal breast cancer HCC1937 cells were treated for 2 hr with MTAs using a concentration that caused a similar degree of microtubule disruption and localization of E-cadherin was evaluated. The microtubule destabilizers eribulin and vinorelbine promoted a localization of E-cadherin to the plasma membrane. In contrast, the microtubule stabilizers paclitaxel, docetaxel and ixabepilone caused E-cadherin to localize to internal structures. β-catenin, a protein bound by E-cadherin, also became enriched at the cell periphery after microtubule destabilizer treatment. In the more epithelial MCF-7 cell line, E-cadherin was constitutively localized at the cell periphery and the MTAs had no effect on this localization, consistent with the epithelial phenotype. Src family kinases (SFKs) are known to promote the internalization and degradation of E-cadherin and to promote EMT. Accordingly, the SFK inhibitor dasatinib caused effects similar to eribulin and vinorelbine by promoting the localization of E-cadherin to the plasma membrane. In contrast, the microtubule stabilizers paclitaxel, docetaxel and ixabepilone caused E-cadherin to localize to internal structures. β-catenin, a protein bound by E-cadherin, also became enriched at the cell periphery after microtubule destabilizer treatment. 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Funding for this work provided by Eisai Inc.
CYC065, a novel CDK2/9 inhibitor: Molecular basis for clinical development in basal-like triple-negative breast cancer


Body: CYC065 is a novel CDK inhibitor, which inhibits CDK2 and 9 with IC\textsubscript{50} values of 5 and 26 nM, respectively. Following completion of IND-enabling studies, CYC065 has been cleared by FDA for first-in-human Phase 1 clinical trials. Triple-negative breast cancers (TNBC), particularly the basal subtype, often exhibit aggressive characteristics. Despite good initial responses to chemotherapy, patients experience early relapse and diminished 5 year survival. Molecular features of basal-like TNBC include amplification or overexpression of cyclin E and MYC, suggesting potential utility for a CDK2/9 inhibitor such as CYC065. CYC065 is effective in cyclin E-overexpressing tumors, such as uterine serous carcinoma\textsuperscript{1} and trastuzumab-resistant Her2+ breast cancer\textsuperscript{2}. Moreover, CDK inhibition has also been reported to be synthetic lethal with overexpressed MYC\textsuperscript{3}. This led us to assess the potency and mechanism of action of CYC065 in basal-like TNBC models to evaluate the potential for CYC065 development in this indication.

\textit{In vitro} cell-based experiments support twice weekly pulse dosing using submicromolar concentrations of CYC065 to achieve maximum impact on cell growth in the majority of breast cancer cell lines tested. Preclinical toxicology data indicate that such levels and durations of exposure are achievable and well tolerated. As a single agent, CYC065 treatment in breast cancer cells resulted in inhibition of RNA-Pol II phosphorylation, down-regulation of Mcl-1, up-regulation of p53 and rapid induction of apoptosis. The impact of CYC065 on CDK2 targets, cyclin E and MYC was also explored. Interestingly immortalized cell lines obtained from non-malignant tissue displayed similar effects on RNA Pol II, Mcl-1, and p53 but did not undergo apoptosis and consequently exhibited relative resistance to CYC065, indicative of a potential therapeutic window. Cell cycle analysis demonstrated that CYC065 treatment induced an increase in G1 population with no significant induction of cell death in non-malignant derived cell lines, compared to cancer cell lines, in which there was significant induction of cell death.

CDKs have a role in DNA repair which can be exploited to enhance the effectiveness of DNA damaging agents. Seliciclib, an oral, first generation CDK2/9 inhibitor, can be effectively combined with DNA damaging agents, such as the oral nucleoside analogue sapacitabine, or its active metabolite CNDAC. A Phase 1 clinical trial is currently underway to evaluate this combination (NCT00999401). Similarly to seliciclib, we demonstrate that CYC065 is synergistic in combination with CNDAC when given sequentially across multiple breast cancer cell lines. CNDAC-induced double strand breaks persisted for longer when cells were subsequently treated with CYC065, supporting the conclusion that these CDK inhibitors suppress DNA double-strand break repair capacity, which may contribute to the observed synergy.

Taken together the data establish CYC065 as a promising anti-cancer agent in basal-like TNBC, with the potential to be combined effectively in this indication with DNA damaging agents.

Title: Niclosamide overcomes cisplatin resistance and inhibits epithelial-mesenchymal transition in triple negative and HER2 positive breast cancer

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Body: Background: Women with triple-negative breast cancer (TNBC) or HER2 positive breast cancer have worse prognosis compared with other breast cancer subtypes. Acquired drug resistance remains an important reason influencing their treatment efficacy. The epithelial-mesenchymal transition (EMT) is one of key development programs associated with cancer progression and drug resistance. Niclosamide has potential therapeutic activities against breast cancer stem cells, so we wished to determine the effect of niclosamide on EMT and stem-like cells elicited by cisplatin resistance and investigate niclosamide as a potential therapeutic agent for TNBC and HER2 positive breast cancer. Methods: TNBC cell line MDA-MB-231 and HER2 positive breast cancer cell line BT474 were continuously exposed to increasing concentrations of cisplatin (5-30 µmol/L) to establish two stable cell lines resistant to cisplatin, 231-CR and BT-CR. Cell proliferation was determined by alama blue and colony formation. Protein expression was determined by western-blotting. Invasion ability was analyzed by transwell assay. Mammosphere formation was conducted to observe the mammosphere forming efficiency. Results: Apoptosis assessment by flow cytometry and alama blue assay showed that niclosamide could induce apoptosis and had cytotoxic effects on both sensitive and resistant cells of MDA-MB-231 and BT474. What's more, combination of niclosamide with cisplatin could reverse cisplatin resistance of both TNBC and HER2 positive breast cancer cell lines. Both of two cisplatin-resistant cell lines, 231-CR and BT-CR underwent EMT confirmed by western-blot and had higher invasion ability compared to naive sensitive cells. Western-blot results showed that niclosamide could inhibit the EMT phenotype of 231-CR and BT-CR with E-cadherin up-regulation and snail, vimentin down-regulation at the concentration of 1 µM. After treatment of 1 µM niclosamide, the inhibition of mammosphere forming efficiency and cell invasion capability was observed in both 231-CR and BT-CR. Conclusion: Our results suggested that niclosamide could overcome cisplatin resistance in both TNBC and HER2 positive breast cancer cell lines. EMT induced by cisplatin resistance could be reversed by niclosamide, as well as reduction of mammosphere formation. Niclosamide may serve as a novel therapeutic strategy for treatment of cisplatin-resistant TNBC or HER2 positive breast cancer.
Title: Selenocystine inhibits triple-negative breast cancer cell proliferation by inducing cell apoptosis and S-phase arrest

Long M, Qiu W, Wu J, Liu R and Su H. Breast Cancer Center, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China and The University of Texas Health Science Center at San Antonio, San Antonio, TX.

Body: Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited effective treatment options. New therapeutic approaches are urgently needed to improve the prognosis of TNBC. Reactive oxygen species (ROS) are inherent byproducts of oxidative metabolism, and forced stimulation of glucose oxidation in cancer cells raises oxidative stress and sensitzes cells to different stresses. Therefore, targeting the antioxidant capacity of cancer cells has become a promising anticancer strategy. As a redox modulator, selenocystine (SeC) has received a great deal of attention and has been shown effective against human melanoma, hormone receptor-positive breast cancer, and cancers of liver, lung and cervical in vitro. However, whether SeC exerts an anticancer effect on TNBC cells has never been explored.

Methods: The dose-response effects and time course of effects of SeC on three different TNBC cell lines, MDA-MB-231, MDA-MB-436 and MDA-MB-468, were investigated in this study. Cellular viability was determined by the CCK-8 assay and cell morphology were recorded under a light microscope. Cellular apoptosis was detected using Annexin V/PI staining assay and cell cycle distribution was analyzed by flow cytometry.

Results: SeC induced cell growth inhibition in all three TNBC cell lines. For 24, 48 and 72 hours of SeC treatments, the IC50 values were 40.8, 12.8 and 9.2 µM for MDA-MB-231 cells; 14.6, 5.4 and 3.0 µM for MDA-MB-436 cells; and 69.6, 29.3 and 19.9 µM for MDA-MB-468 cells. The changes of cellular morphology of TNBC cells in response to SeC treatment were similar to those cells undergoing apoptotic pathway. This result was confirmed by Annexin V/PI staining assays (Table 1). Cell cycle analysis further revealed that SeC also induced S-phase arrest in a dose-dependent manner (Table 2).

Conclusion: In summary, SeC inhibited TNBC cell viability in a dose- and time-dependent manner which was attributed to the induction of apoptosis and S-phase arrest. Our finding indicates that SeC is a potential therapeutic agent for TNBC.

Table 1. Apoptotic rate of TNBC cells after SeC treatment.

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>MDA-MB-231</th>
<th>MDA-MB-436</th>
<th>MDA-MB-468</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>5.8 %</td>
<td>18.1 %</td>
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<td>10</td>
<td>25.5 %</td>
<td>45.8 %</td>
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<td>20</td>
<td>40.1 %</td>
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<td>40</td>
<td>54.7 %</td>
<td>70.7 %</td>
<td>74.5 %</td>
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</tbody>
</table>

Table 2. SeC induces S-phase arrest in TNBC cells in a dose-dependent manner.

<table>
<thead>
<tr>
<th>Conc. (µM)</th>
<th>G0/G1 (%)</th>
<th>S (%)</th>
<th>G2/M (%)</th>
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<td>48.1</td>
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<td>40</td>
<td>41.7</td>
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<tr>
<td>MDA-MB-436</td>
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**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-03-13

**Title:** The anticancer effects of Supinoxin® (RX-5902) in triple-negative breast cancer MDA-MB-231 through phosphorylated p68 on Tyr593

Kim DJ, Yang MY, Lee YB, Remenyi J and Fuller-Pace F. Rexahn Pharmaceuticals, Inc, Rockville, MD and Division of Cancer Research, University of Dundee, Dundee, United Kingdom.

**Body:** Several studies have indicated that the DEAD box RNA helicase DDX5/p68 plays several important roles in cancer (1, 2). In particular, p68 that is phosphorylated on Tyr593 has been shown to be associated with cell transformation, epithelial mesenchymal transition (EMT) and cell migration (3). Therefore, phosphorylated p68 may be a promising target for novel anti-cancer therapeutics. We previously reported that 1-(3,5-dimethoxyphenyl)-4-[(6-fluoro-2-methoxyquinoxalin-3-yl)aminocarbonyl] piperazine (RX-5902, Supinoxin®) inhibits the growth of cancer cells at low nanomolar concentrations by interacting with phosphorylated p68 on Tyr593, interfering with the phosphorylated p68-β-catenin signaling pathway (4). In this study, we sought to determine whether phosphorylated p68 on Tyr593 plays a key role in RX-5902’s ability to inhibit cancer cell growth by knocking down p68. p68-siRNA efficiently down-regulated the expression of phosphorylated p68 on Tyr593 as well as p68 in the triple-negative (TN) breast cancer cell line, MDA-MB-231. Exposure of p68-siRNA-transfected cells to the IC50 concentration of RX-5902 protected MDA-MB-231 cells from the cytotoxic effects of RX-5902, indicating the phosphorylated p68 on Tyr593 is a key molecule for RX-5902 cytotoxic effects. We also examined the tumor growth inhibition (TGI) of RX-5902 in the human TN-breast tumor (MDA-MB-231) xenograft mouse model. Not only did RX-5902 demonstrate potent efficacy in this model but also oral administration with RX-5902 resulted in dose-dependent TGI and extended the overall survival of these animals. Oral administration of 160, 320 and 600 mg/kg of RX-5902 showed 54.4%, 84.4% and 100% TGI, respectively whereas 5 mg/kg of Abraxane (iv) showed only 48.2% TGI at day 29. Further studies demonstrated the inhibitory effects of RX-5902 on cellular motility in MDA-MB-231 in wound healing assays, suggesting the potential function of phosphorylated p68 on Tyr593 in cell migration (5). These data support the potential therapeutic activity of RX-5902 in triple negative breast cancers. A Phase 1 study of RX-5902 on relapse/refractory solid tumors is ongoing.

**References**

1. Fuller-Pace, FV, RNA Biology 10, 121–132 (2013)
Title: MLN0128 regulates survival signaling by AKT and its downstream effectors in HER2+ breast cancer model


Body: Evading apoptosis is considered to be a hallmark of cancers including breast cancer, since mutations in apoptotic regulators invariably accompany tumorigenesis. Chemotherapeutic agents induce apoptosis, and hence disruption of apoptosis during tumor progression may promote drug resistance. AKT is an apoptotic regulator that is activated in HER2+ breast tumor cells and promotes anti-HER2 therapy resistance in vitro. Nevertheless, how mTORC1/C2-AKT signaling disables apoptosis and its contribution to clinical drug resistance are not clear yet. Using HER2 amplified breast cancer cells [BT474 (HER2+/Trastuzumab-sensitive), BT474HerR (HER2+/Trastuzumab-resistant), HCC1954 and MDA-MB453 (both are HER2+/PIK3CA kinase domain mutated)], we show that mTORC1/C2 inhibitor; MLN0128 abrogates AKT (Ser473), Survivin and controls its downstream effectors of apoptotic signaling molecules (e.g. cleaved Caspase 3/9, cleaved PARP, MCL and BIM). MLN0128 also induces annexinV positive cells and regulates cellular proliferation (ON-TOP 3D colony formation and real-time proliferation assay). Additionally, increased cleaved Caspase 3 and decreased MCL1 expression were also observed following MLN0128 treatment in HER2+ xenograft model along with tumor growth inhibition. Our studies provide strong experimental evidence that high apoptotic signaling –specifically reduced MCL1 and increased cleaved-CASPASE3 expression expedite the response of targeted therapy that directly inhibits mTORC1/C2-AKT signaling.
Title: ERβ elicits tumor suppressive effects in triple negative breast cancer through the induction of cystatins and suppression of TGFβ signaling


Body: Background: Triple negative breast cancer (TNBC) accounts for approximately 20% of all breast cancer diagnoses. Clinical management of TNBC is limited to surgery, chemotherapy and radiation due to lack of estrogen receptor alpha and HER2 expression. Recently, we have shown that approximately 40% of TNBCs express estrogen receptor beta (ERβ) and have begun to explore the possibility that this receptor could be utilized as a novel therapeutic target for this disease.

Methods: To examine the biological functions of ERβ in TNBC, novel ERβ expressing TN cell lines (MDA-MB-231 and Hs578T) were developed. In vitro experiments were employed to determine alterations in the global gene expression profiles, biological pathways, proliferation rates, and cell cycle progression following estrogen or ERβ-specific agonist treatment. Cell line xenografts were also established in athymic ovariectomized nude mice to examine tumoral responses to ERβ targeting agents and to investigate gene and protein expression patterns as well as potential serum biomarkers indicative of therapeutic response. Additionally, using the resources of the Mayo Clinic Breast Cancer Genome Guided Therapy Study (BEAUTY), we have identified, and begun to analyze, ERβ+ and ERβ- patient derived xenografts (PDX) established from women with TNBC.

Results: Our studies have revealed that both estrogen and multiple ERβ-specific agonists elicit significant anti-proliferative effects in ERβ+ TNBC cells primarily through a G1/S phase cell cycle arrest. These anti-proliferative effects appear to be mediated by cystatins, a family of small secreted cysteine protease inhibitors which are highly induced following estrogen and ERβ-specific agonist treatment. Conditioned media isolated from estrogen or ERβ-specific agonist treated cells decreased the proliferation rates of multiple non-ERβ expressing cell lines; effects that were completely reversed when cystatins were depleted from the media. In addition, we have shown that activation of ERβ, and the subsequent induction of cystatin gene expression, leads to suppression of canonical TGFβ signaling through multiple mechanisms including suppression of TGFβR2 expression, induction of Smad7 expression and blockade of TGFβ ligand-mediated activation of this pathway both in vitro and in vivo. Finally, ERβ+ TNBC PDXs exhibit significantly decreased tumor growth rates in estrogen-treated mice compared to ERβ- TN breast tumors.

Conclusions: Our in vitro and in vivo data show that estrogen and ERβ-specific agonists elicit anti-cancer effects in ERβ+ TNBC. These effects appear to be mediated, in part, by cystatins through their inhibitory effects on canonical TGFβ signaling, a pathway known to drive TNBC progression. Importantly, these data lay the foundation for studies aimed at examining the ability to therapeutically target ERβ in TNBC patients.
The histone deacetylase inhibitor entinostat enhances the efficacy of the MEK inhibitor pimasertib against aggressive types of breast cancer through Noxa-mediated myeloid cell leukemia 1 degradation

Torres-Adorno AM M, Lee JJ J, Kogawa T, Bartholomeusz C, Pitner MK, Ordentlich P, Lim B, Tripathy D and Ueno NT T. University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX; Section of Translational Breast Cancer Research, Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, The University of Texas MD Anderson Cancer Center, Houston, TX and Syndax Pharmaceuticals, Inc., Waltham, MA.

Purpose: Inflammatory breast cancer (IBC) and triple-negative breast cancer (TNBC) are the two most aggressive types of breast cancer whose molecular mechanisms remain unclear, representing a challenge for the development of effective targeted therapies. Single agent targeted therapies are of limited effectiveness in these types of breast cancer. Therefore, we aim to identify new combination therapeutic candidates for these aggressive diseases by comprehensive genomic screening.

Experimental Design: We screened kinome siRNA libraries with the mitogen/extracellular signal-regulated kinase (MEK) inhibitor [pimasertib], and genome-wide functional mRNA expression with the histone deacetylase (HDAC) type I inhibitor [entinostat] in TNBC and IBC cell lines. We evaluated the relationship between targets of interest and breast cancer patient survival using the IBC consortium database composed of breast cancer patient samples with clinical follow up. After identifying the targets, we assessed the combinational synergistic effect and its mechanism via cytotoxicity assay, flow cytometry, anchorage-independent growth, quantitative real-time polymerase chain reaction, small interfering RNA, western blotting, and mammary fat pad xenograft mouse models.

Results: We identified that knock-down of myeloid cell leukemia 1 (Mcl-1), an anti-apoptotic member of the B-cell lymphoma 2 (Bcl-2) family of apoptosis-regulating proteins, enhanced the anti-proliferative effect of pimasertib. We observed that entinostat induced the expression of Noxa, a pro-apoptotic BH3-only member of the Bcl-2 family that is known to bind and degrade Mcl-1. Interestingly, in a breast cancer patient cohort (N = 389), high Mcl-1/low Noxa co-expression was associated with poorer survival outcomes than low Mcl-1/high Noxa co-expression (P = 0.0038). We found that combination with pimasertib and entinostat enhanced the inhibition of tumor cell proliferation (P < 0.001) compared with entinostat or pimasertib alone. We also observed significant in vivo tumor growth inhibition in both IBC (SUM190, P < 0.0001) and TNBC (SUM149, P < 0.05) xenograft models. Specifically, TNBC and IBC cell lines that overexpressed Noxa after treatment with entinostat were observed to be selectively sensitive to combination treatment with pimasertib. The synergistic antitumor activity of the entinostat-pimasertib combination was due to increased expression of Noxa, which induced the degradation of Mcl-1, resulting in the induction of mitochondrial cell death.

Conclusion: Our data provide evidence that entinostat has enhanced antitumor effect in combination with pimasertib, resulting in the induction of apoptosis by Noxa-mediated Mcl-1 degradation. These findings provide a novel preclinical rationale for developing a clinical trial based on combinatorial HDAC and MEK inhibition therapy for TNBC and IBC with high Mcl-1 expression.
Targeting glycoprotein non-metastatic B (GPNMB) to overcome EGFR-mediated resistance to Mek inhibition in triple negative breast cancer

Rose AA A, Annis MG G, Maric G and Siegel PM M. McGill University, Faculty of Medicine, Montreal, QC, Canada.

Background: Triple negative breast cancer (TNBC) is an aggressive subtype that constitutes ~15% of all BC. Currently there are no targeted therapies available for patients with TNBC and these patients have a poor prognosis. As such, there is much interest in developing targeted therapies for this disease.

Recently we identified GPNMB as a transmembrane protein that promotes breast tumor growth and metastasis. CDX-011 is an antibody drug conjugate that targets GPNMB, and has recently shown promising clinical activity in patients with GPNMB+TNBC. In subset analyses of the EMERGE trial, patients with high GPNMB expressing TNBC had a median OS of 10 vs. 5.5 months for CDX011 versus chemotherapy, respectively. Response rates to CDX011 correlated with degree of GPNMB expression. These findings support the hypothesis that TNBC with high GPNMB will respond better to CDX011. As such, we sought to identify therapies with intrinsic activity against TNBC that would also induce GPNMB expression, in order to synergize with CDX-011.

Recently there has been much interest in targeting the MAPK pathway in TNBC. We have recently shown that MAPK pathway inhibition induces GPNMB expression in melanoma. Therefore we sought to determine whether mek inhibition induced GPNMB in TNBC and the targeted therapies could synergize with CDX-011.

Results: We interrogated the TCGA breast dataset to determine whether the MAPK pathway is more frequently altered in TNBC. Indeed, we find that the MAPK pathway is altered in 93% of basal BC compared to 56%, 82%, and 81% of Lum A, LumB, and Her2 subtypes, respectively.

We used immunoblot and FACS analysis to assess GPNMB expression in response to MAPK-inhibition. We found that mek inhibitors (trametinib, cobimetinib) markedly induced GPNMB protein expression in several TNBC cell lines. RTK upregulation has been proposed as an adaptive resistance mechanism to mek inhibition in TNBC. Indeed, we find that EGFR is upregulated in response to Mek inhibition in MDA-MB-468 and Hs578T cells. Using shRNA to knockdown GPNMB expression in MDA-MB-468 cells or ectopic GPNMB overexpression in Hs578t cells, we found that GPNMB is both necessary and sufficient for enhanced EGFR activation in response to Mek inhibition in TNBC. Interestingly, we also find that Hs578T cells overexpressing GPNMB show less growth inhibition in response to Mek inhibitors compared to control cells in vitro. These in vitro data are corroborated by our analyses of 1097 breast tumors from the TCGA dataset. GPNMB alterations were found in 7% of all BC and correlates significantly with increased EGFR, Mek and Erk activation.

Finally, we are investigating the efficacy of combining trametinib with CDX011 to treat TNBC using in vivo mouse models. Preliminary data from this experiment suggest that MDA-MB-468 tumors treated with both drugs are more growth restricted than tumors treated with either drug alone. Final data will be presented at the meeting.

Conclusions: Mek inhibition induces GPNMB expression in TNBC. GPNMB promotes EGFR activation and protects from mek-inhibitor induced growth inhibition. The combination of a mek inhibitor with CDX011 shows promise in pre-clinical models and warrants further investigation in clinical trials.
Title: Cutting the ties that bind: Targeting chaperonin-containing T-complex (CCT) for therapeutic intervention in the treatment of advanced stage breast cancer

Khaled AS S, Vishnubhotla P, Khaled AR R and Bassiouni R. Orlando VA Medical Center, Orlando, FL and University of Central Florida, Orlando, FL.

Body: Metastatic disease is a principal cause of death from breast cancer. This is due in part to the development of resistance to current therapeutics and the often debilitating side effects that impair quality of life. The challenge is to therapeutically target an essential physiological function of cancer cells not found in normal, non-transformed cells. To this end, we discovered CT20p, a therapeutic peptide that causes cancer-specific death in human breast tumor cells and tumor regression in xenograft models of breast cancer. Using a proteomics approach, we found that CT20p directly binds to multiple subunits of a type II chaperonin called chaperonin containing T-complex or CCT. CCT forms a large macromolecular complex composed of 8 subunits (CCTα, CCTβ, CCTγ, CCTδ, CCTε, CCTζ, CCTη, CCTω). Each subunit contains an ATP binding site as well as substrate binding sites. Inhibition of CCT by CT20p depletes the pool of native actin and tubulin (obligate clients of CCT), impairing the polymerization of cytoskeletal elements needed to support cell adhesion and motility. As a result cancer cells lose the ability to migrate and die from loss of substrate survival signals. We found that expression levels of CCT varied among different triple negative breast cancer (TNBC) cell lines, with the highest expression occurring in those of the mesenchymal stem-like (MSL) subtype with metastatic potential. Lowest levels of CCT were found in normal breast epithelial cells. Sensitivity to killing by CT20p thus correlated with levels of CCT, with cancer cells expressing high amounts of CCT being the most susceptible. Using tissue microarrays (TMAs) of breast cancer progression, we developed an immunohistochemistry staining procedure for CCTβ. Results were interpreted on a scale of 1 to 4 (with 4 being the strongest staining). CCTβ expression was statistically higher in invasive ductal carcinoma (IDC) as compared to normal and cancer adjacent tissue (CAT) (p<0.0001). Within the types of IDC, CCTβ was highest in tumors that exceeded 5 cm across (T3), grew in chest wall or skin and in inflammatory breast cancer (T4) (p<0.05). Examining CCTβ levels in different molecular types showed little correlation with estrogen receptor (ER) positivity but strong correlation with ER and progesterone receptor (PR) positivity or PR alone (p>0.001). Statistical correlations were also observed with Her2 positivity (p>0.05). However, no statistically significant correlations were observed with TNBC tissues, with CCTβ staining ranging from the strongest staining (4) to lowest (1). These results are similar to that observed with the TNBC cell lines and indicate that CCT expression may reflect the heterogeneity of TNBC. These results suggest that CCT is a promising target for therapeutic intervention due to its increased expression in advanced stage breast cancer, independence of molecular identity and dependence by cancer cells to support essential cytoskeletal changes associated with the metastatic stem-like phenotype.
Targeting the pH regulatory mechanisms of breast cancer cells


Background:
The abnormal regulation of H+ ions, leading to a reversed pH gradient in tumor cells in comparison to normal cells, is considered to be one of the hallmarks of cancer. This feature, however, has yet to be exploited as a therapeutic target. The aim of this study was to assess whether targeting proteins (CAIX, NHE1 and V-ATPase) that permit hypoxic cancer cell adaptation to acidosis in the tumor microenvironment can produce an effective therapeutic response in breast cancer, using 2D and 3D models.

Method:
Western blotting and gene expression analysis were performed on MCF-7, MDA-MB-231 and HBL-100 cancer cells to assess target protein expression in differing O2 conditions in 2D, while IHC was used to measure protein levels in 3D using multicellular tumor spheroids. Sulforhodamine B assays were executed to analyze the effects of inhibitors targeting CAIX, NHE1 and V-ATPase on breast cancer cell proliferation in 2D. 3D invasion assays were performed with MDA-MB-231 spheroids and explant tissue derived from human patients to see if CAIX inhibition had any effect on cancer cell invasion. An MDA-MB-231 xenograft model was used to investigate the effects of CAIX inhibition in vivo. Clonogenic assays were performed with MDA-MB-231 spheroids to evaluate whether any of the drugs combined effectively with irradiation.

Results:
2D and 3D expression analysis showed that CAIX levels were extremely responsive to changes in O2 conditions in each of the cell lines, with HBL100 cells exhibiting the largest changes in both mRNA (42-fold increase) and protein (78-fold increase) levels at low (0.5%) O2 concentrations. NHE1 and V-ATPase mRNA/protein levels were, however, much more consistently expressed across the cell lines in different O2 conditions. Drugs targeting CAIX, NHE1 and V-ATPase had anti-proliferative effects on the breast cancer cells in 2D. Normoxic cancer cells were the most sensitive to drug treatment, acute hypoxic cancer cells showed increased resistance to the anti proliferative effects of these drugs, while chronic hypoxic cells had IC50 values more similar to the normoxic cells. The results for the CAIX inhibitor were unexpected, as we had predicted that the increased levels of CAIX in the acute hypoxic cells would make them more sensitive to treatment. CAIX inhibition did, however, significantly reduce the invasion of cancer cells from both MDA-MB-231 spheroids (p≤0.01) and explant tissue (p≤0.001). Targeting pH regulation was also shown to have an effect in vivo on MDA-MB-231 xenografts, with CAIX inhibition significantly reducing the growth (p≤0.05) and proliferation (p≤0.05) of tumors within mice. Finally, clonogenic assays showed that drugs targeting both CAIX and NHE1 led to a significant reduction in colony number when combined with radiation (p≤0.05), compared to either drug individually or radiation treatment alone.

Conclusions:
This study shows that drugs targeting pH regulation molecules have potential in the treatment of breast cancer. This is highlighted by their ability to affect the proliferation and invasion of breast cancer cells, along with their ability to be combined with radiation. Of the 3 pH regulatory molecules, CAIX represents the target with the most promise.
Body: Objective: The six transmembrane epithelial antigen of the prostate (STEAP1) is predominantly overexpressed in human prostate cancer. STEAP1 was first identified as a prostate-specific cell-surface antigen and found to be up-regulated in various cancers including lung, bladder, colon, and ovarian with little expression in normal tissue. An anti-STEAP1 monoclonal antibody linked to an antimitotic agent is currently in Phase I clinical trials for prostate cancer patients. Microarray data from our lab suggested that STEAP1 is also highly expressed in human breast cancers and bone marrow disseminated tumor cells. In this study we evaluate expression STEAP1 in primary tumors, and bone marrow (BM) from breast cancer patients.

Experimental procedures: RNA was isolated from primary tumor, non-malignant breast tissue and bone marrow (BM) from stage II and III breast cancer patients, healthy volunteers, breast cancer cell lines and BM from patient derived xenographs (PDX). Disseminated tumor cells (DTCs) from patient BM were enriched by microfiltration and analyzed by RNA-in situ hybridization (ISH). STEAP1 RNA expression was analyzed by Nanostring nCounter and qRT-PCR using human specific probes. STEAP1 immunohistochemical (IHC) staining of human tissue was performed using standard protocols. Knockdown of steap1 expression was accomplished using a lentiviral system.

Results: STEAP1 mRNA was up-regulated in 77% of tumors (28/36) compared to the corresponding normal tissue. STEAP1 protein was expressed in 100% of tumors (8/8) and was absent in non-malignant breast tissue (7/7) by IHC staining. STEAP1 mRNA was not expressed normal BM, but was detected in 8% (6/74) of BM from patients with early stage breast cancer. STEAP1 expression in the BM was associated with triple negative disease (3/6) and recurrent disease development (4/6, p=.028). STEAP1 expression was observed in individual DTCs isolated from patients BM, while no expression was observed in normal BM. In a PDX model of breast cancer, STEAP1 expression in BM was only observed in mouse who developed metastatic disease associated (7/10, p=.004). Knockdown of STEAP1 in the breast cancer cell line MDA-MB231 cells inhibited cell growth by 80-90%.

Conclusion: STEAP1 is expressed in human breast tumors and disseminated tumor cells found in the bone marrow of breast cancer patients. Expression of STEAP1 in the BM is significantly associated with the development of metastatic disease in patients as well as in a mouse model of breast cancer. Our data indicate that STEAP1 could serve as a therapeutic target for the treatment of minimal residual disease in breast cancer.
CDK8 protein complex as a potential biomarker and therapeutic target in breast cancer


Body: Cyclin-dependent kinase 8 (CDK8) and its paralog CDK19 are transcriptional regulators that, in complex with CCNC, MED12 and MED13, mediate several carcinogenic signalling pathways such as NFκβ, TGFβ/BMP, WNT/β-catenin, HIF1A and serum growth factor network. Using immunohistochemical analysis, we found that CDK8/19 protein is overexpressed in invasive ductal carcinomas of the breast relative to non-malignant mammary tissues. TCGA database analysis showed that gene amplification is the most frequent type of genetic alterations of CDK8, CDK19, CCNC and MED13 in breast cancers, with MED13 appearing as one of the most frequently amplified genes in breast cancer (amplified in 9.7% of samples), whereas point mutations are more common in MED12. CDK8, CDK19 and CCNC expression was strongly increased but MED12 expression was decreased in tumors with mutant p53. Meta-analysis of transcriptome databases revealed that higher expression of CDK8, CDK19, CCNC and MED13 (but not MED12) is associated with shorter relapse-free survival (RFS) in the four molecular subtypes of breast cancer. The RFS correlations were much stronger in patients who underwent systemic adjuvant therapy than in untreated patients, suggesting that CDK8 and its interactive genes impact the failure of systemic therapy. This result is in agreement with the role of CDK8 as a mediator of the chemotherapy-induced paracrine network that promotes drug resistance and metastasis (Porter et al., PNAS, 109, 13799, 2012) and with our finding that a small-molecule CDK8/19 inhibitor augmented the efficacy of doxorubicin in a triple-negative breast cancer xenograft model. The expression levels of CDK8, CDK19, CCNC and MED13 in breast cancer samples were directly correlated with each other and with the expression of MYC but inversely correlated with estrogen receptor (ER)α expression. Since MYC is known to be a positive downstream mediator of the ER activity, we hypothesized that CDK8 may play a similar role, with an increase in CDK8 augmenting estrogen mitogenic signalling in tumors with decreased ER. Confirming this hypothesis, we have found that CDK8 inhibition by selective small-molecule CDK8/19 inhibitors or by shRNA knockdown suppresses estrogen-induced transcription in ER-positive breast cancer cell lines. CDK8/19 inhibition abrogates the mitogenic effect of estrogen on ER-positive cells and synergizes with the ER antagonist fulvestrant. Treatment of estrogen-deprived ER-positive cells with a CDK8/19 inhibitor significantly impeded the outgrowth of estrogen-independent cells, to a greater extent than did mTOR or HER2 targeted drugs. These results indicate that the expression of CDK8 and its interactive genes has a profound impact on the response to treatment in breast cancer and may provide novel biomarkers for relapse-free survival after adjuvant therapy. CDK8/19 inhibition may be useful to augment chemotherapy and hormone therapy of breast cancer and to prevent the development of tumors resistant to estrogen deprivation.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-04-08

Title: Abstract Withdrawn

Body:
Title: Copper chaperons as novel targets for therapy in triple-negative breast cancer (TNBC)

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Body: Background: Copper metabolism is frequently dysregulated in cancer and promotes tumorigenesis. Copper chelation was shown to delay tumor development, attenuate tumor growth, block angiogenesis and inhibit metastases in preclinical breast cancer models. Copper depletion with tetrathiomolybdate (TM) in on-going phase II study for breast cancer patients at high risk for relapse resulted in significant improvement in progression-free survival, especially in patients with TNBC. We hypothesized that targeting ATOX-1 and CCS, copper chaperons that are major regulators of copper trafficking, with novel selective inhibitor may disrupt cellular copper transport and suppress TNBC cell growth, block angiogenic activity, and enhance cytotoxicity of available chemotherapy.

Methods: We measured ATOX-1 and CCS protein expression using western blot in a panel of breast cancer cell lines including TNBC cell lines with basal-like (BL) and claudin-low (CL) subtypes. We compared potency and efficacy of ATOX-1/CCS inhibitor to induce cytotoxicity in MDA-MB231, MDA-MB436, MDA-MB468 and primary normal mammary HMECs. We evaluated ability of the inhibitor to disrupt tubulogenesis of endothelial cells. To determine if blocking copper transport can enhance sensitivity of TNBC to chemotherapy we used novel ATOX-1/CCS inhibitor in combination with Cisplatin to treat TNBC in a schedule-dependent manner.

Results: ATOX-1 protein expression was elevated in all tested TNBC cell lines compared to normal HMEC (1.7±0.2 and 2.1± 0.3 folds higher in BL and CL cells, respectively). Upregulated CCS protein expression was also observed in majority of tested cell lines compared to HMEC (2.8±0.6 and 1.2±0.1 times higher in BL and CL cells, respectively). Treatment of MDA-MB231, MDA-MB436, MDA-MB468 with the inhibitor resulted in reduced cell proliferation. IC50 doses for 72h treatment with single agent were: 0.23±0.02uM (MDA-MB468), 0.29±0.03uM (MDA-MB231) and 0.35±0.02uM (MDA-MB436). Additional cytotoxicity was observed in TNBC when ATOX-1/CCS inhibitor was applied in combination with Cisplatin. Interestingly, sequential treatment resulted in synergistic effect (CI< 1). Treatment with the inhibitor reduced growth of HMECs and HuVECs in vitro, and inhibited angiogenesis in tube formation assay with HuVECs.

Conclusions: Targeting copper trafficking by selective inhibition of chaperons ATOX-1 and CCS is promising and could potentially serve as a therapeutic approach to overcome resistance to chemotherapy in TNBC. In vivo studies investigating efficacy and biological activity of the novel compound in a xenograft model are ongoing and will help to elucidate molecular mechanisms of action, and further estimate potential clinical relevance of this approach.
Title: Phosphodiesterase type 5 promotes the invasive potential of breast cancer cells through Rho GTPase activation

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Body: The impairment of cyclic guanosine monophosphate (cGMP) signaling by overexpression of PDE5 isoform has been recently described in multiple human carcinomas. In addition, accumulating evidences indicate that PDE5 inhibitors could have direct anti-cancer activities as well as they may enhance the sensitivity of certain types of cancer to standard chemotherapeutic drugs. However, despite these studies, neither the expression of PDE5 in breast cancer subtypes nor the underlying regulatory molecular mechanisms by which PDE5 expression may contribute to breast cancer progression have been deeply studied. We demonstrated that PDE5 was expressed in different subtypes of breast cancer cell lines at higher levels than in non-tumorogenic human epithelial breast cell lines. Increased levels were detected in more aggressive endocrine non-responsive basal-like breast cancer cells. Interestingly, PDE5 was expressed at very low levels in luminal A-type breast cancer cell lines, which display low ki67 expression, weak invasive behavior and endocrine responsiveness (MCF-7 and T47D cells) compared to luminal B-like cells (such as ZR-75 cells). These results well correlated with data obtained in immunohistochemistry analyses of human breast cancer tissues, showing PDE5 expression in 30 of 35 tumor entities analyzed, with the highest intensity staining in high-grade tumors. Concomitantly, no cytoplasmic PDE5 staining was observed in non-neoplastic tissues examined (n=5). In addition, retrospective analyses (n=1959, median follow-up time: 25 years) showed that high PDE5 expression in breast cancer patients was correlated with a statistically significant poorer survival compared to low PDE5-expressing patients. A more relevant discrimination is achieved in lymphnode-negative patients, suggesting a role of PDE5 for identifying early patients at high risk of rapid progression.

In order to better ascertain the role of PDE5 in breast tumorogenesis, we selected a breast tumor cell line that express low levels of this enzyme, MCF-7 and engineered stable clones for overexpression studies. Both vector- and PDE5-stable MCF-7 clones demonstrated comparable proliferation rates; whereas, cell motility and invasion were dramatically increased in PDE5-overexpressing cells. RNA sequencing to compare the transcriptomes of vector- and PDE5-overexpressing MCF-7 cells identified differential expression of genes involved in cell migration and invasion. Particularly, based on pathway analysis we found marked changes in the expression of Rho GTPase family members, proteins involved in cell cytoskeleton organization, migration, and metastasis dissemination (Rho A, cdc42 and Rac signaling, activation score= 1.9, 1.342, and 0.302, respectively). Indeed, Rho and cdc42 pull-down assays revealed increased Rho GTPase activity in cells overexpressing PDE5. Moreover, the selective ROCK inhibitor Y-27632 as well as the PDE5 inhibitor sildenafil were able to significantly reduce both migration and invasion of PDE5 clones.

Our data reveal that PDE5 expression enhances motility and invasiveness of breast cancer cells through the activation of the Rho family of GTPases, and highlight, for the first time, a novel role for PDE5 as a marker of poor outcome in breast cancer patients.
Title: Research on specific substrates and function of prolyl hydroxylase in breast cancer by CRISPR-Cas9

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Body: Objective: As an important oxygen sensor in cells, prolyl hydroxylase (PHD) could hydroxylate the proline of its substrates during hypoxia, which results in the ubiquitination and degradation of cells. PHD is a family including PHD1, PHD2 and PHD3. PHD was associated with multiple cell processes and cancer, but no systematic study was performed in breast cancer. This study was to harness CRISPR-Cas9 to knock out PHDs, find their specific substrates and further study the function of PHDs in breast cancer.

Methods: We extracted mRNA from 118 breast cancer tissues and got cDNA by reverse transcription. The expression of PHD1/PHD2/PHD3 was examined by Real-time PCR and the correlation between PHDs mRNA and prognosis of breast cancer patients were calculated by SPSS software. We constructed CRISPR-Cas9 system targeting PHD1/PHD2/PHD3 genes and knock out the three genes in MDA-MB-231 and SK-BR-3 breast cancer cells. T7e1 mutation detection and Sanger sequencing were used to verify the PHDs-knockout cells. Migration and proliferation test were performed to determine the change of function in vitro. Subsequently, we took Immunoprecipitation (IP) to enrich hydroxylased proteins and obtained the hydroxylation substrates of breast cancer cells by MassSpectrometry (MS).

Results: High expression of PHD1 and PHD2 mRNAs were related to better disease-free survival (DFS) (P = 0.013, P = 0.050) and overall survival (OS) (P = 0.016, P = 0.032), but no significant difference was obtained in PHD3. We successfully constructed CRISPR-Cas9 system targeting PHD1/PHD2/PHD3 and got MDA-MB-231 and SK-BR-3 breast cancer cells with PHD1/PHD2/PHD3 knocking out by CRISPR-Cas9, which were verified by T7e1 mutation detection and Sanger sequencing. With PHDs knocking out, cells ability of migration and proliferation increased in contrast to wildtype. IP-MS was performed subsequently and we got hydroxylation substrates of PHD1, PHD2 and PHD3 in breast cancer respectively, including RhoA, RhoC, DOCK1, IKKβ, PGAM1, and so on. Among them, RhoA, RhoC were related with pseudopod formation and cell migration. DOCK1 was guanine nucleotide exchange factor (GEF) and could stimulate the function of rac, which was proved to be an oncogene.

Conclusions: PHD1 and PHD2 mRNAs were related to better prognosis of breast cancer patients. We found more hydroxylation substrates of PHD1, PHD2 and PHD3 in breast cancer through high-throughput screening, which might become the target of breast cancer treatment.
Title: Silencing BMI1 radiosensitizes human breast cancer cells by inducing DNA damage and autophagy

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Body: BMI1, a member of the polycomb group family of repressive complexes, is critical for stem cell renewal and is often overexpressed in many cancer types. Overexpression of BMI1 correlates with advanced stage of disease, aggressive clinico-pathological behavior, poor prognosis and resistance to radiation and chemotherapy. Studies have shown that experimental reduction of BMI1 protein results in inhibition of cell proliferation, induction of apoptosis and/or senescence in tumor cells and increased susceptibility to cytotoxic agents and radiation therapy. Though a role for BMI1 in radioresistance has been implicated, the molecular mechanism by which it mediates this resistance remains to be fully explored. In the present study we investigated the role of BMI1 in radioresistance, and its inhibition in restoring radiosensitivity, using breast cancer as a model. Silencing BMI1 by stably expressing an shRNA against BMI1 radiosensitized the MDA-MB-231 human breast cancer cell line. Clonogenic assay demonstrated that silencing BMI1 reduced the clonogenic survival at 2 Gy (SF2) from 54% ± 2 in the DsRed control cells to 45% ± 1 in shBMI1 (p value=0.01) cells. The involvement of the DNA damage response (DDR) in shBMI1 radiosensitization was examined by determining the number of γ-H2AX foci present in control and shBMI1 cells following irradiation. We observed a 2.15 fold increase in the number of γ-H2AX foci in the shBMI1 cells compared to that of the control cells (p value=0.01) at 24 hours after irradiation suggesting an inhibition of the DNA double-strand break (DSB) repair pathway. These data were further supported by the neutral comet assay which showed a 37% increase in tail length of BMI1 silenced cells over those of the controls (p value=6.05E-06) at 24 hours after irradiation. Additionally, the expression levels of p-Chk2T68, p-ATMS1981, DNA-PK, and Ku80, proteins that are involved in the DDR signaling pathway, were markedly reduced in shBMI1 cells following irradiation compared to the expression levels in the controls. This increase in γ-H2AX expression levels in shBMI1 cells compared to control cells after exposure to ionizing radiation suggests the disruption of DDR signaling takes place upstream of ATM. Since autophagic cell death, has recently emerged as an important mechanism of tumor cell death induced by radiation, we evaluated the contribution of autophagy to shBMI1-mediated radiosensitization in the MDA-MB-231 cells. The induction of autophagy was examined by measuring gene expression of autophagy-related genes by real time RT-PCR (ATG 3, ATG 5, ATG 7, ATG 12, LC3A, LC3B, Beclin-1) and western blot analysis (LC3B I and II, Beclin-1). Elevated expression of autophagy related genes was observed in BMI1 knockdown cells after exposure to ionizing radiation suggesting the involvement of autophagy in BMI-mediated radiosensitization. Collectively, these findings further suggest BMI1 as a therapeutic target for radioresistant breast cancers.
Title: Pin1 negatively impacts Smad3 tumor suppression in triple negative breast cancer cell lines

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Body: Introduction: Triple negative breast cancer (TNBC) is an aggressive subtype associated with poor outcomes. Accordingly, there is an urgent need to develop novel and targeted therapeutics for patients with this disease subtype. Cyclins D and E and the corresponding activation of CDK4/2 represent promising therapeutic targets for the treatment of TNBC. CDK4/2 can non-canonically phosphorylate Smad3, a key TGFβ signaling intermediate, to promote the transition from tumor suppressive to oncogenic TGFβ activity in cyclin-overexpressing breast cancers. We identified a Smad3 interaction with Pin1, a cis-trans isomerase also overexpressed in aggressive breast cancers and associated with CDK-mediated Smad3 phosphorylation. Smad3 interaction with Pin1 can influence protein function and fidelity through recruitment of Smurf2 and subsequent proteasomal degradation. Based on these findings, we hypothesized that inhibition of the CDK-mediated Smad3-Pin1 interaction would stabilize Smad3 protein expression and restore tumor-suppressive Smad3 activity.

Methods: Pin1 expression was knocked-down (KD) in MDA-MB-231 TNBC cells by transfecting with Pin1-targeting siRNA (siPin1) or control non-specific siRNA (siNS). KD efficiency was confirmed by immunoblotting. To assay Smad3 transcriptional activity with Pin1 KD, luciferase reporter studies were performed. Also, following Pin1 KD, immunoblotting was used to determine expression of Smad3 and associated protein targets. MTS assays were utilized to determine cellular proliferation after Pin1 KD. Transwell migration assays were used to assay the effect of Pin1 KD or CDK2 inhibitor treatment, which blocked non-canonical Smad3 Thr179 phosphorylation, on TNBC cell migration.

Results: KD of Pin1 expression in TNBC cell lines resulted in an increase in Smad3 transcriptional activity compared to control cells, and correlated with an increase in expression of cdki p15 and a decrease in c-myc, Smad3-target genes and cell cycle regulators. Additionally, Pin1 KD resulted in a significant decrease in TNBC cell proliferation compared to siNS control TNBC cells. Smad3 protein levels increased following Pin1 KD, suggesting Pin1 action may negatively impact Smad3 stability. We also found that KD of Pin1 or treatment with a CDK2 inhibitor, which blocked Smad3 noncanonical Thr179 phosphorylation, resulted in significantly reduced TNBC cell migration.

Conclusions: Inhibiting the Smad3-Pin1 interaction by knock-down of Pin1 expression in TNBC cells restored Smad3 transcriptional activity, which correlated to an increase in expression of the Smad3 associated protein cdki p15, decrease in c-myc, and a decrease in cellular proliferation. Additionally, Pin1 KD enhanced Smad3 protein levels, suggesting a role of Pin1 in mediating Smad3 stability. Inhibiting the Smad3-Pin1 interaction with Pin1 KD or CDK2 inhibitor treatment also reduced TNBC cell migration. Collectively, these data suggest that the Smad3-Pin1 interaction, facilitated by noncanonical CDK-mediated Smad3 phosphorylation, is associated with pro-tumorigenic and pro-migratory TGFβ signaling, and inhibition of this interaction may provide an important therapeutic option for TNBC patients.
Title: The covalent JNK inhibitor, JNK-IN-8, synergizes with lapatinib to cause cell death in basal-like breast cancer cell lines

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Body: Basal-like and claudin-low breast cancers have the worst prognosis and represent 15-20% of breast cancers diagnosed each year. Endocrine and molecularly targeted therapies, such as trastuzumab, are ineffective due to lack of ER expression or HER2 amplification in the tumors. They also have a high frequency of p53 mutations, low BRCA1 expression and high EGFR expression. Our lab has shown that high expression of JNK2 in human basal-like breast cancers leads to significantly decreased disease-free survival. JNK2 also promotes basal-like tumor progression in mice by increasing EGFR-mediated migration through facilitating internalization of EGFR, upregulating EMT gene expression and promoting metastasis.

The ATP-competitive JNK inhibitor SP600125 has been commonly used by researchers to elucidate JNK-specific mechanisms, but this inhibitor was found to have high affinity to many other intracellular kinases. A new JNK inhibitor, JNK-IN-8, has been developed that binds covalently to all three JNK gene products and is more selective than the SP600125 compound (Zhang, et al. 2012). Using this inhibitor, we have found that basal-like breast cancer cell lines can become sensitized to lapatinib. This combination is synergistic and causes apoptotic cell death, while as single agents at these concentrations, these drugs have little effect on cell viability. Treatment with either lapatinib or JNK-IN-8 decreases transcriptional activity of NF-κB significantly, but combination of the two drugs reduces NF-κB activity to an almost negligible amount compared to vehicle treatment. Combination treatment also led to a 6-fold increase in ROS production that may be activating apoptosis.

We hypothesize that inhibition by both JNK-IN-8 and lapatinib cause independent decreases in NF-κB activation that, when combined, cause a synergistic decrease in NF-κB-mediated survival mechanisms. Use of the JNK-IN-8 inhibitor with lapatinib in humans may increase survival in patients with basal-like or claudin-low breast cancers.

**Title:** Inhibitory effects of calcitriol and the vitamin D analogue paricalcitol in combination with chemotherapy on the growth of HER2 overexpressing breast cancer cells

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**Body:**

**Introduction:** Despite recent advances in therapy, additional options for improving the outcomes of patients with HER2-overexpressing breast cancer (BC) are needed. The role of vitamin D in relation to BC outcomes is controversial. Both calcitriol and synthetic vitamin D analogues have been shown to increase sensitivity to chemotherapy in BC cell lines. In this study, we aimed to determine the effect of combined treatment with either calcitriol or the vitamin D analogue paricalcitol plus doxorubicin or paclitaxel in HER2+ BC cells.

**Methods:** Cell characterization was performed using western blot for estrogen receptor (ER) α, progesterone receptor (PR) A-B, HER2 receptor and vitamin D receptor (VDR) A-B. Flow cytometry was used to determine the quantitative expression of HER2 in an established breast cancer cell line known to express VDR (SKBR3 [ERα-, PR A-B -, HER2+]). The effects of calcitriol and paricalcitol, alone or in combination with doxorubicin or paclitaxel, were evaluated using a Sulforhodamine B growth assay. We calculated IC20 inhibitory concentrations by non-linear regression analysis using sigmoidal fitting of dose-response curves. Results are presented as the mean cell proliferation percentage (%) ± standard deviation. Statistical analyses were carried out using one-way ANOVA and the Holm-Sidak method.

**Results:** Calcitriol, paricalcitol, doxorubicin and paclitaxel inhibited cell proliferation in a dose dependent manner. Calcitriol 100nM in combination with doxorubicin 0.01 µM inhibited the proliferation of SKBR3 cells to 30.9% ± 24.1, compared to 74.6% ± 6.6 with calcitriol alone (p = 0.003) and 86.6% ± 4.1 with doxorubicin alone (p < 0.001). Similarly, the combination of calcitriol 100nM plus paclitaxel 0.001 µM inhibited the proliferation of SKBR3 cells to 11.6% ± 10.4, compared to 77.5% ± 6.1 with calcitriol alone (p < 0.001) and 50.7% ± 8.4 with paclitaxel alone (p < 0.001). Paricalcitol 25nM in combination with doxorubicin 0.01 µM inhibited the proliferation of SKBR3 cells to 49.1% ± 25.2, compared to 72.3 ± 13.2 with paricalcitol alone (p = 0.21) and with doxorubicin alone (p = 0.05). Paricalcitol 10nM in combination with paclitaxel 0.001µM inhibited the proliferation of SKBR3 cells to 26.1% ± 23.1, compared to 72.3% ± 13.2 with paricalcitol alone (p = 0.05) and 43.2% ± 12.1 with paclitaxel alone (p =0.285).

**Conclusions:** Simultaneous treatment of SKBR3 cells with calcitriol plus either doxorubicin or paclitaxel showed a cooperative growth-inhibiting effect when compared with each cytotoxic drug alone. The combination of paricalcitol with each of the cytotoxic agents also showed a trend towards a higher growth-inhibiting effect, but failed to reach statistical significance when compared with each drug alone. Although these results point towards the presence of a cooperative antiproliferative effect of Vitamin D compounds on HER2+ BC cells when combined with chemotherapy, the concentrations needed to reach such an effect were high, which could potentially limit their use in clinical practice.
Title: BRIP1, potential candidate in non-BRCA1/2 breast cancer patients

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Body: In Oman, one of the Middle Eastern countries where the rate of consanguinity is high (~50%), breast cancer (BC) affects younger females (25-40 years) who attend the clinic with advanced stages. These breast tumors do not exhibit any functional mutations in \textit{BRCA1/2}, suggesting other genes to contribute significantly in the onset of BC, either independently or in association with normal \textit{BRCA1/2}. BRIP1 was identified as a potential candidate by gene expression profiling (5 fold induction) in 40 malignant breast tumors compared to their matching control Omani patients (normal/benign breast tissues). The differential expression of \textit{BRIP1} was structurally validated by RT-PCR and immunohistochemistry. Sequencing of the coding region of \textit{BRIP1}, using DNA isolated from the same samples identified major functional mutations at a significantly high rate, the Ser919Pro (60%), found in the BRCA binding domain and c.-141-64G>A (46%) detected in the promoter region. Ongoing validation experiments aim to further unravel the mechanisms underpinning BRIP1-suppressed breast tumour function. In addition to identifying \textit{BRIP1} as a novel potential marker in non-BRAC1/2 Omani BC patients, this study has the potential to pave the way towards the design of specific anti-BC therapeutic strategies for these families.
Title: From transcriptome meta-analysis to targeted therapies in triple negative breast cancer

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Body: Triple-Negative Breast Cancer (TNBC) is a subtype of breast cancer that urgently requires the identification and approval of novel targeted therapies. Even for breast cancer subtypes that have approved targeted therapies such as tamoxifen in ER+ and herceptin in HER2+ patients, there are a proportion of patients that do not respond to these therapies or develop resistance and succumb to metastatic recurrence. Thus, there is a clinical need to identify patients who do not benefit from current standard therapies and developing new strategies for therapy for non-responsive patients across all breast cancer subtypes.

We hypothesised a potential prognostic and drug discovery approach by meta-analysis of multiple global gene expression profiles of breast cancer studies to identify significantly over- and under-expressed genes that associate with clinical outcomes (metastatic and/or death events within five years). These genes were filtered through 3 methods to annotate their predicted functions (1). The third and most generalised method identified a 133-gene signature set that was prognostic in all subtypes of breast cancer. These signatures, particularly with the devised score calculated as the ratio of the average expression of the over-expressed genes to the under-expressed genes, were patented (2). Of the 133 genes, we selected a 21-gene list representing novel and new targets in breast cancer drug development. The 21 genes were selected through an unbiased ranking of least-published cancer and non-cancer studies in PubMed.

This poster reports the characterisation of these 21 genes as drug targets in TNBC. A siRNA screen measuring proliferation and viability (xCELLigence RTCA) was performed (2 siRNAs per gene) on 3 TNBC cell lines (MDAMB231, SUM159PT and BT549) and MCF10A. The top gene hits that show a dependence on cancer proliferation and viability were followed up with clonogenic assays, cell cycle profiles and apoptosis assays. Parallel to these experiments, a negative-selection clustered regularly interspaced short palindromic repeats (CRISPR) screen involving the 21 genes (6 constructs per gene) is currently underway on MDAMB231 cell line in vitro and in vivo to identify cancer dependence on these genes for survival and metastasis.

References
Title: Extracts derived from fungi and plants demonstrate specificity for subtypes of triple negative breast cancer

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Body: New effective therapies are needed for patients with triple negative breast cancers (TNBC). The identification by Lehmann and Bauer\(^1\) of distinct subtypes of TNBC and representative cell lines that are driven by different defects and signaling pathways provided the opportunity, for the first time, to screen for selective activities against these subtypes of TNBC. Using this knowledge, we initiated a screen of diverse natural product extract libraries with the goal of identifying extracts selective for subtypes of TNBC. The compounds with this selective activity will then be purified using bioassay-guided fractionation. Drugs derived from plants and fungi have provided some of the most important pharmaceuticals used today, including numerous anticancer agents.\(^2\) Natural products occupy a biologically validated chemical space that does not overlap with compounds found in most synthetic chemical libraries.\(^3\) Additionally, there are differences in chemical space between plant and fungal-derived compounds\(^4\) and different compound classes are expected to be isolated from these two sources. A total of 1,953 extracts of fungi collected from diverse environments, including Great Lakes sediments and 2,200 plant extracts from tropical environments have been screened for selective cytotoxic activities against cell lines representing 5 subtypes of TNBC. These subtypes are the basal-like 1 and 2 (BL1, BL2), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR). The initial screening using one concentration, 2 µg/ml for fungal extracts and 20 µg/ml for plant extracts, identified many extracts with selective activity against the TNBC subtypes. Detailed dose response curves were then generated with these extracts in each of the TNBC cell lines. A total of 4 fungal extracts and 7 plant extracts with selective cytotoxic activities were identified with selectivity up to 100-fold for 3 of the extracts. Bioassay-guided fractionation is ongoing to identify the active constituents. These results demonstrate that natural product extracts can yield selective actions against TNBC subtypes. We expect that these plant and fungal extracts will yield compounds that target molecular drivers specific to the TNBC subtypes. It is our expectation that compounds with selective, targeted activities will continue to be isolated from these extract collections.

Title: JAK2 copy number and targeted JAK2 inhibition of TNBC cell lines

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Body: Background A subset of triple negative breast cancers (TNBC) has somatic amplification of chromosome 9p24.1, encoding PD-L1, PD-L2, and JAK2 (the "PDJ" amplicon). The JAK/STAT pathway promotes malignant transformation and proliferation, and the JAK1/JAK2 inhibitor ruxolitinib is being evaluated in early phase clinical trials in breast cancer. This study was designed to measure the frequency of PDJ copy number gain in existing TNBC cell lines, and determine the impact of targeted JAK2 inhibition on cellular proliferation.

Methods: Copy number alterations were measured in nine TNBC cell lines (MDA-MB-453, MDA-MB-436, BT549, MDA-MB231, MFM-223, MDA-MB-468, H5578T, MDA-MB-157, HCC1937) by array comparative genomic hybridization (aCGH). JAK2 and pSTAT3 expression was measured by immunoblot in four of the TNBC lines (HCC1937, MDA-MB-468, MDA-MB-231, MDA-MB-436) and compared with MCF10A, SK-BR3, and T47D. To selectively inhibit JAK2, lentiviral vectors encoding five different shRNA targeting JAK2 were generated. Ruxolitinib treatment was performed at 25 uM for 48 hrs and 72 hrs. Cell proliferation was measured by CellTiter-Glo.

Results: All TNBC cell lines had an elevated level of baseline protein expression of JAK2 and pSTAT3 compared to SK-BR3, T47D, and MCF10A. Of the nine TNBC cell lines, only MDA-MB-436 and BT549 had copy number gain of 9p24.1 (log2ratio<1). Four independent shRNA efficiently silenced over 90% of JAK2 expression in MDA-MB-231. The inhibition of JAK2 in MDA-MB-231 was associated with a decrease in both JAK2 and pSTAT3 protein expression and reduction in cell proliferation compared to control shRNA after 96 hrs (p<0.0001). Inhibition of proliferation was also observed for both MDA-MB-231 and MDA-MB-436 after ruxolitinib treatment.

Conclusion These results demonstrate that existing TNBC cell lines have varying copy number alterations of chromosome 9p24.1 encoding JAK2, and targeted JAK2 inhibition in TNBC inhibits cell proliferation.
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Title: TLE1 protein expression in HER2-positive and triple-negative breast cancer


Body: Purpose
Transducin-like enhancer protein 1 (TLE1) is a member of the Groucho (Gro)/TLE family of transcriptional co-repressors that regulate the transcriptional activity of a wide range of genes. TLE1 has been shown to be a specific and diagnostically useful immunohistochemical marker for synovial sarcoma. However, there have been few reports of the status of TLE1 in breast cancers. The purpose of this study was to clarify the nuclear expression of TLE1 in 511 Korean breast cancers by immunohistochemistry (IHC) and to correlate the findings to clinicopathologic variables including prognostic significance.

Methods
IHC was performed on tissue microarrays (TMAs) in 511 cases of breast cancer. Associations between the TLE1 expression and the clinicopathologic characteristics were retrospectively analyzed. Progression-free survival and disease-specific survival was assessed by the Kaplan–Meier method and Cox regression model.

Results
Of 511 cases of breast cancer, high expression of the TLE1 was detected in 63 (12.3%) cases. TLE1-high expression was strongly associated with HER2-positive breast cancer phenotype \( (p < 0.001) \) and triple-negative breast cancer phenotype \( (p < 0.001) \). Furthermore, high TLE1 was significantly associated with high histologic grade \( (p < 0.001) \), node-negative \( (p =0.011) \), estrogen receptor negativity \( (p < 0.001) \), progesterone receptor negativity \( (p < 0.001) \), CK5/6 positivity \( (p < 0.001) \), epidermal growth factor receptor positivity \( (p < 0.001) \), high ki-67 proliferative index \( (p < 0.001) \) and high p53 expression \( (p < 0.001) \).

Survival analysis demonstrated no significant association between TLE1 expression and disease progression and cancer-related death \( (p =0.112 \) and \( p =0.068 \), respectively).

Conclusion
Our results show that high TLE1 expression is significantly associated with HER2-positive and triple-negative breast cancer. TLE1 may represent a potential therapeutic target for this aggressive disease, which warrants further investigation.
Prevalance of crown-like structures of the breast, a histologic biomarker linked to obesity: A retrospective study of 99 cases

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Body: Introduction: Breast cancer risk is multifactorial, and depends partly on obesity and related metabolic imbalances, including inflammation. Obesity is increasing worldwide, and is a known cancer risk albeit with complex mechanisms. Previous reports (Morris et al., 2011; Iyengar et al., 2015) indicate that local inflammation can be seen histologically as a rings of macrophages around necrotic adipocytes (“crown-like structures of the breast”, CLS-B). Our goal was to determine the prevalence of CLS-B in routine specimens from a cohort of patients with known BMI.

Methods: We retrieved archival H&E slides from a breast cancer cohort (N=99) previously characterized for BMI and fasting plasma/serum metabolic factors. Two pathologists reviewed all available sections of white adipose tissue not adjacent to tumour (median 7 blocks/case), excluding fat necrosis and mastitis, blinded to correlative data/BMI. We recorded the presence/absence and numbers of CLS-B, defined as a continuous ring of macrophages surrounding an adipocyte. Paraffin blocks were available in a subset (N=72) and a representative block was immunostained for CD68 to highlight CLS-B. For all cases, the average fat vacuole size was determined by digital image analysis (NIH ImageJ Software). We performed correlative statistics between CLS-B status and clinical data ($\chi^2$, Wilcoxon rank-sum tests).

Results: CLS-B were present in 37 of 99 cases (37%). When present the total number of CLS-B ranged from 1 to 18 (mean=4.3, median=3). CLS-B were detected in 7/10 (70%) patients with BMI >30 vs. 30/89 (34%) with BMI ≤ 30 (p=0.02). CLS-B also trended to higher prevalence in women over 60 compared to women under 60 (12/20, 60% vs. 25/79, 32%, p = 0.063). There was no significant association of CLS-B status with tumor T- and N-stage or grade (all P>0.4). The median C-reactive protein in the group with CLS-B was 1.5 mg/L vs. 0.8 mg/L in the group without CLS-B (P=0.10) There was no significant association of CLS-B with insulin, glucose, HOMA, leptin, adiponectin, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, or IGF-1 (all P>0.27). The average fat globule area determined by image analysis correlated significantly with BMI (Spearman correlation 0.54, p<0.0001) but not to the presence of CLS-B (p=0.102).

Within the subset immunostained for CD68, 32/72 (44%) had CLS-B on the original H&E sections, whereas 13/72 (18%) had CLS-B on the representative CD68-stained section. This corresponded to a false negative in 22/59 (37%) CD68-negative cases, and increased detection in 3/13 of the CD68-positive cases.

Conclusion: In our cohort, obesity is correlated with elevated tissue inflammation as seen by the presence of CLS-B, but CLS-B is not correlated with metabolic markers. CLS-B are well appreciated on routine H&E sections; however, more work is needed to find a practical approach to both ancillary testing (e.g. CD68) and quantitation. Our work independently confirms the association of CLS-B with obesity, and supports the concept that CLS-B is a tissue biomarker of obesity-related inflammation.

(Z.E. was co-principal author.)
Title: Regulation of glucose metabolism by the RUNX2 transcription factor has a negative impact on mitochondrial function in breast cancer cells

Kim MS, Choe M, Cho H, Polster BM M, Girmun GD D and Passaniti A. University of Maryland, Baltimore, Baltimore, MD and Stony Brook University, Stony Brook, NY.

Body: The RUNX2 transcription factor regulates breast cancer (BC) metastasis to bone and is itself a target of IGF-1 signaling pathways. We have shown that glucose can activate RUNX2 phosphorylation through IGF-1 receptor signaling and that RUNX2 was associated with inhibition of pyruvate dehydrogenase (PDHA1) activity and repression of mitochondrial respiration in BC cells. However, the mechanisms by which RUNX2 alters mitochondrial function and supports an oncogenic phenotype are not completely known. RUNX2 expression in a luminal BC cell line increased expression of glycolytic genes, and sensitivity to glucose starvation. However, RUNX2 knockdown in a triple-negative BC cell line inhibited expression of glycolytic genes. RUNX2 also repressed mitochondrial oxygen consumption rates (OCR), a measure of oxidative phosphorylation while overexpression of SIRT6, a NAD-dependent histone deacetylase, increased respiration in RUNX2-positive cells. RUNX2 repressed SIRT6 expression directly at the transcriptional and post-translational levels. High SIRT6 expression was observed in normal mammary tissue and cells that did not express RUNX2 but endogenous SIRT6 expression was low in malignant BC tissues or cell lines, which expressed high levels of RUNX2. These results suggest that SIRT6, a known tumor suppressor, was a critical mediator of these RUNX2-regulated metabolic changes. These results support a hypothesis whereby RUNX2 upregulation during BC progression leads to inactivation of the SIRT6 tumor suppressor to promote tumorigenesis. It will be important to investigate further changes in mitochondrial function to understand the precise mechanisms by which RUNX2 regulates BC metabolism.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-05-03

Title: Targeting hypoxia-induced cancer cell metabolism in breast cancer

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Body: Rational: Hypoxia contributes to high tumor grade in human breast cancer and is associated with resistance to radiation and chemotherapy. Hypoxia is generally encountered in perinecrotic regions throughout the heterogeneous tumor microenvironment and may be due to rapid tumor growth, insufficient neovascularization or as a result of antiangiogenic and chemoradiation therapies which cause acute vascular thrombosis. Cancer stem-like cells residing within these hypoxic zones compensate for the low concentration of oxygen through alterations in metabolism that favor aerobic glycolysis and de novo fatty acid synthesis. These metabolic derangements are known to enhance proliferation and survival, although the contributing molecular mechanisms are not fully understood. Here we evaluate a panel of FDA approved azole drugs for alternative use as glycolytic inhibitors with and without the fatty acid synthase (FASN) inhibitor, TVB-3166. TVB-3166 is currently in phase II clinical trials and is the first FASNi approved for human testing. METHODS: IC50 values of single or combined agents were determined by MTT under conditions of normoxia and hypoxia using sister plates of estrogen receptor positive or triple negative breast cancer cells, MCF-7, SKBR3 and MDA-MB-231. RESULTS: Because the combination of Itraconazole and TVB-3166 displayed a synergistic enhancement in cell death at a low nanomolar concentrations in MCF-7 cells, we next assessed the efficacy of these drugs in an in vivo xenograft model of breast cancer. Here, 3.6 million MCF-7 cells were implanted into the right flanks of (n=16) athymic nude mice, which were then randomized by tumor volume into 4 groups. Itraconazole and TVB-3166 were administered at 60 mg/kg daily by oral gavage as single agents or combined throughout the duration of the study. After three weeks, mice treated with combined inhibitors exhibited best response with a statistically significant decrease in tumor volume compared to the vehicle control group. CONCLUSION: Given the significant tumor response to the combination of metabolic inhibitors, further studies that evaluate the radiosensitizing effects under conditions of hypoxia are warranted. By targeting hypoxia-induced metabolic pathways during radiotherapy, we may enhance cancer stem-like cell death to dramatically improve patient outcome.
Title: MALDI mass spectrometry imaging profile of low molecular metabolites in breast carcinoma tissues embedded in frozen tissue microarray

Torata N, Kubo M, Miura D, Ohuchida K, Miyazaki T, Fujimura Y, Hayakawa E, Kai M, Oda Y, Mizumoto K, Hashizume M and Nakamura M. Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan; Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Kyushu University Hospital Cancer Center, Fukuoka, Japan and Advanced Medicine Innovation Center Kyushu University, Fukuoka, Japan.

Body: [Background]
Metabolomics is now widely utilized for searching disease markers or identification of new drug targets. In common method, tissue samples originated in human resected specimens are stored by formalin-fixed, paraffin-embedded (FFPE) blocks. However, these samples are inadequate to measure low molecular metabolites or lipids. Furthermore, extraction process that is required for conventional mass-spectrometry causes the loss of information on the spatial localization of the metabolites. In this study, we directly analyzed breast carcinoma tissues embedded in frozen tissue microarray (fTMA) using MALDI mass spectrometry imaging (MALDI-MSI). With our original method, we could obtain profiles of low molecular metabolite and mapping images of several tissues at one time.

[Method]
Six fTMA blocks were constructed by 119 breast tissues (carcinoma 84, normal 35) from 99 patients and sectioned at 10 um thickness. MADLI-MSI were performed by AXIMA Confidence (Shimadzu, Japan) with 9-aminoacridine as a matrix (m/z range: 50–1000, Negative Ion mode). Carcinoma and normal area in individual tissues were confirmed by H&E staining of slide grasses after MADLI-MSI analysis. Acquired MSI data were processed with the freely available software BioMap (Novartis, Switzerland).

[Result]
We could detected 1,915 peaks derive from endogenous metabolite by direct tissue MALDI-MSI analysis of breast carcinoma fTMA. Among them, 185 peaks that could be commonly detected were subjected to further analysis. Among these peaks, we could identify 18 metabolites related to energy metabolism such as ATP. By comparison of metabolite profiles obtained from carcinoma with normal tissues, we found that the energy charge (EC; which is related to ATP, ADP and AMP concentrations) and the sum of adenosine phosphate compound intensities (AXP) were significantly higher than that of normal tissue (EC; T : N = 0.56 : 0.35, AXP; 17453 : 2066, p<0.0001), but there were no significant difference with lymph node metastasis, tumor histological type and tumor size. In comparison with tumor subtype, higher EC was observed in ER(+) / Her2(-) tumor than others but AXP showed no significant among all subtypes including Ki-67 labeling index.

[Conclusion]
A combination of fTMA and MALDI-MSI is promising approach for biomarker discovery because it can achieve high throughput metabolic mapping without obvious artifact or other problem. In this study, even though high EC value were indicated in carcinoma tissue than normal but newly biomarker candidate was indeterminate at this moment. Identification of the candidates of novel carcinoma tissue biomarker is now underway.
Title: Inhibition of enhanced glucose uptake and glycolysis by KU-55933 as a novel strategy against aggressive breast cancer


Body: The ability of cancer cells to produce large amounts of lactate through aerobic glycosis (Warburg effect) is coupled to high rates of glucose uptake. Enhanced glucose uptake and glycolysis are closely correlated to increased breast tumor aggressiveness and poor prognosis. However, despite the importance of glucose uptake in supplying energy and preventing apoptosis of cancer cells, the majority of current efforts in searching for therapeutic agents targeting glucose metabolism have been aimed at modulating activities of different metabolic enzymes that are involved in glycolysis. Very limited studies have been done in developing novel therapeutic agents against glucose uptake in breast cancer cells.

Ataxia-telangiectasia (A-T) is a monogenic, autosomal recessive disorder characterized by cerebellar ataxia and oculocutaneous telangiectasias. The gene mutated in this disease, ATM (A-T, mutated), encodes a 370-kDa protein kinase. Although ATM is traditionally considered to be a nuclear protein that functions as a signal transducer in the cellular response to DNA damage, it is now known that ATM is also present in the cytoplasm and has important cytoplasmic functions. We previously discovered that ATM activates Akt, a main regulator of glucose uptake, by stimulating its phosphorylation at Ser473 following insulin treatment. We also found that ATM participates in insulin-mediated glucose uptake in muscle cells, and KU-55933, a specific inhibitor of ATM, strongly inhibits this process.

Recently, we found that KU-55933 inhibits cell proliferation by inducing apoptosis in MDA-MB-231, a triple-negative breast cancer cell line. We have also found that KU-55933 inhibits migration of MDA-MB-231 by a cell invasion assay. Furthermore, we found that these cancer cells exhibit enhanced glucose uptake in response to insulin and the addition of KU-55933 leads to a dramatic reduction of insulin-mediated glucose uptake in these cells. To further test whether KU-55933's ability to induce apoptosis is linked to its inhibition of glucose uptake, we performed a cell death ELISA assay in MDA-MB-231 cells treated with KU-55933 and different concentrations of glucose. Our results show that KU-55933 induces apoptosis of MDA-MB-231 cells, resulting in a similar degree of cell death as glucose starvation, while cells treated with glucose in conjunction with KU-55933 have decreased apoptosis. Moreover, we performed a cell migration assay and found that KU-55933 strongly inhibits the migration of MDA-MB-231 cells (similar to that caused by glucose starvation), which is almost fully rescued by the extra glucose supplemented in the cell culture medium. We have also established a positional isotope labeling-based targeted metabolomics method that can directly measure the conversion from glucose to lactate through glycolysis in cancer cells. Our results show strong production of lactate from glucose in MDA-MB-231 cells even under normal aerobic growth conditions, and KU-55933 strongly inhibits this process. Our findings may lead to the development of KU-55933 and its analogs as a new generation of therapeutic agents against aggressive breast cancer.
Title: Cobalamin metabolism in breast cancer

Collins DA A. Mayo Clinic, Rochester, MN.

Body: Cobalamin (Cbl) has two canonical co-enzymatic functions within mammalian cells. In the cytoplasm methylcobalamin promotes the synthesis of methionine, thymidine, and S-adenosylmethionine to sustain the anabolism of proteins, DNA, and multiple methylation reactions, respectively. Within the mitochondria, 5-deoxyadenosylcobalamin (AC) facilitates the final step in the catabolism of branched chain amino acids, odd chain fatty acids and cholesterol for ATP production.

Methods and Findings:
To assess Cbl metabolism within breast tumors, Cbl plasma levels were obtained in 20 women with suspicious breast lesions. Next 0.25 ug of an Indium-111 (In-111) radiolabeled AC analog was intravenously administered and quantitatively imaged at 2-5 hours after injection with single photon emission computed tomography. Within the cohort, 17 were found to have tumors at biopsy, while 3 had benign pathology. Of the 17 breast cancers, 94.1% (16/17) had increased In-111 AC metabolism. Most of the In-111 AC avid breast cancers were in foci of invasive, high nuclear grade ductal carcinoma and were imaged independent of their estrogen (ER), progesterone (PR), or Herceptin (HER2) receptor status. Foci of high grade ductal carcinoma in situ (DCIS), and single cases of invasive lobular, inflammatory and triple negative (TN) tumors were also depicted. Excluding one patient with a large, 7.5cm breast tumor, the average tumor size was 1.9 cm (range 0.7 - 3.4 cm).

The average T:B ratio of the 16 true positive (TP) In-111 AC avid breast cancers was 5.8 (range 2.0-22.5). Four TP patients had ingested Cbl or dexamethasone (DEX) 24 hrs prior to their scans. The average T:B ratios in their tumors was 12.8 (range 5.9-22.5) with an average Cbl plasma concentration of 1,150 (range 710-2000 ng/L). In the 12 TP patients that had not ingested Cbl or DEX 24 hrs prior to tracer injection, the T:B ratio was 3.5 (2.0-5.6) with an average Cbl plasma concentration of 400 (range 70-580 ng/L). The 3 true negative (TN) scans had an average T:B ratio of 1.2 (range 1.0-1.5). The one false negative (FN) scan was a low grade well differentiated tubular carcinoma with a T:B ratio of 1.8. In these four patients the average Cbl plasma concentration was 402 (range 350-488 ng/L).

Pulse-chase (PC) studies in 8-12 weeks old, 20-25g nude mice burdened with the MDA-MB-231 TN breast tumors demonstrated that the administration of 2.0 ug of non-labeled AC at 2, 8, or 24 hrs prior to the injection of 0.5 ug of In-111 AC increased the In-111 AC uptake within the TN tumors by an average of 21.5%, 67.6%, and 90.6%, respectively. This occurred despite the doses of AC and In-111 AC being 20 and 5 times the respective maximum murine recommended daily allowance for Cbl. The DEX 24 hr PC interval increased In-111 AC uptake within the MDA-MB-231 tumors by 44.8%.

Conclusion:
The Cbl metabolic pathway is upregulated within aggressive breast malignancies and can be biochemically targeted by a simple pulse-chase technique in vivo. The definitive mechanisms underpinning the up-regulated mitochondrial metabolism of Cbl within human breast tumors and the PC methodology remain to be elucidated. Further investigation of Cbl metabolism within benign and malignant breast tissue could potentially yield new molecular targets to detect and treat aggressive breast tumors.
Title: The role of thyroid hormones in breast tumorigenesis: A translational study utilizing mouse models, cell culture and patient data

Franco A, Jolly LA Ann, Russell S, Goldstein L and DeHart J. University of Arkansas for Medical Sciences, Little Rock, AR and City of Hope, Duarte, CA.

Body: Breast cancer and thyroid hormone signaling have been linked since the 1960s. Breast cancer patients have a higher incidence of thyroid cancer, and thyroid cancer patients have a higher incidence of breast cancer than would be predicted by chance alone, supporting a link between thyroid hormone signaling and breast malignancy. Despite many correlative studies, the role and mechanism of thyroid hormone signaling in mammary tumorigenesis has not been elucidated. Past studies have not comprehensively evaluated thyroid hormones (T3, T4) and thyroid stimulating hormone (TSH) levels with breast cancer status in the same individual. The results are further confounded by issues of temporality; studies have either assessed thyroid hormone levels before or after diagnosis, prohibiting conclusions regarding whether thyroid disruption is a cause or effect of breast cancer. In vitro models demonstrate that mammary cells have thyroid hormone receptors and respond to thyroid hormones; however these studies have not been extended to in vivo models.

In the current study, we took a translational approach by combining data from cell culture, mouse models and breast cancer patients from the City of Hope Cancer Registry (CHCR). We used the murine MMTV-PyMT model of breast cancer and treated mice with the anti-thyroid drug PTU; lowering T4 levels and increasing TSH. These mice developed significantly larger mammary tumors than untreated animals or those treated with T4 (p= 0.0012 and p=0.0183, respectively). Next, we showed that MCF10a cells in vitro are sensitive to both T4 and TSH added to the culture medium. We hypothesize that even subtle perturbations in normal thyroid hormone levels can stimulate mammary cell growth, increasing risk of transformation. We extended these findings to a small pilot study of 879 invasive and 136 in situ female breast cancer patients with comprehensive thyroid hormone lab analyses in the CHCR (total=984). Pre-treatment results were available for 44 women. TSH was significantly elevated for invasive versus in situ patients (Pt-test =0.016), regardless of the timing of the test. Pre-treatment TSH levels were significantly increased as the severity of disease increased (Pt-test =0.026). Women diagnosed stage 1 disease with no recurrence had a mean TSH level of 1.68 ± 1.87 (Standard Deviation: SD), whereas women diagnosed with stage 1 disease who returned with metastasis had a mean of 2.64 ± 1.72 SD. In the same women, free T4 and total T4 levels were lower and T3 levels were higher (Pt-test< 0.05) in women with invasive versus in situ disease.

These studies support the hypothesis that even minimally dysregulated thyroid hormone levels may increase the risk of breast cancer development and progression to aggressive disease. Many women over the age of 50 suffer from sub-clinical hypothyroidism, and our results suggest that sub-clinical hypothyroidism increases breast cancer risk and disease progression. Interestingly, our data indicate that as disease progresses, dependence on TSH lessens, and the most striking differences may be seen in early and low grade disease. Collectively, our data highlight a need to further investigate the role of sub-clinical hypothyroidism in breast cancer.
Title: Proliferative breast disease identified by nipple aspirate fluid cytopathology has the laterality and asymmetry characteristics of breast cancer, supporting the thesis it is a cancer precursor

Kylstra JW, Kalnoski MH, Vo T, Lee ML, Chen S-C and Quay SC. Atossa Genetics, Inc., Seattle, WA; National Reference Laboratory for Breast Health, Seattle, WA and UCLA, Los Angeles, CA.

Body: Introduction. Within the basket of conditions known as Benign Breast Disease, Proliferative Breast Disease (PBD) is a finding at biopsy or aspiration of either benign hyperplasia or atypical hyperplasia (ADH). PBD is known to confer increased risk of future breast cancer [Hartmann, NEJM 2015;372:78-89]. However, there is some controversy as to whether these lesions are precursors to breast cancer (BCa), in which their synchronicity and asymmetry should match BCa, or alternatively, if they are biomarkers of risk, in which future cancers would not necessarily be linked in time and space to earlier PBD lesions. The Left-dominant asymmetry was first described over half a century ago, but its molecular origins in embryonic development have only recently been characterized [Wilting. J. Current Medic Chem 2011; 18:5519-27]. This study was conducted to explore if a similar pattern of asymmetry and unilateral v. bilateral incidence might exist in PBD. This could help to resolve the question of whether PBD is a precursor to BCa or merely a biomarker.

Methods. We examined Nipple Aspirate Fluid (NAF) for evidence of proliferative cytopathology, defined as a finding of either hyperplasia or ADH. NAF was collected using either one of two models of aspirator devices (ForeCYTE™ or HALO™). NAF is aspirated, dispersed on a flower filter, sprayed with fixative and stained with the Pap stain prior to cytologic characterization. Filters mounted whole on glass are placed directly under the microscope. This method allows for a diagnostically interpretable specimen in NAF-droplets as little as 0.007 µL, 1000-fold smaller than the unassisted visual detection limit. A central lab evaluated all specimens.

Results. Between 1/2012 and 6/2013, 1154 women without prior BCa (Age range 18-85; median 48, mean 47.9) consented to submit bilateral NAF specimens; 99.7% of breasts yielded NAF adequate for analysis, evidenced by presence of a duct-selective protein assay on the filter. PBD was found in 149 women, of whom 24 bilateral. The distribution is shown in the table below.

<table>
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<tr>
<th></th>
<th>Left-only</th>
<th>Right only</th>
<th>Bilateral</th>
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<tr>
<td>CCR 4</td>
<td>59</td>
<td>42</td>
<td>18</td>
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<tr>
<td>CCR 5</td>
<td>16</td>
<td>8</td>
<td>4</td>
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<tr>
<td>CCR4 (L) and CCR5 (R)</td>
<td>-</td>
<td>-</td>
<td>2</td>
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<tr>
<td>Total Women</td>
<td>75</td>
<td>50</td>
<td>24</td>
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King Classification: CCR4 = Benign hyperplasia; CCR5 - Atypical Hyperplasia (ADH)

Benign hyperplasia was found in 121 women: 10.5% of those tested. It was bilateral in 13% of cases and left-sided only in 57%. ADH was found in 30 women or 2.6% of those tested. When present, ADH was bilateral in 13% (4/30), L-sided only 53% (16/30) and R-sided only 33% (10/30) of the time. Total PBD identified by NAF cytopathology occurred in 13% (149/1154) of this population, was bilateral in 16.1% (24/149), L-only in 50.3% (75/149) and R-only in 33.5% (50/149). The excess left-sided occurrence of 17% was significant (p=0.048 by chi-square).

Conclusion. This is the first report describing laterality and symmetry of PBD as made by non-invasive NAF collection and demonstrates that the pattern observed is similar to what is seen with invasive breast cancer and in situ lesions, supporting the hypothesis that PBD are precursor lesions to breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-06-03

Title: Effect of obesity on molecular characteristics of invasive breast tumors: Gene expression analysis of 405 tumors by BMI

Ellsworth RE E, Toro AL L, Costantino NS S, Shriver CD D and Ellsworth DL L. Windber Research Institute and Murtha Cancer Center.

Body: Background: Obesity is a risk factor for breast cancer in postmenopausal women and weight gain after diagnosis is associated with decreased survival and less favorable clinical characteristics such as greater tumor burden, higher grade, and poor prognosis, regardless of menopausal status. Despite the negative impact of obesity on clinical outcome, the molecular mechanisms by which excess adiposity influences breast cancer are not well-understood.

Methods: Affymetrix U133 2.0 gene expression data was available for 405 primary breast tumors and 20 tumor-adjacent adipose tissues; RNA was isolated from tumor epithelia or adipose using laser microdissection. Patients were classified as lean (BMI<25), overweight (BMI 25-29.9) or obese (BMI>30). Statistical analysis was performed by ANOVA using Partek Genomics Suite version 6.6.

Results: Obese patients were significantly more likely to be diagnosed >50 years and to be of African American ancestry compared to lean or overweight women, while pathological characteristics including tumor stage, size or grade, lymph node status and intrinsic subtype did not differ significantly between groups. Principal component analysis could not effectively cluster the tumors by weight and no genes were differentially expressed using a false discovery rate <0.05. Using a less stringent unadjusted P-value of <0.01, 329 genes were differentially expressed, however, this gene expression profile could not discriminate patient tumors by BMI. In contrast, BMI was effective in clustering tumor-adjacent adipose samples by BMI based on differential expression of 156 genes.

Conclusions: Although tumor epithelial cells from obese women do not differ significantly from those of lean and/or overweight women at the gene expression level, tumor-adjacent adipose did differ by weight. These data demonstrate that less favorable outcomes in obese patients are not be attributable to the tumor itself but to influences from the microenvironment and suggest that decreasing breast adiposity may be an effective strategy to reducing risk and improving outcomes in obese women.
Title: Synergistically elevated hallmarks of cancer induced in non-malignant human breast cells by mixtures of environmental chemicals

Dairkee SH H, Luciani-Torres G, Moore DH H and Goodson WH H. California Pacific Medical Center Research Institute, San Francisco, CA.

Body: BACKGROUND: Estrogenic overexposure is a well-accepted risk factor in the etiology of breast cancer. Synthetic chemicals that simulate natural estrogens, i.e., xenoestrogens (XEs), occur widely in the environment entering the human body through multiple routes. Consequently, mixtures of XEs are known to circulate in the blood at any given time. Current protocols to evaluate chemical safety rely primarily on testing chemicals individually, ignoring the fact that the general population is exposed daily to mixtures of XEs. We asked whether mixtures of low dose XEs would intensify the cellular effects of individual XEs at the same concentrations.

METHODS: We exposed non-malignant breast epithelial cell cultures (HRBECs), obtained from high-risk human donors by random periareolar fine needle aspiration (RPFNA), to the high volume chemicals - bisphenol-A (BPA), methylparaben (MP) and perfluorooctanoic acid (PFOA). Cells were exposed to individual XEs or mixtures of all three at a range of concentrations encompassing environmentally relevant levels. Breast cancer associated phenotypes were quantitated as test endpoints using fluorescence-activated cell sorting (FACS) and/or Western blotting. The endpoints included: total and phosphorylated estrogen receptor (ER)\(\alpha\), ER\(\beta\), S-phase fraction, and 4-hydroxytamoxifen (OHT) induced apoptotic fraction. We fit a log-linear dose-response model to the data generated from 3 doses for each chemical, individually and as a mixture. Evidence of synergism was tested by comparing the observed data for mixtures with that predicted by an additive-in-dose model for single doses.

RESULTS: Our data demonstrates that the degree of perturbation induced by the chemical mixture tested here is not merely an additive effect of each chemical; instead it reflects considerable synergism in the mode of action. In 3/3 SNP-authenticated non-malignant HRBEC cell lines exposed to the chemical mixture at the lowest test dose, total and phosphorylated ER\(\alpha\) levels were consistently elevated. Concurrently, ER\(\beta\) level was reduced. A dose-dependent increase was observed in the S-phase fraction, together with a marked reduction of the OHT-induced apoptotic fraction in HRBECs exposed to BPA, MP, and PFOA individually (\(p<0.001\) for each exposure). A significantly greater effect, or synergism, was detected for these endpoints upon exposure to a mixture of the chemicals at the same concentrations (\(p<0.001\) for the combination vs. the predicted sum of individual doses). Primary HRBEC cultures from 7 additional human volunteers displayed a similar pattern of response.

CONCLUSION: Routine tests conducted to assess the carcinogenic potential of chemicals overlook possible synergism among different chemicals. We show that the combined effects of low doses of various chemicals can be much stronger than predicted by addition of their effects as single agents. Despite being cost, time, and labor-intensive, current testing of individual chemicals fails to inform regulators about the safe dose range for human exposure. It is thus imperative to expand present methods to include combinatorial approaches pertaining to test chemicals, endpoints, and target cells.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-06-05

Title: MED12 exon 2 mutations in phyllodes tumors of the breast

Nagasawa S, Ohta T and Tsugawa K. Division of Breast and Endocrine Surgery, St.Marianna University School of Medicine, Kawasaki, Japan and Division of Translational Oncology, St.Marianna University School of Medicine, Kawasaki, Japan.

Body: Exon 2 of MED12, a subunit of the transcriptional mediator complex, has been frequently mutated in uterine leiomyomas and breast fibroadenomas; however, it has been rarely mutated in other tumors. Although the mutations were also found in uterine leiomyosarcomas, the frequency was significantly lower than in uterine leiomyomas. Here, we examined the MED12 mutation in phyllodes tumors, another biphasic tumor with epithelial and stromal components related to breast fibroadenomas. Mutations in MED12 exon 2 were analyzed in nine fibroadenomas and eleven phyllodes tumors via Sanger sequencing. A panel of cancer- and sarcoma-related genes was also analyzed using Ion Torrent next-generation sequencing. Six mutations in fibroadenomas, including those previously reported (6/9, 67%), and five mutations in phyllodes tumors (5/11, 45%) were observed. Three mutations in the phyllodes tumors were missense mutations at Gly44, which is common in uterine leiomyomas and breast fibroadenomas. In addition, two deletion mutations (in-frame c.133_144del12 and loss of splice acceptor c.100-68_137del106) were observed in the phyllodes tumors. No other recurrent mutation was observed with next-generation sequencing. Frequent mutations in MED12 exon 2 in the phyllodes tumors suggest that it may share genetic etiology with uterine leiomyoma, a subgroup of uterine leiomyosarcomas and breast fibroadenoma.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-07-01

Title: Successful whole transcriptome analysis of 25-year-old breast tumor samples from the phase III trial SWOG-8814 by next generation sequencing (NGS): Standardized analytical methods for exploratory and validation studies

Cherbavaz DB B, Hayes DF F, Qu K, Crager MR R, Barlow WR R, Goddard AD D, Beasley EM M, Jeong J, Collin F, Liu M-L, Rae JM M, Ravdin PM M, Tripathy D, Gralow JR R, Livingston RB B, Osborne CK, Ingle JN N, Pritchard KI I, Davidson NE E, Carey LA A, Sing AP P, Baehner FL L, Hortobagyi GN N, Shak S and Albain KS S. Genomic Health, Inc., Redwood City, CA; University of Michigan, Ann Arbor, MI; Cancer Research and Biostatistics, Seattle, WA; Cancer Therapy & Research Center (CTRC) of The University of Texas Health Science Center, San Antonio, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; University of Washington, Seattle Cancer Care Alliance, Seattle, WA; University of Arizona Cancer Center, Tuscon, AR; Baylor College of Medicine, Houston, TX; Mayo Clinic, Rochester, MN; Sunnybrook Odette Cancer Centre and the University of Toronto, Toronto, ON, Canada; University of Pittsburgh Cancer Center, Pittsburgh, PA; University of North Carolina at Chapel Hill, Chapel Hill, NC; Genomic Health, Inc. and University of California, San Francisco, Redwood City and San Francisco, CA and Loyola University Chicago Stritch School of Medicine, Maywood, IL.

Body: BACKGROUND: We previously reported that low 21 gene Recurrence Scores (RS) identify patients with ER-positive, lymph node-positive breast cancer who may not benefit from anthracycline-based adjuvant chemotherapy added to tamoxifen (SWOG-8814 A NCI correlative science study; Albain et al. Lancet Oncol 2010). New exploratory and comprehensive quantitative analyses now permit whole transcriptome NGS on residual RNA extracted from FFPE blocks 12-18 years post-fixation. Herein, we report methodology details and feasibility results (see companion abstract, Albain et al., for clinical outcomes correlations).

METHODS and RESULTS: Sequencing was carried out in Illumina HiSeq 2000 instruments, yielding 4.2 trillion data points. Messenger RNA expression was quantified using 3rd quartile normalization. Both Library (RNA) and Sequencing Standards showed high quality coverage as measured by median uniquely mapped reads over a 13 month window (168M and 182M, respectively, including duplicate reads). The median absolute deviation (MAD) of the relative log expression (RLE) of mapped reads for the Library Standard was 0.22 and the Sequencing Standard was 0.05, respectively. The Library Standard variation was greater than the Sequencing Standard, as library preparation was manual. Of 360 patient samples with sufficient RNA (≥ 100 ng total RNA), 354 (98.3%) were successfully sequenced and included in the final analysis data set. Average library yield was 39 ng/µL. Only 5 libraries failed yield requirements and one library failed expression quality metrics. The median insert length was 120 bp with the first and third quartiles 93 and 152 bp, respectively. After removal of duplicate reads, 82% of reads were uniquely mapped, and the median library size was 8.95M (number of unique mapped reads). Sequences with counts <10 for all 354 patients were excluded. The medians of the 1st, 2nd and 3rd quartiles for exons mapped to the RefSeq database were 20, 40 and 78 counts, respectively. The majority of exonic (86.7%) and intronic (95%) sequences were mapped. There were 20,101 RefSeq mapped exons with counts ≥10. Of these exons, 988 passed additional filtering criteria and were subjected to hierarchical clustering, with Gene Ontology and pathway analysis performed on selected gene expression patterns (for results, see companion abstract of Albain et al.).

CONCLUSIONS: High quality whole transcriptome NGS is feasible from decades-old clinical trial FFPE specimens that have not been stored in any special fashion. Controlled laboratory, bioinformatics and biostatistics methods, with inclusion of appropriate process controls, ensure robustness and reliability of the NGS process. This in turn results in the discovery and validation of biologically and clinically relevant variations from prior landmark clinical trials.

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Title: Systematic analysis and modulation of Ki67 interobserver variance in 9069 patients from three clinical trials – How much pathologist concordance is needed for meaningful biomarker results?

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Body: Background: Ki67 has been suggested as a marker for diagnosis of luminal A and B breast carcinomas. Interestingly, on one hand a multitude of studies have described significant results for Ki67 as a prognostic marker, while on the other hand the analytical validation and standardization of this marker has been a challenge. The best parameter for Ki67 interobserver performance is the interclass correlation coefficient (ICC). ICC values between 0.59 and 0.92 have been reported. Recently a minimum ICC of 0.8 has been suggested as a goal for the international ring trial and as a prerequisite for introduction of Ki67 into clinical practice. However, this suggested ICC is not derived from analysis of data, and the amount of pathologist variance that is allowed for meaningful biomarker results is still not defined.

Methods: This study is based on a total of 9069 tumor samples from three large clinical cohorts (IBCSG VIII+IX, BIG1-98, and GeparTrio). In a systematic modeling approach, we introduced different amounts of variance to previously generated central pathology Ki67 datasets by simulation of a total of 1800 different pathologist evaluations for each study cohort. These evaluations were grouped into groups with defined ICCs, ranging from very good concordance (ICC=0.9) to extremely poor concordance (ICC=0.1). For each of the simulated pathologist evaluations, all possible Ki67 cutoffs were systematically evaluated using the web-based software Cutoff Finder (http://molpath.charite.de/cutoff/). As endpoints, we used DFS for all three study cohorts as well as pCR for the neoadjuvant cohort.

Results: For the neoadjuvant GeparTrio study, the different groups with ICCs of 0.8, 0.6 and 0.4 showed a very similar performance resulting in significant analyses for prediction of pCR across a wide range of cutoffs. The odd ratios for pCR were slightly lower with lower ICC. Even with an extremely low ICC of 0.2, 99% of the analyses had one or more significant cutpoints. The survival endpoint DFS was shown to be very stable despite increased interpathologist variance in all three clinical cohorts. Even with a poor ICC of 0.4, the majority of cutpoints were significant for DFS. For IBCSG VIII+IX 85% of the analyses with an ICC of 0.4 had one or more significant cutpoints for Ki67. In the large BIG 1-98 dataset (n=6090) even an ICC of 0.2 resulted in one or more significant DFS cutpoints in 100% of the analyses. Comparable results were obtained if the analysis was restricted to luminal tumors.

Conclusion: Our results suggest that Ki67 is extremely robust to pathologist variation. Even if less than 40% of the variance is attributable to true Ki67-based proliferation (ICC<0.4), this percentage of information is sufficient to obtain statistically significant differences. This stable performance of Ki67 might provide an explanation for the observation that many Ki67 studies achieve significant results despite the interobserver variance and heterogeneity issues. It might also suggest a relevant clinical utility for Ki67 despite considerable variation introduced in the evaluation. Ongoing efforts to further reduce interobserver variability, however, should be continued.
Prosigna® results impact on adjuvant decision making in early breast cancer (EBC): Final analysis of the prospective WSG study

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**Body: Background:** Prosigna is a standardized test measuring expression levels of 50 classifier genes (PAM50) in breast tumor tissue using nCounter® Technology (Nanostring Technologies, Inc.). It provides intrinsic subtype and risk of recurrence (ROR) score predicting 10-year recurrence probability. WSG prospectively evaluated Prosigna’s impact on therapy decisions in EBC and the quality assurance at its implementation in clinical routine. **Methods:** The study recruited 201 consecutive postmenopausal patients in 11 centers with ER+ HER- N0 EBC (10/2013 to 10/2014). Its primary objective was to assess the extent to which the Prosigna (Breast Cancer Intrinsic Subtype Test (BCIST)) affects the oncologists’ treatment recommendations regarding adjuvant chemotherapy (CT) and actual treatments received for EBC patients. Changes in treatment recommendations include (1) endocrine therapy alone, (2) endocrine therapy plus CT, and (3) changes in types of CT before/after the test and confidence in this treatment decision from physician and patient at a 6-month follow up. Secondary objectives included information on (1) physicians’ confidence in the recommendations before/after the test, and by cancer recurrence risk groups, (2) rate of CT related adverse events stratified by administration of CT, and (3) patients’ decisional conflict status, anxiety levels, and functional status before/after Prosigna’s results. As a secondary endpoint for quality assurance, we repeated Prosigna in a second decentralized pathology laboratory in Germany for inter-observation control. **Results:** In the total evaluable cohort (n=198), 114 (57.6%) of tumors were classified by Prosigna as Luminal A, 79 (39.9%) Luminal B, 3 (1.5%) Basal-like and 2 (1%) HER2-E. Median Prosigna ROR score was 45 (0-94). There was a 29.3% discordance in intrinsic subtyping between Prosigna and IHC. Concordance between central pathology and the second lab regarding molecular subtype was 95.5% with only 9 discordant samples, all within the luminal group. Concordance regarding ROR risk group classification was 92.9%; no clinically relevant differences (low-high or vice versa) were seen. Overall, there was a change of treatment choice (change in CT indication and change in regimens) in 18.2% compared to the pre-Prosigna decision. Post-Prosigna, 87.8% of physicians felt more confident with their prognostic assessment and 89.4% with their intended treatment. 94.8% of the patients expected to stick to their decision after discussing the Prosigna results. Six-month follow up (actual CT administered, its morbidity, perceptions by physicians and patients) will be presented at SABC. **Conclusion:** Overall, there was a change of treatment choice (change in CT indication and change in CT regimens) in 18.2% compared to the pre-Prosigna decision. Substantial discordance between Prosigna (PAM50) and local IHC underlines the importance of molecular subtyping prior adjuvant treatment decisions. High concordance of Prosigna results between central and decentralized lab testing were found. Results of WSG study can be pooled with two similar European studies and may thus help to improve our understanding of treatment decision making and adherence in EBC.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-07-04

Title: Pathology data predicts MammaPrint result- The Magee MammaPrint equation

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Body: Introduction
Prognosis for breast cancer patients may be determined using clinical-pathologic data (CPD) or gene expression profiling (GEP). We have previously reported a combined morphologic and immunohistochemical (IHC) method (Magee Equations) that can be used to estimate the Oncotype DX (ODX) recurrence score in clinical practice (http://path.upmc.edu/onlineTools/mageequations.html ). MammaPrint (MP) is a 70 gene GEP assay that is used to assess prognosis. Our goal was to develop similar equations to estimate the MP result based on CPD.

Methods
The study included 344 patients who had MP and complete CPD in an IRB approved research setting. Using the available CPD, a logistic regression model was constructed to predict MP risk group classification. Odds ratios and corresponding 95% confidence intervals were calculated. Model estimates were used to create an equation to predict MP risk category. The risk group cut-off for predicted scores was chosen to give 0.90 sensitivity with resulting specificity of 0.51 based on the observed MP scores. This corresponds to 10% of subjects in the observed MP high risk group being predicted as low risk. The sensitivity was chosen to minimize the misclassification rate of the high risk group.

Internal validation of the prediction model was determined using the bootstrap method. For each of the 1,000 iterations, the bootstrap sample was used to generate the prediction equation and the cutoff value as described above. Subsequently, this equation was used to predict the risk group for the observations not contained in the bootstrap sample (out-of-bag observations) and the resulting sensitivity and specificity were recorded.

Results
The logistic regression model from the observed data is:
\[ y = 0.7495(Age < 50) - 1.4135(Nottingham Grade 1) + 0.4644 (Nottingham Grade 2) + 1.7437 (Nottingham Grade 3) -0.0038*(ER H-Score)– 0.0044* (PR H-score) + 1.9220 (HER2 Positive) \]

The probability of being classified as high risk is then given by:
\[ p=\frac{\exp(y)}{1+\exp(y)} \]

The corresponding predicted risk group is defined as:
Low Risk: \( p < 0.18 \)
High Risk: \( p \geq 0.18 \)

The 0.18 cut-off was chosen to yield 90% sensitivity. For subjects with a known MP result of high risk, the probability that the model will predict high risk is 0.90. As a result of this sensitivity, the specificity is 51%. For subjects with a known MP result of low risk, the probability that the model will predict low risk is 0.51. The sensitivity was chosen to minimize the misclassification rate of the high risk group.

The median with 1st and 3rd quartiles for the cut-off value from the bootstrap prediction equations is 0.18 (0.16, 0.20). The median with 1st and 3rd quartiles for the sensitivity and specificity for the predicted scores of out-of-bag observations are 0.89 (0.85, 0.93) and 0.50 (0.45, 0.54), respectively.

Conclusion
The pathology data can predict for high risk MP result with a high sensitivity and moderate specificity.
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Publication Number: P5-07-05

Title: Development of individual adjuvant chemotherapy benefit estimating program for breast cancer patients management


Body: Background
To predict the individual benefit of adjuvant chemotherapy (CT) is challenging especially in estrogen receptor tumors HER2 negative tumors. Existing tools are based on data from randomized trials or on genomic tests with benefit calculated for outmoded CT. The aim of this study was to develop an exportable model to predict from real life data the individual benefit of adjuvant anthracyclines/taxanes based CT.

Patients and methods
Women with estrogen receptor-positive HER2-negative tumors without metastasis at the time of the diagnosis treated at the Institut Curie - Centre René Huguenin (St Cloud, France) between 2000 and 2008 were included. Clinical characteristics, pathological data and information concerning the treatments and outcomes had been prospectively registered. We divided the study population into 2 groups: patients who did not receive adjuvant CT and patients who did. Multivariate survival analysis and prediction were performed according to the Cox model (proportional hazard model). Non-informative variables in the Cox model were excluded from the final model. The individual benefit of CT was calculated by comparing predicted distant disease-free survival without chemotherapy (using a model constructed in patients treated without adjuvant CT) and modeled distant disease-free survival with CT. Benefit of chemotherapy was validated by cross validation and compared to Adjuvant online predictions.

Results
Data from 3385 women were available: 2137 treated without adjuvant chemotherapy and 1248 with. The models to predict survival without and with CT were based on tumor size, number of metastatic nodes, grade and KI67 (all patients had ER-positive-HER2 negative tumors). The discrimination and the calibration of the models were excellent: C-index were 0.81 and 0.76 for patients treated without and with CT. In this population, the mean 10-year benefit provided by CT was 6.2% (median: 1.5%, min: -4%, max: 35%). The accuracy of the model was internally validated and over-performed predictions of adjuvant online because Ki67 and HER2 status are included in our model (p<.05). A web-based interface is available.

Conclusion
We constructed a tool to evaluate the benefit of adjuvant anthracycline / taxane at an individual level. This tool is based on a model that outperform currently available methods.
Background: Abnormal levels of glucose and lipids may be linked to survival after breast cancer (BC) diagnosis, but their association to other causes of mortality such as cardiovascular (CV) disease may result in a competing risk problem and invalidate conventional analyses.

Methods: We assessed serum glucose, triglycerides (TG) and total cholesterol (TC) measured prospectively three months to three years before diagnosis in 1,798 women with BC in the Swedish Apolipoprotein Mortality Risk Study (AMORIS). In addition to using multivariable Cox proportional hazards regression, we employed latent class proportional hazards models to capture any heterogeneity of associations between these markers and BC death. The latter method was extended to include the primary outcome (BC death) and competing outcomes (CV death and death from other causes), allowing latent class-specific hazard estimation for cause-specific deaths.

Results: No association between prediagnostic glucose, TG or TC with BC death was observed with Cox regression. With latent class proportional hazards model, two latent classes (Class I and II) were identified in the cohort. Class I, comprising the majority (81.5%) of BC patients, had an increased risk of BC death following higher TG levels (HR: 1.87, 95% CI: 1.01-3.45 for every log TG increase). Lower overall survival was observed in Class II, but no association for BC death was found. On the other hand, TC positively corresponded to CV death in Class II, and similarly, glucose to death from other causes.

Conclusion: Higher TG was associated with an increased risk of BC death in the majority of BC patients. Our study also identified a subgroup of BC patients at higher risk of early death likely driven by other metabolic-related diseases, which adds to our understanding into BC survival in presence of competing outcomes.
Title: Fluorescence quantitative image analysis of HER2 evaluation against current clinical HER2 assays in breast cancer testing

Kornaga EN N, Feng X, Klimowicz AC C, Dean ML L, Guggisberg N, Morris DG G and Magliocco AM M. Translational Research Laboratory, Alberta Health Services, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada; Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT and H Le Moffitt Cancer Center & Research Institute, Tampa, FL.

Body: BACKGROUND: Presently, therapy for treatment of breast cancer is based on the evaluation of formalin fixed, paraffin embedded (FFPE) pathological specimens using a combination of immunohistochemistry (IHC) and gene copy assessment by in-situ hybridization (-ISH) techniques, following current testing guidelines from the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). Patients with specimens found to have marked overexpression and amplification of the human epidermal receptor 2 (HER2) are approved for treatment with trastuzumab. While IHC and –ISH assays represent the current clinical standard, these assays are subject to pre-analytical variation, which could lead to false negative results. There are novel laboratory technologies which may improve the accuracy of HER2 measurements and lead to improved patient selection for therapy.

AIM: In this comparative study, we assessed the utility of testing HER2 expression by the fluorescence IHC using AQUA® - a novel computer assisted platform to enable quantitative assessment of protein expression in FFPE tissue specimens – against current clinical assays (IHC and –ISH).

METHODS: Local cases from 2008-2010 clinically evaluated for HER2 were identified for further pathological review. FFPE tissue specimens were retrieved from a total of 207 cases with sufficient tumor present, and placed into a tissue microarray (TMA). TMA sections underwent assessment for IHC HER2 (Clone 4B5, Ventana), HER2/Chromosome 17 gene copy number (Inform HER2 dual-ISH DNA Probe Cocktail Assay, Ventana), and fluorescence IHC (Clone SP3, Thermo Fisher).

RESULTS: HER2 results were available for 142 patients for IHC, and 134 patients for dual–ISH and fluorescence IHC. A comparison of the current clinical methods revealed 11 discordant cases. The average median fluorescence IHC cytoplasmic HER2 expression (cAQUA) was found to be 225.65, (70.65-419.95). HER2 cAQUA was strongly correlated with dual-ISH, and cases with low level amplification had low cAQUA expression. There were cases having high cAQUA expression that did not show amplification by dual-ISH. Only a few amplified cases demonstrated low cAQUA expression. Dichotomizing cAQUA at the 256 showed an improvement of the receiver operating characteristic compared to the clinical HER2 IHC assay (cAQUA=0.903, p<0.001; IHC=0.833, p=0.006).

CONCLUSIONS: Measurement of HER2 expression using human interpretation can be imprecise as there is still some discordance between HER2 IHC and dual-ISH assays. Evaluation of HER2 protein expression using the novel AQUA assay showed correlation with IHC and dual-ISH, and AQUA may present a more precise way to quantify HER2 protein expression. HER2 cAQUA used a different antibody clone than the clinical IHC assay; however, previous studies have shown strong correlation between these two antibodies. The AQUA assay may identify a previously unrecognized group of breast cancers with elevated HER2 expression. The significance of this finding requires further investigation, particularly in regards with cAQUA HER2 serving as a marker for response to anti-HER2 therapy.
Title: The clinical utility of alternative chromosome 17 probes in equivocal HER2 results

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Body: Background
Accurate assessment of HER2 status in patients with breast cancer is critical in selecting patients for targeted therapy. In 2013, the American Society of Clinical Oncology-/College of American Pathologists (ASCO/CAP) released new guidelines for HER2 testing in an attempt to more accurately identify all breast cancer patients who are eligible for HER2 targeted therapy; these guidelines included new cutoff points for HER2/CEP17 ratio by in situ hybridization (ISH) or average HER2 copy number/cell and recommended the use of a reflex test with alternative ISH chromosome 17 probe for resolving cases with equivocal HER2 ISH results. We sought to determine the clinical utility of alternative chromosome 17 probes in such equivocal cases.

Patients and Methods
Our institution's database of HER2 dual-probe ISH results was searched for cases of invasive breast cancer with a HER2/CEP17 ratio of <2 and an average HER2 copy number of ≥4 and <6 signals/cell (i.e., cases meeting the new ASCO/CAP definition of equivocal). Of these, 30 consecutive cases with available corresponding sections were selected for additional chromosome 17 studies using probes for Smith-Magenis syndrome (SMS) and retinoic acid receptor alpha (RARA) genes. A eusomic copy number exhibited in one or both of these loci was used to calculate a revised HER2/chromosome-17 ratio by using the eusomic gene locus as the reference.

Results
Of 5489 cases of invasive breast cancer tested by dual-probe ISH for HER2 gene status, 316 (5.7%) cases were found to have equivocal results according to the 2013 ASCO/CAP guidelines. Further analysis of the 30 equivocal consecutive cases using the alternative chromosome 17 probes (SMS/RARA), 17 cases were upgraded from equivocal to positive HER2 status, and 13 cases remained unchanged.

Conclusion
The use of alternative chromosome 17 probes effectively determines true HER2 status in most equivocal HER2 ISH cases. Equivocal results are common; therefore, we advocate testing for alternative chromosome 17 genes to better categorize equivocal cases under the new ASCO/CAP guidelines. Additional analysis of the remaining equivocal cases and their relation to disease progression is underway and will be presented at the meeting.
Title: Heterogeneity of tumor infiltrating lymphocytes in breast cancer and its impact for use as a biomarker

Mani NL L, Schalper K, Hatzis C, Chagpar A, Pusztai L and Rimm DL L. Yale University School of Medicine, New Haven, CT.

Body: Background: In breast cancer, elevated tumor infiltrating lymphocytes (TILs) is associated with PD-L1 expression, hormone receptors negativity, and better outcome. The presence of numerous CD8+ cytotoxic T cells in pre-treatment specimens is associated with clinical benefit from PD-1 axis blockade in melanoma and lung cancer, suggesting its predictive value. Despite recent efforts to standardize the pathologist evaluation of TILs in breast cancer, objective determination of lymphocyte subpopulations and their distribution/uniformity within tumor tissues remains largely unexplored. Here, we simultaneously measured diverse TIL subpopulations using quantitative immunofluorescence (QIF) in different areas of breast tumors to determine the heterogeneity of TILs and its possible impact for use as biomarker.

Methods: Using a multiplexed QIF-based assay for simultaneous detection of DAPI (all cells), Cytokeratin (epithelial cells, M3515-DAKO), CD3 (T lymphocytes, E272Â–Novus), CD8 (cytotoxic T cells, C8/144B-Â–DAKO), and CD20 (B cells, clone L26-DAKO), we measured the levels of TIL subpopulations in whole tissue section slides of 3 tumor cores obtained from different areas of 31 breast carcinomas. The levels of the markers were measured using the AQUA method of QIF and the heterogeneity was studied using numerical correlations of log2 transformed scores and variance component analysis with linear mixed effects (LME). The concordance (kappa index [κ]) between binarized scores obtained measuring 1 vs 3 cores of the same tumor was also evaluated.

Results: As expected, we found a positive correlation between CD3 and CD8 levels across all patients (Pearson correlation coefficient [CC]=0.827). The levels of CD3 and CD8 showed weaker association with CD20 signal (CC=0.446 and 0.363, respectively). For all the TIL markers, the intra-tumor variation was higher than the inter-tumor differences with intraclass correlation coefficients (ICC) of 0.411 for CD3, 0.324 for CD8, and 0.252 for CD20. In the variance component analysis, 66-69% of the variance was attributable to signal differences between areas of the same tumor core and 30-33% was due to differences between cores from different areas. Consistent with this and using the median score as cutpoint to stratify cases in high/low marker levels, the concordance of measuring TILs in 1 vs 3 cores of the same tumor was κ=0.705 for CD3, κ=0.655 for CD8, and κ=0.603 for CD20.

Conclusion: Objective measurement of TIL markers indicates that T and B lymphocytes show heterogeneity in breast cancer. The tumor variation of the markers is driven predominantly by differences within the same tumor core. The data from our study suggests that although a single core biopsy of tumors provides considerable information regarding the degree of lymphocyte infiltration in breast cancer patients, caution should be taken when using this as a clinical biomarker.
Title: Quantification of circulating tumor cells using NanoFlares in breast cancer patients

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Body: Background: NanoFlares enable the detection of intracellular mRNA targets in live-cells and can be used to fluorescently detect genetic markers of circulating tumor cells (CTCs) in whole blood. Unlike the current technology which tracks CTCs by protein markers located on the cell surface, NanoFlares allow for the detection of mRNA targets in live-cells at the single-cell level. This technique was recently validated in a murine model of metastatic breast cancer. In this study, we aimed to utilize the NanoFlares to quantify the number of CTCs in patients with breast cancer.

Methods: We enrolled a prospective cohort of patients with stage 1-4 breast cancer. After obtaining informed consent, donation of blood samples for CTC analysis was collected at study entry. Blood samples were treated with control scrambled NanoFlares or vimentin NanoFlares to a final concentration of 6nM. Samples were incubated for approximately seven hours at 37°C then processed to remove red blood cells and stained with a fluorescent anti-CD45 antibody. Data was acquired via flow cytometry on a LSRFortessa cell analyzer. CD45+ peripheral blood mononuclear cells were gated out, and vimentin-positive cells were enumerated by identifying populations in the vimentin Flare-treated samples which were above the baseline fluorescence found in the control Flare-treated samples. Vimentin is a reported marker of mesenchymal cells and aggressive breast cancer cells. Cells with a high vimentin NanoFlare fluorescent response were presumed to be CTCs for this study. Wilcoxon rank sum test was used to compare median CTCs between subgroups.

Results: Fourteen patients were included in this analysis. The median age was 52.6 years (range 30.1-81.5). Eight patients had early stage disease (stage 1: 2, stage 2: 3, stage 3: 3) and 6 patients had stage 4 breast cancer. Among the stage 4 patients, all had visceral metastases and 5 had progression of disease noted on imaging at blood draw. In the entire cohort, 12 (86%) patients had estrogen receptor (ER) and/or progesterone receptor (PR)-positive disease and 5 (26%) were HER2-positive. CTCs were observed in all patients on study. Patients with stage 4 breast cancer had higher median number of CTCs compared to stage 1-3 patients (139 vs 42, p=0.09). Patients with ER/PR negative disease had a higher median number of CTCs compared to ER and/or PR positive (191 vs 62, p=0.58). HER2 status did not appear to affect the median number of CTCs (67 in HER2-positive vs 56 in HER2-negative, p=0.84). Among stage 4 patients, there were no differences in CTCs in those who had progression of disease at the time of the blood sample versus those who had stable disease.

Conclusions: NanoFlares was successful in detecting CTCs in each patient. Though our data set is currently limited by the small number of patients, this analysis suggests that differences exist in the amount of CTCs by stage and/or receptor status with a trend for higher CTCs in stage 4 patients and those with ER/PR-negative disease. This technology is the first approach for detecting, isolating, and characterizing live cancer cells in patient blood based upon intracellular mRNA targets, and may have the ability to impact clinical decisions in patients.
Title: CDCP1 as a new marker of aggressiveness in triple-negative breast cancers

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Body: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype with high recurrences and mortality rate, for which no therapies besides chemotherapy are available to date. Lacking specific markers for an effective targeted therapy, TNBCs continue to represent the most important challenge for clinical oncologists. Here, we investigated the expression of CDCP1, a transmembrane non-catalytic receptor reportedly associated with poor prognosis in some solid tumors (e.g., lung and pancreatic cancer), and its association with tumor aggressiveness in a cohort of 115 human TNBC primary specimens obtained from women surgically treated in our Institute from the beginning of 2002 to the end of 2006 and selected based on immunohistochemical (IHC) criteria (<1% cell positivity for estrogen receptor, progesterone receptor and HER2 expression classified as 0 or 1+). CDCP1 was overexpressed in 56.5% of human primary TNBCs. FISH analysis of 75 TNBCs for which material was available delineated four different genetic categories: 1) disomic, with only two copies of CDCP1 and centromere (CDCP1<3, CEP3<3) (50/75, 67%); 2) amplified CDCP1 (CDCP1 ≥3, CEP3<3) (4/75, 5%); 3) polysomic CDCP1 (CDCP1≥3, CEP3≥3) (15/75, 20%); and 4) CDCP1 deleted of its centromere (CDCP1<3 CEP3≥3) (6/75, 8%). FISH positivity (polysomy or amplification) was significantly associated with IHC positivity (p=0.003). Permutation accuracy variable importance estimated by Random Survival Forests identified both CDCP1 protein expression and FISH positivity for CDCP1 as prognostic factors for DFS (HR=2.67, 95%CI 1.25-5.71, and HR= 2.95, 95%CI 1.33-6.53, respectively) and DDFS (HR=2.40, 95%CI 1.01-5.73, and HR= 3.40, 95%CI 1.44-8.04, respectively), together with age, lymph node involvement, tumor size, DCIS and Ki67 expression. Multivariate Cox survival analysis revealed a synergistic interaction between CDCP1 FISH/IHC status and N-status in DFS and DDFS. Indeed, while the 5-year relapse probability in N-negative patients did not differ according to CDCP1 IHC expression in tumor cells (18% and 13% in CDCP1 IHC negative and positive, respectively), the probability of developing distant metastases at 5 years of follow-up was 82% in N-positive/CDCP1 IHC-positive patients versus only 29% in N-positive/CDCP1 IHC-negative patients. Similarly, the probability of developing distant metastases at 5 years in the N-positive subgroup was 88% for CDCP1 FISH-positive versus 35% for CDCP1 FISH-negative patients, but only 16% and 14% in N-negative/CDCP1 FISH negative and positive, respectively.

Together, our results strongly suggest that CDCP1 is a marker of aggressiveness able to identify cases with poorer prognosis among N-positive TNBCs and, noticeably, overexpression of CDCP1 in human primary TNBCs can reflect a CDCP1 genetic gain. Supported by AIRC.
**Title:** Local nuclear architecture features from H&E images predict early versus distant recurrence in lymph node negative, ER+ breast cancers

Ali S, Rimm D, Ganesan S and Madabhushi A. Case Western Reserve University, Cleveland, OH; Yale University, New Haven, CT and Rutgers University, Piscataway, NJ.

**Body:**

**Introduction:** Breast cancer (BCa) Patients with ER+ tumors that are lymph node negative (LN-) typically receive hormonal therapy. There is a need to identify ER+ LN- patients that will not benefit from adjuvant chemotherapy and will respond to hormonal therapy alone. Oncotype DX, a quantitative prognostic and predictive gene assay, provides a recurrence score that has been correlated with distant and early recurrence. In this work we present an approach that employ computer extracted features of nuclear architecture and morphology from routine H&E slides alone that can distinguish early and distant recurrence in ER+ breast cancers. By constructing graph networks within epithelium and stroma regions, built using nuclei as vertices and edge connections between proximal nuclei, local nuclear architecture can be quantitatively characterized. Hosoya index (HI) (originally introduced for analysis of chemical bonds) is a measure of a bond (in this context nuclei connections in a graph). In this work, we leverage HI to measure structural similarities of graphs across the populations that are indicative of recurrence in LN- ER+ breast cancer tissue microarray (TMA) images.

**Design:** In this study we considered two tissue microarrays (TMAs) comprising 453 early-stage lymph-node negative (LN-) estrogen receptor positive (ER+) breast cancer (BCa) patients (diagnosed with invasive ductal carcinoma), with a total of N=90 patients experiencing lifetime distant recurrence and N=343 patients who did not. All TMA cores were digitized at 20x magnification (0.33 um/pixel spatial resolution) using a digital whole-slide scanner. Each nucleus was identified via an automated computerized image analysis algorithm developed by our group. Then, using a cluster cell graph that encodes a link between a pair of nodes based on proximity, a series of graphs are constructed for a TMA. A HI value was then assigned to each local graph. A support vector machine classifier was trained in conjunction with the distribution of HI values for the early and distant recurrence cases on the training TMA (n=243, 50 early recurrences). Independent validation of the SVM classifier was performed on the second TMA (n=210, 40 early recurrences).

**Results:** For the LN- ER+ breast cancer dataset, our method was able to distinguish tumors with early and distant recurrence with an accuracy of 75.4%, a positive predictive value of 78.6% and a negative predictive value of 76.4%. The separation between the Kaplan-Meier curves for early and distant recurrence of LN-, ER+ breast cancers on the validation set was statistically significant (p < 0.00102).

**Conclusion:** Based only on tiny H&E punches, a computer-aided morphometric classifier appears to identify lymph node negative, ER+ breast cancers with a low likelihood of recurrence. With further validation, this approach could be developed into an image based assay which could serve as a lower cost alternative to Oncotype DX.
Title: Multi-gene fluorescence in situ hybridization analysis of cell cycle gene copy number aberrations in breast cancer with metastatic lymph nodes

Zhang S, Li J, Hu Y, Bai J, Yuan W, Hu L, Cheng T, Zhang J and Zetterberg A. Tianjin Medical University Cancer Institute and Hospital, Tianjin, China; Beijing Union Medical College Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Tianjin, China and Karolinska Hospital, Karolinska Institute, Solna, Sweden.

Body: Background: Metastasis in the axillary lymph node confers a high risk for recurrence and is the most important predictor of prognosis in early breast cancer. Aberrations occurring in cancer frequently involve components of the cell cycle machinery, and it has been proposed that this may be a prerequisite for tumorigenesis and axillary lymph node metastasis. The primary goals of this study were to investigate analyze the genes involved in the G1-S checkpoint signaling pathway (p16, Rb1, p53, Mdm2, c-myc, CCND1, p21, and CHEK2) and to examine the relationship between the CNAs of these genes and metastatic lymph nodes by multi-gene fluorescence in situ hybridization (M-FISH).

Materials and methods: The CNAs of these genes involved in the G1-S checkpoint signaling pathway were investigated in formalin-fixed, paraffin-embedded tissues from 60 samples of lymph node-negative cases and 60 samples of lymph node-positive cases and 40 samples of their corresponding lymph node metastases by means of M-FISH.

Results: There were 118 gene variation events in the lymph node-negative cases with the ratio of 42.9%, and 157 cases in lymph node-positive cases with the ratio of 57.1%. In comparison with the lymph node-negative cases, CNAs of the eight genes involved in the G1-S checkpoint signaling pathway occurred more often in lymph node-positive cases (P=0.000). In the meantime, we also used M-FISH to detect 40 paired lymph node tumors. CNAs of the genes in the G1-S checkpoint signaling pathway the primary tumor reflected the status of the axillary lymph nodes. Univariate survival analysis revealed that the gene amplification of C-Myc (P=0.021) and the gene deletions of Rb1 (P=0.026) and p16 (P=0.001) were independent prognostic factors that affected the 5-year overall survival (OS) for lymph node-positive cases. COX multivariate analysis revealed that the gene amplification of C-Myc (P=0.025) and the gene deletions of p16 (P=0.011) were significantly associated with a poor prognosis for lymph node-positive cases and were independent predictive factor of 5-year OS for lymph node-positive patients.

Conclusions: The CNAs of eight genes involved in the G1-S checkpoint signaling pathway occurred more often in the primary tumors of patients with lymph node metastases. The genes c-Myc and p16 were correlated with the prognosis of lymph node-positive cases. The data at hand thus indicate that the genomic characteristics of a primary tumor may help predict the likelihood that metastases have already occurred in patients with clinically localized disease. And the molecular classification based on the M-FISH could guide patient-tailored therapy.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-07-14

Title: Value of CYP2D6 genotyping based on germline-tumor polymorphisms and the cancer genome atlas outcomes data

Gonzalez A, Sathyan P, Hashemi-Sadraei N, Wachtler C, Zhang S, Dehghani M and Rosenblatt KP P. University of Texas Health Science Center at Houston, Houston, TX and CompanionDx Reference Lab, Houston, TX.

Body: Tamoxifen is used for the treatment and prevention of ER (estrogen receptor)-positive BrCa (Breast Cancer). Controversy exists in the value of CYP2D6 genotyping in tamoxifen responsiveness. We hypothesize that BrCa tissues will harbor numerous mutations, due to a mutator phenotype inherent in most cancers, including within the CYP450 family of genes and, specifically, within the CYP2D6 gene. We expect that comparisons between germline DNA isolated from peripheral blood, and mutated DNA isolated from BrCa (somatic DNA) specimens within the same patient will reveal extensive differences in CYP2D6 genotypes. Towards the correlation of tumor Vs germline genotypes with Tamoxifen metabolic phenotypes and patient outcomes, we assessed CYP2D6 phenotypes in breast cancer patients using the Cancer Genome Atlas (TCGA) database and compared them with breast cancer outcomes from tamoxifen therapy population.

The study extensively genotyped 70 BrCa patients using a retrospective cohort with matched blood and tumor tissue from an existing biobank at UTHealth. We also looked for discrepancies in genotypes and phenotypes and determine the magnitude of genetic bias possible in such cohorts.

Method: DNA samples were extracted from matched archived tumor cells, dissected by laser microdissection microscopy and blood. Genotyping was performed by clinically validated Taqman assays on the most common alleles for CYP2D6. We also studied the genotype status of these paired samples for CYP2C9, VKORC1, Factor II, Factor V, MTHFR, CYP3A4 and CYP3A5 genes. CYP2D6 gene copy number and gene rearrangement with CYP2D7 pseudogene were also assessed by Taqman copy number assays at 3 three different sites within gene. Thus far, the genotyping results for 44 matched samples were analyzed. Genotype-phenotype conversion was performed using an in-house developed, clinically validated genotype-phenotype translator package. Genotype agreement was assessed between the two DNA sources. Clinical data for TCGA samples was downloaded through the TCGA data matrix (https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm). The clinical patient file was used for this analysis. Variation data for the TCGA samples was restricted to only variants found in genes of interest for the analysis based on Ensembl canonical transcripts. All tumor variants were removed to produce a CYP2D6 genotype list based only on germline. These variants were annotated with Gene Symbol, SIFT and Polyphen scores, and variant type using a custom R script.

Results: 31.8% non-concordant results between DNA from breast tumors and blood were observed in the genotyping of polymorphisms in the CYP2D6 gene as well as 4.5% for CYP3A4 and 9% for CYP3A5 genotyping. Additionally, our TCGA data analysis indicates a statistically significant survival benefit for an ER positive population that lacks damaging CYP2D6 variations. Interestingly, our data also indicates a lack of survival benefit in on ER negative breast cancer population based on the presence or absence of damaging CYP2D6 variations.

These results strengthen the value of assessing germline CYP2D6 genetic polymorphisms to predict tamoxifen efficacy. Further research in this important area in pharmacogenetically guided therapy is needed.
Body: Rationale: Tissue microarrays (TMAs) have become a valuable resource for biomarker expression in translational research. Immunohistochemical (IHC) assessment of TMAs is the principal method for analyzing protein expression in large numbers of patient samples efficient with conservation of tissue. However, manual IHC assessment of TMAs remains a challenging and laborious task. With advances in image analysis, computer generated analyses of TMAs have the potential to lessen the burden of expert pathologist review. Computerized ER scoring relies on tumor localization.

Aim: The objective of this study was to compare the effectiveness of a locally developed automated invasive tumor location system with the skills of specialist breast pathologists.

Methods: In this study, tumor localization for estrogen receptor (ER) scoring was evaluated comparing computer-generated segmentation masks with those of two specialist breast pathologists. Automated tumor localization was achieved using a novel image analysis algorithm, which labeled compact groups of pixels called superpixels. Machine learning techniques were adopted to model color, shape and textural properties of superpixels in a rotation invariant manner, suitable for histopathology images. The resulting automatically and manually-obtained segmentation masks were used to obtain IHC scores for thirty-two ER stained invasive breast cancer TMA samples using FDA-approved IHC scoring software.

Results: Pixel-level comparisons showed lower agreement between automated and manual segmentation masks ($\kappa = 0.84$) than between pathologists' masks ($\kappa = 0.91$). However, this had little impact on computed IHC scores (Allred method; $\kappa = 0.91$, Quickscore method; $\kappa = 0.92$).

Conclusion: The automated system provides sufficiently consistent measurements for standardized IHC analysis of nuclear staining in TMAs from large clinical trials.
Title: Contemporary local and regional recurrence rates in very young breast cancer patients

Aalders KC, Postma EL, Strobbe LJ, van der Heiden-van der Loo M, Sonke GS, Boersma LJ, van Diest PJ, Siesling S and van Dalen T. Diakonessenhuis, Utrecht, Netherlands; Canisius Wilhelmina Hospital, Nijmegen, Netherlands; Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, Netherlands; Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; University Hospital Maastricht-GROW Maaastro Clinic, Maastricht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands and MIRA Institute for Biomedical Technology and Technical Medicine-University of Twente, Enschede, Netherlands.

Body: Introduction: Historically, young breast cancer patients proved to have a poorer prognosis regarding survival and locoregional recurrence. Over the last two decades, the survival of breast cancer patients has improved substantially, while at the same time locoregional recurrence rates decreased. The diminishing recurrence rates in the overall breast cancer population and acknowledgement of tumor biology and intrinsic subtypes in relation to age, raise the question whether the historically high locoregional recurrence risk in young women has decreased over a time where systemic treatment has evolved, particularly for the aggressive tumor types that occur frequently in young women. The aim of this study was to evaluate contemporary local and regional recurrence rates in very young breast cancer patients in relation to tumor biology in the shape of intrinsic subtypes.

Methods: Women <35 years of age who were operated for primary unilateral invasive breast cancer between 2003-2008 were selected from the Netherlands Cancer Registry. Patients were categorized according to intrinsic subtypes using hormone receptor and HER2 status. The 5-year risks of developing local recurrence (LR) and regional lymph node recurrence (RR) were estimated using Kaplan Meier statistics. The prognostic influence of different clinicopathological and treatment factors was assessed.

Results: A total of 1,000 patients were identified. The overall 5-year LR and RR rates were 3.5% and 3.7% respectively and a decreasing trend for both rates was observed over time.

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Rate</th>
<th>N</th>
<th>Rate</th>
<th>N</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>213</td>
<td>8</td>
<td>4.2%</td>
<td>11</td>
<td>6.1%</td>
<td>36</td>
</tr>
<tr>
<td>2004</td>
<td>212</td>
<td>10</td>
<td>5.6%</td>
<td>10</td>
<td>5.1%</td>
<td>38</td>
</tr>
<tr>
<td>2005</td>
<td>182</td>
<td>3</td>
<td>2.0%</td>
<td>5</td>
<td>3.1%</td>
<td>25</td>
</tr>
<tr>
<td>2006</td>
<td>170</td>
<td>5</td>
<td>3.2%</td>
<td>2</td>
<td>1.2%</td>
<td>13</td>
</tr>
<tr>
<td>2007</td>
<td>117</td>
<td>2</td>
<td>2.1%</td>
<td>1</td>
<td>0.9%</td>
<td>9</td>
</tr>
<tr>
<td>2008</td>
<td>106</td>
<td>3</td>
<td>3.2%</td>
<td>4</td>
<td>4.4%</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1,000</td>
<td>31</td>
<td>3.5%</td>
<td>33</td>
<td>3.7%</td>
<td>131</td>
</tr>
</tbody>
</table>

Intrinsic subtype proved to be a prognostic factor for both LR and RR (P=0.0556 and P=0.0141, respectively). Particularly HR-/HER2+ tumors were associated with high LR and RR rates. Patients with lymph node metastases at time of diagnosis had a higher RR-risk in both the total population (P=0.0349) as well as within the different intrinsic subtypes, although only significantly in the triple negative group (P=0.0401). Type of surgery did not influence the rate of LR and RR in this study.

Conclusions: Overall, the LR and RR rates in very young breast cancer patients were relatively low and decreased over time. The higher recurrence rates in this population were associated with the presence of more aggressive intrinsic subtypes. We emphasize that tumor biology should guide decision-making towards optimal treatment in this specific population. Although longer follow-up is needed, especially for this very young patient population, the results of this study provide important insight in the...
locoregional recurrence risks for this historically high-risk population.
Title: Real-life analysis evaluating 1594 N0/Nmic breast cancer patients for whom treatment decisions incorporated the 21-gene recurrence score result: 5-year KM estimate for breast cancer specific survival with recurrence score results \( \leq 30 \) is >98%

Stemmer SM M, Steiner M, Rizel S, Soussan-Gutman L, Geffen DB B, Nisenbaum B, Ben-Baruch N, Isaacs K, Fried G, Rosengarten O, Uziely B, Svedman C, Rothney M, Klang SH H, Ryvo L, Kaufman B, Evron E, Zidan J, Shak S and Liebermann N. Davidoff Center, Rabin Medical Center, Petah Tikva, Israel; Lin Medical Center, Haifa, Israel; Teva Pharmaceutical Industries Ltd, Shoam, Israel; Soroka University Medical Center, Beer Sheva, Israel; Meir Medical Center, Kfar Saba, Israel; Kaplan Medical Center, Rehovot, Israel; Ha'emeek Medical Center, Afula, Israel; Rambam Health Care Campus, Haifa, Israel; Shaare Zedek Medical Center, Jerusalem, Israel; Hadassah Hebrew University Medical Center, Jerusalem, Israel; Genomic Health Inc., Redwood City, CA; Clalit Health Services, Tel Aviv, Israel; Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel; Sheba Medical Center, Ramat Gan, Israel; Assaf Haroeh Medical Center, Zerifin, Israel and Ziv Medical Center, Safed, Israel.

Body: Background: The 21-Gene Recurrence Score® Assay (Oncotype DX®) has been validated as a prognostic and predictive tool in estrogen receptor (ER)+ breast cancer in multiple studies using archival specimens of clinical trials with long term follow up. Prospective outcome data from patients where treatment decisions incorporated the Recurrence Score results have not been reported. We evaluated treatments and clinical outcomes in patients undergoing Recurrence Score testing in 9 medical centers within Clalit Health Services (CHS), the largest HMO in Israel.

Methods: Medical records of patients with N0/Nmic ER+ HER2-negative disease undergoing testing from 12/2004 to 12/2010 in 9 medical centers (Rabin, Lin, Soroka, Meir, Kaplan, Hadassah, Ha'emek, Rambam, and Shaare Zedek) within CHS were individually reviewed to verify treatments given, recurrence, and survival status. 5-year Kaplan-Meier (KM) and standard error estimates for distant recurrence and breast cancer specific survival were determined.

Results: 1594 patients were evaluated with 5.9 years median follow-up. Median age, 61 (25-85) years; N0/Nmic (90%/10%); Grade I (16%), II (48%), III (16%), N/A (19%); histology, IDC (80%), lobular (13%), other (7%). Distribution of Recurrence Score risk groups (Recurrence Score results of <18, 18-30, \( \geq 31 \)): low (51%), intermediate (38%), and high (11%), with chemotherapy (CT) use of 1%, 26%, and 89%, respectively. Distant recurrence was reported in 17/813, 33/612, and 24/169 patients in the low, intermediate, and high Recurrence Score groups, respectively. The 5-year KM estimate for distant recurrence rate was 1.4% (95% CI: 0.9-2.3%) for the entire cohort, and 0.5% (95% CI: 0.2-1.6%), 1.2% (95% CI: 0.6-2.8%), and 6.9% (95% CI: 3.7-12.9), for the low, intermediate, and high Recurrence Score groups, respectively. The 5-year KM estimate for breast cancer specific survival was 98.4% (95% CI: 97.6-98.9%) for the entire cohort, and 99.9% (95% CI: 99.0-99.98%), 98.5% (95% CI: 97.1-99.2%) and 90.6% (95% CI: 84.5-94.4%), for the low, intermediate, and high Recurrence Score groups, respectively.

Conclusions: These are the first prospective long term clinical outcome data from approximately 1600 patients for whom the 21-gene Recurrence Score assay has been incorporated in real-life clinical decision making. The documented use of CT was appropriately based on the Recurrence Score result, and the outcomes for recurrence and survival are consistent with previously reported prospective-retrospective studies of the 21-gene assay. The 5 year KM estimates for distant recurrence rate in patients with low and intermediate Recurrence Score results who were treated based upon their Recurrence Score results were very low (0.5% and 1.2%, respectively).
Title: Prognostic impact of the combined risk groups by breast cancer index and HOXB13/IL17BR ratio in hormonal receptor positive, node negative breast cancer: A TransATAC study

Zhang Y, Sestak I, Schroeder BE E, Dowsett M, Cuzick J, Schnabel CA A and Sgroi DC C. BioTheranostics, Inc., San Diego, CA; Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University London, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom and Massachusetts General Hospital, Boston, MA.

Body: Background: Breast Cancer Index (BCI) is a gene expression-based assay that reports two distinct results: 1) BCI predictive based on HoxB13/IL17BR ratio (H/I), and 2) BCI prognostic based on an algorithm incorporating H/I with the Molecular Grade index (MGI). Both biomarkers have been validated independently in randomized trial cohorts. However, integrated results to better correlate recurrence risk with endocrine response have not been evaluated. The aim of this post-hoc analysis was to examine patient outcomes within BCI prognostic and predictive groups using the translational arm of the Arimidex, Tamoxifen, Alone or in Combination trial (TransATAC).

Methods: Primary tumor samples (N=742) from hormonal receptor-positive, N0 breast cancer patients treated with 5 years of tamoxifen (TAM) or anastrozole (ANA) in the ATAC trial were examined. Kaplan-Meier analysis was used to examine the risk of distant recurrence (DR) in patient subgroups derived from BCI and H/I results. A separate series of clinical cases submitted for BCI testing (N=853) were analyzed to determine distribution of the combined BCI and H/I groups in clinical practice.

Results: Summary of patient distribution across the 6 BCI clinical subgroups showed that a large number of patients (331/742, 45%) were BCI low risk with low likelihood of benefit, whereas 108/742 (15%) of patients with endocrine responsive disease (High H/I) were classified as BCI low risk (Table 1). Kaplan-Meier analysis demonstrated that patients classified as BCI low risk had a very similar 10-year risk of DR irrespective of H/I status (H/I low: 5.5% vs. H/I high: 4.0%), indicating that prognosis was largely determined by BCI vs H/I.

Table 1: Distribution of BCI and H/I risk groups in TransATAC

<table>
<thead>
<tr>
<th>BCI: Prognostic</th>
<th>H/I: Predictive</th>
<th>Low Risk</th>
<th>Intermediate Risk</th>
<th>High Risk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Likelihood</td>
<td>331</td>
<td>87</td>
<td>17</td>
<td></td>
<td>435 (59%)</td>
</tr>
<tr>
<td>High Likelihood</td>
<td>108</td>
<td>95</td>
<td>104</td>
<td></td>
<td>307 (41%)</td>
</tr>
<tr>
<td>Total</td>
<td>439 (59%)</td>
<td>182 (25%)</td>
<td>121 (16%)</td>
<td></td>
<td>742</td>
</tr>
</tbody>
</table>

In 853 node negative cases submitted for BCI clinical testing, the distribution of BCI and H/I risk groups were similar to that from the TransATAC cohort (Table 2).

Table 2: Distribution of BCI and H/I risk groups in clinical cases submitted for BCI testing

<table>
<thead>
<tr>
<th>BCI: Prognostic</th>
<th>H/I: Predictive</th>
<th>Low Risk</th>
<th>Intermediate Risk</th>
<th>High Risk</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Likelihood</td>
<td>364</td>
<td>105</td>
<td>23</td>
<td></td>
<td>492 (58%)</td>
</tr>
<tr>
<td>High Likelihood</td>
<td>96</td>
<td>107</td>
<td>158</td>
<td></td>
<td>361 (42%)</td>
</tr>
<tr>
<td>Total</td>
<td>460 (54%)</td>
<td>212 (25%)</td>
<td>181 (21%)</td>
<td></td>
<td>853</td>
</tr>
</tbody>
</table>
Discussion: Both prognostic and predictive components reported from the BCI assay may be used to stratify patients into 6 clinical subgroups based on prognostic risk of distant recurrence and endocrine responsiveness. Findings from this analysis indicate that patients classified as BCI low risk, regardless of H/I status, had sufficiently low DR rates and identifies patients that may be adequately treated with 5 years of endocrine therapy.
Title: Bioscore: A novel staging system for breast cancer patients receiving neoadjuvant chemotherapy


Background: We previously described a novel breast cancer staging system, the CPS+EG score, which incorporates pretreatment clinical stage, post-treatment pathologic stage, estrogen receptor (ER) status and nuclear grade to create an ordinal scale that is predictive of disease-specific survival (DSS) after receipt of neoadjuvant chemotherapy. The prior work predated (1997-2005) routine use of trastuzumab for patients with HER2+ disease. The current study was undertaken to update the staging system with a more contemporary cohort of patients to include patients with HER2+ disease receiving trastuzumab. The impact of using 1% as the cutoff for ER-positivity was also assessed.

Methods: A cohort of 2377 patients treated with neoadjuvant chemotherapy from 2005-2012 was identified. Clinicopathologic characteristics, treatment regimens and patient outcomes were recorded. Patient scores were computed using two versions of the CPS+EG staging system with ER status categorized as positive if >10% or if >1%. Fits of the Cox proportional hazards (PH) model for the two sets of prognostic scores were compared using the Akaike Information Criterion (AIC). HER2 status was then added to the model and the likelihood ratio test was used to determine the improvement in fit.

Results: Median follow-up time was 4.2 years (range, 0.5 to 11.7). Five year DSS was 89% (95% CI: 87%-90%). This cohort validated our previous finding that the CPS+EG score facilitates more refined categorization into prognostic subgroups than initial clinical or final pathologic stage alone (table). The AIC demonstrated that the CPS+EG model fits were nearly identical for ER status categorized using either cutoff, though the fit was slightly better for the >1% cutoff. There were 591 HER2+ patients included; all of them received trastuzumab-based chemotherapy. The improvement in the fit of the model when HER2 status was added was highly significant (p=0.00005) and incorporation of HER2 into the CPS+EG staging system by adding one additional point for HER2-negative status defined the bioscore (table) which again stratified patients with respect to prognosis.

Conclusion: The current study demonstrates a novel bioscore that significantly improves a previously validated prognostic score in patients receiving neoadjuvant chemotherapy and allows the staging system to be applied to patients with HER2+ disease. We recommend that biologic markers and response to treatment be incorporated into the forthcoming revision of the AJCC staging system.

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>5-yr DSS (95%CI)</th>
<th>Pathologic Stage</th>
<th>5-yr DSS (95%CI)</th>
<th>CPS+EG Score (1% cutoff for ER+)</th>
<th>5-yr DSS (95%CI)</th>
<th>Bioscore</th>
<th>5-yr DSS (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>98% (92-100%)</td>
<td>0</td>
<td>97% (78-10%)</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>96% (75-99%)</td>
<td>IA</td>
<td>95% (92-97%)</td>
<td>1</td>
<td>98% (96-99%)</td>
<td>1</td>
<td>99% (95-100%)</td>
</tr>
<tr>
<td>IIA</td>
<td>96% (94-97%)</td>
<td>IB</td>
<td>90% (76-98%)</td>
<td>2</td>
<td>94% (91-95%)</td>
<td>2</td>
<td>97% (95-98%)</td>
</tr>
<tr>
<td>IIB</td>
<td>90% (87-92%)</td>
<td>II A</td>
<td>91% (87-94%)</td>
<td>3</td>
<td>87% (84-90%)</td>
<td>3</td>
<td>93% (90-95%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>85% (80-89%)</td>
<td>II B</td>
<td>86% (81-90%)</td>
<td>4</td>
<td>75% (69-80%)</td>
<td>4</td>
<td>86% (82-89%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>78% (70-85%)</td>
<td>III A</td>
<td>80% (75-84%)</td>
<td>5</td>
<td>52% (40-63%)</td>
<td>5</td>
<td>71% (64-77%)</td>
</tr>
<tr>
<td>IIIC</td>
<td>76% (70-81%)</td>
<td>III B</td>
<td>64% (42-80%)</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>48% (35-60%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIC</td>
<td>64% (55-72%)</td>
<td></td>
<td></td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>
Title: Abstract Withdrawn
Title: Comparison of risk prediction with the 21-gene recurrence score (oncotype DX) and the 70-gene signature (MammaPrint) in patients with estrogen receptor-positive early stage breast cancer

Jiang H, Denduluri N, Majure M, Favret A and Rugo HS S. Peiking University Cancer Center, Beijing, China; Virginia Cancer Specialists Research Institute, US Oncology Research, Fairfax, VA and University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Background: Gene expression profiling assays estimate prognosis & predict benefit from adjuvant chemotherapy in patients (pts) with estrogen receptor positive (ER+) early stage breast cancer (ESBC). The 21-gene recurrence score (RS) and 70-gene signature (MP) are widely used assays. Pts may have both tests performed to adjudicate discordant results. We compared RS and MP in pts with both tests and evaluated their impact on adjuvant treatment decisions.

Methods: This was a retrospective clinical cohort study from 5 centers from 2007-2014. Eligibility included ER+ ESBC with RS and MP results from the same tissue sample; exclusions included carcinoma in situ, primary metastatic disease, & bilateral breast cancer. Two definitions of RS risk were evaluated (Genomic Health [GH] or TAILORx [TRx]): low risk (GH:0-17) or (TRx:0-10); intermediate risk (GH:18-30) or (TRx:11-25); and high risk (GH:>31) or (TRx:>26). Demographic, clinicopathologic (CP) data, and treatment decisions were extracted from medical records. Agreement was assessed between RS and MP using weighted kappa scores. CP factors were assessed with the t-test and chi-square test.

Results: 123 pts were identified from UCSF or 4 US Oncology Network practices. 60 pts had low risk RS (GH); 27 were low risk by MP (45% agreement). 15 cases had a low risk RS (TRx); 9 were low risk by MP (60%). 10 pts had high risk RS (GH); 8 were high risk by MP (80.0%). Similarly, 18 cases had high risk (TRx) RS;16 were high risk by MP (88%). Using the RS GH risk definition, there was poor agreement for low risk RS/MP and high risk RS/MP (kappa=0.169). However, using the TRx RS risk definition, there was good agreement for low risk RS/ MP and high risk RS/MP (kappa=0.509). 53 pts had intermediate risk RS by (GH); by MP 16 were low risk (30%), and 37 were high risk (70%). 90 pts had an intermediate risk RS by TRx; by MP 34 were low risk (37.8%), and 56 were high risk by MP (62.2%). Using TRx RS increased the number of pts classified as intermediate risk compared to GH RS (73.2 vs 43.1%). Complete adjuvant treatment data were available for 90 patients. Of 40 pts with low risk RS (GH), 25% received chemotherapy associated with node status but not tumor size, grade, or MP. Of 10 pts with high risk RS (GH), 70% received chemotherapy without association with CP features or MP. For the 45 pts with intermediate risk RS (GH), 53% received chemotherapy, without correlation with CP features. Receipt of chemotherapy was positively associated with MP high risk, but this was not significant (odds ratio 2.08, 95% CI 0.54-8.01); p=0.29.

Conclusion: Concordance in risk prediction between RS and MP was greater using RS defined by TRx compared to GH, and RS using TRx increases those categorized as intermediate risk. There appeared to be no correlation between CP features and decisions to use chemotherapy across risk groups. Interestingly, in those with intermediate risk RS (GH or TRx), there was a non-significant trend toward use of chemotherapy in those with high risk by MP. Although gene expression tests are used frequently to aid in treatment decisions in ESBC, considerable variation exists in their application in clinical practice.
Title: Appropriate use of the 21-gene recurrence score (RS) assay across Michigan

Ali HY Y, Munir K, Braun T, Griggs JJ J, Silver SM M, Gorski DH H, Breslin TM M and Henry NL. Henry Ford Health System; Michigan Breast Oncology Quality Initiative; University of Michigan Comprehensive Cancer Center; Karmanos Cancer Institute and Northwestern Memorial Hospital.

Body: Background: The 21-gene RS assay is used to assess prognosis and to predict response to adjuvant chemotherapy in patients with early stage hormone receptor positive, Her2 negative invasive breast cancer. The National Comprehensive Cancer Network (NCCN) first recommended consideration of testing of appropriate patients with the RS assay in 2008. We examined trends in the use of testing with the RS assay in hospitals across Michigan from 2006 through 2013 using data from the Michigan Breast Oncology Quality Initiative (MiBOQI), a Blue Cross Blue Shield of Michigan/Blue Care Network-sponsored quality initiative. Methods: Demographic, pathologic, and treatment data for women with breast cancer treated at all 25 hospitals participating in MiBOQI were abstracted from the medical record. Patients were excluded if they had stage 0 or IV disease at diagnosis, received neoadjuvant therapy, had bilateral breast cancer, or had a prior history of breast cancer. The primary outcome was the percentage of patients eligible for testing according to NCCN criteria (version 2010) who underwent testing with the RS assay. Analyses were performed using the statistical software R, Version 3.0.1. Results: Of the 18,046 patients in the MiBOQI Registry from 2006-2013 who met inclusion and exclusion criteria, 7133 (39.5%) met the NCCN criteria for testing (eligible). The rate of testing increased from 2006 to 2013 in both the eligible and ineligible cohorts, and varied by site.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Eligible (n=7133)</th>
<th>Ineligible (n=10913)</th>
</tr>
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<tbody>
<tr>
<td>Tested with RS assay</td>
<td>3920 (55.5%)</td>
<td>1424 (13.0%)</td>
</tr>
<tr>
<td>Testing rate in 2006</td>
<td>43.8%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Testing rate in 2013</td>
<td>62.3%</td>
<td>19.6%</td>
</tr>
<tr>
<td>Testing by site, 2006-2013 (range)</td>
<td>35.8% - 73.3%</td>
<td>6.8% - 26.2%</td>
</tr>
</tbody>
</table>

Testing of the eligible cohort was statistically significantly associated with younger age, lower tumor grade, and lack of nodal involvement. Overall, 73.4% of patients whose tumors were tested with the RS assay met the NCCN criteria for testing and were deemed appropriately tested. This rate of appropriate testing ranged from 60.8% to 85.4% across sites. Of all patients who underwent testing, 498 (9.3%) had 1 or more positive lymph nodes (>0.2 cm). Receipt of chemotherapy was lower in eligible patients who were tested compared to those not tested (25.5% vs 29.9%, p<0.001). Of the 2387 eligible patients with RS < 18, 117 (5.5%) received chemotherapy, which ranged from 0% - 13.6% across the 25 sites. Of the 341 patients with RS > 30, 56 (9.8%) did not receive treatment with chemotherapy, which ranged from 0% - 50% across the sites. Of the 1192 patients with RS 18-30, 502 (45.7%) received chemotherapy, ranging from 14.5% for RS 18 to 72.5% for RS 30. Conclusions: In sites across Michigan the majority of patients whose tumors were tested with the RS assay were in accordance with the NCCN guidelines, although there was considerable variability across sites. The rate of testing for patients who do not meet the NCCN criteria is increasing. There is very low inappropriate use of the recurrence score for making chemotherapy treatment decisions.
Title: Use across Michigan of the 21-gene recurrence score (RS) assay in lymph node positive patients with breast cancer


Body: Background: Standard of care for women with lymph node positive breast cancer includes treatment with chemotherapy. The 21-gene RS assay is indicated to assess prognosis and to predict response to adjuvant chemotherapy in patients with early stage hormone receptor-positive, HER2-negative invasive breast cancer. Findings from the SWOG S8814 clinical trial, published in 2010, suggested utility for using the RS assay for treatment decision making in node-positive patients in order to withhold chemotherapy. The Center for Medicare and Medicaid Services subsequently approved coverage for the use of the RS assay in women with up to 3 involved lymph nodes, and the National Comprehensive Cancer Network recently recommended consideration of testing in this population. We examined trends in the use of testing of patients with node positive breast cancer with the RS assay in hospitals across Michigan from 2006 through 2013 using data from the Michigan Breast Oncology Quality Initiative (MiBOQI), a Blue Cross Blue Shield of Michigan/Blue Care Network-sponsored quality initiative.

Methods: Demographic, pathologic, and treatment data for women with breast cancer treated at all 25 hospitals participating in MiBOQI were abstracted from the medical record. Patients were excluded if they had stage 0 or IV disease at diagnosis, received neoadjuvant therapy, had bilateral breast cancer, or had a prior history of breast cancer. The primary endpoint was the percentage of patients with lymph node positive, hormone receptor-positive, HER2-negative breast cancer who underwent testing with the RS assay. Analyses were performed using the statistical software R, Version 3.0.1.

Results: Of the 30,992 patients included in the MiBOQI Registry from 2006-2013, 2526 (10.7%) had hormone receptor positive, HER2 negative, lymph node positive disease and met the eligibility criteria. The rate of testing with the RS assay in this patient cohort increased from 0% in 2006 to 32.5% in 2013, including an increase from 15.4% in 2010 to 28.3% in 2011. Median age of the tested cohort was 60 (range 26-87). On multivariate analysis, testing was statistically significantly associated with older age, smaller tumor size, 1-3 involved lymph nodes, and lower Charlson Comorbidity Index. Receipt of chemotherapy was lower in those patients who underwent testing compared with those not tested (40.0% vs 82.0%, p<0.001). Chemotherapy was administered to 105 (27.4%) of the patients with RS < 18, 91 (51.4%) of the patients with RS 18-30, and 49 (92.4%) of the patients with RS > 30.

Conclusions: Use of the RS assay for assessment of women with involved lymph nodes is increasing over time, primarily in older patients and patients with lower risk disease, and is associated with decreased treatment with chemotherapy. Results of the ongoing SWOG S1007 clinical trial, which is assessing the impact of use of the RS assay on breast cancer outcomes, are eagerly awaited.
Title: Differential patient stratification by the breast cancer index HoxB13/IL17BR ratio vs recurrence score (RS) plus quantitative ER expression in hormone receptor positive, node negative breast cancer

Naughton MJ J, Schroeder BE E, Operana TN N, Zhang Y and Schnabel CA A. Washington University in St. Louis, St. Louis, MO and BioTheranostics, Inc., San Diego, CA.

Body: Background: Second generation genomic biomarkers for patients with early stage breast cancer are based on integration of proliferative and estrogen signaling-related gene expression, which has led to data applicable in the extended (post-5 year) endocrine therapy setting. The Breast Cancer Index (BCI) assay interrogates these two signaling pathways, significantly stratified patients into high (13.4%) or low (3.5%) risk of late (5-10y) distant recurrence in TransATAC, and includes a gene expression signature (HoxB13/IL17BR, H/I) that predicted benefit from extended endocrine therapy in MA.17. The 21-gene assay has recently been investigated in combination with quantitative estrogen receptor (qER) expression, wherein a subset of approximately 20% of patients with a high recurrence score (RS) and qER above 9.1 had a higher prognostic risk for late distant recurrence (12.6%) than those with Low RS (4.7%) or intermediate RS (4.1%) (Wolmark, ASCO 2014). The objective of this study was to compare patient stratification with H/I and RS+qER.

Methods: Consecutive cases submitted for BCI clinical testing from lymph node-negative breast cancer patients with available RS scores and qER >9.1 abstracted from pathology reports (N=115) were analyzed. Cohen's kappa statistic was used to test agreement between H/I and RS+qER for patient stratification.

Results: No statistically significant agreement was observed between H/I stratification and RS+qER prognostic risk stratification with respect to identifying patients for extended endocrine therapy (Cohen's kappa = -0.002, p = 0.51). H/I identified 36 cases (34%) as High likelihood to benefit from extended endocrine therapy compared to 3 cases (3%) classified as having high risk of late recurrence (Table). Of the 69 cases (66%) classified as RS+qER Low risk, 19 were identified as High likelihood to benefit from extended endocrine therapy by H/I.

Table

<table>
<thead>
<tr>
<th></th>
<th>Low H/I Predictive</th>
<th>High H/I Predictive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS+qER Low Risk</td>
<td>50 (48%)</td>
<td>19 (18%)</td>
<td>69 (66%)</td>
</tr>
<tr>
<td>RS+qER Inter Risk</td>
<td>17 (16%)</td>
<td>16 (15%)</td>
<td>33 (31%)</td>
</tr>
<tr>
<td>RS+qER High Risk</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Total</td>
<td>69 (66%)</td>
<td>36 (34%)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: This retrospective analysis of clinical cases shows that H/I and RS+qER identify distinct subsets of patients, and highlights that the underlying biology of risk stratification differs from that of endocrine responsiveness. Comparatively, findings indicate that the H/I identifies additional patients that may be considered for extended endocrine therapy.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-08-10

Title: The breast cancer index as a tool in decision making for adjuvant hormonal therapy in early luminal breast cancer: Initiation, withdrawal and continuance

Link JS S, Buck LJ J and Kapoor NS S. Breastlink, Orange, CA.

Body: Background: Most women with newly diagnosed breast cancer are of luminal type and will be offered 5-10 years of adjuvant endocrine therapy. Many women will not have a survival benefit from this therapy and 30% or more will struggle with side effects and quality of life issues. Breast Cancer Index (BCI) is a genomic biomarker for early-stage, ER+ breast cancer and has been validated to assess risk of late (5-10 yr) distant recurrence and predict likelihood of benefit from extended (>5y) endocrine therapy utilizing the HoxB13/IL17BR(H/I) ratio. H/I has also been shown to predict benefit from endocrine therapy in the early adjuvant setting. The objective of this study was to characterize the impact of BCI on endocrine therapy decision-making for early-stage breast cancer patients.

Methods: Data was collected retrospectively from patients with early-stage luminal breast cancer treated at Breastlink who underwent BCI testing between 10/2014-5/2015. The impact of BCI test results were analyzed for 3 indications: 1) initiation of endocrine therapy for patients considering no adjuvant treatment; 2) patients 6 mo-4y post diagnosis struggling with side effects and desiring to discontinue endocrine therapy; and 3) patients beyond 4 years of adjuvant hormonal therapy deciding whether to extend therapy for an additional 5 years.

Results: One hundred patients underwent BCI testing with median age 53 (range 35-77). The BCI assay was utilized in 14 cases at diagnosis, 54 cases at 6 mo-4y during therapy, and 32 cases at >4y post-diagnosis. In patients tested at time of diagnosis, 10/11 that were low risk for late recurrence and low likelihood of benefit from endocrine therapy chose not to initiate therapy, and 2/2 patients were high risk/high likelihood to benefit initiated therapy. One patient, a 72 year-old with low risk and high likelihood to benefit, declined therapy. In patients tested between 6 mo-4y on therapy, 30/30 patients were low risk and low likelihood of benefit chose to stop endocrine therapy, and 11/11 patients were high risk and high likelihood of benefit chose to continue. Of 7 patients that were low risk but high likelihood of benefit, 5 continued therapy. All 6 patients that were high risk but low likelihood of benefit chose to stop therapy. Of 32 patients tested after 4 years of adjuvant therapy, 13/13 were low risk and low likelihood of benefit chose to stop endocrine therapy, and 8/8 were high risk and high likelihood of benefit chose to extend therapy to 10 years. All 5 patients that were high risk but low likelihood of benefit elected to stop, and 3/6 patients that were low risk but high likelihood to benefit extended therapy. In total, endocrine therapy treatment decision making aligned with predictive (H/I) results in 93/100 patients.

Conclusion: The BCI test was instrumental in assisting almost all women in their decision to receive or maintain adjuvant hormonal therapy and 67% of women discontinued or declined hormonal therapy based on test results. All patients with high risk and high predictive benefit on BCI assay chose to pursue adjuvant endocrine therapy. Oncologists can use BCI in their algorithms of delivering personalized cancer care.
Objective: The Oncotype DX® Breast Cancer Assay is a multigene assay which quantifies the risk of distant recurrence at 10 years and predicts the potential benefit of chemotherapy in women with early stage I/II, lymph node negative, estrogen receptor positive breast cancer. This retrospective study examines utilization of Oncotype DX® for appropriate patients in a community hospital system. We hypothesize that chemotherapy administration follows the Oncotype DX® score recommendation that patients with low recurrence scores not receive chemotherapy and patients with high recurrence scores receive chemotherapy and intermediate recurrence scores receive chemotherapy approximately half of the time.

Methods: In this retrospective study, 302 female patients from January 1, 2012 – January 1, 2014, were identified by the breast cancer diagnosis codes (ICD9 codes 174.0174.9) from the hospital's cancer registry. Data including imaging findings, pathology, and treatment information was then compared using chi square tests for categorical variables and two sample t tests or Wilcoxon rank sum tests for continuous variables.

Results: Three hundred two female patients diagnosed with stage I/II, lymph node negative, HR+, HER2/neu, breast cancer from a community hospital system were identified as appropriate candidates by NCCN guidelines, for Oncotype DX® testing between January 1, 2012 - January 1, 2014. In patients receiving Oncotype DX®, mass sizes were larger and were a higher proportion of stage 2 cancers (p<0.001). Utilization of Oncotype DX® in our CoC and NAPBC accredited hospital system was lower than hypothesized with only 73% of patients receiving testing. Following Oncotype DX® testing, appropriate treatment ensued 94% of the time (low and high recurrence risk). Intermediate recurrence scores received treatment 36% of the time.

Conclusion: In our CoC and NAPBC accredited community hospital system, utilization of Oncotype DX® testing was lower than hypothesized. For the patients who received Oncotype DX® testing, expected treatment was administered. Oncotype DX® testing when performed influences patient care by assisting in the decision making process for administration of chemotherapy, however with 27% of patients not receiving testing and 25% of those patients not receiving testing due to patient preference, this observation supports that this test not be ordered reflexively, but rather with patient input and appropriate consent.
Introduction
Immune modulating therapies offer an attractive novel approach in the treatment of breast cancer. There is a growing body of literature demonstrating that immune-related expression signatures predict breast cancer prognosis and chemo- and/or targeted therapy responsiveness. However, it is unclear how these signatures relate to one another. Here we evaluated 58 immune signatures in breast cancer and generated co-expression modules to classify patients into immune subtypes.

Methods
We evaluated 58 published expression signatures related to immune function in 5 breast cancer gene expression datasets (TCGA (n=817), METABRIC (n=1992), EMC344 (n=344), pooled triple negative: GSE31519 (n=579), pooled neoadjuvant chemotherapy treated: GSE25066 (n=508)). For each dataset, consensus clustering was used to subset the signatures based on their co-expression pattern. Signatures in the same consensus cluster across all 5 datasets were used to define immune modules. Module scores were computed as the average across their constituent signatures. Patients were classified into immune subtypes based on their module scores using consensus clustering. Overall survival (OS) differences between immune subtypes were assessed using Cox proportional hazard modeling in basal breast cancers from the METABRIC dataset (n=329).

Results
Consensus clustering of the 58 expression signatures consistently yields four distinct co-expression modules across the five datasets. These modules appear to represent distinct immune components and signals, with constituent signatures relating to: 1) T-cells and/or B-cells (T/B-cell), 2) interferon (IFN), 3) transforming growth factor beta (TGFB), 4) core serum response, dendritic cells and/or macrophages (CSR). Of note, the T/B-cell module contains 20 of the 58 signatures evaluated; and the CSR module is highly correlated to proliferation (r=0.81). Subtyping of patients based on these co-expression modules consistently yields subsets that fall into five major immune subtypes. The expression pattern of the four modules within each immune subtype is summarized below:

<table>
<thead>
<tr>
<th>Immune Co-expression Modules (columns); Immune Subtypes (rows)</th>
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<tbody>
<tr>
<td><strong>T/B-cell</strong></td>
</tr>
<tr>
<td>T/B-cell/IFN High</td>
</tr>
<tr>
<td>IFN/CSR High</td>
</tr>
<tr>
<td>Immune Low</td>
</tr>
<tr>
<td>CSR High</td>
</tr>
<tr>
<td>TGFB High</td>
</tr>
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</table>

These immune subtypes are associated with differences in overall survival in the METABRIC basal breast cancer cases, where the CSR High subtype has the worst outcome (10-year OS: 23%). In comparison, the subsets corresponding to the T/B-cell/IFN High subtype have better outcomes (Hazard ratio: 0.43, p = 0.018). In contrast, no significant outcome differences were observed between the poor outcome CSR-High subtype and the remaining three immune subtypes (p>0.05).

Conclusion
Our exploratory study identified four distinct immune co-expression modules (T/B-cell, IFN, TGFB, or CSR) from a collection of published immune signatures. Using these modules, we identified 5 immune subtypes with prognostic significance in basal breast cancers. We propose to test representative signatures from the 4 modules and the combined immune subtypes as predictive biomarkers of response to immunotherapies.
Title: Tumor infiltrating lymphocytes and pathological response are prognostic biomarkers in inflammatory and non-inflammatory breast cancer

Arias-Pulido H, Colpaert C, Chaher N, Qualls C, Kaufman PA A, Marotti JD D, Vermeulen P, Dirix L, van Laere S and Kuppusamy P. Geisel School of Medicine at Dartmouth College; 5Hematology/Oncology and 6Pathology, Dartmouth-Hitchcock Medical Center, Lebanon, NH; Oncology Centre, GZA Hospitals, Iridium Cancer Net, Antwerp, Belgium; Centre Pierre et Marie Curie, Algiers, Algeria and University of New Mexico, Albuquerque, NM.

Body: Background: Tumor-infiltrating lymphocytes (TILs) have been associated with pathologic complete response (pCR) to neoadjuvant chemotherapy (NACT) as well as disease-free (DFS) and overall survival (OS) in certain breast cancer subtypes. pCR has been shown to be predictive of long-term outcome in several neoadjuvant studies and is therefore a potential surrogate marker for patient outcome. The aim of this study was to determine whether TILs and pCR can be used as a prognostic biomarker in inflammatory and non-inflammatory breast cancer.

Materials and Methods: Stromal lymphocytic infiltration (strTILs), defined as the percentage of tumor stroma containing infiltrating lymphocytes (lymphocyte predominant breast cancers (LPBC) cut-off: ≥50%), and pCR, defined as the absence of any residual invasive cancer on the resected breast specimen and all sampled ipsilateral lymph nodes following completion of NACT, were evaluated in 383 (221 Inflammatory (IBC) and 162 non-IBC Locally-advanced (LABC)) breast cancer patients. Tumors were categorised into molecular subtypes and Ki-67 status based on immunohistochemistry. Correlations with clinico-pathological variables, breast cancer-specific (BCSS) and disease-free survival (DFS) were made.

Results: strTILs were present in all patients (median: 15%, IQR: 5% to 30%). There was no difference in the frequency of strTILs between IBC and LABC cases. Thirty three (15%) IBC and 18 (11%) LABC tumors were LPBC. strTILs were significantly more frequent in triple negative (TNBC) (median, 25%) than in HER2+, Ki-67-high (15% for both) and ER/PR+ (10%) (p<0.001; Kruskal-Wallis One Way Analysis of Variance on Ranks). There was a significant association of strTILs with pCR (p<0.001). strTILs median was 27.5%, 15% and 10% for pCR, partial response and no response, respectively (p<0.001). pCR was obtained in 4 (9.1%) of patients with strTILs <10%, in 25 (56.8%) of patients with strTILs between 10 and 40% and in 15 (34.1%) of patients with strTILs >40% (p=1.09E5). strTILs did not predict either DFS or BCSS in the overall breast cancer population. pCR was negatively associated with ER+ (p=0.002), positively with TN (p=0.02) and strongly associated with both DFS & BCSS (p<0.0001, for both). Multivariate analysis showed that, in IBC patients, pCR (p<0.0001) and lymph node rate (p=0.034) were independent predictors for DFS and pCR (p<0.0001), lymph node rate (p=0.034) and LPBC (p=0.024) were independent predictors for BCSS. In LABC, DFS was independently predicted by pCR (p<0.0001) and LPBC (p=0.042) and BCSS by pCR (p<0.0001), LPBC (p=0.005) and ER (p=0.029). LPBC was associated with negative outcome in both IBC and LABC cases.

Conclusion: strTILs showed a strong association with TNBC tumors and with pCR. pCR is a strong prognostic factor for both IBC and LABC. The negative association of LPBC with outcome is unexpected and warrants additional studies.
Title: Tumor Infiltrating lymphocytes (TIL) related genomic signature associated with chemotherapy response and prognosis in subtypes of breast cancer

Kochi M, Niikura N, Iwamoto T, Bianchini G, Mizoo T, Nogami T, Shien T, Motoki T, Taira N, Masuda S, Doihara H, Fujiwara T, Tokuda Y and Matsuoka J. Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan; Okayama University Hospital, Okayama, Japan; Tokai University School of Medicine, Isehara, Japan; San Raffaele Hospital, Milan, Italy; Nihon University School of Medicine, Tokyo, Japan and Okayama University Hospital, Okayama, Japan.

Body: Background: Tumor infiltrating lymphocytes (TIL) in subtypes of breast cancer may provide clinically important information on chemotherapy response and prognosis. However, the standardized methodology for immunohistochemical (IHC)-TIL has not yet been established, reproducible and objective method of evaluation of TIL such as gene expression profiles is warranted. We evaluated whether IHC-TIL level was associated with gene expression profiles and whether such profiles could be used to predict chemotherapy response and prognosis according to subtypes of breast cancers.

Methods: To select TIL associated genes, we used 40 samples with both IHC-TIL information and gene expression profiling data. The degree of TIL at the edges of the tumor mass, in the tumor mass, or in the stroma surrounding the expanding mammary ducts packed by carcinoma cells was evaluated as score 0, 1, and 2, when TIL was not recognizable (0%), sparse (0 << 50%) and dense (50% ≤), respectively. We selected 22 genes as the TIL-gene signature (GS), by comparing expression profiles between TIL score 2 and 0 tumors. We showed the associations between the TIL-GS levels and subtypes of breast cancers (Estrogen receptor: ER / Human Epidermal growth factor 2: HER2). The chemotherapy sensitivity analysis was performed on cohorts of 625 patients with stage I–III breast cancer who received neo adjuvant chemotherapy (NAC) based on Anthracycline and Taxane containing regimen. Data from 1,586 tumors were used to evaluate the association between distant metastasis free survival (DMFS) and the TIL-GS in a Kaplan-Meier analysis.

Results: The TIL-GS for ER negative (-)/HER2- and HER2 positive (+) cases were significantly higher expression level than luminal types (p-value <0.001). All breast cancer subtypes except luminal-low proliferation had significantly higher differential TIL-GS level in cases with pathological complete response (pCR) after NAC than residual disease (luminal-high: p-value = 0.013, HER2+: 0.005, and ER-/HER2-: 0.016). With no adjuvant chemo or only tamoxifen treated breast cancer data set, the TIL-GS had no prognostic power in luminal cases regardless of proliferative level. In HER2+ breast cancers, cases with the high TIL-GS had significantly better prognosis than low cases (p-value =0.001), but no significance in ER-/HER2- cases (p-value = 0.621).

Conclusions: Higher TIL-gene signature of 22 genes appeared to be associated with aggressive subtypes and pCR rate (except luminal-low) of breast cancers. This approach may improve the reproducibility of assessment on tumor TIL level and thus serve the clinical applications for breast cancers.
Title: Prognostic value of aldehyde dehydrogenase 1 (ALDH1) and tumor infiltrating lymphocytes (TIL) to predict the late recurrence in ER positive, HER2 negative breast cancer

Miyoshi Y, Shien T, Ogiya A, Ishida N, Yamazaki K, Horii R, Horimoto Y, Masuda N, Yasojima H, Inao T, Osako T, Takahashi M, Tomioka N, Hagi K, Endo Y, Hosoda M and Yamashita H. Okayama University Hospital; Cancer Institute Hospital, Japanese Foundation for Cancer Research; Hokkaido University Hospital; Juntendo University School of Medicine; NHO Osaka National Hospital; Graduate School of Medical Science Kumamoto University; Kumamoto City Hospital; NHO Hokkaido Cancer Center and Nagoya City University Graduate School of Medical Sciences.

Body: Introduction: Aldehyde dehydrogenase 1 (ALDH1) is known to be cancer stem cell marker. Also, tumor infiltrating lymphocytes (TILs) are known to be prognostic factor for triple negative breast cancer. It is reported that these factors have the correlation with chemosensitivity. Meanwhile, the late recurrence (LRec; 5 years after primary surgery) of ER positive breast cancer is the major problem. Significance of expressions of ALDH1 and TILs in primary tumor as predictive factors for late recurrence in ER positive, HER2 negative breast cancer is still unknown.

Methods: ER-positive, and HER2-negative breast cancer patients who underwent surgery or received neoadjuvant chemotherapy between January 2000 and December 2004 were registered from nine institutes belonging the Collaborative Study Group of Scientific Research of the Japanese Breast Cancer Society. For each LRec patient, approximately two matched control patients without relapse for more than ten years were selected. Expression of ALDH1 was assessed by immunohistochemistry. Positive ALDH1 was defined as tumor including more than 1% cancer cells with ALDH1 expression. TIL was assessed by single whole section according to Denkert's definition. A tumor showing high ki67 and/or low PgR expressions was categorized into Luminal B-like group.

Results: 639 patients (184 with early recurrence (ERec), 134 with LRec and 321 with no recurrence (NoRec)) were analyzed. The rates of positive ALDH1 in ERec, LRec and NoRec groups were 18%, 13% and 8%, respectively. ALDH1 positivity was significantly higher in ERec compared with NoRec group (p<0.01). There was no significant difference between LRec and NoRec group (p=0.12). Positive ALDH1 showed significantly shorter DFS and OS in multivariate analyses (DFS: p=0.03, OS: p<0.01). Especially, that was the significantly prognostic factor in the Luminal B like tumor with adjuvant or neoadjuvant chemotherapy (p=0.01), but not in those without any chemotherapy (p=0.53). High TILs in ERec, LRec and NoRec was 1.1%, 1.5% and 3.7%, respectively. There was no significant difference among three recurrent groups (p=0.13). High TILs was not significantly associated with DFS (p=0.09) and OS (p=0.72). However, there was significant correlation between High TILs and DFS in Luminal B like group (p=0.04) and ALDH1-negative group (p=0.02).

Conclusion: In ER-positive, and HER2-negative breast cancer, ALDH1 was an independent prognostic factor (a predictor of ERec, but not LRec). ALDH1 might be a predictor of benefit from chemotherapy in Luminal B like subtype. TILs was neither a predictor of ERec nor LRec. However, significance of TILs as prognostic factor might differ depending on subtypes and cancer stemness.
Title: Local immunologic environment related with tumor infiltrating lymphocytes (TIL) and PD-1/PD-L1 expression in early stage breast cancer

Okabe M, Toh U, Iwakuma N, Mishima M, Kawahara A, Kage M, Itoh K and Akagi Y. Kurume University School of Medicine, Kurume, Fukuoka, Japan; Research Center for Innovative Cancer Therapy, Kurume University, Kurume, Fukuoka, Japan and Kurume University Cancer Vaccine Center, Kurume, Fukuoka, Japan.

Body: Purpose:
Recent studies have shown that local immune environment revealed with programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) and tumor infiltrating lymphocytes (TIL) affects the tumor-growth and prognosis. In this study, we evaluated the tumor local immune environments using immunohistological staining for analysis of PD-1/PD-L1 expression and TIL of tumor local in operable early-stage breast cancer.

Method:
A total of 100 surgical specimens of stage I-III invasive breast carcinoma paraffin embedded between 1995 and 2005 were analyzed. Immunohistological staining for PD-1, PD-L1, PTEN, CD3, CD8, and CD163 were performed by the conventional PAP method. In addition, intratumoral and intrastromal TILs and macrophages were simultaneously stained by anti-CD3, CD8, CD163 antibodies and measured by 'Win ROOF' computer software (version 5.7, Mitani Corporation, Japan).

Results:
Intratumoral PD-1 expressed significantly higher in triple negative breast cancer (TNBC) compared to other subtype BC (p=0.0094), intratumoral and intrastromal CD3+ lymphocytes and CD163+ macrophages were also significantly higher in TNBC, respectively (CD3: p=0.0002; 0.0139 and CD163: p=0.0043; 0.0270). PTEN loss was also more frequently observed in TNBC (p=0.0475). In addition, after a median 5-year follow-up, patients of luminal A subtype with lower PD-L1 and PTEN expression showed better disease free survival (DFS) with a significant difference (p=0.0148, p=0.0475).

Conclusion:
Local expression of PD-1/PD-L1 antigens on tumor cells, CD3+ lymphocytes, CD163+ macrophages infiltration significantly increased in early-stage TNBC. PTEN expression on tumor local might be associated with DFS in patients with early-stage BC.
Title: A study of c-Jun N-terminal kinase (JNK) and c-Jun as biomarkers in early breast cancer

Palmieri C, Rudraraju B, Giannoudis A, Moore D, Shaw J, Chan S, Ellis IO O, Caldas C, Coombes RC Charles, Carroll JS S, Ali S and Abdel-Fatah TMA MA. University of Liverpool, Liverpool, United Kingdom; University of Leicester, Leicester, United Kingdom; Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; University of Nottingham, Nottingham, United Kingdom; Cancer Research UK Cambridge Institute, Cambridge, United Kingdom and Imperial College, London, United Kingdom.

Body: Background

The AP-1 transcription factor c-Jun is a key downstream target of c-Jun N-terminal kinase (JNK) which mediates intracellular signalling associated with a variety of cellular functions. The JNK pathway in breast cancer (BC) can be attenuated via loss of function mutations in MAPK kinases as well as via PIK3CA mutations; however, there is contradictory information about the role of JNK pathway and its clinical implications in BC.

Methods

In the current study, the clinicopathological implications of JNK and JUN mRNA expressions were evaluated in multiple independent BC datasets: a) Training-set (Uppsala cohort; n=249), b) Test-set (human genome atlas database; n=540), c) External validation-set (METABRIC cohort; n=1952) and d) Multicentre pooled databases (n=5530). The clinicopathological associations of their phosphorylated proteins (p-Jnk and p-c-Jun) were assessed in the Nottingham Tenovus Primary BC Series (n= 1650) and in an ER negative cohort (n=450).

Results

Both JNK and c-JUN mRNA high expressions were significantly associated with PAM50-Luminal A and ER+/HER2-/low proliferation molecular BC subtypes, tubular/lobular types, and integrative molecular clusters 4 (IntClust.4), ps<0.001. Whereas BC that had both low JNK and c-JUN mRNA, were significantly associated with large tumour size, high grade, absence of hormonal receptors (HR), HER2 overexpression, PAM50 HER2 and PAM50 Basal molecular subtypes, and IntClust.1, 9 and 10 BCs; ps<0.001.

There was a significant positive correlations between p-Jnk and p-c-Jun protein levels (p<0.0001), however; our data suggested that differential p-Jnk/p-c-Jun expression may influence BC phenotypes. BC with p-Jnk-ve/p-c-Jun-ve were associated with the most aggressive phenotypes including largest tumour size, highest grade, lympho-vascular invasion, absence of HR, basal-like-phenotype, HER2 overexpression, and loss of double strand , single stand and base excision DNA repair proteins (ps<0.0001). In addition p-Jnk-ve/ pc-Jun-ve phenotype was associated with the lowest levels of p-38, ATF2, and p-ATF2; ps<0.001. Interestingly, low levels of either c-JUN-mRNA or pc-Jun protein, was associated with, PAM50-luminal B, epithelial mesenchymal transition and TP53 mutation and loss of its downstream proteins such as MDM2, MDM4, Bcle2 and p21; ps<0.05. JNK+ (mRNA and p-Jnk) and c-JUN+ (mRNA and p-c-Jun) individually were associated with prolonged BC specific survival (ps<0.001). Multivariate cox regression models that included other validated prognostic factors and therapies revealed that c-JUN-mRNA (Uppsala: p=0.005 and METABIRIC: p=0.036) and p-c-Jun (HR: 0.69; 95% CI = 0.55-0.88; p=0.002) were independently associated with clinical outcome. Furthermore, in ER+ high risk BC, exposure to tamoxifen was associated with decreased risk of death from BC in those patients with p-c-Jun-ve BC (HR: 0.65; 95% CI: 0.45-0.95; p=0.025).

Conclusion

JNK and c-JUN mRNA as well as p-Jnk and p-c-Jun protein levels are associated with luminal BC, with p-c-Jun being found to be an independent prognostic factor. The interaction between p-Jnk, p-c-Jun and TP53 mutation could predict response to endocrine therapy in ER+ BC. The role of the transcriptionally active form of c-JUN warrants further investigation with regard to its role in BC.
Title: Biomarker (BM) results from MERiDiAN, a double-blind placebo (PLA)-controlled randomized phase 3 trial of 1st-line paclitaxel (PAC) with or without bevacizumab (BEV) for HER2-negative metastatic breast cancer (mBC)

Das Thakur M, Bais C, Estay I, Vaidyanathan R, O'Shaughnessy J, Cameron D, Hubeaux S, Quah C and Miles D. Global Development BioOncology, Genentech Inc., South San Francisco, CA; Baylor Charles A Sammons Cancer Center, US Oncology, and Texas Oncology, Dallas, TX; University of Edinburgh and Cancer Services, NHS Lothian, Edinburgh, United Kingdom; F Hoffmann-La Roche Ltd, Basel, Switzerland and Mount Vernon Cancer Centre, Northwood, United Kingdom.

Body: Background: In the MERiDiAN trial, progression-free survival (PFS) was significantly improved with the addition of BEV to 1st-line PAC for mBC in both the ITT population and the subgroup with high baseline plasma (p) vascular endothelial growth factor (VEGF)-A, meeting both co-primary objectives. However, a predictive effect of pVEGF-A was not seen (PFS pVEGF-A-by-treatment interaction p=0.46; secondary endpoint). We report exploratory analyses of additional candidate BMs.

Methods: Patients (pts) with HER2-negative mBC previously untreated with chemotherapy for mBC were randomized to receive PAC (90 mg/m², days 1, 8 & 15 q4w) combined with either PLA or BEV 10 mg/kg q2w until disease progression or unacceptable toxicity. Plasma, blood and archival tumor sampling was mandatory. The BM-evaluable population (BEP) comprised all ITT pts with a baseline sample for ≥1 BM. Prespecified exploratory BM analyses included: tumor (t) CD31 (marker of microvascular density) and tVEGF-A (molecular target of BEV) by immunohistochemistry; tPAM50 gene expression; and pVEGF receptor (R)-2 by ELISA. For CD31, tVEGF-A and pVEGFR-2 analyses, the BEP was dichotomized using the median of each BM as the cutoff between low and high subgroups. BEV effect on PFS was assessed within these subgroups (unstratified analyses). Similar subgroup analyses were done for each tPAM50 molecular subtype. No adjustment was made for multiplicity of testing as the analyses were exploratory.

Results: The BEP included 467 (97%) of the 481 randomized pts. There was no correlation between CD31, tVEGF-A and pVEGFR-2. Correlations between BMs and PFS are shown below. The hazard ratio (HR) point estimate for BEV effect was lower in luminal B (0.59) than luminal A (0.96) or other smaller tPAM50 subgroups, but 95% CIs overlapped. pVEGFR-2 showed borderline significance for predictive potential using the median (10.2 ng/mL) as the cutoff. In further exploratory analyses using the 1st quartile (Q1; 8.7 ng/mL) as the cutoff, the PFS HR was 1.19 (95% CI 0.75–1.89) in the low (≤Q1) subgroup vs 0.60 (95% CI 0.46–0.79) in the high (>Q1) subgroup (interaction p=0.01).

<table>
<thead>
<tr>
<th>BM</th>
<th>Subgroup</th>
<th>No. of events/pts</th>
<th>Median PFS, mos</th>
<th>PFS HR (95% Wald CI)</th>
<th>Interaction p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>PAC–PLA</td>
<td>PAC–BEV</td>
<td>PAC–PLA</td>
<td>PAC–BEV</td>
</tr>
<tr>
<td>tPAM50 (N=421)</td>
<td>Luminal A</td>
<td>65/103</td>
<td>67/106</td>
<td>10.9</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
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<td>46/63</td>
<td>32/56</td>
<td>9.0</td>
<td>11.0</td>
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<tr>
<td></td>
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<td>9/10</td>
<td>5.5</td>
<td>8.3</td>
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<tr>
<td></td>
<td>Basal like</td>
<td>29/37</td>
<td>27/35</td>
<td>5.6</td>
<td>8.5</td>
</tr>
<tr>
<td>tCD31 (N=410)</td>
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<td></td>
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<td>64/94</td>
<td>9.2</td>
<td>11.0</td>
</tr>
<tr>
<td>tVEGF-A (N=434)</td>
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<td>78/107</td>
<td>77/110</td>
<td>7.4</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>High</td>
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<td>65/106</td>
<td>9.2</td>
<td>10.9</td>
</tr>
<tr>
<td>pVEGFR-2 (N=436)</td>
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<td>70/107</td>
<td>73/111</td>
<td>9.2</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>83/109</td>
<td>68/109</td>
<td>7.9</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Conclusions: Of the 4 candidate BMs explored here, potential predictive value was suggested only for pVEGFR-2. Correlations
between pVEGFR-2 levels and outcome have been observed in previous retrospective analyses of breast cancer trials. However, similar levels in healthy donors and breast cancer pts, as well as the narrow dynamic range, may limit the utility of pVEGFR-2 as a potential predictive BM for BEV efficacy.
Title: A single-nucleotide polymorphism in the 3’-UTR region of the adipocyte fatty acid binding protein 4 gene is associated with prognosis of triple negative breast cancer

Yuan P, Wang W, Wu C, Lin D and Xu B. Chinese Academy of Medical Sciences, Cancer Hospital, Beijing, China.

Objectives: Triple negative breast cancer (TNBC) is a subtype with poor prognosis and high heterogeneity. The aim of this study was to screen single nucleotide polymorphisms (SNPs) associated with the prognosis of TNBC patients and to explore the potential mechanism.

Methods: We selected SNPs (MAF ≥ 1%) located on the 3’ untranslated region (3’UTR) of differentially expressed genes in breast cancer through databases (Nextbio, ensemble, NCBI, MirSNP). We investigated the possible associations between 111 SNPs and progression risk among 323 TNBC patients using two-step case-control study with discovery cohort (n=162) and validation cohort (n=161). The Benjamini Hochberg false discovery rate (FDR) method was used to assess the statistical significance after correction for multiple comparisons. Kaplan-Meier analysis was applied in order to assess the association between disease free survival (DFS) and positive SNPs, as well as Body Mass Index (BMI). We also investigated the expression of FABP4 in adipocytes adjacent to TNBC tissues (n=35) using immunohistochemistry. The integrated optical density (IOD) was measured as an approximation of FABP4 expression. Mann-Whitney U test was used in an attempt to assess the correlations among SNPs in FABP4, FABP4 expression and TNBC prognosis.

Results: SNPs in FABP4, KRAS and NTRK2 genes associated with the recurrence risk of TNBC throughout the discovery cohort and validation cohort, while the statistical significance was retained after multiple comparisons only for FABP4 rs1054135. The G allele of rs1057035 was associated with decreased risk of disease progression (aHR 0.14, 95%CI 0.03-0.66), as well as an increased DFS (P<0.05). Furthermore, the expression of FABP4 was statistically significant lower in patients with rs1054135-GG genotype and those in disease-free group (P=0.0464, P=0.0061 respectively).

Conclusions: Our study found a lipid metabolism related gene and an important SNP in the 3’UTR of FABP4 associated with TNBC prognosis, which may aid the screening of high-risk patients with TNBC recurrence and the development of novel chemotherapeutic agents.
Title: Breast cancer patient survival prediction based on the signature derived from DNA methylation and mRNA expression

Yang HH H and Lee MP P. NCI/NIH, Rockville, MD.

Body: We analyzed a subset in the TCGA breast cancer data set for the samples with both expression and methylation assays. We first used the three Immunohistochemistry (IHC) markers for ER, PR and HER2 to find methylation sites that showed significant association to the IHC markers with Bonferroni adjustments. We obtained 2616 methylation sites in 2148 genes in the union of these significant methylation sites. On the other hand, based on the gene expression of samples, we selected top one percent most variable genes (179 genes). Intersection of the 2616 methylation markers with the 179 genes resulted in 58 methylation markers in 43 genes. We found 339 samples which have both expression and methylation data from which we can compute the correlation between the expression of a gene and the methylation of a marker in this gene. We defined a methylation expression index (MEI) which was a weighted sum of the expression of the 43 genes using the negative Spearman correlations as the weights. The expression index was applied in the survival analysis in three independent data sets GSE6532, NKI and METABRIC using Kaplan-Meier method and found the significant difference between the patient groups with high and low MEI index. Compared to the signature derived from the first principal component, the MEI gave better survival prediction across different data sets. We used Cox proportional hazards regression model to evaluate the hazard ratio of the MEI with adjustment of the other variables including age, grade, tumor size, ER and node and found HR=1.58 for the MEI with 95% confidence interval [1.06, 2.35].
Expression of EZH2 and its downstream effectors pEZH2 and H3K27 in pregnancy associated breast cancer (PABC)

Blanco Jr LZ Z, Parini V, Dhamne SA A and Siziopikou KP P. Northwestern University, Chicago, IL and Robert H. Lurie Comprehensive Cancer Center, Pathology Core Facility, Chicago, IL.

Background: Pregnancy associated breast cancer (PABC) arises during or after pregnancy, is typically triple negative, and is associated with a poor prognosis. Enhancer of Zeste Homolog 2 (EZH2), a core protein of the polycomb-repressive complex, plays a vital role in the epigenetic maintenance of histone H3 lysine (H3K27) repressive chromatin mark and has been found to be aberrantly expressed in various cancers including the breast. EZH2 is involved in stem cell renewal and is involved the initiation, progression and maintenance of cancer cells. EZH2 has been reported to be highly expressed in triple negative breast cancers and to be associated with poor prognosis. In this study, we assessed expression of EZH2 and its downstream markers phosphorylated EZH2 (pEZH2) and H3K27 and correlated their expression with clinicopathologic characteristics in this aggressive type of breast carcinoma.

Design: 23 patients diagnosed with PABC within 2 years of pregnancy (mean age=35.8, range=26-48) and control age-/stage-matched nulliparous women (mean age=37.5, range=29-51) were evaluated. Slides were reviewed and pathologic tumor characteristics were noted. Immunohistochemical stains for EZH2, pEZH2, H3K27 and the cancer stem cell marker ALDH1 were performed on 23 PABC and 15 control cases. Extent (1=1-25% positive tumor cells, 2=26-50%, 3=51-75% or 4=76-100%) and intensity (1=weak, 2=moderate or 3=strong) of staining were assessed. A composite score (CS) was calculated by multiplying the extent by intensity (range=0-9; weak=1-3; moderate=4-6 and strong=7-9).

Results: PABCs were more likely than controls to have moderate to strong immunoreactivity for EZH2 (69.6% vs. 33.3%, p=0.04). All PABC and control cases expressed EZH2 and pEZH2, while 60.9% of PABC and 40% of control cases expressed H3K27. Within the PABC group, moderate to strong EZH2 expression was seen more frequently in grade 3 tumors (86.7% in grade 3 tumors vs. 37.5% in grades 1-2, p=0.03). Of interest, all triple negative (TN) PABC cases had moderate to strong EZH2 (100% vs. 43.8%, p=0.02). All five PABC cases with the strongest EZH2 (CS=9) were grade 3 tumors (3 TN, 1 HER2+ and 1 luminal B), 60% of which had positive lymph node metastasis. Although 83.3% of ALDH1+ cases also had moderate to strong EZH2, no significant correlation was observed.

Conclusions: 1. PABCs express EZH2 and pEZH2 consistently. 2. PABCs are more likely than controls to have moderate to strong immunoreactivity for EZH2. 3. Within the PABC group, all triple negative tumors and the majority of grade 3 tumors have high EZH2 expression. 4. PABC cases with the strongest EZH2 are grade 3 tumors that have positive lymph nodes. Increased expression of EZH2 in PABC may contribute to the poor prognosis in these cases. Our findings add to the understanding of the molecular pathways that operate in different subtypes of breast carcinomas.
**Title:** DUSP4 is associated with increased resistance against anti-HER2 therapy in breast cancer

Györffy B, Munkacsy G, Esteva FJ J, Miquel TP P and Menyhart O. MTA TTK Lendlet Cancer Biomarker Research Group, Budapest, Hungary; Semmelweis University, Budapest, Hungary; Clinical Cancer Center, NYU Langone Medical Center, NY and Molecular Oncology, Facultat de Medicina, Girona Institute for Biomedical Research, Universitat de Girona, Girona, Spain.

**Body:**

**Background:** The majority of patients develop resistance against suppression of HER2-mediated signaling by trastuzumab in HER2 positive breast cancer (BC). HER2 overexpression activates multiple signaling pathways, including the mitogen-activated protein kinase (MAPK) cascade. MAPK phosphatases (MKPs) are essential regulators of MAPKs and participate in many facets of cellular regulation, including proliferation and apoptosis. We aimed to identify whether differential MKPs are associated with resistance to targeted therapy in patients previously treated with trastuzumab.

**Methods:** Using Affymetrix HGU133plus2 gene chip data of 88 HER2-positive, trastuzumab treated BC patients, candidate MKPs were identified by Receiver Operator Characteristics analysis performed in R. Genes were ranked using their achieved area under the curve (AUC) values and were further constricted to those markers significantly associated to worse survival. Functional significance of the two strongest predictive biomarkers was evaluated in vitro experiments after gene silencing in the HER2 overexpressing, trastuzumab resistant breast cancer cell lines SKBR-3-TR and JIMT-1.

**Results:** Out of 10 investigated MKPs, the strongest predictive genes were DUSP4/MKP2 (AUC=0.75, p=0.0096) and DUSP6/MKP3 (AUC=0.77, p=5.29E-05). Furthermore, higher expression for these correlated to worse survival in 221 HER2 positive BC patients (DUSP4: HR=1.6, p=0.04 and DUSP6: HR=1.8, p=0.0053). Silencing of DUSP4 had significant sensitization effects - viability of DUSP4 siRNA transfected, trastuzumab treated cells decreased significantly compared to scramble-siRNA transfected, trastuzumab treated controls (SKBR-3-TR: p=0.016; JIMT-1: p=0.016). In contrast, simultaneous treatment with DUSP6 siRNA and trastuzumab did not alter cell proliferation.

**Conclusions:** Our findings suggest that DUSP4 is involved in the development of trastuzumab resistance in HER2 positive BC.
**Title:** Ki-67 expression is not a valuable predictive prognostic factor when progesterone receptor expression is high in estrogen receptor-positive breast cancer

Han JH, Kang YJ, Han W, Lee H-B, Kim Y, Yoo T-K, Moon H-G and Noh D-Y. Seoul National University College of Medicine, Seoul, South Korea, Korea and Cancer Research Institute, Seoul National University College of Medicine, Seoul, South Korea, Korea.

**Body:**

**Background**
Immunohistochemistry markers are recognized as a predictive prognostic factor for women with breast cancer. Ki-67 and progesterone receptor (PgR) expression are reported to be independently associated with breast cancer prognosis. Some studies report high Ki-67 expression as a negative predictive marker. Whereas other studies report tendency of similar survival between high and low Ki67 cancers when PgR expression is high. In this study, we examined the prognostic significance of Ki-67 expression under PgR expression status.

**Methods**
The records of 2,366 patients were retrospectively reviewed. The patients underwent surgery for primary breast cancer from July 2009 to December 2012 at a single institution. We studied the prognostic significance of Ki-67 expression under PgR expression. We used 20% and 10% as the cut-off value for PgR and Ki-67, respectively. The end point was recurrence-free survival (RFS) evaluated by use of Kaplan-Meier analysis.

**Result**
Of the 2,366 analyzed patients, the median follow-up time was 43 months. During follow-up, 44 patients had recurrence, loco-regional recurrence developed in 23 patients and distant recurrence developed in 21 patients. In patients with low PgR expression, high Ki-67 expression group showed significantly worse prognosis compared to low Ki-67 expression group (p=0.005). On the other hand, no significant difference was shown between low and high Ki-67 expression group when PgR expression was high (p=0.637). Also multivariate analysis demonstrated that high Ki-67 expression was an independent prognostic factor only when PgR expression was low. (95% confidence interval [CI], 1.35-10.48; p=0.011)

**Conclusion**
This is the largest reported study that prognostic significance of Ki-67 expression is defined by PgR expression. Our study presents that high Ki-67 expression is inversely correlated with recurrence risk in early breast cancer patients only under low PgR expression. At high PgR expression, Ki-67 expression has no influence on breast cancer prognosis. Therefore, attention should be paid to correlation between PgR and Ki-67 expression.
Title: Evaluation of single nucleotide polymorphisms (SNPs) as predictive factors of progression (PFS) and metastatic (MFS) free survival in adjuvant breast cancer (BC)


Body: Background: Adjuvant chemotherapy (ACT) using anthracyclines and taxanes is a standard treatment for BC. Usual criteria as age, SBR grade, HER2 or hormonal status (HS), estrogen receptor (ER), triple negative BC, histological type, lymphovascular invasion (LVI), Ki67, tumor size and lymph node involvement are not yet sufficient for ACT decision. As SNPs located in genes involved in metabolism or transport of cytotoxic drugs may affect efficacy of ACT, we investigated the potential of 49 SNPs to predict response to ACT in BC.

Methods: From 01/2008 to 01/2012, 418 patients (pts) with BC treated with ACT were included. 309 pts received FEC100-Docetaxel regimen (cohort 1), and 109 pts with HER2 overexpression FEC100-Docetaxel-Trastuzumab regimen (cohort 2). Genotyping of 49 SNPs was performed on germline DNA using real time PCR with SNPType (Fluidigm) or Taqman probes (Life technologies) on BioMark Platform (Fluidigm). PFS, MFS and overall survival (OS) were estimated by Kaplan-Meier method. Association of clinicopathologic features (CPF) with PFS/MFS was evaluated by log-Rank test. After ensuring Hardy-Weinberg equilibrium was respected, PFS/MFS were correlated to CPF and genotypes, using univariate and multivariate Cox logistic regression. A prognostic score was established.

Results: PFS, MFS and OS rates were respectively 81.8%, 83.4% and 87.3% in cohort 1 (3.4 years of median follow up (FU)) and 90.1%, 90% and 93.8% in cohort 2 (4 years of FU). In cohort 1, univariate analysis revealed that 5 SNPs, SLCO1B3 (rs11045585), NOS3 (rs1799983), CYB2B6 (rs2279345), BRCA1 (rs799917) and CYP2D6 (rs3892097) were associated with MFS. Genotypes, HR, 95%CI and p value are as follows: SLCO1B3 GG 7.73 (1.83-32.7) p=0.001, NOS3 GT 0.32 (0.14-0.76) p=0.006, CYB2B6 TT 2.29 (1.02-5.13) p=0.04, BRCA1 CT 0.41 (0.19-0.89) p=0.02, and CYP2D6 AG 2.14 (1.05-4.36) p=0.03. Multivariate analysis revealed that 4 SNPs remained associated with metastatic risk: CYB2B6; TT 2.38 (1.05-5.41) p=0.038, NOS3; GG-TT 3.11 (1.33-7.27) p=0.009, BRCA1; CC-TT 2.21 (1.01-4.85) p=0.047, CYP2D6; AG 2.14(1.04-4.40) p=0.039. No CPF was associated with survival. Prognostic model revealed a metastatic risk of 10.25 (1.29-81.31) if these four adverse genotypes coexist. In cohort 2, subject to limited number of events, age (p=0.03), HS (p=0.06), ER (p=0.05), LVI (p=0.02) tumor size (p=0.003) and 2 SNPs were associated in univariate with PFS: CYB2B6 (rs2279345); CT 5.73 (1.22-27) p=0.01, MTHFR (rs1801133); CT 4.61 (0.98-21.7) p=0.03. Multivariate analysis showed unfavorable PFS for heterozygous patients for CYB2B6 or for MTHFR: 9.67 (1.82-51.28) p=0.008 and 5.62(1.19-26.59) p=0.03 respectively and if tumor size was ≥T2 10.78 (2.12-54.90) p=0.004.

Conclusion: SNPs of genes involved in oxidative stress (NOS3 rs1799983; GG-TT), docetaxel transport (SLCO1B3 rs11045585; GG) cyclophosphamide (CYB2B6 rs2279345; TT in cohort 1 ou CT in cohort 2) and 5FU metabolism (MTHFR rs1801133; CT), or DNA repair (BRCA1rs799917; CC-TT) are associated with survival in pts treated with ACT. BRCA1, CYB2B6 and SLCO1B3 represent potential attractive tools for guiding ACT indication.
Title: CKAP2 (cytoskeleton associated protein 2) is a new prognostic marker in HER2-negative luminal breast cancer

Sim SH, Bae C-D, Kwon Y, Park IH, Lee KS, Jung S-Y, Lee S, Kang H-S, Lee ES, Kim H-S, Hong K-M and Ro J. Center for Breast Cancer, National Cancer Center, Goyang, Korea; Research Institute, National Cancer Center, Goyang, Korea; Sungkyunkwan University School of Medicine, Suwon, Korea and Inje University Ilsan Paik Hospital, Goyang, Korea.

Body: Background: Ki-67 has been increasingly used as a prognostic marker in spite of debates on the evaluation methods and inconsistent results on its clinical values. CKAP2 is a microtubule-associated protein which plays key roles in microtubule assembly and disassembly. In the present study, the clinical significance of CKAP2-positive cells was evaluated and compared with the results of Ki-67 positive cells.

Methods: A total of 579 early breast cancer patients who underwent surgery at the National Cancer Center Hospital between 2001 and 2005 were accrued. The proliferation activity was measured by CKAP2-positive cell count (CPCC) and Ki-67 labeling index (Ki-67 LI) using CKAP2 and Ki-67 antibodies, respectively, by immunohistochemical staining on FFPE tumor tissue. The correlation of CPCC or Ki-67 LI with recurrence free survival (RFS) was analyzed. The immunofluorescent staining was performed on HeLa cells after synchronization by double thymidine block to compare the patterns between CKAP2 and Ki-67.

Results: The CPCC (median, 8 with the range of 0- 170) and Ki-67 LI (median, 10.2 with the range of 0%- 91.7%) were highly correlated (R = 0.754, P < 0.001). While CPCC was marginally significant in multivariate analysis for RFS in all cases, it was a significant variable for RFS in the subset analysis with HER2-negative luminal breast cancer patients (HR, 3.154; 95% CI, 1.154-10.693; P = 0.027). On the contrary, Ki-67 LI failed to show any correlation with RFS in all or any subgroups. In the analysis on HeLa cells, CKAP2 staining was more specific to cells in metaphase than Ki-67 staining.

Conclusions: CPCC can be an independent prognostic factor specifically in a HER2-negative luminal type of breast cancer. In addition, CPCC appears to be superior to Ki-67 LI as a survival indicator which may be related to the restricted expression pattern of CKAP2 in metaphase cells. Further study is warranted.
Title: CYP1A2– A novel genetic marker for aromatase inhibitor response in the treatment of breast cancer patients

Simonsson M, Veerla S, Markkula A, Rose C, Ingvar C and Jernström H. Division of Oncology and Pathology, Lund, Lund University, Lund, Sweden; Lund University, Lund, Sweden and Division of Surgery, Lund, Lund University and Skåne University Hospital, Lund, Sweden.

Body: Background: Endocrine resistance is a major obstacle for optimal endocrine treatment in breast cancer (BC). Several genetic markers for tamoxifen response have been proposed. Although some genetic markers have been proposed for response to aromatase inhibitors (AI), data is insufficient. The aim of this study was 1) to perform an exploratory analysis of genes involved in Absorption, Distribution, Metabolism, and Elimination (ADME) to find new predictive markers in a subset of the cohort and 2) examine these potential markers in relation to risk for events in the extended cohort.

Materials and methods: In Lund, Sweden, 190 AI-treated primary breast cancer patients with estrogen receptor positive tumors, who underwent breast cancer surgery between 2002 and 2008, were followed until December 31st 2012. Clinical data were obtained from medical records and population registries. The impact of single nucleotide polymorphisms (SNPs) on risk for breast cancer events was conducted on a subset of the cohort followed until December 31st 2011 (13 cases, 11 controls) and analyzed with data from a DMET (TM) chip including 1931 SNPs in 225 ADME-related genes. Secondly, four SNPs in CYP19A1 and the significant SNPs from the first analysis were reanalyzed concerning disease-free survival in the extended cohort of 190 patients.

Results: A CYP1A2 SNP was significantly associated with risk for early events among the 24 AI-treated BC patients both in the subset of the cohort, (P=0.0007) and in the extended cohort, adjusted HR 3.83 (95% CI 1.40-10.42). SNPs in CYP19A1 alone were not significantly associated with disease-free survival in any of the analyses. The impact of the CYP1A2 SNP was modified by a SNP in CYP19A1, where patients with both SNPs had increased risk for early events, adjusted HR 5.21 (95% CI 2.05-13.23) compared to other patients.

Conclusion: This study identified a potential and new predictive marker in BC patients: CYP1A2 alone and CYP1A2 in combination with CYP19A1. If confirmed, these results may provide a way to more personalized medicine.
2015 San Antonio Breast Cancer Symposium

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Title: Estrogen receptor beta expression is prognostic among chemotherapy-treated patients – Results from a population-based breast cancer cohort

Elebro K, Borgquist S, Rosendahl A, Markkula A, Simonsson M, Jirström K, Rose C, Ingvar C and Jernström H. Lund University and Skåne University Hospital, Oncology and Pathology, Lund, Sweden; Lund University, Lund, Sweden and Lund University and Skåne University Hospital, Lund, Division of Surgery, Lund University, Lund, Sweden.

Body: Background and aim

Estrogen receptor beta (ERβ) expression has been suggested to hold prognostic and predictive information, especially for endocrine treatment. The role of ERβ may depend on the expression of estrogen receptor alpha (ERα). The aim of this study was to evaluate ERβ expression in relation to tumor characteristics and risk for early events (disease-free survival, DFS) – overall and in different treatment groups, in a population-based cohort of primary breast cancer patients.

Materials and methods

Patients with primary breast cancer were invited at the preoperative visit between October 2002 and June 2012. After exclusion of patients who received preoperative treatment and patients with in situ carcinoma, the study population consisted of 1026 patients. Tumor specimens mounted in tissue microarrays were analyzed for ERβ expression using immunohistochemistry (antibody PPG5/10, Dako, dilution 1:20). Two cut-offs for positivity were evaluated (>10% and >75% of stained nuclei, respectively). Cox regression analyses yielding hazard ratios (HRs) with 95% confidence intervals (CIs) were adjusted for ERα status, tumor size, axillary lymph node involvement, histological grade, age at inclusion and body mass index. Events were defined as ipsi- or contralateral recurrences, regional or distant metastases. DFS was calculated from inclusion to event, non-breast cancer related death or last follow-up by June 30 2014, whichever came first. Patients were followed for up to 11 years (median follow-up 5.0 years for patients still at risk).

Results

ERβ expression was available for 911 patients (89%). ERβ positivity defined as >10% (ERβ10+, 92.1%) was positively associated with ERα (P<0.0001). ERβ10+ was not associated with DFS, overall or in patients who received tamoxifen and/or aromatase inhibitors. ERβ positivity defined as >75% (ERβ75+, 72.7%) was associated with ERα and PR positivity, lower histological grade, smaller invasive tumor size and no involvement of axillary nodes (all Ps≤0.03). In survival analyses among all patients, ERβ75+ was inversely associated with risk of early events (LogRank $P=0.0005$, adj HR 0.62: 95% CI 0.42-0.90; $P=0.013$). The magnitude of the association was larger in patients with ERα negative tumors (LogRank $P=0.011$, adj HR 0.42: 95% CI 0.17-1.02; $P=0.05$) compared to patients with ERα positive tumors (LogRank $P=0.069$, adj HR 0.74: 95% CI 0.48-1.14; $P=0.18$). In analyses stratified for chemotherapy, ERβ75+ expression was significantly associated with lower risk of early events among the 232 patients who had received chemotherapy (Log Rank $P=0.0005$, adj HR 0.31: 95% CI 0.15-0.63; $P=0.001$), but not among the 670 patients without chemotherapy (Log Rank $P=0.14$, adj HR 0.81: 95% CI 0.50-1.31; $P=0.38$). ERβ75+ was not associated with early events in patients with ERα+ tumors who received tamoxifen and/or aromatase inhibitors.

Conclusion

This study provides support for high tumor ERβ expression as a prognostic marker in breast cancer, for patients who received chemotherapy. Previous reports of ERβ as a predictor of endocrine therapy response could not be confirmed.
Title: ERβ1 inversely correlates with PTEN/PI3K/AKT pathway and predicts a favorable prognosis in triple-negative breast cancer


Purpose
In contrast to the well established role of estrogen receptor alpha (ERα) in breast cancer, the significance of estrogen receptor beta (ERβ) remains controversial, especially in triple-negative breast cancer (TNBC). We aimed to investigate the clinical importance of wild-type ERβ (ERβ1) in TNBC based on a large population, and to explore the potential molecular pathways involved in.

Methods
A total of 571 patients with invasive TNBC undergoing curative surgery were included in this study. Immunohistochemical staining for ERβ1, pAKT, PTEN, pERK, β-catenin, EGFR, p53 and E-cadherin was performed on tissue microarray (TMA). Prognostic determinants for OS and DFS, as well as the risk factors for distant metastasis-free survival (DMFS) and locoregional recurrence-free survival (LRRFS), were evaluated in univariate and multivariate analysis.

Results
Overexpression of ERβ1 was detected in 30.4% of TNBCs. Patients with ERβ1 tended to be postmenopausal, and less likely to develop lymphatic metastasis. Multivariate analysis demonstrated that ERβ1 conveyed a better OS, DFS, and DMFS independently. Regarding other biomarkers, only pAKT was identified as an independent negative predictor for survival. Additionally, ERβ1 expression was inversely associated with pAKT and the loss of PTEN. Notably, further survival analysis according to status of ERβ1/pAKT indicated that ERβ1(+)/pAKT(-) predicted the most favorable prognosis for TNBC. On the contrary, ERβ1(-)/pAKT(+) was associated with the worst outcomes.

Conclusions
In summary, our findings indicate that ERβ1 independently predicts a better prognosis for TNBC, and potentially interacts with the PTEN/PI3K/pAKT pathway. The role of ERβ1-specific agonist combined with inhibitor of pAKT merits further investigation.
Title: Tamoxifen or aromatase inhibitors: Should smoking status impact on selection of endocrine therapy in breast cancer patients?

Jernström H, Persson M, Simonsson M, Markkula A, Rose C and Ingvar C. Lund University, Lund, Division of Oncology and Pathology, Lund, Skane, Sweden; Lund University, Lund, Skane, Sweden and Lund University and Lund University Hospital, Lund, Division of Surgery, Lund, Skane, Sweden.

Body: Introduction: The association between smoking and breast cancer prognosis has been investigated in several studies but remains unclear. To our knowledge, no study has investigated whether the response to different endocrine treatments differs between smokers and non-smokers. Smoking can suppress aromatase activity, but also increase inflammation, which may lead to higher activity. The aim was to investigate whether preoperative smoking was associated with risk of breast cancer events in endocrine-treated patients.

Patients and methods: This population based cohort consisted of 1026 female breast cancer patients with invasive tumors and no preoperative treatment who were enrolled in an ongoing prospective cohort study at Skåne University Hospital in Lund between October 2002 and June 2012. Pre- and postoperative questionnaires regarding lifestyle factors, including smoking status, and treatments were completed. Information on tumor characteristics, treatments, and dates for new breast cancer events or deaths was obtained from pathology reports, patients' charts and population registers. A breast cancer event was defined as local or regional recurrence, contralateral breast cancer, or distant metastasis.

Results: For the survival analyses, two patients were excluded due to missing information on smoking and eight patients were excluded due to metastatic spread within 0.3 years of inclusion, leaving 1016 patients of which 206 (20.3%) reported preoperative smoking. Less than 1% of the 810 preoperative non-smokers reported smoking at either the 3-6-months or 1-year postoperative visits, while about 10% of the patients who smoked preoperatively reported not to smoke during the follow-up visits. Thus, the majority of the patients did not switch smoking status. Patients were followed for up to 11 years (median 5.1 years for patients still at risk). Overall, there was no significant association between smoking and risk of breast cancer events (adjusted hazard ratio (HR) 1.45: 95% CI 0.95-2.20; P=0.08) adjusted for patient and tumor characteristics. For the 408 tamoxifen-treated patients aged 50 years or older with estrogen receptor positive tumors, smoking was not significantly associated with risk for early events (adjusted HR 1.58: 95% CI 0.76-3.30; P=0.22). However, for the 309 aromatase inhibitor-treated patients aged 50 years or older with estrogen receptor positive tumors, smoking was significantly associated with a 3-fold increased risk of breast cancer events (adjusted HR 2.97: 95% CI 1.44-6.12; P=0.003). Some patients had been treated with sequential tamoxifen and aromatase inhibitor therapy.

Conclusions: Preoperative smoking was associated with a significantly increased risk for breast cancer events among patients treated with aromatase inhibitors, but not among tamoxifen-treated patients. If confirmed, smoking status should be taken into consideration when selecting endocrine therapy.
Title: Androgen receptor in early breast cancer: Distribution and prognostic value

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Body: Purpose:
Androgen receptor (AR) status in breast cancer has received renewed interest over the last years especially in triple-negative disease (TNBC), but the prognostic value is still under debate. The aim of this study was to assess the distribution and prognostic value of AR in early breast cancer patients with or without adjuvant endocrine treatment.

Patients and methods:
AR was assessed on tissue microarray with the AR 441 antibody (Thermo Scientific) on a cohort consisting of 471 patients derived from two clinical studies: (1) 208 premenopausal node-negative patients of which 87% had received no adjuvant medical treatment and (2) 263 estrogen receptor (ER)+ and ER-, node-positive and –negative patients treated with 2 years of adjuvant tamoxifen. Nuclear AR was divided in 5 groups: 0-1%, 2-10%, 11-50%, 51-75%, and 76-100% positive cells, scored as 0-4. Cox proportional hazards regression, stratified by study, was used to model the impact of the prognostic factors on distant disease-free survival (DDFS), both using trend tests and a cut-off for positivity set at >10%, and log-rank tests to compare survival in different strata. Due to non-proportional hazards, the analysis was restricted to the first 5 years after diagnosis, a time period during which 95 patients developed distant recurrences.

Results:
76% of all patients were AR+, and 89%, 48%, and 23% of the ER+, ER-, and TNBC, respectively. Positive associations were observed between AR, ER and progesterone receptor status (PgR), negative associations with Ki67, and histological grade, but no associations with tumour size, age or Human Epidermal Growth Factor Receptor 2 (HER2). In univariable analysis, when divided into 5 groups, AR was a prognostic factor for DDFS with a Hazard Ratio (HR) of 0.86 per step in fraction score (95% Confidence Interval (CI): 0.76-0.98, p=0.018), as was HER2, age, size, grade, node-status, PgR, and ER status. In the Kaplan-Meier curves for each study, a similar but weaker trend was found (log-rank test for trend p=0.14 and 0.057 for cohort 1 and 2, respectively). With a cut-off at 10%, a similar HR was found (HR=0.67, 95% CI:0.43-1.05, p=0.078). In multivariable analysis, adjusted for grade, tumour size, HER2, ER, node-status, and age, AR did not retain independent prognostic value (HR 1.04 95% CI:0.88-1.23, p=0.66). In the TNBC patients there were no significant differences in DDFS in the AR+ vs AR-patients, possibly due to few events and a small population (n=20/75).

Conclusion:
This study demonstrates that AR is a weak prognostic factor for recurrence in a cohort consisting of node-negative premenopausal patients without endocrine treatment and patients who have received adjuvant endocrine treatment. There was however no independent value in multivariable analyses. It is noteworthy that there were 23% AR positive TNBC patients, for whom there is currently no available targeted treatment. There are several ongoing studies with AR-targeted treatment in the metastatic setting, which if proven effective, may be transferred to studies in the adjuvant setting with the goal of improving long-term prognosis for TNBC. Taken together, AR may be clinically helpful for prognostic considerations and for selection of adjuvant treatment.
Body: Background
Early breast cancer (BC) outcomes are mainly estimated based on clinicopathological parameters and rarely include proliferation markers: (Ki-67), and multigene signatures (MGS), which are typically measured in tumors from resection specimens (RS). We believe that tumor proliferation can change within days of anti-estrogen use. However, little is known how proliferation changes after withdrawal of hormone replacement therapy (HRT) or oral anti-conception (OAC) after a core needle biopsy (CNB) shows BC. Hence, this study compares the Ki-67 labeling index and the MGS results in CNB and RS collected from a cohort of patients under OAC/HRT.

Patients and Methods
This retrospective study included consecutive women diagnosed with a grade 1-2, any pTN0-1, primary operable estrogen receptor-positive, human epidermal growth factor receptor 2-negative, and invasive ductal carcinoma between January 2013 and July 2014 at the Multidisciplinary Breast Center of University Hospitals Leuven were selected from a prospectively managed database. Ki-67 staining was performed on RS (Ki-67\textsuperscript{RS}) to compare those who used HRT/OAC for at least 3 months at diagnosis and those not using OAC/HRT at diagnosis. OAC/HRT was always stopped between CNB and RS. Subsequently, we compared Ki-67 of the CNB (Ki-67\textsuperscript{CNB}) with the matched RS in 15 patients with a low Ki-67\textsuperscript{RS} (14%) stopping OAC/HRT after CNB; revised standard pathology confirmed absence of tumor heterogeneity in all samples. In addition, in a subset of patients (≥50 years, Ki-67\textsuperscript{RS} ≤5%) we compared Ki-67 index and MGS results (MammaPrint (MP) and BluePrint, Agendia) from the CNB versus RS.

Results
193 patients with a known Ki-67\textsuperscript{RS} were included; 38 patients (mean age of 55 years) were on OAC/HRT at CNB and 155 patients (mean age of 64 years) were not. The median time between stopping OAC/HRT and resection was 23 days (range 8-48 days). Ki-67\textsuperscript{RS} was <6%, 6-14% and ≥15% in 30.0%, 36.8% and 33.2% in the whole group of patients, respectively. These figures were 44.7%, 44.7% and 10.5% in patients on OAC/HRT at CNB diagnosis and 26.5%, 34.8% and 38.7% in patients not on OAC/HRT. This difference was significant (p<0.05) for patients ≥50 years on HRT at CNB diagnosis. Four of 15 patients, which stopped OAC/HRT after the CNB (26.7%), showed a low Ki-67\textsuperscript{RS} (≤14%) and had a high matched Ki-67\textsuperscript{CNB} (≥15%). In another subset of four out of 15 patients we compared CNB and RS (table 1) where we observed changes in both Ki-67 and MGS.

MP and Ki-67 results from matched CNB and RS.

<table>
<thead>
<tr>
<th>Patient</th>
<th>CNB</th>
<th>RS</th>
</tr>
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<tbody>
<tr>
<td>Ki-67 (%)</td>
<td>MP index</td>
<td>Ki-67 (%)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.06</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>-0.70</td>
</tr>
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</table>

Low risk is defined as MP index ≥0.0575 and/or Ki-67 ≤14.

Conclusion
Women on OAC/HRT, at diagnosis of an early luminal BC, are more likely to have a lower Ki-67\textsuperscript{RS} as those not using OAC/HRT.
Our findings are likely explained by a sudden decrease in sex steroids after BC diagnosis, resulting in lower proliferation markers. As such, a lower hormonal environment by withdrawing HRT/OAC at BC diagnosis might underestimate proliferation markers used for prognostic and predictive purposes. Therefore we are currently testing this hypothesis in larger cohorts.
Title: Stratifying risk in ER+/HER2- early breast cancer using routine pathological markers

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Body: Gene expression profiling (GEP) and expanded immunohistochemistry (IHC) tests are increasingly utilised for guiding difficult chemotherapy decision making in ER+ early breast cancer patients. IHC4 has the advantage of being a locally processed, potentially cost effective test that has been reported to be a clinically valid, robust predictor of distant recurrence. IHC4 requires quantitative assessment of ER and issues regarding reproducibility of detecting Ki67 remain. This study aim was to develop a pragmatic analytically reproducible, cost effective clinical outcome score (COS) equivalent that identifies risk in early ER+ breast cancer using routine pathological markers. A combined allred ER and PR IHC score was used to represent ER signalling, grade as a marker of proliferation, HER2 IHC score for HER2 signalling and patient age to calculate COS (score range 2-10). Statistical analysis was performed with accurate 15 year patient follow up data.

Results: For the entire cohort (n=517) there was a highly significant linear distribution of COS scores (2-10) associated with 15 year breast cancer specific survival (p=2.7x10-5), 10 year disease free survival (DFS) (p=3x10-5) and 5 year DFS (p=3.8x10-8). A clear division was observed between low (2-4) and high scores (5-10) and in multivariate analysis this was independently associated with 15 yr breast cancer survival (high COS HR 3.75 CI 2.2-6.6, p=1x10-8) and early recurrence (high COS HR 6.5 CI 3.1-13, p=5x10-8). Similarly in the ER+/HER2- endocrine treated cohort (n=279), COS was independently associated with 15 yr breast cancer survival (high COS HR 3.5 CI 1.9-6.4 p=1.7x10-5) and 5 year recurrence (high COS HR 5.6 CI 2.3-13, p=1.9x10-5).

In addition low COS was associated with highly significant improved outcome in terms of 5 year recurrence and breast cancer specific survival when analysed in the intermediate ('challenging') prognostic subgroups of ER+/HER- endocrine treated patients: grade 2 (n=167, p<0.001) size 20-50mm (n=101, p=0.004) and 1-3 lymph node + (n=84, p=0.024).

Conclusion: COS can be tested locally utilising routine pathological markers. Our results suggest COS can identify risk in ER+/HER2- early breast cancer. Importantly it appears to clearly define low risk in the challenging ER+/HER2- prognostic subgroups.
Title: Decreased overall survival in "young" breast cancer patients with renal insufficiency

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Body: Background
Data still remain scanty on the potential impact of chronic kidney disease (CKD) on the mortality of breast cancer (BC) patients. The results of 3 clinical studies we conducted (IRMA-2, CANDY and MARS) were pooled. In all 3, the methodology was the same regarding CKD, allowing pooled analysis on the prevalence of CKD and on the potential association between RI and overall survival (OS), and to stratify the risk, if any, depending on the GFR.

Material and methods
The KDIGO definition and classification of CKD was used. GFR was estimated with the MDRD formula. RI was defined as GFR<60 ml/min/1.73 m². Multivariate analysis was conducted using patients with a GFR ≥ 90 ml/min/1.73m² as the reference. This sub-group analysis presents the results for young (<65 years) BC patients.

Results
The total population included 5908 solid cancer patients, among them, 1716 were young BC patients. Median age: 53.0 years, median body mass index: 23.4 kg/m², bone metastasis: 27.7%, visceral metastasis: 38.7%. 75.9% of these patients were alive at the end of the 1-year follow-up. In these young BC patients, prevalences of a GFR<90 and <60 mL/min/1.73m² were 46.6% and 5.4%, respectively (no dialysis patients). Multivariate Cox model adjusted for age, and metastasis reported that GFR was significantly associated with OS with an increased risk of mortality at a GFR of 71 mL/min/1.73m² (HR=1.51 [1.10-2.08]; p=0.04). This risk gradually increased with the decrease in GFR (table), except for patients below 30 mL/min/1.73m², however still significantly increased.

Conclusion:
This pooled analysis reported that: 1) abnormal GFR was frequent in young BC patients, 2) reduced GFR was a statistically significant prognostic factor for reduced OS. 3) This reduced OS began at an early stage of CKD in young BC patients, for a GFR<71 ml/min/1.73m². These results underline that assessing, monitoring and managing renal function in BC patients is crucial, and for relatively high levels of GFR. Preventing the reduction in renal function requires early care, multidisciplinary, or a transfer of good practices from nephrologists to oncologists involved in the care of these patients. Recommendations from the Cancer & the Kidney International Network (C-KIN), recently founded, are to be released.

Table. Multivariate Cox model regressions in BC patients

<table>
<thead>
<tr>
<th>GFR</th>
<th>n, %</th>
<th>HR [95% CI]; p-value as compared to GFR ≥ 90</th>
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<tbody>
<tr>
<td>GFR&lt;90</td>
<td>799 (46.6%)</td>
<td>HR=1.22 [0.79-1.89]; p&gt;0.05</td>
</tr>
<tr>
<td>GFR&lt;80</td>
<td>501 (29.2%)</td>
<td>HR=1.11 [0.84-1.47]; p&gt;0.05</td>
</tr>
<tr>
<td>GFR&lt;70</td>
<td>238 (13.9%)</td>
<td>HR=1.60 [1.15-2.22]; p=0.005</td>
</tr>
<tr>
<td>GFR&lt;60</td>
<td>92 (5.4%)</td>
<td>HR=1.80 [1.14-2.86]; p=0.012</td>
</tr>
<tr>
<td>GFR&lt;50</td>
<td>33 (1.9%)</td>
<td>HR=2.38 [1.22-4.63]; p=0.01</td>
</tr>
<tr>
<td>GFR&lt;40</td>
<td>12 (0.7%)</td>
<td>HR=2.77 [1.27-6.03]; p=0.011</td>
</tr>
<tr>
<td>GFR&lt;30</td>
<td>5 (0.3%)</td>
<td>HR=1.67 [1.04-2.69]; p=0.04</td>
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</table>
Concordance of histologic grade of invasive breast cancer between core needle biopsy and surgical excision specimen; A systematic review and meta-analysis

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Body: Introduction
Histologic grade is one of the most important prognostic factors for breast cancer and one of the determinants of the need for adjuvant systemic treatment. Grading is usually based on surgical excision (SE) specimen. However, with the increasing use of neoadjuvant chemotherapy and minimally invasive ablative therapies, pre-treatment assessment of grade is needed. Core needle biopsy (CNB) is an accurate tool for diagnosing breast cancer. It is unclear whether CNB provides sufficient tissue for accurate grading. We conducted a systematic review and meta-analysis of the literature to derive a reliable estimate of the agreement in grading between CNB and SE.

Methods
This study was conducted according to the Preferred method for Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) criteria. A search in EMBASE, PubMed and the Cochrane Library was conducted. Studies that provided data on grade of both CNB and SE were included. Proportion of agreement between CNB and SE, agreement beyond chance (Cohen's kappa) and the proportion of over- and underestimation of CNB grading were calculated for each study and pooled. Agreement of grade components (tubular formation, nuclear pleomorphism and mitotic rate), was also pooled. Random effect models were applied because of substantial heterogeneity (tested by $I^2$ test). Meta-regression was used to explore determinants of the level of agreement between CNB and SE.

Results
The search retrieved 1232 papers, of which 34 articles were included in the systematic review (6029 patients) and 33 studies were used for meta-analysis (4980 patients). Pooled absolute agreement was 71.1% (95%CI 68.9-73.3%), pooled Cohen's kappa was 0.54 (95%CI 0.5-0.58). Underestimation of grading by CNB occurred more frequently than overestimation; 19.1% (95%CI 17.1-21.3%) and 9.3% (95%CI 7.7-11.4%), respectively. Fourteen studies reported nuclear pleomorphism scores, with a pooled agreement of 70.2% (95%CI 65.7-74.3%). Pooled agreement of tubule formation (12 studies) was 74.5% (95%CI 68.4-79.5%). For mitotic count (13 studies), pooled agreement was 62.4% (95%CI 57-67.6%). Meta-regression showed a positive association between agreement of histologic type on CNB and SE and agreement of grading. Higher proportion of ER positive patients was negatively associated with agreement.

Conclusions and discussion
Grading on CNB corresponds moderately with grading on SE. CNB underestimates grade in one of five patients. Of the three grade components, mitotic count is most frequently discordant. However, incorrect tumor grading does not have clinical implications in all patients, since the indication for adjuvant systemic therapy is decided by several other factors as well and in a substantial proportion of cases not affected by differences in grade. Finally, considerable inter-observer variability of grading on only SE exists, which may also affect treatment decision making.
Title: Correlation of predictive markers in invasive breast carcinoma in core and excision specimens: A retrospective review

Music J and Sahoo S. UT Southwestern Medical Center, Dallas, TX.

Body: Background:
Testing of biomarkers in biopsies is a critical driver of determining therapy in patients diagnosed with invasive breast cancer. In the past, open surgical biopsy was considered to be the gold standard, but has been supplanted by the less invasive core needle biopsy. It is therefore imperative to know if such biopsies are representative of disease existing within the entirety of the tumor. This is especially the case now that neoadjuvant therapy is offered to patients with early stage tumor prior to surgical excision. In order to confirm that core biopsies are sufficient to serve as the launch point for treatment decision, we have conducted a retrospective review to compare the biomarker results of core biopsies with their corresponding excisional specimens. Previous studies have contained a smaller sample size, thus this is the largest single-site study of its kind.

Methods:
Data from patients with invasive breast cancer diagnosed between 2006 and 2013 have been retrospectively studied. Results for estrogen receptor (ER), progesterone receptor (PR) and Her2 status were analyzed. When the core biopsy and excisional specimens are either both "positive" or both "negative," the pair is considered to be "concordant." Otherwise, the pair is categorized as "discordant." As per the current ASCO/CAP guideline, hormone receptor status (estrogen and progesterone receptors) is considered positive if there were at least 1% tumor cells positive for either ER or PR. Both IHC and FISH concordance are evaluated for Her2 status. Patients who received neoadjuvant therapy of any kind were excluded from this study.

Results:
A total of 571 pairs of data totaling 1142 samples were analyzed in this study. Concordance for ER status is 97%, and for PR status is 92%. Concordance for hormone receptor status (when results of ER and PR are combined) between core biopsy and excision is 99%. Concordance for Her2 status, using FISH as the gold standard is 99%.

Discussion:
Typically, tumors that are ER+ are often also PR+. Nevertheless, they are often treated the same with hormonal therapy whether ER+PR+, ER+PR-, or, less commonly, ER-PR+. Almost all laboratories perform both ER and PR as a standard testing protocol for all breast carcinomas. Therefore, the lower concordance rate for only PR status without taking into consideration ER result is less meaningful. This study shows very high concordance rates for hormone receptor and Her2 status between core needle biopsy and excisional specimens, reiterating that treatment of invasive breast cancers based on the marker results obtained from core needle biopsies is appropriate.
Contemporary risk of local breast cancer recurrence after neo-adjuvant chemotherapy: Results of a population-based cohort study

Aalders KC C, Sonke GS S, van der Heiden-van der Loo M, Boersma LJ J, van Diest PJ J, Siesling S and van Dalen T. Diakonessenhuis, Utrecht, Netherlands; Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Netherlands Comprehensive Cancer Organization (IKNL), Utrecht, Netherlands; GROW Maastro Clinic-University Hospital Maastricht, Maastricht, Netherlands; University Medical Center Utrecht, Utrecht, Netherlands and MIRA Institute for Biomedical Technology and Technical Medicine-University of Twente, Enschede, Netherlands.

Body: Introduction
Neo-adjuvant chemotherapy (NAC) is increasingly used in breast cancer to enable less extensive surgery and monitor the response to systemic therapy. Little is known about local recurrence (LR) in patients who received NAC. However, this information is important when deciding on optimal local treatment in these patients, especially since NAC is increasingly being offered to patients with smaller tumors. The aim of this study is to assess the contemporary rates of local breast cancer recurrence in patients that received NAC.

Methods
All women treated with NAC for primary invasive breast cancer in the years 2003-2008 were selected from the Netherlands Cancer Registry. The first event within five years after NAC was included for analyses. The 5-year local (LR) recurrence rate was calculated using Kaplan Meier estimates and the prognostic value of various clinicopathological and treatment factors was evaluated.

Results
A total of 2,457 patients were identified of whom 43% had cT1-2, 25% cT3 and 29% cT4 tumors. Two-thirds of the patients had metastatic lymph node involvement and 85% received adjuvant radiotherapy. The overall 5-year risk of LR was 6.7% and decreased from 2003-2008.

Table 1. Overall 5-year rate of local breast cancer recurrence in 2,457 breast cancer patients that received neo-adjuvant chemotherapy in the period 2003-2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>412</td>
<td>30</td>
</tr>
<tr>
<td>2004</td>
<td>429</td>
<td>28</td>
</tr>
<tr>
<td>2005</td>
<td>549</td>
<td>39</td>
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<tr>
<td>2006</td>
<td>604</td>
<td>23</td>
</tr>
<tr>
<td>2007</td>
<td>406</td>
<td>16</td>
</tr>
<tr>
<td>2008</td>
<td>489</td>
<td>24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,457</strong></td>
<td><strong>160</strong></td>
</tr>
</tbody>
</table>

The LR-rate was lower in hormone receptor positive (HR+) than HR-negative (HR-) tumors (3.3% vs. 12.9%) and increased with larger residual tumor size (from 1.2% in ypT0 to 13.0% in ypT3 and 16.1% in ypT4 tumors). The LR-rate also increased with the ypN-stage (4.1% in ypN0, 5.7% in ypN1 and 11.3% in ypN>1 patients) and was lower following breast-conserving surgery (BCS) than after mastectomy (4.8% vs. 7.2%).

Currently, we are working on the multivariate analyses, which will be available at the San Antonio Breast Cancer Symposium.

Conclusions
The rate of LR in patients treated with NAC has decreased over time. This will most likely be caused by enhanced imaging and
radiotherapy techniques, as well as by increased insight in tumor biology resulting in improvements in both the development and application of systemic treatment modalities. Multivariate analyses will have to provide further insight into the risk of developing LR in patients treated with NAC, as well as into the prognostic value of different clinicopathological factors.
Intraductal papillomas: Risk of cancer, immediate and delayed

Khan S, Diaz A, Archer KJ J, Lehman RR R, Mullins TC C, Cardenosa G and Bear HD D. Virginia Commonwealth University, Richmond, VA and Massey Cancer Center, Richmond, VA.

Introduction

The management of Intraductal papillomas (IP) of the breast diagnosed on core needle biopsy (CNBx) is still controversial. For IP with atypia, excision is generally recommended. For IP w/o atypia, data for excision vs. observation are variable. A clearer understanding of the risk of the presence of invasive or in situ malignancy (IDC or DCIS) coincident with IP, as well as the long-term risk for cancer would be helpful in managing these patients. The aim of this study is to evaluate the rate of malignancy on immediate excision or with prolonged follow-up. We hypothesized that IP w/o atypia do not require excision, as the risk of malignancy is low. Conversely, we hypothesized that IP w/ atypia should be excised because of a significant rate of concomitant malignancy. We also evaluated the long-term risk of malignancy in either breast with excision or observation of women with IP.

Methods

266 women who underwent a CNBx between 1995 and 2010 were identified from surgical pathology and breast imaging records. Four groups were defined based on the CNBx diagnosis (IP w/o atypia, IP with atypia, IP + ADH/ALH and Papillomatosis) and were also separated on the basis of immediate excision versus observation. For the 15-year period, it was generally the policy to excise IP lesions with atypia or ADH/ALH. Management of IP w/o atypia was more variable, but in the most recent 5 years, patients with IP w/o atypia were usually observed. For those who underwent immediate excision, the proportions with IDC or DCIS were calculated and compared using Fisher's exact test. Kaplan Meier curves were determined for each group's estimated time to cancer diagnosis, and significance was evaluated by the log-rank test.

Results

When surgical excision was performed for IP w/ atypia or IP + ADH/ALH on CNBx, cancer was found in 32% and 38.5% respectively. Of the 109 excisions for IP w/o atypia, cancer was found in 8.3%, significantly different from IP w/ atypia (p=0.004) and IP + ADH/ALH (p=0.007). For patients without atypia or ADH/ALH at the time of biopsy and no cancer on excision, the probability of remaining cancer-free was not significantly different for patients who had immediate surgical excision versus those that were observed (93.8% and 91.5% cancer-free at 10 years, p= 0.773). For patients with atypia or ADH/ALH at the time of biopsy but no cancer on excision, the probability of remaining cancer-free in both breasts was 85.9% at 10 years, and did not differ between patients who were excised or observed (p= 0.518). However, those w/atypia or ADH/ALH were significantly less likely to remain cancer-free than those w/o atypia (85.9% versus 92.8% at 10 years, respectively, p=0.008).

Conclusions

After a CNBx showing IP w/ atypia or IP + ADH/ALH, surgical excision is clearly justified, based on a 30-40% risk of concomitant invasive or in situ cancer. For IP w/o atypia, the likelihood of cancer is much lower. Moreover, even with excision, the finding of IP with atypia carries a significant risk of developing cancer long-term, and such patients should be followed carefully and perhaps should be considered for chemoprevention.
Title: Low lactate dehydrogenase B expression correlates with decreased distant-metastasis free- and recurrence-free survival post-chemotherapy in basal-like breast cancer

Dean-Colomb W, Tan M, Tang W, Ambs S and Yates C. University Hospital and Clinics, Lafayette, LA; Tuskegee University, Tuskegee, AL; Mitchell Cancer Institute, Mobile, AL and National Cancer Institute/NIH, Bethesda, MD.

Body: Background:
Metabolism is an important differentiating feature of cancer cells. Lactate dehydrogenases (LDH A/B) are metabolically important proteins involved in the critical inter-conversion of pyruvate to lactate and vice versa. Several reports suggest that LDHB levels are elevated in TNBC, compared to other breast cancer subtypes. However, we recently published that LDHB levels are low in TNBC cell lines and restoring LDHB results in decreased cell proliferation, oxidative phosphorylation, and reversal of EMT. Furthermore, in a small patient cohort, we have shown that although LDHB levels are higher in TNBC patients compared to non-TNBC patients, LDHB levels where consistently lower when compared to LDHA levels. Thus, we set out to determine if either "Hi" or "Low" LDHA and LDHB levels effect patient survival.

Methods:
Utilizing the publically available datasets contained within kmplot, which contains gene expression data and relapse free and overall survall, we determined mean levels of LDHA and LDHB in breast cancer patients. To analyze the prognostic value, patient samples were split into two groups based upon expression above the mean (considered high expressors, "Hi") or below the mean (considered low expressors, "Low"). The two patient cohorts were compared by a Kaplan-Meier survival plot, and the hazard ratio with 95% confidence intervals and logrank P value calculated. Groups were further stratified based upon LDH levels prior to- and post-chemotherapy.

Results:
We found that in patients with luminal A and luminal B breast cancer, there were no significant changes in either LDHA (p=0.1) or LDHB (p=0.21) on distant metastasis-free (DMFS) or recurrence-free (RFS) survival. However, in the basal subtype (i.e. patients with ER negative and PR negative breast cancer), low levels of LDHB was significantly associated with poorer DMFS (p=0.025) (n=240) prior to chemotherapy and both DMSF (p=0.048) (n=176) and RFS (p=0.0082) (n=388) post-chemotherapy. Examining the mean expression values for each of these patient populations, we did not observe any significant changes in DMSF or RFS pre or post-chemotherapy, suggesting an intrinsic feature of basal-like patients with low LDHB expression to have a more aggressive phenotype. Interestingly, we did observe significance in RFS (n=581, p=0.0043) in patient with "Hi" LDHA expression pre-chemotherapy, however there was no significant associations of LDHA with RFS (p=0.19) (n=388) or DMSF (n=176, p=0.75) post-chemotherapy.

Conclusion:
These findings, coupled with our cell line data, showing overexpressing LDHB in TNBC cell lines results in decreased proliferation with increased mitochondrial damage and apoptosis, suggests that lower levels of LDHB expression is indeed associated with an aggressive breast cancer phenotype that undergoes EMT and the Warburg effect. This could contribute to the lack of pathological response after chemotherapy and thus increased risk for later metastasis. Additionally, given the very large number of patients examined within these independent datasets, these findings further suggest that low LDHB expression is a robust prognostic biomarker of clinical outcome in patients with a basal-like phenotype.
**Title:** Low risk of contralateral breast cancer rates in Asian women- A case for not recommending contralateral prophylactic mastectomy

Taib NA, See MH, Bhoo Pathy N, Yahya A, Tan GH, Jamaris S and Yip CH. University Malaya Medical Centre, Kuala Lumpur, Malaysia.

**Body:**

**Introduction**
The rates of contralateral prophylactic mastectomy (CPM) appears to be higher in the west as compared to the east. There is a suggestion of an improved survival benefit of CPM. The trends are increasing in the west and little is known in Asia. In western data a rate of 0.5 to 1% per year is often quoted. However, there is scarcity of epidemiological data on rates of contralateral breast cancer (CBC) in Asia.

Known predictors of CBC in the west include high stage at diagnosis, early age at diagnosis, presence of family history, genetic predisposition, time from first diagnosis and hormone receptor status. Little is known in Asia. Population based registries are not available in Malaysia as in many Southeast Asian countries. This study aims to provide CBC rates to provide evidence for informed decision making of CPM in Asians.

**Method**
A prospective database of newly diagnosed Stage I and II breast cancer patients diagnosed in University Malaya Medical Centre between 1993 to 2012 were analysed. Data on CBC were obtained from institutional database and medical records. Multivariate analysis used Cox regression was applied to observe for any associations with multiple variables such as age at diagnosis, time from diagnosis, presence of family history, menopausal status, ethnic groups, parity and hormone status.

**Results**
A total of 3060 Stage I and II patients were prospectively followed up. 127 (4.2%) women developed contralateral breast cancer. At 5 years, 57 (1.9%) and at 10 years, 100 (3.4%) developed CBC. Using a multivariate model, the only independent predictor of developing contralateral breast cancer at 5 years was a positive family history OR 1.97 (CI 1.02-3.80). However, at 10 years we found the effect of positive family history diminished OR 1.57 (CI 0.94-2.64) and younger women were more likely to develop cancer, those aged 51 to 64 years OR 0.29 (CI 0.11-0.73), >65 years OR 0.20 (CI 0.07-0.60) compared to those less than 35 years. The other factors in the model were not significant.

**Discussion**
There is an increasing rate of CPM at 22% from 9% in the west. A study in Singapore showed a low CPM rate of 1.25%. Although uptake of CPM in Asia has not been reported extensively and appears low, the trends may rise and that evidence is needed to counsel patients on the benefits and harms of CPM if patients request for CPM. Given the lower incidence of breast cancer in Malaysia i.e. 5% average risk compared to 10% in the west, an urgent need to document the risk of contralateral breast cancer, and this data from a single institution is the first to report these rates in Southeast Asia.

**Conclusion**
The data shows that risk of contralateral breast cancer is low in this Southeast Asian country. Any recommendations or practice of CPM should be dealt with extreme caution.

**References**
Title: Abstract Withdrawn
Title: Prognostic contribution of mammographic breast density and HER-2 overexpression to the Nottingham prognostic index in patients with invasive breast cancer

Masarwah A, Sudah M, Sutela A, Päivi A and Ritva V. Kuopio University Hospital, Kuopio, Finland.

Body: Purpose: To examine the associations between very low mammographic breast density (VLD), HER2, ER and PR status in a more homogenized patient group within matched NPI categories. We also aimed to investigate whether those variables could be added to the NPI to enhance its prognostic and predictive values and better categorize which patients are at higher risk.

Materials and methods: A total of 270 patients with invasive breast cancer were included in the final analysis. Patients with mammographic breast density of <10% were considered as VLD. We compared the performance of NPI with and without ER, PR, VLD and/or HER2. Factors first underwent univariate and then multivariate analysis to assess the factors combined until the best fit was obtained. Cox multivariate analysis, time-dependent receiver operating characteristic curve (tdROC), concordance index (c-index) and prediction error (i.e. 0.632+ bootstrap estimator) were used to derive an updated version of NPI. Patients' survival was assessed by the Kaplan-Meier method using a log rank (Mantel-Cox) test.

Results: The average NPI value was 4.66 (range 2.12-7.40), the percentage of HER2 positive patients progressively increased from the low to the high prognostic groups of NPI (p<0.001). While MBD, ER and PR status showed no such difference in distribution. Patients showed survival differences in the intermediate and high risk groups of NPI according to VLD and HER2 status (p<0.001 and p=0.02, respectively). Outcome analysis showed that the addition of both factors combined provides improved patient outcome stratification superior to the traditional NPI helping to significantly decrease the percentage of patients in the intermediate patient groups.

Conclusion: VLD breasts and HER2 positivity are prognostic factors for breast cancer independent of the NPI. Moreover, their addition to the NPI helps increase its prognostic accuracy and reduces the percentage of patients in the intermediate prognostic group.
Title: Development and validation of personalized ex vivo platform mimicking patient heterogeneous tumor microenvironment to enable personalized treatment for breast cancer

Majumder PK K, Majumder B, Mehrotra DG, Ghosh, Radhakrishnan P, Baraneedharan U, Agarwal S, Sengupta S and Babu G. Kidwai Memorial Institute of Oncology, Bangalore, Karnataka, India; Mitra Biotech, Bangalore, Karnataka, India; Mitra Biotech, Waltham, MA; Indian Institute of Science, Bangalore, Karnataka, India and Harvard-MIT Division of Health Sciences and Technology, Cambridge, MA.

Body: Tumour heterogeneity is emerging as a key driver of drug response. Understanding the heterogeneity is therefore a critical milestone towards the prediction of clinical outcome of anti-cancer drugs. There is a need for technology that can predict treatment outcome with high fidelity by contextually integrating tumor heterogeneity and phenocopying the tumor microenvironment.

Tumor grade-matched matrix support, activated immune compartments and autologous ligands from individual patients were used to engineer a novel personalized ex vivo platform named CANScript. We evaluated functional outcomes as a measure of response to a panel of anticancer drugs in this CANScript platform. Histopathological and molecular characterization of the tumor explants cultured in CANScript revealed a close approximation to the parental tumor at baseline as confirmed by tumor cell viability, proliferation, critical phosphoproteomic status, global transcriptomic profiles and balance in active components of tumor and stromal phenotypes (Majumder. B et al Nat. Commun, 2015). Using more than 100 patients clinical outcome of anticancer drugs (training set) we have further built a machine learning algorithm that takes all functional outcome data from the CANScript platform and provides outcome data in a linear scale showing clinical prediction. This algorithm when applied to the test cohort of more than 40 patients with refractory breast cancer assessed in the CANScript achieved more than 90% sensitivity while keeping specificity in a desired high range for predicting short term clinical outcome.

The high specificity and sensitivity observed in predicting clinical outcomes using the CANScript support the use of this novel platform to select treatment for patients with breast cancer.
Title: CASCADE study: Longer overall survival in the novo versus recidivant patients with locally advanced/metastatic breast cancer

Servitja S, Zamora P, Santaballa A, García J, de Paz L, Plata Y, Garau I, Florian J, Chacón I, de la Haba J, García P, San José B, Rodríguez-Villanueva J, Orcajo L, Martínez E and Segui MA A. Hospital del Mar, Barcelona, Spain; Hospital La Paz, Madrid, Spain; Hospital La Fe, Valencia, Spain; Complejo Hospitalario, Orense, Spain; Hospital Arquitecto Marcide, Ferrol, A Coruña, Spain; Hospital Médico Quirúrgico, Jaén, Spain; Hospital Son Llàtzer, Son Ferriol, Palma de Mallorca, Spain; Hospital de Barbastro, Barbastro, Huesca, Spain; Hospital Virgen de la Salud, Toledo, Spain; Hospital Reina Sofía, Córdoba, Spain; Hospital San Agustín, Avilés, Asturias, Spain; OXON Epidemiology, Madrid, Spain; EISAI Pharmaceuticals, Madrid, Spain; Hospital Provincial, Castellón de la Plana, Spain and Hospital Parc Taulí, Sabadell, Barcelona, Spain.

Body: BACKGROUND: Current treatment strategies for locally advanced and/or metastatic breast cancer (LA/MBC) are meant to prolong survival while maintaining or improving the quality of life. Nevertheless, there is a lack of recent data regarding the actual clinical management and its impact on the prognosis of these patients. It is unknown whether prior diagnosis and treatment of early breast cancer (EBC) make any difference in the outcome of the advanced disease. CASCADE is an epidemiological, retrospective, and multicenter study aimed at retrieving this information from a representative cohort of LA/MBC patients treated within the Spanish National Healthcare System.

MATERIALS AND METHODS: Thirteen Spanish public hospitals covering nearly 5’000’000 inhabitants (>10% of the national population) applied several combined systematic strategies to identify patients firstly diagnosed with LA/MBC between 01/2007 and 12/2008. Once identified, patients were followed throughout their metastatic lifetime until death, lost to follow-up, or until December 2013, whichever occurs first. Data collected included demographical, pathological, diagnostic, and therapeutic information for each line of treatment. Descriptive statistics were applied.

RESULTS: We identified 443 LA/MBC patients; median age at diagnosis was 59 years (CI95%: 49.5 - 71.6). Previous history of early BC was reported in 69.3% of them with a median disease-free interval of 38 months. Median Overall Survival (OS) for the whole study population was 33.5 months, while numbers for advanced cases originally diagnosed as EBC or the novo LA/MBC were 31.7 (CI95%: 26.8 - 36.0) and 38.8 months (CI95% 32.8 - 45.3; p = 0.0138) respectively. Main tumor immunohistochemical subtypes for EBC and the novo LA/MBC were: HER2+/HR- 11.3% and 15.3%, HER2+/HR+ 16.2% and 19.1%, HER2-/HR+ 41.2% and 51.1%, and Triple-negative 17.9% and 11.5%, respectively.

At the end of the study follow-up (Dec 2013) 78.2% of the patients had died. Breakdown of the decaying percentage and OS for the entire study population, early-, and the novo diagnosed LA/MBC from the beginning of each line of treatment was:

<table>
<thead>
<tr>
<th>Treatment line</th>
<th>1L</th>
<th>2L</th>
<th>3L</th>
<th>4L</th>
<th>5L</th>
<th>6L</th>
<th>7L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole pulation Patients (%)</td>
<td>95.3</td>
<td>70.2</td>
<td>53.5</td>
<td>38.4</td>
<td>24.2</td>
<td>15.1</td>
<td>9.5</td>
</tr>
<tr>
<td>Whole pulation OS (months)</td>
<td>32.6</td>
<td>22.6</td>
<td>16.6</td>
<td>13.5</td>
<td>13.3</td>
<td>12.4</td>
<td>8.5</td>
</tr>
<tr>
<td>Early diag. LA/MBC OS (months))</td>
<td>30.9</td>
<td>21.0</td>
<td>15.6</td>
<td>12.9</td>
<td>12.4</td>
<td>9.1</td>
<td>7.5</td>
</tr>
<tr>
<td>The novo diag. LA/MBC OS (months)</td>
<td>37.6</td>
<td>25.9</td>
<td>21.6</td>
<td>18.7</td>
<td>14.0</td>
<td>16.9</td>
<td>13.8</td>
</tr>
</tbody>
</table>

CONCLUSION: Our study's OS data supports the hypothesis that highly effective current neo/adjuvant treatment may cure most treatment-sensitive early tumors, allowing only those more aggressive to develop to LA/MBC, which then will fare worse than those of the novo metastatic diagnosis.
Title: Neuroendocrine differentiation in breast carcinoma: Clinicopathological features and outcome


Body: Background: Primary neuroendocrine (NE) breast carcinoma (BC) is an entity lacking definite diagnostic criteria with a reported wide range of prevalence and poorly defined clinical behavior. We evaluated the prevalence, clinicopathological features and the clinical outcome of NEBC.

Patients and methods: Immunohistochemical staining for synaptophysin and chromogranin A was carried out on whole section from archival specimens of 1232 consecutive cases of invasive BC. We divided NEBC in focal (10-49% positive cells) and diffuse (≥50% positive cells). We compared outcome of patients with NEBC with strictly matched patients with non-NEBC, using 12 clinicopathological parameters.

Results: One hundred twenty-eight BC showed NE differentiation: 84 were diffuse and 44 focal. NE differentiation showed a significant association with T4 stage (P=0.001), solid papillary and mucinous histological type (P<0.0001), G2 grading (P=0.002), positive ER (P=0.003) and PR (P=0.002). Kaplan-Meier analysis revealed that patients with NEBC had a trend towards a statistical significance for worse clinical outcome for DFS (P=0.04) but not for cancer specific survival (P=0.20). We did not find significant differences for clinicopathological features, DFS and cancer specific survival between diffuse and focal NEBC.

Conclusions: We showed that NEBC represent 7-10% of invasive BC; 74% of NEBC are no special type invasive BC, however the more frequent histotypes with NE differentiation are solid-papillary (70%) and mucinous carcinoma (20%). Almost 90% of NEBC are ER+/HER2- and more than half ER+/HER2-/Ki-67>15%. NE differentiation shows a trend towards a statistical significance for a worse prognosis for DFS but not for cancer specific survival and the extent of NE marker expression does not seem to correlate with clinical behavior.
Title: The predictive potential of metastatic serum markers and circulating tumor cells in advanced HER2-positive breast cancer patients - Focused on CNS metastases

Mikulova V, Jancikova M, Tesarova P and Zima T. Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic and General University Hospital in Prague and First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic.

Body: Introduction: We performed a prospective study of advanced HER2-positive breast cancer (HER2-aBC) patients in palliative Trastuzumab therapy. We studied the presence of circulating tumor cells in the peripheral blood and matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) levels in the blood serum. We correlated these known metastatic markers primarily with the development of central nervous system (CNS) metastases, a very serious risk factor in HER2-positive BC patients. Further, we correlated these markers with disease progression and overall survival.

Patients and Methods: Our study group comprised 44 women with HER2-aBC and 42 healthy volunteers. Eighty-three percent of patients had metastases, mostly visceral and bone. Twelve patients developed CNS metastases, 3 before and 9 after study enrollment.

An immunomagnetic separation (AdnaGen, Germany) from 5 ml of whole blood, PCR-based methods and an on-chip capillary electrophoresis was used for the determination of CTC presence in BC patients. MMP-2, MMP-9 and VEGF serum levels were determined by ELISAs (Quantikine, R&D System, USA) of patients' sera - aliquoted and frozen at -20Â°C.

Results: CTCs were determined in only 17% of HER2-aBC patients. CTC positive patients had significantly shorter overall survival (p=0.04). The patients' MMP-9 and VEGF serum levels did not significantly differ from the control group. MMP-2 serum levels were significantly higher in the CTC-positive patients (p=0.02) and in the patients with bone metastases (p<0.01). The patients with an MMP-2 serum level exceeding the 344 ng/ml had a 53times higher risk of CNS metastases (specificity 97%, sensitivity 64%). The HER2-aBC patients already suffering from bone metastases with an MMP-2 serum level above 350 ng/ml had a 204times higher risk of developing CNS metastases (specificity 97%, sensitivity 86%).

Conclusion: The presence of CTCs in the blood of HER2-aBC is a negative prognostic marker for overall survival. The HER2-aBC patients with the highest risk of CNS metastases development are those with an MMP-2 serum level above 350 ng/ml and bone metastases present. Our results indicate MMP-2 serum level as a possible predictive marker for CNS metastases development. Since CNS metastases represent a very serious complication in HER2-aBC patients our findings warrant verification in a larger study on a more stratified patient group.

This work was supported by the Grant Agency of Charles University GAUK, 539512, PRVOUK-P-27/LF1/1 and RVO-VFN 64 165.
Title: Competing causes of death among women with breast cancer in South East Asia: Effects of ethnicity, and age at diagnosis and stage at diagnosis


Body: Background: Over the past decades, breast cancer survival has improved substantially and many patients die of other, non-breast cancer related causes. In South East Asia, where large ethnic differences exist in stage at presentation and overall survival, causes of death of breast cancer patients have been understudied.

Aim: To examine cause-specific mortality among breast cancer patients in multi-ethnic Singapore and investigate effects of ethnicity, and age and tumor stage at diagnosis.

Methods: Data of women diagnosed with breast cancer between 1990 and 2011 at the National University Hospital in Singapore were retrieved from the hospital-based breast cancer registry. Cause of death was categorized as breast cancer (ICD8 174; ICD9 174; ICD10 C50), cardio- and cerebrovascular disease (ICD8 390 to 459; ICD9 390 to 459; ICD10 I00 to I99), other malignancies (ICD8 140 to 239; ICD9 140 to 239; ICD10 C00 to D48 except codes for death resulting from breast cancer), and death from other causes (all ICD codes except those already listed). Patients with unknown cause of death (n=6) were classified as death from other cause. Chi square statistics were used to compare cause of death distributions.

Results: Of 4108 patients, median age at diagnosis was 51 years (range 21-98 years). The majority of women were Chinese (n=3223, 78%), followed by Malay (n=517, 12%), Indian (n=257, 6%) and other ethnicities (n=111, 3%). After a median follow-up of 7 years, 1125 (27%) patients died: 910 (81%) patients died of breast cancer, 70 (6%) of cardio- and cerebrovascular disease, 71 (6%) of other malignancies, and 83 (7%) of other causes. Compared with other ethnicities, Malay women most frequently died as a result of breast cancer (n=178, 90%). The highest percentage CVD deaths was observed among Indians (n=7, 11%). Breast cancer accounted for 92% of deaths in women younger than 50 years at diagnosis and for 60% in women older than 65. The proportion of deaths as a result of CVD, other cancer or other causes increased with age. Patients with higher tumor stages at diagnosis were more likely to die of breast cancer (96% of deaths of patients with TNM4 were breast cancer related).

Conclusion: The present study showed that breast cancer is the most important cause of death in breast cancer patients among all ethnic groups, and ages and stages in South East Asia. The highest risk of death due to breast cancer in Malay women might be explained by their presentation at advanced stages and young age at diagnosis. Breast cancer is less likely to be the cause of death in women of Chinese ethnicity, older age at diagnosis and early tumor stage. In these groups more attention for competing causes of death, in particular CVD, should be a priority for the future.
Clinical outcome of pathological T1N0 breast cancer according to the hormone receptor and HER2 status and adjuvant therapy

Tokunaga E, Akiyoshi S, Koga C, Nakamura Y, Taguchi K, Ishida M and Ohno S. National Hospital Organization Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka, Japan; National Hospital Organization Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka, Japan and Breast Oncology Center, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo, Japan.

Background: The outcome of T1N0 breast cancer is relatively good, however, a subpopulation of this stage has a high population of relapse. It is important to clarify the factors associated with the outcome in order to determine the adequate adjuvant systemic therapy for T1N0 breast cancer.

Aims: To investigate the prognosis of pathological T1N0 (pT1N0) breast cancer by receptor (estrogen receptor; ER, progesterone receptor; PgR, human epidermal growth factor receptor 2; HER2) status and adjuvant systemic therapy, and thus to identify the factors associated with the outcome.

Methods: Among 2164 women who underwent surgery for primary breast cancer in our department, 925 had pathological T1N0 tumors (130 T1a, 234 T1b and 561 T1c). The associations between clinicopathological characteristics, adjuvant therapy and relapse-free survival (RFS) were examined.

Results: Of 908 patients with known hormone receptor (HR; ER and PgR) and HER2 status, 675, 79, 64 and 90 had HR+/HER2-, HR+/HER2+, HR-/HER2+ and HR-/HER2- (triple negative; TN) tumors, respectively. The prognosis of patients with T1c tumors was significantly poorer than that of the patients with T1a and T1b tumors (5-year DFS of T1a, T1b and T1c: 97.5%, 97.9%, 94.5%, p=0.0201). HR+/HER2- subtype was significantly associated with better prognosis than other subtypes (5-year DFS: 96.9% vs. 93.6%, p=0.0194). Patients younger than 40 year old or older than 74 years old had significantly shorter PFS (p=0.0039). Lymphovessel invasion (ly), high histological grade (HG2, 3) were associated with poor outcome in all cohort (p=0.0026, p=0.0356). In HR-positive tumors, ly, high HG and omission of the adjuvant endocrine therapy were associated with shorter RFS (p=0.0009, p=0.0306 and p=0.0016, respectively). Adjuvant chemotherapy was not associated with RFS regardless of HR and HER2 status. However, in T1c with nuclear grade (NG) 3 tumors, use of the adjuvant chemotherapy was associated better prognosis. The prognosis of patients with HER2+ tumors was not significantly different among patients with or without trastuzumab in this cohort.

Conclusion: In spite of the excellent prognosis of pT1N0 breast cancer, adjuvant endocrine therapy is important for patients with HR+ tumors. However, the use of adjuvant chemotherapy or trastuzumab did not significantly improve the prognosis. Thus, the indication of chemotherapy or anti-HER2 therapy should be determined in consideration of the several prognostic factors for pT1N0 breast cancer.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-08-48

**Title:** Vitamin D deficiency in various breast cancer subtypes and its impact on response to neoadjuvant chemotherapy in operable breast cancer

Raman R, Link BK K, Mott SL L, Schroeder MC C and Thomas A. University of Iowa, Iowa City, IA.

**Body: Introduction:**
Pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) is associated with improved long-term outcomes. Vitamin D (VD) deficiency has been associated with carcinogenesis as well as poorer outcomes in breast cancer (bc) particularly in luminal-type bc. In addition, replacement of VD during adjuvant chemotherapy was found to improve disease-free survival. However, VD deficiency was not associated with pCR following NAC in one series which included patients (pts) with human epidermal growth factor receptor-2 (HER2) negative bc only. We report the relationship between VD deficiency and pCR in a cohort that includes all bc subtypes and report how VD deficiency varied by clinical and pathologic parameters.

**Methods:**
Patients (pts) prospectively enrolled in the University of Iowa Breast Molecular Epidemiologic Resource between 2010-14 with invasive bc, receiving at least one cycle of NAC, and who had serum collected at enrollment or during NAC were eligible. VD deficiency was defined as < 20 ng/dl. pCR was defined as no residual invasive disease in breast and lymph nodes. Patients were stratified by their BMI as normal (≤ 25 kg/m2) or overweight-obese (>25 kg/m2). To investigate the relationship between VD, clinical parameters, and NAC outcomes, chi-square, Fisher's exact, and t-tests were used.

**Results:**
73 pts were eligible. 52 (71%) pts had Stage I-II bc. All pts received a combination of taxanes with other agents (Cytoxan, 5FU, Carboplatin, Gemcitabine) with or without an anthracycline or anti-HER 2 therapy. 62/73 (85%) received an anthracycline. 65/73 (89%) received at least 75% of intended NAC. pCR was achieved in 25/73 (34%) pts. VD deficiency was found in 17/73 (23%) pts and was more frequent among overweight-obese pts (Table 1). VD deficiency occurred significantly more often in estrogen receptor (ER)+/progesterone receptor (PR)+/HER2- and ER+ or PR+ bc but not in HER2+ bc. Results suggest VD deficient pts may be at increased odds of not achieving a pCR (OR=3.02, p=0.10). In this cohort, VD deficient pts were at increased odds of having residual disease in the breast (OR=3.76, p=0.05), but not in the nodes (OR=1.43, p=0.53), (Table 2).

**Conclusion:**
Being overweight-obese was associated with VD deficiency and may warrant screening in this group. VD deficiency was significantly associated with ER+/PR+/HER2-bc and ER+ or PR+ disease. Notably, these pts are candidates for bone-injuring anti-estrogen agents as part of their overall course of therapy. VD deficiency was not significantly associated with pCR after NAC though a trend was suggested.

**Table 1: VD and clinical parameters**

<table>
<thead>
<tr>
<th></th>
<th>VD Sufficient</th>
<th>VD Deficient</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N=56</td>
<td>N=17</td>
<td></td>
</tr>
<tr>
<td>Age (mean in years)</td>
<td>52</td>
<td>47</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
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<tr>
<td>≤25</td>
<td>26(46%)</td>
<td>3(18%)</td>
<td>0.03</td>
</tr>
<tr>
<td>&gt;25</td>
<td>30(54%)</td>
<td>14(82%)</td>
<td></td>
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<tr>
<td>Ellis-Ellison grade</td>
<td></td>
<td></td>
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<tr>
<td>1-2</td>
<td>24(43%)</td>
<td>7(41%)</td>
<td>0.90</td>
</tr>
<tr>
<td>3</td>
<td>32(57%)</td>
<td>10(59%)</td>
<td></td>
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<tr>
<td>Tumor receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/PR+/HER2-</td>
<td>15(27%)</td>
<td>10(59%)</td>
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<tr>
<td>ER+ or PR+/HER2+</td>
<td>6(11%)</td>
<td>3(18%)</td>
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</tr>
<tr>
<td>ER-/PR-/HER2+</td>
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<td>0(0%)</td>
<td>0.10</td>
</tr>
<tr>
<td>ER-/PR-/HER2-</td>
<td>17(31%)</td>
<td>3(18%)</td>
<td>0.36</td>
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<tr>
<td>ER+ or PR+</td>
<td>29(52%)</td>
<td>14(82%)</td>
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</table>
Table 2: VD and NAC Outcomes

<table>
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<th>VD Deficient</th>
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<tbody>
<tr>
<td></td>
<td>N=56</td>
<td>N=17</td>
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<tr>
<td>pCR</td>
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<tr>
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<td>34(61%)</td>
<td>14(82%)</td>
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<tr>
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<td>Breast Residual Disease</td>
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<td>25(45%)</td>
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<td>31(55%)</td>
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<tr>
<td>Node Residual Disease</td>
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<td></td>
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<tr>
<td>No</td>
<td>28(50%)</td>
<td>7(41%)</td>
<td>0.52</td>
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<tr>
<td>Yes</td>
<td>28(50%)</td>
<td>10(59%)</td>
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</table>
Title: Incidence and prognostic importance of hyponatremia in a cohort of patients with breast cancer

Castillo JJ, Glezerman IG, Boklage SH, Lamerato LE, Chiodo JA, Tidwell BA and Schulman KL. Dana-Farber Cancer Institute, Boston, MA; Memorial Sloan Kettering Cancer Center, NY, NY; Otsuka America Pharmaceutical, Inc., Princeton, NJ; Henry Ford Health System, Detroit, MI and Outcomes Research Solutions, Inc., Waltham, MA.

Body: Background: It has been suggested that hyponatremia (HN) may be a negative prognostic factor in patients with cancer but little research has been conducted specifically in breast cancer (BC). We measured the incidence of hyponatremia (hypervolemic and euvolemic) after BC diagnosis and its prognostic importance for progression free (PFS) and overall survival (OS).

Methods: This retrospective cohort analysis utilized data from the Henry Ford Health System electronic medical record, tumor registry, and administrative databases. Study data were collected electronically and via medical record abstraction. Adults diagnosed (2002-2010) with incident invasive BC were selected if they had a known disease stage at the time of tumor registration, were classified as an analytic case, had ≥ 1 administration of chemo/radiation therapy ≤ 6 months from diagnosis, met continuous enrollment thresholds, and did not experience hypovolemic HN post index. Only the first tumor registered from each patient was considered study-eligible. Hypervolemic or euvolemic HN incidence (serum sodium ≤ 135 mEq/L) was measured per 1000 person-years (PY) of observation and classified as mild (131–135 mEq/L), moderate (125–130 mEq/L) or severe (<125 mEq/L) based on the lowest observed value. A Cox proportional hazards model was used to assess the prognostic value of HN as a time-varying covariate on PFS and OS while controlling for age, race, income, morphology code, diagnosis year, cancer stage at diagnosis, performance status at diagnosis, and hormone receptor status.

Results: 527 patients were eligible (mean [SD] age 56.4±11.3 years, 61% Caucasian). Mean (SD) follow-up was 3.7±2.8 years. Eighty-five percent of patients had infiltrating ductal carcinoma; 72% and 65% had estrogen or progesterone sensitive tumor, respectively; 35% were HER2 positive; and 15% had triple negative disease. Eighty-two percent of patients had early stage (I, II) disease at time of diagnosis. HN episodes (n=377) occurred in 204 patients (39%) at a rate of 193 per 1000 PY (95% CI, 174–213.5), with 89% of the total episodes (337/377) classified as mild (131–135 mEq/L), moderate (125–130 mEq/L) or severe (<125 mEq/L) based on the lowest observed value. A Cox proportional hazards model was used to assess the prognostic value of HN as a time-varying covariate on PFS and OS while controlling for age, race, income, morphology code, diagnosis year, cancer stage at diagnosis, performance status at diagnosis, and hormone receptor status. Results: 527 patients were eligible (mean [SD] age 56.4±11.3 years, 61% Caucasian). Mean (SD) follow-up was 3.7±2.8 years. Eighty-five percent of patients had infiltrating ductal carcinoma; 72% and 65% had estrogen or progesterone sensitive tumor, respectively; 35% were HER2 positive; and 15% had triple negative disease. Eighty-two percent of patients had early stage (I, II) disease at time of diagnosis. HN episodes (n=377) occurred in 204 patients (39%) at a rate of 193 per 1000 PY (95% CI, 174–213.5), with 89% of the total episodes (337/377) classified as mild, 10% (36/377) as moderate, and 1% (4/377) as severe. Additionally, 7% of all BC patients (37/527) had at least one episode of moderate/severe HN. Median time to first HN episode was 174.5 days and the median HN episode duration was 24.0 days. Five year OS in patients developing HN was 92%, compared to 97% in patients who never developed HN. Hazard ratio (95% CI, p-value) for OS in the HN group was 4.4 (1.5-12.7; p=0.006) after controlling for age, diagnosis year, race, income, morphology, cancer stage, performance status, and hormone receptor status. Fifty patients had progressive disease during follow-up with a mean (SD) time to progression of 763.4 (758.1) days. Hazard ratio (95% CI, p-value) for PFS in the HN group was 1.4 (0.8-2.7; p=0.262) after controlling for age, race, income, morphology, cancer stage, performance status, and hormone receptor status.

Conclusions: Incidence of hypervolemic or euvolemic HN is high (39%) after a BC diagnosis, and the occurrence is associated with significantly poorer OS. A significant impact on disease progression was not observed.
Title: Associations of MYC protein expression and gene status with breast cancer subtypes and outcome in patients treated with anthracycline-based adjuvant chemotherapy


Body: Background-Aim: Breast cancer is a heterogeneous disease and despite recent scientific progress there is still need for the identification of biomarkers associated with risk for relapse, as well as for markers identifying patients who will benefit from specific treatments. The aim of the present study was to investigate the role of MYC, as a clinically meaningful biomarker, in the outcome of breast cancer subtypes.

Patients and Methods: We have pooled the patients and the respective breast carcinomas from two randomized anthracycline-based adjuvant phase III trials, consecutively conducted by the Hellenic Cooperative Oncology Group (HE10/97 and HE10/00). The HE10/97 trial included a non-paclitaxel arm. Tissue microarrays were constructed from 1,060 formalin-fixed paraffin-embedded tumor tissue samples that were collected retrospectively in the first and prospectively in the second trial. MYC was evaluated by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) in 986 cases.

Results: In total 61.0% of the cases showed positive cytoplasmic MYC immunostaining, while 26.5% showed positive nuclear staining. 65-80% of the patients were characterized as non-amplified or loss/normal-low gain in all FISH cut-offs examined. A weak association was observed between FISH and nuclear protein expression of MYC. High histological grade was associated with MYC protein overexpression and gene amplification. In terms of disease-free survival (DFS), low (2.5-5 copies) and high (≥5 copies) gain of MYC was of adverse prognostic value compared to loss/normal (<2.5 copies) MYC (HR=1.50, 95% CI 1.13-1.98, Wald's p=0.004 and HR=1.45, 95% CI 1.07-1.97, p=0.016, respectively). Comparable results were observed for overall survival (OS) (HR=1.51, 95% CI 1.09-2.08, p=0.013 and HR=1.65, 95% CI 1.17-2.33, p=0.005, respectively). The comparison of neoplasms with CEP8 ratio ≥1.3 and polysomy 8 for MYC versus all others resulted in worse survival prognosis (HR=1.44, 95% CI 1.13-1.83, p=0.004), while tumors with nuclear protein overexpression were associated with better DFS (HR=0.77, 95% CI 0.60-0.99, p=0.039) and OS (HR=0.73, 95% CI 0.55-0.98, p=0.034). In HER2-enriched patients, MYC amplification was found to be an adverse prognostic factor for DFS (HR=2.11, 95% CI 1.09-4.07, p=0.026) and OS (HR=2.41, 95% CI 1.12-5.15, p=0.024). Treatment with paclitaxel was found to differentiate the effect of MYC: CEP8 ratio ≥1.3 and polysomy 8 in terms of DFS and OS in our total cohort. Among patients with CEP8 ratio ≥1.3 and polysomy 8, those treated with paclitaxel performed significantly better than those not treated, while among patients not treated with paclitaxel, those with CEP8 ratio ≥1.3 and polysomy 8 performed much worse than those with CEP8 ratio <1.3 or no polysomy 8.

Conclusions: Our data suggest that MYC has prognostic and predictive value in patients with breast cancer. MYC amplification and MYC protein overexpression are detected in breast cancer patients and are of adverse prognostic value for DFS and OS. Polysomy 8 is also associated with worse prognosis. Treatment with paclitaxel in the adjuvant setting benefits breast cancer patients with MYC:CEP8 ratio ≥1.3 and polysomy 8.
Title: SIAH2 protein expression is inversely correlated with the ER status and outcome to tamoxifen therapy in metastatic breast cancer patients

van der Willik KD D, Timmermans MM M, Look MP P, Reijm EA A, van Deurzen CHM HM, den Bakker MA A, Westenend PJ J, Martens JWM WM, Berns EMJJ MJJ and Jansen MPHM PHM. Erasmus MC Cancer Institute, Rotterdam, Zuid-Holland, Netherlands; Erasmus MC Cancer Institute, Rotterdam, Zuid-Holland, Netherlands; Maasstad Hospital, Rotterdam, Zuid-Holland, Netherlands and Laboratory for Pathology, Dordrecht, Zuid-Holland, Netherlands.

Body: Introduction: In a previous study we observed a positive correlation between Seven in Absentia Homolog 2 (SIAH2) and Estrogen Receptor (ER) mRNA levels. Additionally, high SIAH2 mRNA levels were related to a favorable progression-free survival (PFS) after first-line tamoxifen. In contrast, others showed high SIAH2 protein levels in ER-negative breast cancer associated with an unfavorable relapse-free survival. In this study, we investigated the above discrepancy between SIAH2 protein and mRNA findings and evaluated the prognostic and predictive value of SIAH2 protein in breast cancer patients.

Patients and methods: Tissue microarrays (TMAs) of formalin-fixed, paraffin-embedded primary breast tumors were immunohistochemically stained for SIAH2 protein. The TMAs contained core specimens of 759 patients with early disease and of 245 ER-positive patients with advanced disease treated with first-line tamoxifen. SIAH2 protein staining was scored for its intensity and proportion positive cells and subsequently evaluated for its relationship with metastasis-free survival (MFS) and PFS in uni- and multivariate analyses including traditional prognostic or predictive factors, respectively.

Results: The proportion SIAH2-positive cells had a relationship with MFS and PFS, whereas staining intensity and a previous described score for SIAH2 combining intensity and proportion were not related with clinical outcome. Based on these results, tumors with more than 20% positive cells were considered as SIAH2-positive. In early disease, 267 patients (35%) had SIAH2-positive tumors, which were further characterized by decreased expression of ER at protein and mRNA levels (P <0.001 and P = 0.003, respectively). These SIAH2-positive tumors correlated with significant unfavorable MFS in lymph node negative, ER-positive breast cancer patients, but only in univariate analysis. In advanced disease, 86 patients (35%) had SIAH2-positive tumors which was associated with an unfavorable PFS after first-line tamoxifen in both uni- and multivariate analyses (HR = 1.45; 95% CI, 1.07 to 1.96; P = 0.015).

Conclusions: SIAH2 protein expression is especially observed in ER-negative tumors and has no additional prognostic value in breast cancer. The proportion SIAH2-positive cells in ER-positive tumors can be used as biomarker to predict tamoxifen treatment failure in breast cancer patients with advanced disease. Future studies should establish if expression of certain microRNAs explain the observed discrepancy in SIAH2 mRNA and protein levels.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-09-01

Title: Simulation in continuing professional development in oncologic care: Advancing evidence-based decisions in the management of HER2-positive metastatic breast cancer


Body: Background: Breast cancer is the second leading cause of cancer death among women. The growth factor receptor HER2 is overexpressed in 20% to 30% of invasive breast cancers, and use of HER2-targeted therapies have improved responses and survival in patients with metastatic breast cancer (MBC). However, the choice of the most appropriate agents and their sequencing is crucial to maximizing beneficial patient outcomes. A study was conducted to determine if simulation-based educational interventions to address underlying clinical practice gaps could improve competence and performance of oncologists in the management of HER2-positive breast cancer.

Methods: A cohort of US-practicing oncologists who participated in online simulation-based education was evaluated. The interventions consisted of two cases presented in a platform that allowed physician learners to assess the patient and choose from an extensive database of diagnostic possibilities matching the scope and depth of actual practice. Clinical decisions made by participants were analyzed using a sophisticated decision engine, and instantaneous clinical guidance was provided at each decision point employing current evidence-based and expert faculty recommendations. Participant decisions were collected after clinical guidance and compared with each user's baseline data using a 2-tailed paired T-test to provide P values for assessing the impact of simulation-based education on the clinical decisions made by participants as of 12/4/2014.

Results: The assessment sample consisted of 123 oncologists who made clinical decisions within the simulation and proceeded to the concluding debrief section. As a result of clinical guidance provided through simulation, significant improvements were observed in several areas of management of patients with HER2-positive MBC, specifically:
• 35% improvement (P<.001) in the selection of the preferred treatment regimen (trastuzumab, pertuzumab, with a taxel) in the first-line setting
• 21% improvement (P=.003) in evidence-based treatment selection for individuals whose disease progressed on first-line therapy
• A 35% decrease was seen in the number of participants who selected trastuzumab for individuals whose disease progressed on first-line therapy, which demonstrated an improvement in oncologists ability to select the most appropriate selection based on the current evidence-base
• 39% (P<.001) improvement in the number of oncologists who ordered adverse event counseling for the patient
• Similarly, 33% more participants (P<.001) referred a patient for psychosocial counseling after clinical guidance

The data gathered during simulation also provided insights into the remaining gaps, including the choice of the most appropriate, evidence-based first line HER2-targeted regimen in patients with MBC.

Conclusion: This study showed improvements in evidence-based practice patterns of oncologists who were selecting therapeutic protocols for patients with HER2-positive MBC, thus demonstrating that simulation-based instruction can result in an increase in evidence-based clinical decisions and, therefore, may play a role in improving the quality of care and patient outcomes.
Impact of therapeutic complexity on practice patterns for metastatic breast cancer (MBC) in the United States: Results of a 2-phase national study

Quill TA A, Jahanzeb M, Obholz KL L, Brady E, Howson A, Rasulina M, Willis C and Hurvitz S. Clinical Care Options, Reston, VA; University of Miami Sylvester Comprehensive Cancer Center, Deerfield Beach, FL; M Consulting, Birmingham, AL; Thistle Editorial, Snoqualmie, WA; Annenberg Center for Health Sciences, Palm Desert, CA and University of California - Los Angeles, Los Angeles, CA.

Background: The rapidly changing clinical management of MBC has challenged the ability of clinicians to understand and integrate new data, which relates directly to the quality of clinical care and is a key determinant of patient outcomes. This study was designed to determine the potential impact of the increased clinical complexity of decision making in MBC on optimal patient care by quantifying professional practice gaps and barriers among oncology specialists at academic medical centers and community clinic settings in the United States.

Methods: From October 2014 to February 2015, 216 actively practicing US oncology specialists with a caseload of ≥1 patient/year with MBC were recruited to participate in a 2-phase national needs assessment study. In the first, qualitative phase, 35 participants consented to a 45-minute telephone interview focused on the personal, contextual, and behavioral factors that influence their clinical reasoning process in diagnosis and treatment of MBC. Findings from this initial phase informed the second phase of the study. This quantitative phase included an online survey comprising specific multiple choice questions, semantic differential rating scales, and case vignettes. Respondents’ (N = 181) answers to these questions were compared with optimal answers, as identified by treatment guidelines and MBC experts.

Results: Eight core practice gaps were identified through combined analysis of data from the in-depth interviews and online surveys. Of note, only 15% of respondents agreed with the experts' choice of letrozole + palbociclib as initial treatment for a postmenopausal patient with HR+ MBC with bone and visceral lesions after a prolonged response to adjuvant endocrine therapy. Survey respondents indicated that they use chemotherapy substantially more frequently than experts when treating patients with HR+ MBC. Only 36% of respondents' current practice matched expert recommendations regarding management of toxicity associated with exemestane + everolimus and 32% opted for management strategies with a risk of worsening treatment-related toxicity. Just over 30% of respondents agreed with the expert choice of ado-trastuzumab emtansine as second-line therapy for HER2+ MBC after progression on trastuzumab/paclitaxel. In addition, a minority of respondents knew the mechanisms of action of newly approved (palbociclib [45%]) and investigational agents, including dovitinib (19%), neratinib (30%), pembrolizumab (49%), and pictilisib (28%).

Conclusions: A significant percentage of US oncology specialists are not applying optimal care in patients with MBC. Most notably, this study indicated that participants overuse chemotherapy in patients with HR+ MBC, suboptimally manage treatment-related toxicities, and are challenged to select optimal therapy for HER2+ MBC patients who progress on previous therapy. Finally, a lack of familiarity with mechanisms of action of approved and promising investigational agents in MBC may lead to delays in the appropriate integration of new agents or indications into clinical practice. A full review of the study results and recommendations will be presented.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-09-03

Title: Education and integration of medical breast nurse practitioners in a multidisciplinary breast cancer program

Iyer PH H, Kline M, Oliphant D, Crowe J, Grobmyer S and Pederson H. Cleveland Clinic, Cleveland, OH.

Body: Background:
Work-up of patients with breast symptoms and radiographic abnormalities, risk management, and survivorship care are vital elements in a multidisciplinary breast cancer program. Breast surgeons have traditionally provided for these needs, but with intense clinical demands and with efforts to reduce treatment time, access for patients with these problems can be a challenge. The medical breast specialist (MBS) is defined as a primary care physician who has been provided with additional training in benign and malignant breast disease [Smedira, AIM 2008]. With increasing need for providers, a fellowship for training MBS was designed for board eligible or board certified internists, family practitioners, obstetricians/gynecologists, and has recently been expanded for board certified primary care nurse practitioners, one of the first of its kind in the country. In our practice, medical breast visits (MBV) are defined as clinical visits for evaluation or follow-up of patients with breast symptoms, high risk management, and/or survivorship.

Methods:
The MBS Program at our institution was established in 1997, with expansion involving a formalized training for Nurse Practitioners in 2013. The medical breast fellowship uses a defined curriculum and offers nurse practitioners a six month multidisciplinary program with multiple rotations including medical breast clinic, surgical breast clinic, breast imaging, medical oncology, radiation oncology, genetics, pathology, plastic surgery, physical therapy, and psych-oncology. We analyzed the impact of trained NPs on overall medical breast and breast center volumes and clinical workflow. Our prospectively maintained Electronic Medical Records (EMR) system, which allows the acquisition of volume and practice pattern data, was utilized for this analysis.

Results:
The number of MBV has significantly increased between 2008 and 2014 (p<0.05). MBV accounted for 54% of total visits seen within the breast surgery program in 2014. The total number of medical breast visits in 2014 was 7146 with 5543 (78%) seen by MBS and 1603 (22%) seen by surgeons. Of the medical breast visits seen by MBS, 37% are seen by medical breast staff physicians and 63% are seen by medical breast nurse practitioners. The recent completion of the medical breast fellowship by a NP resulted in the addition of 1654 MBV over the course of 1 year.

Conclusions:
Medical breast visits account for a large percentage of patients seen within a multidisciplinary breast cancer program. The development of a curriculum for training NP medical breast specialists enables the rapid incorporation of a NP into a multidisciplinary cancer program. The training of a NP within the fellowship can increase patient access, timeliness of care, and can facilitate streamlining of a breast cancer practice to optimize patient care.
Title: Consensus and disagreement between experts and community practitioners asked to make therapeutic recommendations for early breast cancer (EBC)

Obholz KL L, Rosenthal KM M, O'Regan RM M, Swain SM M, Yardley DA A and Brady ED D. Clinical Care Options, LLC, Reston, VA; University of Wisconsin School of Medicine and Public Health, Madison, WI; Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC, DC and Sarah Cannon Research Institute; Tennessee Oncology, PLLC, Nashville, TN.

Body: Intro. Most patients with stage II BC will receive surgery along with systemic therapy, but no consensus exists among experts on optimal use of neoadjuvant vs adjuvant therapy in many cases. Furthermore, treatment guidelines list multiple reasonable regimens for EBC, but lack patient-specific recommendations. We have shown previously that online decision support tools can affect treatment decisions of community practitioners. In this study, we sought to determine areas of consensus and disagreement among expert faculty providing treatment recommendations for a 2015 decision support tool on EBC as well as those using the online tool.

Methods. An online decision support tool was developed with input from 5 experts on systemic therapy recommendations for 235 patient scenarios in EBC. Tool users were asked to enter specific patient criteria and their intended management for each case before displaying the 5 expert recommendations for the user-entered case. Users were asked to indicate if the expert recommendations changed their intended approach.

Results. At interim analysis, 406 individuals used this tool, with 674 patient scenarios entered. Among users reporting on the tool's clinical impact, 88% indicated expert recommendations either confirmed or changed their intended therapy. Expert recommendations in the tool showed areas of consensus and disagreement in treating patients with EBC. For example, expert recommendations varied in the choice of systemic therapy prior to surgery and when to continue directly to surgery before systemic treatment.

Expert recommendations for initiating systemic neoadjuvant therapy in HER2-, HR+ EBC

<table>
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Experts did agree on starting with surgery in patients with node-negative, T1a disease; however, only 30% of tool users agreed. Both experts and users agreed in recommending systemic neoadjuvant therapy for patients with HER2+, node-positive T2 disease. In patients with HER2+ EBC, experts always chose to include dual HER2-targeted therapy in neoadjuvant systemic therapy but only included trastuzumab in adjuvant regimens. However, only 51% of tool users selected dual HER2-targeted therapy as part of neoadjuvant therapy and 13% use dual HER2-targeted therapy in the adjuvant setting. Expert opinion varied on when to use adjuvant chemotherapy in patients with HR+, HER2- EBC, particularly for those with intermediate or unknown recurrence scores and no lymph node involvement. Detailed comparison of expert consensus and disagreement, analysis of
practice pattern information from user responses, and perceived impact of the expert recommendations will be presented. **Conclusions.** This EBC tool highlights specific clinical scenarios having either consensus or disagreement among experts and community practitioners. Education that includes online decision support tools may increase the number of clinicians making optimal treatment decisions for patients with EBC.
Title: Communication design toolkit for metastatic breast cancer patients and their health care professionals


Body: Worldwidebreastcancer.com is an online resource designed to increase understanding of the basics of breast cancer detection through good design. Tools are used in 8 countries by 500+ advocacy groups reaching 1MM+ people in English, Spanish, Turkish, Arabic, Japanese, Gujarati and Samoan.

The Metastatic Breast Cancer Alliance (MBCA) is an advocate-led coalition aiming to transform and improve the lives of people living with metastatic breast cancer (MBC). Different from early breast cancer, MBC is not curable and is the cause of virtually all breast cancer deaths; ~40,000 die annually of MBC. People with MBC are always in treatment, switching drug regiments as their disease progresses. Survival and quality of life depends on patients' treatment decisions; yet many do not have a high level of engagement during in-office conversations with their oncologists.

Objective: Develop a visual tool kit to help MBC patients, with little or no engagement in a treatment decision-making process, communicate on equal ground with their health care professionals (HCPs) about their diagnosis, treatment, and quality of life. Low literacy rates, fear of cancer and the cultural taboos associated with breast cancer, create hurdles difficult to overcome. Despite a large number of education campaigns, none offer a multilingual, multicultural solution leaping these hurdles for MBC patients.

Methods: A USER Design Thinking Model Framework (Beaumont, 2011) with 4 cyclical phases: (1) USER: understand the needs of patients and stakeholders; (2) SYSTEM: understand the communication along the patient pathway; (3) ESTABLISH: determine patient centered needs; (4) REALIZE: develop physical tools to address needs and, going back to the USER phase, test with patients and stakeholders, evaluating their usefulness along the patient pathway to determine recommendations for the next round of improvements. Tools are being developed at a National Cancer Institute, before being trialed in two different clinical settings.

Results: Based on this project's ground breaking Stage 1 targeting women with early stages of breast cancer, we anticipate the results will show that visual metaphors overcome health literacy and communication issues for MBC patients not engaged in treatment plans. Stage 1 results showed the majority of participants (n=67) were able to interpret visuals accurately without the use of text in terms of symptoms (65%) and anatomy (86%) of breast cancer. Health practitioners using the materials reported the images were effective in communicating symptoms.

Conclusion: A visual approach to improving communication between MBC patients and their HCPs seems possible based on positive results of patient interpretation and practitioner feedback from research and testing with early stage breast cancer. Visual tools help HCPs engage patients with information to enable them to understand their disease, and goals and nature of treatments, so they can make informed decisions right for them.

1. www.mbcalliance.org/
Title: Patient education for targeted therapies with apps

Younus J and Lyn K.  London Health Sciences Centre - Regional Cancer Clinic, London, ON, Canada.

Body: Background: Explaining targeted therapies to oncology patients is a challenging task for most health care professionals (HCPs). This discussion may need to include the schedule, side effects, benefits, mechanism of action and the rationale of these medications. Despite the high frequency with which HCPs counsel these patients, there is no standardized set of information that serves to meet the challenge of providing this information in a simple, easy to understand format yet, is also amenable to individualization. Informal pictorial patient education by simply drawing on the exam table paper (a picture is better than thousand words) had been used to bridge this gap of information. This experience formed the basis for development of the hand held computer applications.

Design/Methods: Three applications (Apps), one each for tamoxifen, aromatase inhibitors (AI's) and Herceptin™ were created, utilizing the IPhone platform with subsequent conversion to the I Pad. The content of these Apps was approved through the LRCP Breast Disease Site Team and Patient Education Committee. The study was approved by the Ethics Committee at the University of Western Ontario. All adult patients with breast cancer undergoing adjuvant treatments with tamoxifen, aromatase inhibitors and/or Herceptin™ were considered eligible. The study was described to patients and consent obtained.

The Apps use animated cartoons with limited text to designate the receptors or the medications. The information was verbally reviewed with patients as the HCP showed the animated cartoons through the App. In order to gauge the impact of using these apps as an education tool with patients, a satisfaction survey was designed with 5 questions, using a visual analogue scale where 1 indicated poor agreement and 7 complete agreement. To evaluate the level of understanding achieved, the patients were asked two "exam questions" with a multiple-choice answer format after each App was used by a HCP.

Results: A total of 64 patients participated, 33 with the AI App, 19 with the tamoxifen App and 12 with the Herceptin™ App. For the "exam questions" segment, there were no wrong answers given by any of the patients using one of the three Apps. The survey questions evaluated the patient's understanding of the mechanism of action, benefits, side effects and the dosing schedule. The App was evaluated for overall preference for the pictorial App presentation format, likelihood of recommending the App to other patients, and feeling more knowledgeable post presentation. The vast majority of patients rated the Apps very highly on all questions with the mean for these questions ranging between 6.67 to 7 on the visual analogue scale.

Conclusions: The use of Apps is a novel and effective approach to educate oncology patients regarding complex molecularly targeted treatments. These Apps are quick, easy to use, readily available and demonstrable with smart phones or an I-pad. It also reminds and helps HCPs to provide pertinent information in simple language. From our results, it is quite apparent that patients liked this approach of providing education and counseling and actually understood the contents explained to them.
Title: Using the power of the internet to deliver breast teaching in Africa

Shaaban AM M, Abdulkareem F and Rotimi O. Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom; College of Medicine, University of Lagos, Lagos, Nigeria and St James's University Hospital, Leeds, United Kingdom.

Introduction:
Delivering high quality breast teaching to African countries is of utmost importance. Our previous audit of breast cancer reporting in Africa has identified areas that would benefit from teaching and education. However, time and resource issues limit the ability to deliver teachings to a large number of African pathologists in person.

Method
As part of a comprehensive national in house teaching for senior trainees in Nigeria, a distant module of teaching was delivered by a team of UK pathologists using a freely available internet application.

Results
Distant teaching formed part of a two week residential postgraduate histopathology course that was designed for trainees preparing for their exit Histopathology exam. Sixty African pathologists attended the teaching. Previous auditing of breast cancer reporting indicated the need for education on macroscopic specimen handling, typing and grading of breast cancer. Other topics included an update on inflammatory breast cancer which has a high incidence in Africa and columnar cell lesions that are increasingly being encountered by African pathologists in the context of breast screening. A set of anonymous virtual slides were scanned and distributed to delegates before the course date.

Teaching was delivered using Skype application that only required a computer with internet access at the UK and Nigeria. We utilised video-conferencing which allowed trainees to see their lecturers and vice versa. The new data sharing facility of the application allows simultaneous sharing of Power Point presentations, with annotation which facilitates interactive discussion. A slide seminar using the previously circulated virtual slides, covering areas of diagnostic challenges morphologically and by immunohistochemistry was also delivered. An approach to diagnosing these lesions was delivered with re-focussing on tumor typing and grading. Participants were able to ask questions and interact with the UK-based tutors thorough the sessions. The feedback collected from attendees was excellent.

Conclusion
Distant teaching using freely available internet applications is a valuable, time and cost effective method that allows sharing educational material including lectures and slide seminars in an interactive manner. It is particularly useful in delivering teaching to resource-poor countries.
Title: Information needs and Internet use of breast cancer patients in Mexico

Villarreal-Garza C, Platas A, Bargalló-Rocha JE, Aguilar-González CN, Ortega-Leonard LV, Martínez-Cannon BA, Ramos-Eliás P and Soto-Perez-de-Celis E. Instituto Nacional de Cancerología, Mexico City, Mexico; Centro de Cancer de Mama, Tecnologico de Monterrey, Monterrey, Mexico; Programa Para la Atencion e Investigacion de Mujeres Jovenes con Cancer de Mama, Mexico City, Mexico and Cancer Care in the Elderly Clinic. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.

Body: Introduction: Information seeking is critical for the decision making of individuals undergoing cancer diagnosis and treatment. The information needs of breast cancer (BC) patients and whether they use online resources to obtain it are largely unknown, particularly in developing countries with scarce educational resources and poor Internet access. Additionally, the influence of age on the use of online BC resources has not been explored. In this study, we aim to describe the information needs of BC patients in Mexico, and to analyze the difference in such needs between age groups.

Patients and Methods: A cross-sectional survey was conducted from March to May 2015 amongst women with BC undergoing treatment or follow-up at the National Cancer Institute in Mexico City. Demographic data (age, place of residence, occupation and level of education), Internet use and access (e-mail, social networks, tablet and/or smartphone use) and need of information sources (printed and online materials) were collected. Patients were asked which particular aspects of BC they would like to see highlighted in online educational resources. Patients were divided into three age groups (≤ 40 years [y], 41-64 y and ≥ 65 y). Chi-square test was used for comparison between group characteristics, and Student’s t test to detect differences in information needs between groups.

Results: 325 patients were invited, 15 refused to answer and 310 provided completed surveys. Median age was 47 y (19-87), 220 (72.6%) were housewives, and 181 (58.4%) had less than undergraduate education. 163 (52.6%) had Internet access, 139 (45%) had e-mail, 133 (43%) had a social network profile and 178 (57.6%) owned a smartphone/tablet. Regarding information needs, 94.5% (n = 241) believed printed materials should be available, while 93.2% (n = 287) thought online resources would be useful and 78% (n = 241) expressed the desire to have an online forum with other patients. Women ≤ 40 y were more likely to have Internet access (p < 0.0001), e-mail account (p < 0.0001), social network profiles (p < 0.0001) and tablets/smartphones (p < 0.0001). Although there was no difference in the perceived need for printed materials between age groups (p = 0.26), women ≥ 65 y were less likely to believe online resources would be useful (90.8 vs. 97.3%; p = 0.01). 21.6% (n = 67) of patients mentioned diet and exercise as the most important aspect they would like to see in a website, followed by survivorship (19.7%; n = 61), and treatment (14.5%; n = 45). Women ≤ 40 y were significantly more interested in learning about the adverse effects of treatment than their older counterparts (12.4 vs. 3%; p < 0.001). There was no difference between age groups regarding other aspects of perceived information needs.

Conclusions: Despite having a poor educational background and low rates of Internet access, almost all the surveyed women were interested in obtaining online BC information, regardless of age. Perceived information needs and preferences do not differ greatly between age groups, although young women are more likely to want information about adverse effects. Supplying patients with appropriate and accessible online educational resources addressing their information needs is essential, and healthcare providers should strive to accomplish this goal.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-09-09

Title: Preferences in educational topics of interest for women with breast cancer: Does age influence topic preferences?


Body: Purpose: To ensure that people with breast cancer receive materials and programs that fit their needs for education, information, and support by identifying clinical, treatment, demographic, socioeconomic, and emotional characteristics targeting their specific needs and concerns.

Respondents and Methods: Women diagnosed with breast cancer responded to an online 80-question survey to identify education and support needs. Participants self-reported age at time of the survey, and were asked to rate their level of interest in 35 educational topics, including treatment issues and practical, financial, and psychosocial concerns. Potential ratings were "Not Interested," "Interested," and "Very Interested." This analysis examined whether age was associated with interest level in these topics. Point biserial correlations were calculated to determine bivariate association. Interest ratings were collapsed into "Not Interested" and "Very Interested or Interested," and the age variable was continuous (mean = 53.37 years; standard deviation = 10.65). A false discovery rate adjustment was applied to the significance threshold (p < .029) to account for multiple comparisons.

Results: The response rate to each item/topic was approximately 75.4% (2,636/3,496). Topics that received high levels of interest, regardless of age, included new treatments and research and long-term health impacts. However, advancing age was negatively associated with interest in fertility preservation (p < .001), career and work (p < .001), early menopause (p < .001), and breast reconstruction (p < .001). Advancing age was positively associated with interest in hospice and end-of-life care (p < .001) and communication with your doctor and healthcare team (p < .01).

Conclusions: Age can be a determining factor in women's preferences for breast cancer educational topics. All women, regardless of age, agreed that staying informed about new treatments and research and long-term health impacts are of great interest. Younger women, as might be expected, were significantly more interested than older women in fertility preservation, career and work, early menopause, and breast reconstruction, while older women were significantly more interested in hospice and end-of-life care and communicating with their doctor and healthcare team. Understanding the impact of age on educational topic preference could enable the healthcare team to focus on those issues of greatest interest at a time when face-to-face interactions continue to be reduced, maximizing the effectiveness of these interactions by tailoring discussion to the individual's educational concerns. Living Beyond Breast Cancer and several other breast cancer organizations have some age-specific educational materials and programs available. Oncology practices and cancer centers might consider reviewing them in light of these data, which point to the need to enhance these face-to-face interactions and overall quality of life.
**Title:** Preferences in educational topics of interest for women with breast cancer: Does income level influence topic preferences?

**Body:**

**Purpose:** To ensure that people with breast cancer receive materials and programs that fit their needs for education, information, and support by identifying clinical, treatment, demographic, socioeconomic, and emotional characteristics targeting their specific needs and concerns.

**Respondents and Methods:** Women diagnosed with breast cancer responded to an online 80-question survey to identify educational and support needs. Participants self-reported income level and were asked to rate their level of interest in 35 educational topics, including treatment issues and practical, financial, and psychosocial concerns. Potential ratings were "Not Interested," "Interested," and "Very Interested." This analysis examined whether income level was associated with interest level in these topics. Interest ratings were collapsed into "Not Interested and “Very Interested or Interested.” Contingency tables were generated, and phi coefficients were calculated to determine bivariate association. A false discovery rate adjustment was applied to the significance threshold (p < .021) to account for multiple comparisons.

**Results:** The response rate for each item/topic was approximately 75.1% (2,625/3,496). Topics that received high levels of interest, regardless of income, included prevention of other illnesses after cancer and new treatment and research. However, higher income was negatively associated with interest in clinical trials (p < .01), dating (p < .001), pain management (p < .001), and health insurance (p < .001). It was positively associated with interest in sexuality (p < .001) and parenting issues (p = .02).

**Conclusions:** Income can be a determining factor in women's preferences for breast cancer educational topics. Women across all income levels agree that prevention of other illnesses after cancer and staying informed about new treatments and research are of great interest, but topic preferences for women with incomes of <$50k annually differed from those with higher income levels. In this analysis, lower income women were more interested in clinical trials, dating, pain management, and health insurance, and were less interested than women with higher incomes in sexuality and parenting issues. Lower income women are often diagnosed with higher staged cancers and have poorer outcomes overall. They may have less access to information about the clinical trials process and be less likely to ask about trial availability. Marginal insurance may drive their interest in pain management since they have fewer resources to manage their discomfort and other treatment side effects. Understanding the unique educational needs of lower income women and developing interventions targeted to address them could encourage greater participation in clinical trials for which they would qualify, appropriate pain management and enhanced access to resources to alleviate insurance concerns. Attention should be paid to differences in the educational needs of lower income women. Empowering them by meeting these needs could enhance quality of life and may improve overall outcomes.
Title: Hear my voice: Preparing women with metastatic breast cancer for community engagement

Ormerod CL L, Fawzy Morales JL L, Guglielmino J, Hanson AA and Creme Henry CM M. Living Beyond Breast Cancer, Bala Cynwyd, PA and Triage Cancer, Culver City, CA.

Body: Background:
Findings from the Metastatic Breast Cancer Alliance’s 2014 Landscape Analysis indicate that women living with metastatic breast cancer (MBC) face challenges in accessing needed education and support resources, feel isolated from the wider breast cancer community, and encounter public misconceptions about the diagnosis, its causes and its ability to be treated.

To address these areas of need, Living Beyond Breast Cancer (LBBC) created the Hear My Voice: Metastatic Breast Cancer Outreach Volunteer training program to provide intensive training to a small group of women living with MBC. Program goals for participants included:
• to become leaders in their physical and digital MBC communities
• to connect themselves and others to MBC resources
• to raise public awareness about the realities of the MBC

Methods:
LBBC launched online recruitment in conjunction with its Annual Conference for Women Living With Metastatic Breast Cancer. More than 80 women applied for 25 seats. LBBC provided financial assistance to offset travel and lodging costs for participants. During an intensive 1-day training, facilitators shared information about the current landscape of MBC information, research, and psychosocial needs. Participants learned about the spectrum of advocacy and public speaking. They began identifying areas of advocacy and practiced sharing their stories. Following the training, the conference provided medical and quality-of-life information and allowed for additional networking and brainstorming.

Results:
The 3-day program enrolled 31 women from 18 states. Of participants, 26% were women of color; 15% lived in rural areas, 29% were diagnosed with de novo metastatic breast cancer, and 71% were diagnosed before age 45 (age range: 24 -68). Participants connected with others who could relate to their unique diagnosis and treatment situation and brainstormed ideas to begin addressing gaps in services, information, and support. Participants commit to two outreach projects in either their physical or digital communities, help LBBC plan for and lead an MBC awareness day across social media platforms on September 21, 2015, and participate in monthly conference calls through December 2015.

Since the training, participants have applied for positions on research review committees, launched blogs, testified before government bodies, collaborated with local nonprofits and health systems to improve their inclusion of MBC needs, participated in training as peer support mentors, and shared vital resources with their healthcare providers and peers. Participants continue to advise and support one another through a closed Facebook group where they share outreach ideas as well as health status, treatment, and well-being updates.

Conclusions:
Providing a physical and digital workspace and a shared goal for a small group whose diagnosis often marginalizes them creates a supportive atmosphere that allows participants to explore ideas and interests and create initiatives that have an immediate impact in their home and online communities. National nonprofit organizations can effect change in discrete and diverse communities by empowering women living with MBC to identify gaps in services, information, and support and begin addressing them with guidance from their national partner.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-09-12

Title: The importance of education for adherence to mammographic screening in the Brazilian public health services

Blanco EC C, Ribeiro IM M, Nascimento CCP CP, da Silva CA A, Abrahão CF F and Borges MM M. Hospital Alemão Oswaldo Cruz, Sao Paulo, Brazil; Hospital Alemão Oswaldo Cruz, Sao Paulo, Brazil; Hospital Alemão Oswaldo Cruz, Sao Paulo, Brazil; Hospital Alemão Oswaldo Cruz, Sao Paulo, Brazil and Hospital Alemão Oswaldo Cruz, Sao Paulo, Brazil.

Body: Introduction: A third of the registration of new cases of breast cancer in Brazil corresponds to locally advanced or late diagnosed disease. The benefits of screening in this sense become evident and measurable in terms of their temporal extent and adherence of the target population to its recommendations. Unlike the organized mammographic screening where there is call, breast health education, monitoring and enforcement of the intervals between examinations, in the opportunistic model, individuals are subject to the recommendations from the spontaneous demand by health services. Objective: To determine the influence of education in breast cancer for adherence to mammographic screening in brazilian public health services. Methods: This is a cross-sectional, descriptive and comparative study between two groups of women with (group A, n=136) and without (group B, n=128) knowledge about breast cancer, who appeared in the Center of Detection, Diagnosis and Monitoring (CDDM) for organized screening. For this were interviewed 264 women in relation to the realization of previous mammograms in the last three years. The repetition of examinations, respecting the range of 24 months was defined as criteria to classify participants in adherent or not to screening. Results: Both groups of women had a similar mean age (50.2 ± 9.2 and 49.5 ± 9.5 years; p>0.05). Of the respondents, 77.2% (n=204) reported mammographic screening before joining the CDDS, however highest proportion was registered in group A (64.8%). Sixty women denied conducting prior mammography with predominance in the Group B (93.4% vs 6.6%, p<0.05). One hundred forty-four women were adherent to screening, 90.2% (n=130) of these belonged to group A. The remaining variables (education, occupation, family income, distance between place of residence and mammography center and personal / family history of breast cancer) were not associated with adherence to screening (p>0.05). Conclusion: The decrease in the adherence to conducting periodic mammography observed in group B reflects the opportunistic character of the screening in the country, where it loses the chance to sensitize women through education to improve their effective adhesion. Acknowledgements to the Brazilian Ministry of Health for their support to the study.
**Title:** The effect of bone pain–specific education vs general chemotherapy side-effect education on reported bone pain in patients (pts) with breast cancer receiving chemotherapy and pegfilgrastim

Maxwell CL L, Guinigundo AS S, Vanni L, Morrow PK, Reiner M, Shih A, Klippel Z and Blanchard E. Miami Cancer Institute, Miami, FL; Oncology Hematology Care Inc., Cincinnati, OH; Providence Hospital, Southfield, MI; Amgen Inc., Thousand Oaks, CA and Southcoast Health, New Bedford, MA.

**Body:**

**Background:** Mild-to-moderate bone pain is the most commonly reported adverse event (AE) associated with pegfilgrastim, but pt education has not been specifically studied in the management of pegfilgrastim-related bone pain. We investigated the effect of pt education on reported bone pain in pts with breast cancer receiving adjuvant or neoadjuvant chemotherapy and pegfilgrastim.

**Methods:** In this single-blind study, female pts ≥ 18 years of age with newly diagnosed stage I–III breast cancer, planning ≥ 4 cycles of neoadjuvant or adjuvant chemotherapy with pegfilgrastim support starting in cycle 1, were randomized 1:1 to view one of two 2-minute educational DVDs: a general educational DVD (GE-DVD) on chemotherapy side effects or a more specific DVD on bone pain following chemotherapy and pegfilgrastim (BP-DVD). Pts were excluded if they were not able to understand English, were scheduled to receive weekly chemotherapy, had ongoing chronic pain requiring treatment, had received chemotherapy for cancer within the last 5 years, or had previously received G-CSF. Pts were required to watch the DVD on 2 separate days during clinic visits up to and including the visit for pegfilgrastim administration in cycle 1. In each of the four cycles of the study period, pts completed a brief bone pain survey once per day for 5 days, beginning the day they received pegfilgrastim; severity of pain was rated on a scale of 0–10. Pts also recorded any medications taken to alleviate bone pain. Pts were asked about AEs at the beginning of each chemotherapy cycle and at the safety follow-up visit.

**Results:** Of the 312 pts screened, 304 were enrolled, and of those, 300 received pegfilgrastim in cycle 1: 149 in the GE-DVD arm and 151 in the BP-DVD arm. Baseline demographics and characteristics were largely balanced between the arms, but fewer pts in the GE-DVD arm were Hispanic/Latino (3.4% vs 7.9%). Fewer pts in the GE-DVD arm were ER positive (59.1% vs 69.5%) and PR positive (46.3% vs 59.6%), while more were HER2 positive (30.2% vs 18.5%). Receipt of taxane-based chemotherapy regimens was balanced between the arms. Pt-reported maximum bone pain was similar in the GE-DVD arm vs the BP-DVD arm (cycle 1, 3.2 vs 3.5, \(P = .3479\); across all cycles, 4.1 vs 4.6, \(P = .2196\)). Pt-reported mean bone pain was also similar between arms (cycle 1, 1.6 vs 1.8, \(P = .3188\); across all cycles, 1.5 vs 1.6, \(P = .5846\)) as was area under the curve for pt-reported bone pain (cycle 1, 6.7 vs 7.6, \(P = .3346\); across all cycles, 6.3 vs 6.6, \(P = .6255\)). All-grade bone pain and grade 3/4 bone pain from AE reporting were similar between the arms. Pt-reported bone pain and bone pain from AE reporting were highest in cycle 1; pain decreased thereafter and remained stable in cycles 2, 3, and 4. Bone pain medication usage was similar between the arms; usage was highest in cycle 1 and decreased with each subsequent cycle. Pain therefore appeared to be truly stable in cycles 2, 3, and 4, not just better medicated.

**Conclusions:** Our bone pain–specific educational program did not improve perceptions of bone pain reported by this pt population. Bone pain was highest in cycle 1, decreased in cycle 2, and then remained stable.
Title: Understanding and attitudes towards cancer clinical research among breast cancer patients compared to the general population: Prospective cross-sectional study

Kelly CM M, McCaffrey JA A and Kelly CM M. Mater Misericordiae University Hospital, Dublin, Ireland and Irish Platform for Patients' Organisations, Science and Industry (IPPOSI), Dublin, Ireland.

Body: Background: In 2009 the Irish Platform for Patients' Organisations, Science and Industry (IPPOSI) commissioned research to ascertain the Irish public's understanding and opinion of clinical research (CR) and clinical trials (CT). The aim of this study was to compare attitudes and understanding regarding participation in cancer CT in breast cancer pts treated in a disadvantaged area to those of the general population surveyed by IPPOSI.

Methods: Eligibility criteria included: a cancer diagnosis (dx), attendance at the Mater Hospital, able to complete a questionnaire. We examined: a) demographics b) cancer dx, c) understanding and attitudes towards CR, 4) discussion regarding participation and 5) experience on a CT. Comparison was made between this study and the IPPOSI survey of 1000 members of the Irish public.

Results: 356 pts completed the questionnaire. The majority (58%, n=206) had a history of breast cancer and these results focus on this group. The median age was 56 yr (range: 28-81 yr), 19% were ≥ 65 yr. 87% had adjuvant disease, 13% had advanced cancer. 36% did not complete 2nd level education and 38% reported inaccurate cancer details. Most reported understanding the terms 'CR' (91%) and 'CT' (84%). Nearly half (46%) expressed ambivalence or would decline participation in a CT. Reasons offered for this attitude include: fear of side effects (46%), requiring more information (24%), and inappropriate candidate/age (10%). There was a significant association between the decision to participate in a CT and participant's level of education (p=0.02) and cancer stage (p=0.01). Participants with a low level of education were more likely to decline participation (57%). Participants with advanced cancer were more likely to agree to participate (79%). 47% of the study population used support to help in the decision making process. Family (40%), GP (7%) and members of the CT team (10%) were the forms of support most commonly accessed. 33% had the option to participate in a CT. 86% (n=59) accepted. 9% (n=6) were ineligible. The majority of pts who took part in a CT reported a positive experience (89%) and a positive impact on their quality of life (82.5%). Over 90% of pts would recommend or take part in another CT.

In comparison with the IPPOSI study, breast cancer pts displayed a greater understanding than the Irish public of the terms 'CR' (91% vs 52%) and 'CT' (84% vs 75%). They were more willing than the Irish public to participate in CR (73% vs 42%) and more specifically research involving the donation of blood (78% vs 71%) or supply of medical details (92% vs 67%). However, there was a similar level of reluctance to participate in a 'CT' amongst both groups.
Title: Patient advocates as partners in breast cancer research at Georgetown University Lombardi Comprehensive Cancer Center


Body: The integration of patient advocate input into biomedical research grant proposals is a relatively new phenomenon and represents a paradigm shift for basic, translational and clinical researchers seeking funding for their proposed studies. In 2011, the Lombardi Comprehensive Cancer Center (LCCC) at Georgetown University (GU) established the Georgetown Breast Cancer Advocates (GBCA) to facilitate collaboration between researchers and advocates. The mission of GBCA is to ensure research is patient-centered, innovative, evidence-based, and accessible. Working with researchers and clinicians at GU-LCCC early in proposal development, the members of the GBCA evaluate the feasibility of research, emphasizing the need for bench-to-bedside studies, the importance of quality of life, health care disparities and a reduction in breast cancer mortality. This integrated and early approach has resulted in GU-LCCC researchers being awarded a PCORI Grant, a DOD Idea Expansion Award, and a prestigious NIH U01 award.

The GBCA consists of survivors and community stakeholders from diverse ethnic, racial, and age groups. It ranges from women at high risk for breast cancer to both short- and long-term survivors of various sub-types of breast cancer and those with recurrent disease. Several advocates were trained by the National Breast Cancer Coalition’s scientific education program, Project LEAD, and others participate in the Susan G. Komen for the Cure Advocates in Science Program. Members have served as consumer reviewers for the DOD’s Breast Cancer Research Program at both the peer review and programmatic review levels, and as patient representatives on American Society of Clinical Oncology (ASCO) clinical practice guideline panels. GU-LCCC researchers and oncologists serve as advisors for the group.

This poster describes the evolution and work of the GBCA and how the group has influenced breast cancer research at GU-LCCC. GBCA works with researchers in the pre-award phase, providing input to investigators regarding methodologies to increase participant recruitment, retention, and adherence to research protocols. The advocates also provide input on study designs and patient education strategies. Through their contributions, the advocates have become an integral and respected part of the breast cancer research community at GU-LCCC.
Title: Clinical trials: "A holistic approach"


Body: Goal:
New and improved treatments depend on the completion of successful clinical trials. Our goal is to impact all aspects of the clinical trial process to enhance accrual and outcomes.
1) To provide patient focused feedback at the design phase of clinical trials
2) To increase health literacy about clinical trials at both the national and community level
3) To provide decision aids for individual trials
4) To provide patient focused staff communication trainings

Strategy: CISN principals are currently working with the following groups
National Clinical Trial Network
Academic Medical Centers: UCSF and Mayo
Industry: Genentech, Pfizer, Novartis, and Lilly
Nonprofit Organizations: AACR, Faster Cures, Susan G. Komen, SOCRA
CISN principals are working within the NCTN to accomplish strategies one and two. The goal is to branch out to more community venues in the future. A 2001 study by Lara et. al. reported that the consent process with its legalistic and confusing forms is itself a barrier to patient participation with 49% of eligible patients declining enrollment. We address these concerns by accomplishing strategies 3 and 4 above.

Action Taken
CISN is introducing the medical community to an array of issues affecting patients considering participation in clinical trials. They are also developing patient-centered, study specific, educational materials included as part of the informed consent process. These interventions may enhance patient literacy, improve patient satisfaction and advance public trust in the research enterprise, leading to responsible increased accrual and retention. Additionally, CISN has worked as a contractor for several biotech companies to develop patient educational materials for several studies.

Work done at the Clinical Trial Summit documented that 67% of professionals consenting patients have less than 6 hours of psychosocial training. To address that issue, CISN developed a training program for those professionals who administer consent. Two PhD psychologists where brought onboard as consultants to assist in the development of the training. To date CISN has conducted many trainings for various groups and will soon submit grants to partner with other organizations to translate the materials into Spanish and work with patient navigators to further ensure proper training in all communities.

Outcome
Interest in our methods and materials continues to grow. CISN was identified by Faster Cures as having "best practice" methods in the area of informed consent. We work closely with academic, government, other non-profits and industry researchers to help foster public awareness about the importance of medical research to daily life. CISN addresses these issues and presents various strategies that might be applied to NCI network group and industry trials so as to bridge the research gap, move research forward, and adopt the best course to serve the needs of the community, researchers and patients.
Title: XRAYS (eXamining Relevance of Articles to Young Survivors) program survey of information needs and media use by young breast cancer survivors and young women at high risk for breast cancer


Body: Women age 45 or under with breast cancer, or who are at high risk for breast cancer, have distinct health risks and needs when compared to their older counterparts. Young women with breast cancer or at high risk for breast cancer need evidence-based, high-quality information to help them make informed decisions about their specific health needs. Interpreting media reports on research findings, including determining the study implications for younger women is often challenging. To help women better understand media coverage of new research, Facing Our Risk of Cancer Empowered (FORCE) developed the CDC-funded XRAYS (eXamining Relevance of Articles to Young Survivors) program. To assure that the XRAYS program is responsive to the community's needs, FORCE launched a survey to assess where young women turn for information about breast cancer and to identify their information needs. The survey examines: how frequently women visit various media sources and health- or cancer-related websites for information on breast cancer screening, treatment, surgery, prevention, genetics, or survivorship; how much the women trust these information sources; whether they have ever tried to share media articles with their health care team and how the team received the information; and at what point(s) during the process of screening, diagnosis, treatment, survivorship and/or risk management respondents actively seek out information from the media. FORCE launched the survey nationally through its network of 50 outreach groups, partner organizations that serve young breast cancer survivors, and via a social media campaign targeting women age 45 and under with, or at high risk for breast cancer. The survey was open March 15 - June 30, 2015. We will report results from over 800 women, age 45 or younger, including those with breast cancer, who have previously had breast cancer, or who are at high risk for breast cancer. Analysis will determine response frequencies and whether information needs and utilization correlate with key demographic variables such as race/ethnicity, education, and income level. We will use correlation and multiple regression analysis to assess patterns in the types of information needed and channels where information is sought. These results will ensure XRAYS materials and dissemination efforts are efficient and responsive to the young breast cancer population's needs, and will inform the broader medical, media and patient advocacy communities about the distinct information needs of this group.
Care guidelines for young women diagnosed with breast cancer

DeCoteau MJ. Rethink Breast Cancer, Toronto, ON, Canada.

Background: Rethink Breast Cancer has been offering educational resources and front-line psychosocial and practical support to younger women with breast cancer for over ten years. We have heard first-hand the unique issues that these women face. Some of these challenges include diagnosis during pregnancy, effects of chemotherapy on fertility, risk of menopausal symptoms or osteoporosis, feelings of isolation, questions about sexuality, childcare, relationships, dating, employment and money.

Purpose: Health Canada and the Canadian Medical Association have established comprehensive Clinical practice guidelines for the care and treatment of breast cancer and our Provincial Cancer Agencies, the Canadian Association of Nurses in Oncology (CANO) and the Canadian Association of Psychosocial Oncology (CAPO) all have developed excellent Standards of Care. However, our report, Breast Cancer in Young Women in Canada – A Needs Assessment, finds that younger women's needs continue to fall through the cracks. The report also shows that despite a growing array of peer support based interventions and community resources for young women with breast cancer, their needs are often not being met by current healthcare systems. In response to the feedback from young women experiencing breast cancer in Canada provided in our report, Rethink Breast Cancer has developed a set of recommended Care Guidelines for Young Women with Breast Cancer to ensure their special needs are addressed (in a timely matter). They serve as a compliment to the guidelines mentioned above.

Methods: The care guidelines have been developed in consultation with Rethink's Young Women's Network, a group comprised of young women across the Canada that have personal experience with breast cancer and are interested in Rethink's unique role within the breast cancer community. The network provides important input and feedback on our resources and campaigns for young women dealing with breast cancer. The care guidelines were also developed through consultation with our professional advisors in oncology, nursing and psychosocial support. They are based on original work by UK specialist breast cancer support charity Breast Cancer Care.

Results & Conclusions: These guidelines provide information about key issues individuals may want to discuss with their healthcare providers involved in their breast cancer treatment and care. They are recommendations on the kind of information and support to which individuals should be given access to as a younger woman with breast cancer. They will also serve to guide the care these individuals receive from healthcare professionals.
Title: Preferences for chemotherapy side effect profiles in breast cancer- The view of oncologists

Beresford MJ J and Makris A. Royal United Hospital, Bath, United Kingdom and Mount Vernon Cancer Centre, Northwood, United Kingdom.

Body: Background
The best sequencing of chemotherapy in metastatic breast cancer is unclear and the choice of chemotherapy agent is often influenced by side effect profile. A number of studies have examined patient preferences for chemotherapy toxicities but none have looked specifically at the preferences of prescribing oncologists. There is clear potential for the preferences of individual oncologists to bias discussions about chemotherapy and it is important to take into account any differences in the relative weighting of potential toxicities to help inform a more reasoned discussion with patients. The aim of this study was to survey the opinions of a group of consultant breast cancer oncologists asking what they deemed to be the most and least severe chemotherapy side effects, and to compare with similar patient opinion studies.

Methods
Consultant breast cancer oncologists at the UK Breast Cancer Meeting (UKBCM) 2014 were asked to vote using an electronic keypad voting system. A slide listed nine chemotherapy side effects: nausea/vomiting, hand-foot syndrome, sensory neuropathy, motor neuropathy, myalgia, mucositis, diarrhoea, fatigue and alopecia. Delegates were asked to vote on which was the worst side effect, assuming grade 3/4 toxicity. The vote was then repeated asking the question which was the most acceptable side effect.

Results
106 electronic keypad voting responses were received to both questions. Sensory neuropathy (21.7%), nausea/vomiting (19.8%), motor neuropathy (17.9%) and fatigue (17.9%) were voted the most troublesome side effects. When asked about the most acceptable side effects, alopecia was the most popular response (40.6%) followed by fatigue (22.6%). Sensory (<1%) or motor (0%) neuropathies were not felt to be acceptable.

A previous study in breast cancer patients found that diarrhoea (34%), nausea/vomiting (15%) and hand-foot syndrome (13%) were considered the worst side effects. In contrast only 4.7% of oncologists ranked diarrhoea as the worst symptom. Both the patient and oncologist studies ranked alopecia as the most acceptable side effect (34% and 40.6% respectively).

Conclusions
The effect of fatigue in cancer treatment is often underestimated. Only 9% of patients recognised fatigue as the worst side effect compared with 17.9% of oncologists, although a similar proportion of oncologists (22.6%) felt it to be most acceptable - even professionals have a varying understanding of severe fatigue. Oncologists might underestimate the impact of diarrhoea caused by drugs such as capecitabine on quality of life, but tend to rank longer term toxicities such as neuropathy higher than patients. This is the first study to assess the concerns of oncologists with regard to chemotherapy side effects and as such gives some insight into how certain regimes might be chosen or presented to patients.
Title: Survival benefit needed to undergo chemotherapy: Patients and physicians preferences

Vaz Luis I, O'Neill A, Sepucha K, Miller KD D, Baker E, Dang CT T, Northfelt DW W, Winer EP P, Sledge GW W, Schneider BP P and Partridge A. Dana Farber Cancer Institute, Boston, MA; Massachusetts General Hospital, Boston, MA; Indiana University Cancer Center, Indianapolis, IN; Memorial Sloan Kettering Cancer Center, NY, NY; Mayo Clinic, Scottsdale, AR and Stanford University, Stanford, CA.

Body: Background: Data regarding patients (pts) and physicians' preferences for modern adjuvant chemotherapy (CT) are limited. Prior studies suggested that most pts with early stage breast cancer were willing to receive 6 months of adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) for modest survival benefits (e.g. most women would have accepted 3-6 months extension of life).

Methods: E5103 was a phase III trial which randomized node positive or high risk node negative breast cancer pts to receive adjuvant CT (doxorubicin, cyclophosphamide and paclitaxel) with either placebo or bevacizumab. Telephone based surveys were administered to all pts enrolled on E5103 between 01/Jan/10 and 08/Jun/10, as part of a Decision-Making/Quality of Life component. Results presented here are part of the 18 months post-enrollment follow-up. Pts were asked to rate the survival benefit needed to justify 6 months of CT. A complementary survey was sent to all physicians who registered at least one pt on E5103.

Results: 465 out of 519 eligible pts (90%) responded to this survey at 18 months. Main reasons for non response were: inability to reach the patient (6%) or patient refusal (2%). Median pts age was 51 (25-76); 42% of pts had at least a college degree. The majority had at least Stage II cancer.

179 (16%) physicians participated, among whom median age was 50 (35-70). The median years in practice was 17 (3-38); 78% of physicians worked on large size practices, 72% saw at least 5 new breast cancer pts/month, and 77% enroll between 1-4 pts on trials/month.

We found considerable variation in pts preferences particularly for modest survival benefits: a substantial minority of pts (24%) would consider 6 months of CT definitely worthwhile for 1 month survival benefit, 18% would possibly consider it and 56% would not. The percentage considering CT definitely worthwhile increased with greater benefit, but did not reach 100%, even with 24 months survival benefit. About half of pts considered 6 months of CT definitely worthwhile for 9 months benefit, 70% for 12 months and 84% for 24 months.

Physicians were less likely to accept CT for a small chance of benefit (34% of pts vs. 5% of physicians would definitely consider CT worthwhile for 2 months of benefit). For longer benefit, pts and physicians choices were similar (84% of pts vs. 92% of physicians would definitely consider CT worthwhile for 24 months benefit).

Table

<table>
<thead>
<tr>
<th>Consider 6 months of CT to live:</th>
<th>Yes, definitely worthwhile</th>
<th>Yes, maybe</th>
<th>No, not worthwhile</th>
<th>No answer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pts</td>
<td>Physicians</td>
<td>Pts</td>
<td>Physicians</td>
</tr>
<tr>
<td>1 month longer</td>
<td>24%</td>
<td>3%</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td>2 months longer</td>
<td>34%</td>
<td>5%</td>
<td>23%</td>
<td>32%</td>
</tr>
<tr>
<td>6 months longer</td>
<td>44%</td>
<td>32%</td>
<td>35%</td>
<td>54%</td>
</tr>
<tr>
<td>9 months longer</td>
<td>53%</td>
<td>51%</td>
<td>34%</td>
<td>42%</td>
</tr>
<tr>
<td>12 months longer</td>
<td>70%</td>
<td>75%</td>
<td>23%</td>
<td>22%</td>
</tr>
<tr>
<td>24 months longer</td>
<td>84%</td>
<td>92%</td>
<td>12%</td>
<td>5%</td>
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</table>

n Pts= 465; n Physicians= 179; * equal results in both groups
Conclusions: This subgroup of pts who had undergone modern adjuvant CT in a large multicenter randomized controlled trial and these physicians who registered pts on the same trial had different cutoffs for acceptable levels of benefits and risks when considering adjuvant chemotherapy. It is important to engage pts in determining whether CT is or is not a "reasonable" option for treatment.
Title: Development of an interactive text messaging tool to improve adherence with adjuvant endocrine therapy: Breast cancer endocrine therapy adherence (BETA) pilot study


Body: Introduction: Approximately 75% of stage I-III breast cancers are hormone receptor (HR) positive for which the standard of care is 5-10 years of adjuvant endocrine therapy, which has been shown to reduce recurrences and improve survival. Unfortunately, up to 40% of patients may not take the prescribed medication daily or may discontinue it early. Mobile health technology provides an opportunity to develop new innovative tools to identify women who are not taking medication as prescribed, to understand their barriers for adherence and to facilitate communication with providers to improve adherence.

Methods: The objective of the BETA study was to develop a new bi-directional text messaging application that simultaneously assesses patient adherence to endocrine therapy and provides direct communication to the provider team. Our primary endpoint was to assess feasibility of the application and the secondary endpoints included adherence, side effects and their severity, and quality of life (QOL). The intervention consisted of 3 types of text messages to which patients responded: 1) daily, evaluating adherence, 2) weekly, evaluating medication-related side effects and their severity, and 3) monthly, evaluating barriers to taking the medication. After 3 months of participation, patients completed surveys assessing the tolerability and financial burden of the intervention and adherence to medication. Patients were eligible if they had stage I-III, HR-positive breast cancer, owned a cell phone, and were initiating endocrine therapy. Target enrollment is 100 patients. For comparison, 100 consecutive patients meeting the above criteria were identified retrospectively as historical controls; adherence was assessed via chart review.

Results: Between November 2014 and May 2015, 62 patients (mean age 53.5 years) were enrolled and 25 had completed the study. Of those approached, 66% participated. Of those who completed the study, the application was found to be helpful by 63%; specifically, 76% felt the intervention was a reminder to take the medication, 96% felt it was easy to use, and 71% wanted to continue receiving text messages after the study ended. On average, patients spent 12 minutes with the application per week, 0% felt it took up too much time, and only 1 patient incurred text messaging fees. No patients withdrew from the study and only 1 patient did not adhere to treatment (as defined by ≥ 80% adherence). None of the enrolled patients discontinued endocrine therapy, compared to 9% of historical controls. Side effects were common: hot flashes/night sweats (61% of patients), joint aches/pains (56%), and vaginal symptoms (29%) were reported. Severe side effects (reported by 29% of patients) prompted a return phone call to the patient. The study is ongoing and final results will be available by December 2015.

Conclusion: We developed a new bi-directional text messaging intervention to assess adherence to endocrine therapy that provides real-time feedback to providers. Patients found the application helpful, easy to use, and not time consuming. Our tool is scalable for large population-based trials.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-11-04

Title: A focus group study on women and physicians' receipt of a prescription assistance software dedicated to personalized breast cancer risk assessment and prevention

Pourtau L, Ragusa S, Gauthier E, Taleb S, Tlemsani C, Helin V, Yung M-F and Delaloge S. Gustave Roussy, Personalized Prevention Unit, France and Statlife, Villejuif, France.

Body: Background: Mass screening and mass prevention interventions have as yet been moderately efficient in breast oncology. "Personalized prevention" including risk communication, personalized screening and primary prevention recommendations is very promising. Women's and physicians awareness about individual breast cancer (BC) risks and personalized prevention are heterogeneous and generally low. Prevention is a time-consuming activity for GPs. We have developed a physician-oriented prescription assistance software (PAS) dedicated to personalized breast cancer risk assessment and prevention. Objectives: the main objectives of this focus group (FG) study were to evaluate women's and physician's receipt of BC risk evaluation, of personalized prevention in this field, and of the usefulness of the proposed PAS.

Methods: Women were recruited through "Les Seintinelles" website to participate to 4 FG. The only requirement was that they had not been treated for cancer. Radiologists, gynecologists were recruited for 4 other physician-oriented FG. During each FG, a short overview on recent knowledge in the field was provided, the PAS presented and a structured discussion conducted by a senior sociologist. All participants were question about risk evaluation, risk perception, their view on personalized prevention and their detailed opinion on several aspects of the PAS (graphics, velocity, perceived usefulness, expectations…). All FG were transcribed and analysed.

Results: Women globally considered positively the personalized prevention concept, though some of them feared under-evaluation. Most women accepted BC risk assessment well, if transmitted by a (their) physician and accompanied with practical prevention advice. Most women considered risk communication through a number (percentage) inadequate, while the assignment to a general risk category seemed much more acceptable (low, moderate, high, very high). They considered the PAS may be useful, but mostly for non-specialist physicians. They globally trust their physician more than any software. They acknowledge the use of such tool to guide decisions anyway. Physicians also globally acknowledged the concept of personalized prevention. They considered BC risk must be communicated by physicians. They were very uncomfortable with risk communication if numbers were to be used, whereas categories were again more acceptable. GPs were more interested than specialists in the PAS, some of the latter considering that they did not absolutely need such tool in today's practice. Physicians were worried about the potential legitimacy conflict between themselves and the PAS regarding individual decisions, as well as patients' anxiety and loss of trust it might be associated with. Shared decision was rarely mentioned.

Conclusion: The receipt of breast cancer risk assessment and personalized prevention information through a PAS is good among women and physicians. Both considered this PAS shall remain in the medical domain. Individual risk should be verbalized as categories. To be largely used, such PAS must avoid potential conflicts with the physicians' legitimacy.
Title: Making a difficult conversation easier: Estimating and explaining scenarios for survival time in patients with HER2 positive, metastatic breast cancer

Vasista A, Stockler M, West T, Wilcken N and Kiely B. NHMRC Clinical Trials Centre, Sydney, NSW, Australia; Sydney Medical School, University of Sydney, Sydney, NSW, Australia and Westmead Hospital, Sydney, NSW, Australia.

Body: Aim
Advances in the treatment of HER2 positive metastatic breast cancer (MBC) have resulted in significant improvements in survival time. Therefore, when estimating survival time, HER2 positive MBC now needs to be considered as a separate entity to HER2 negative MBC. Using data from trials of HER2 targeted therapies for HER2 positive MBC, we aimed to estimate worst-case, typical, and best-case scenarios for survival time to provide oncologists with a simple method to estimate and explain survival time to their patients in this situation.

Method
We sought randomised trials of HER2 targeted therapies in MBC published from 2001 to 2014. We recorded median progression free survival (PFS), median overall survival (OS), and extracted the following percentiles (represented scenario) from each OS curve: 90th (worst-case), 75th (lower-typical), 25th (upper-typical), and 10th (best-case). We also estimated these scenarios for each OS curve by multiplying its median by four simple multiples: 0.25 (worst-case), 0.5 (lower-typical), 2 (upper-typical), and 3 (best-case). Estimates were deemed accurate if within 0.75 to 1.33 times the actual value.

Results
We reviewed 10 first-line and 5 subsequent-line trials of HER2 targeted therapies: 8 of trastuzumab, 6 of lapatinib, 2 of trastuzumab-emtansine (TDM-1), 1 of pertuzumab and 1 of neratinib. The mean for median PFS was 11.1 months (interquartile range [IQR], 8.1 – 12.9) for first-line trials and 5.6 months (IQR 3.6 -6.6) for subsequent-line trials. The scenarios for overall survival are tabulated. Follow-up was insufficient for the best-case scenario (10th percentile) to be extracted from any curve. Simple multiples of the median OS provided accurate estimates of the worst-case scenario in 75% of OS curves, lower-typical in 100% and upper-typical in 88%. Characteristics associated with a longer median OS in first-line trials were: dual HER2 targeted therapy (adding a second agent increased the median OS by 25 months [95% CI 12-38, p = 0.001], and more recent year of publication (for each 1 year increase in year of publication, median OS increased by 1 month, [95% CI 0.2 to 2, p=0.02]

Table 1: Scenarios for overall survival

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>Worst-case (IQR)</th>
<th>Lower-typical (IQR)</th>
<th>Median OS (IQR)</th>
<th>Upper-typical (IQR)</th>
<th>Best-case</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st-line HER2 trials</td>
<td>2676</td>
<td>9.4 (7.7-11.0)</td>
<td>18.8 (16.4 - 20.8)</td>
<td>34.2 (29.1-38.4)</td>
<td>56.2 (47.1-63.3)</td>
<td>Not evaluable</td>
</tr>
<tr>
<td>Subsequent-line HER2 trials</td>
<td>2122</td>
<td>5.7 (4.1-7.3)</td>
<td>10.7 (8.6-12.8)</td>
<td>19.9 (15.2-24.4)</td>
<td>21.7 (20.0-23.0)</td>
<td>Not evaluable</td>
</tr>
</tbody>
</table>

Conclusions
The median OS for women starting first-line HER2 targeted therapies for HER2 positive MBC was on average 34 months. For most OS curves, simple multiples of the median provided accurate estimates of the worst-case (one-quarter of the median) and typical (half to double the median) scenarios for survival. Longer follow-up is needed to determine the best-case scenario, which is likely to be greater than triple the median OS. For a woman starting first-line HER2 targeted therapy, survival time could be explained as a worst-case scenario of less than 9 months, typical scenario of 18 months to 4.5 years, and a best-case scenario of 8 years or longer. This framework will help oncologists discuss survival times with their patients.
Title: The attitudes of breast cancer patients and their accompanying caregivers to the disclosure of cancer information in Nigeria

Nwankwo KC C and Okwor V. University of Nigeria,Nsukka, Enugu, Nigeria and University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, Nigeria.

Body: Background: The cancer disclosure practice of some physicians is influenced by the opinions of the accompanying caregivers.
Objective: to study and report the attitudes of breast cancer patients and their accompanying caregivers to disclosure of cancer information to the patients.
Methods: Interviewer administered questionnaire was used on all the consenting breast cancer patients that presented for radiotherapy and their accompanying caregivers between September 2012 and May 2014. The data collected were recorded and analyzed using Statistical Package for Social Sciences (SPSS) PASW Statistics 18.
Results: Out of 315 breast cancer patients that presented for radiotherapy, 285 were eligible for survey. The mean age of the accompanying caregivers was less than the patients (41 years versus 48 years) and proportionately more females than males. Almost all the patients preferred full disclosure of cancer diagnosis to the cancer patients as opposed to the accompanying caregivers (96.0% versus 71.5%, p<0.001). Less number of patients and accompanying caregivers prefer disclosure of terminal prognosis to the patients (65.3% versus 34.2%). When the family insist that the doctor should not disclose the information to the patient, most of the patients disagreed significantly more than the accompanying persons (93.1% versus 71.0%, p<0.002).
Conclusion: Almost all the breast cancer patients desire disclosure of their cancer information unlike their accompanying caregivers. The physicians that rely on the opinions of the accompanying caregivers to decide on the information to disclose to the patients should have a change of attitude.
Key words: cancer patients, caregivers, disclosure, diagnosis, prognosis.
Body: Background: Due to insufficient data regarding patient needs and preferences, health professionals remain concerned about correct the amount, type and extent of information to be communicated to cancer patients. The study intended to explore whether and to what extent patients want to participate in treatment decision making if provided with complete diagnostic and treatment information and clearly defined goals. 

Aim: The objective of this study was to assess the needs and preferences of breast cancer patients for information regarding their disease and to explore preferences for involvement in treatment decision making among patients with cancer. 

Methods: The study was carried out at a regional cancer centre and a tertiary care hospital in Multan. Patients were interviewed using a structured pretested questionnaire to determine their desire and preferences for information about their illness, selected from a heterogeneous sample of 232 individuals visiting Multan Institute of Nuclear Medicine and Radiotherapy and Nishtar Medical College Hospital, Multan.

Results: Two hundred thirty two patients having a mean age of 44.44±17.24 years participated in the study. About two third of the patients (71.9%) wanted to know all the information about their condition regardless of its nature, good or unfavorable. Information about prognosis of disease and chances of cure was desired by 94.3% of the respondents. Most of the patients wanted to know about all the possible treatments (68.7%), about the action of treatment in body (57.6%) and its side effects (68%). Majority of the patients interviewed were likely to let the physician make decisions regarding their disease management. The overall proportion of patients preferring active, collaborative and passive roles were 35.4%, 2.6% and 62% respectively. Majority of the patients thought that cancer patients should be involved in decisions regarding their treatment, although paradoxically 75% were of the view that all the cancer patients do not have the ability to get involved in deciding about their treatment. Half of the patients (50.4%) opined that if a patient does not want to be involved in deciding about treatment, the physicians should nevertheless try to involve him in deciding about his treatment.

Conclusions: Majority of the patients with cancer want to know about their diagnosis, effect of illness on daily functioning, prognosis and examples of cases in which treatment they are receiving was effective. The results of the study suggest that oncologists should individually assess each patient to determine the type of role they prefer in making decisions about their treatment.
Title: Adjuvant endocrine therapy and risk of contralateral breast cancer among a cohort of U.S. women with breast cancer

Gierach GL L, Curtis RE E, Pfeiffer RM M, Mullooly M, Hoover RN N, Nyante SJ J, Feigelson HS S, Glass AG G and Berrington de Gonzalez A. National Cancer Institute, Bethesda, MD; University of North Carolina at Chapel Hill, Chapel Hill, NC; Kaiser Permanente Institute for Health Research, Denver, CO and Kaiser Permanente Northwest Center for Health Research, Portland, OR.

Body: Background: The increasing incidence of estrogen receptor (ER)-positive breast cancer in the U.S. in concert with the aging population and improved survival have resulted in an increased number of women at risk of developing a second contralateral primary breast cancer. Results from randomized clinical trials have suggested a reduced risk of contralateral breast cancer among women taking tamoxifen or aromatase inhibitors. However, little is known about the duration of beneficial effects of endocrine therapy within the context of real life treatment scenarios, where gaps in treatment and varying durations of use may influence risk.

Methods: We assessed contralateral breast cancer risk associated with adjuvant tamoxifen treatment among a cohort of 7,541 women, ages 24-85 years, who were members of Kaiser Permanente (KP) Northwest or Colorado, and were diagnosed with invasive breast cancer between 1990 and 2008 and remained at risk of contralateral breast cancer for at least one year. We also assessed risk in relation to aromatase inhibitor use, though statistical power was somewhat limited due to the relatively recent introduction of aromatase inhibitors in this older cohort. Use of tamoxifen, aromatase inhibitors and other treatments was ascertained from KP prescription and medical records. Relative risks (RR) and 95% confidence intervals (CI) were estimated using multivariable Poisson regression adjusting for study site, age at and year of diagnosis, stage at diagnosis, ER status, chemotherapy, and radiotherapy.

Results: Over a median (range) of 6.3 (1.0-20.9) years of follow-up, 248 women developed contralateral breast cancer. Among patients surviving at least five years (n=4,668), 58% were prescribed tamoxifen with a median (range) duration of use of 4.2 (0.25-16.2) years. In models evaluating joint effects of tamoxifen duration and time since last use, we observed a statistically significant reduced risk of contralateral breast cancer among current tamoxifen users (RR=0.47, 95% CI: 0.30, 0.74) and among former users with 4+ years of tamoxifen (RR=0.39, 95% CI: 0.24, 0.63) as compared with women not treated with tamoxifen. Former users with 1-4 years of tamoxifen demonstrated a suggestive reduction in risk (RR=0.71, 95% CI: 0.45, 1.10), but there was no evidence of risk reduction for former users with <1 year of tamoxifen (RR=0.96, 95% CI: 0.56, 1.64). The reduced risks associated with 4+ years of tamoxifen persisted among patients surviving at least 7 years but were attenuated among those with more than 10 years since their first primary diagnosis. Aromatase inhibitor use was also associated with reduced contralateral breast cancer risk (RR=0.46, 95% CI: 0.22, 0.97). In subgroup analyses restricted to women whose first primary cancer was ER-positive (n=5,951), findings were consistent with those observed in the overall cohort.

Conclusions: Adjuvant tamoxifen and aromatase inhibitor therapy considerably reduce the risk of contralateral breast cancer. Furthermore, our data suggest that tamoxifen protects against contralateral breast cancer while women are being treated and that the protective effect appears to continue after cessation with longer durations of use.
Title: Effect of 5 vs 2.5 mg/day letrozole on residual estrogen levels in post-menopausal women with high BMI - A prospective crossover study

Body: Background: Some studies have suggested that women with high BMI have less benefit from aromatase inhibitors (AI) vs. tamoxifen as adjuvant treatment for early breast cancer. One possible mechanism for this observation is that complete suppression of estrogen is not achieved in these women with the standard flat dose of AI. We evaluated whether a doubling of letrozole to 5 mg/day for 4 weeks affected residual estrogen levels in this population.

Methods: Post-menopausal women with early breast cancer and BMI>25 already taking adjuvant letrozole for at least 3 months were recruited from medical oncology clinics at 4 sites in Toronto, Canada. Fasting blood samples were collected 24 hours following the last dose at baseline (routine use of own letrozole), after 28 days of monitored adherence to a provided supply of letrozole (Femara) 2.5 mg/day (Part A), and after an additional 28 days of letrozole (Femara) 5 mg/day (Part B). Symptom/quality of life questionnaires were completed at the same timepoints. Estradiol and estrone were measured using a high sensitivity liquid chromatography-tandem mass spectrometry assay. One interim analysis for futility and efficacy was planned after 31 eligible patients had completed the study, using estradiol and O'Brien-Fleming boundaries with an inner wedge.

Results: 36 patients were enrolled and started on study, and 31 eligible patients completed Parts A and B. The 5 non-completers withdrew because of adverse events (n=4, unlikely related to drug) or withdrawal of consent (n=1). Median age was 62 (range 48 to 77) and BMI 28.3 kg/m\(^2\) (Range 25.2 to 42.2 kg/m\(^2\)). One patient had non-postmenopausal estrogen levels at Day 29 and Day 57 and one patient's blood assay was unsuccessful; both were excluded from further analyses. The predetermined stopping rule for futility was met. Estradiol levels (mean±standard deviation) changed from 2.68±0.40 pg/mL at baseline to 2.67±0.59 pg/mL at Day 29 to 2.70±0.53 pg/mL at Day 57. Mean change from Day 29 to Day 57 was 0.03±0.48 pg/mL (95% confidence interval -0.15 to 0.21 pg/mL). Four patients reported new or increased arthralgias (to NCI CTCAE Grade 2 or 3) while taking letrozole 5 mg/day in Part B. There was no association between changes in estradiol levels and either study non-completion or the development of arthralgias. Estrone results were similar.

Conclusion: Increasing letrozole from 2.5 to 5 mg/day did not further suppress estrogen levels in women with BMI>25. It is unlikely that letrozole dosing tailored to body size would improve clinical outcomes. The letrozole 5 mg/day intervention was terminated based on the results of the interim analysis for futility.
Prevalence of bone loss among nonmetastatic breast cancer patients treated with aromatase inhibitors in the United States

Pirolli M, Hernandez RK K, Reich A and Liede A. IMS Health, Plymouth Meeting, PA and Amgen Inc., CA.

**Background:** Adjuvant endocrine therapy compromises bone health in patients (pts) with breast cancer, leading to osteopenia, osteoporosis, and fractures (Forbes 2008). For postmenopausal women with estrogen receptor (ER) positive (+ve) breast cancer, aromatase inhibitors (AI) have emerged as the standard of care because of superior efficacy over selective ER modulators such as tamoxifen, shown in several large clinical trials. As rates of utilization and duration of AI therapy are expected to continue to increase, we report estimates of the prevalence of women with nonmetastatic breast cancer treated with AI in the United States and examine evidence of bone loss before and after AI exposure.

**Methods:** The Oncology Services Comprehensive Electronic Records (OSCER) database, an electronic medical record database on >500,000 cancer pts from oncology practices across the US, was used to identify women with breast cancer (ICD-9 174*), ≥1 clinic visit, and confirmed AI therapy (anastrozole, letrozole, or exemestane) in 2014, excluding pts with evidence of metastases (ICD-9 196-198, stage IV, or M1 disease). OSCER is projected nationally through methods of direct estimations utilizing claims data for 1-year period prevalence. Bone loss was defined by diagnosis of osteoporosis (ICD-9 733.0), osteopenia (ICD-9 733.90), or receipt of bone therapy in 2014. Bone therapies used as proxy for bone loss were intravenous (IV) (ibandronate, zoledronic acid), or oral bisphosphonates (BPs) (risedronate, ibandronate, etidronate, alendronate), or subcutaneous anti-RANK ligand antibody (denosumab [60 mg Q6M]).

**Results:** It is nationally estimated that 538,630 (95% CI 519,839 - 557,422) women with nonmetastatic breast cancer were treated with an AI in 2014, representing a median of 6 prescriptions (mean 6.8). Of these, most (94%) were treated with anastrozole or letrozole, 55% were ≥65 years, and 11% took tamoxifen prior to first AI. Among women taking AI in 2014, 39% started in 2014, 24% started in 2013, and 37% have been on AI therapy since 2012 or earlier. Overall, 285,543 (53%) pts on AI therapy had evidence of bone loss, 30% of whom developed bone loss after exposure to AI therapy. Among the 338,784 women with no evidence of pre-existing bone loss, 25% (85,697) developed bone loss after initiating AI therapy (table), with 21% treated with oral and 1% IV BPs, or 9% with denosumab.

<table>
<thead>
<tr>
<th>2014 prevalence</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmetastatic pts on AI therapy</td>
<td>538,630</td>
</tr>
<tr>
<td>At least 2 AI prescriptions in lifetime</td>
<td>468,608 (87)</td>
</tr>
<tr>
<td>Bone loss before starting AI</td>
<td>199,846 (37)</td>
</tr>
<tr>
<td>No bone loss before AI, with bone loss after AI</td>
<td>85,697 (16)</td>
</tr>
</tbody>
</table>

**Conclusions:** This study provides current estimates of the prevalence of nonmetastatic breast cancer treated with AI in the US using real-world data, indicating that more than 535,000 women were treated with AI in 2014. Considering that 37% of pts have been on endocrine therapy since 2012 or earlier, continued use of AI will likely lead to increased pts with bone loss. These women face an increased risk of fractures, highlighting the need for intervention with antiresorptive treatments (i.e., BPs have been studied, and denosumab has a labeled indication in this setting) that may help build bone mass and counteract detrimental effects on the bone.
Title: Directed exercise intervention in breast cancer patients with arthralgias receiving aromatase inhibitors: A randomized pilot study

Varadarajan R, Helm E, Arnold C, Huelsenbeck-Dill L, Ingraham-Lopresto B, Sonaad S, Swanson P, Sims-Mourtada J and Dickson-Whitmer D. Helen F Graham Cancer Center, Christiana Care Health Services, Newark, DE.

Body: Purpose: Early discontinuation or non-adherence of AI therapy occurs in 25-50 % of patients due to treatment associated toxicities, including musculoskeletal symptoms such as arthralgias and myalgias. The goal of this pilot study is to evaluate the effect of a directed physical therapy regimen including joint mobility and stretching exercises on musculoskeletal symptoms resulting from AI therapy, in comparison to normal physical activity alone.

Patients and methods: Eligibility criteria included post-menopausal women with histological evidence of hormone receptor positive breast cancer who were currently receiving adjuvant AI therapy and experiencing significant joint discomfort/stiffness when attempting activities of daily living which began or significantly increased after initiation of AI therapy. Patients were excluded if they had preexisting rheumatoid arthritis or fibromyalgia, systemic metastasis or ECOG performance status of greater than 2. Patients were randomly assigned to an 8 week directed exercise program under the supervision of a physical therapist (Group A, n=15) or were told to participate in moderate physical activity (walking) (Group B, n=12). Before beginning the study and at the end of the 8 week intervention, patients in both groups underwent a physical therapy evaluation including performance based measures such as functional lower extremity strength and grip strength and a Patient Specific Functional Scale (PSFS) to assess functional ability to complete specific activities. Additionally, all patients answered a series of questionnaires including a pain disability index (PDI), pain scale (PS) and the PHQ4 depression scale. Effects of the directed exercise regimen were evaluated using non-parametric analysis to determine differences between the two groups.

Results: Significant improvement was observed in both left and right grip strength and right pinch in the intervention group (Group A) as compared to the control group (Group B). Additionally, significant improvement in lower body measures including 2-minute step tests and chair raises were observed in patients who underwent the directed exercise program compared to the control group. No significant differences were observed in PS, PDI or PHQ4 between the groups. However, Group A showed a slight improvement on the PS and PDI.

Conclusions: Directed exercise regimens involving joint mobility and stretching exercises may have benefits over moderate physical activity alone for joint pain and arthralgia associated with AI therapy.
Title: CYP3A4*22 polymorphism is associated with increased exemestane concentrations in postmenopausal breast cancer patients


Body: Background: Exemestane is a second generation steroidal aromatase inhibitor (AI) used for the treatment of estrogen receptor (ER) positive breast cancer in postmenopausal women. Variability in AI treatment efficacy and side effects seen across patients may be due, in part, to inter-patient differences in drug exposure. This exposure variability is likely caused by patient genetics factors, such as single nucleotide polymorphisms (SNPs) in drug metabolizing enzymes, or clinical factors such as patient body size, organ function, and comorbidities. The objective of this secondary correlative analysis was to identify genetic and clinical characteristics that affect steady state exemestane concentration, with a specific focus on the influence of inherited genetic variants and baseline hepatic function.

Methods: 500 patients were enrolled on the Exemestane and Letrozole Pharmacogenetics (ELPh) Study and randomized to either drug. Clinical data and DNA were collected at baseline and blood samples were collected after 1 or 3 months of treatment to measure steady-state exemestane concentration via HPLC/MS. Genotyping was performed on a custom Sequenom MassARRAY iPLEX that included the recently discovered low activity CYP3A4*22 (rs35599367) SNP and several other SNPs with putative functional consequence in enzymes thought to be involved in exemestane metabolism (CYP1A1/2, CYP1B1, CYP3A4, CYP4A11, AKR1C3/4, AKR7A2). Our primary hypothesis was that patients carrying CYP3A4*22 variants would have higher serum exemestane concentrations. Other SNPs and clinical characteristics (hepatic and renal function, age, body mass index (BMI), time of sample collection, prior chemotherapy) were assessed for independent association, and then adjusted for in a multivariable tobit regression model for CYP3A4*22 on log-transformed censored exemestane concentration.

Results: 246 (225 randomized to exemestane arm, 21 crossed-over from letrozole arm) patients had exemestane steady state levels and were evaluable in this analysis. As hypothesized, the CYP3A4*22 polymorphism (minor allele frequency=0.06) was associated with a 54% increase in exemestane concentration (95% CI: 14% - 109%, p<0.01). Exemestane concentration was 44% greater in patients who had evidence of hepatic impairment (AST or ALT>40) at baseline (95% CI: 2% - 104%, p=0.02), 1% lower per unit increase in BMI (95% CI: 0% - 3%, p=0.05), and 20% lower in patients who received prior chemotherapy (95% CI: 4% - 34%, p=0.03). Age, renal impairment, and other SNPs were not associated with exemestane concentration. After adjustment for significant clinical covariates the CYP3A4*22 SNP remained significant (p<0.01).

Conclusions: Genetic and clinical predictors of exemestane concentration were discovered in a large cohort of prospectively enrolled estrogen responsive breast cancer patients. Ongoing analyses will determine whether the variability in exemestane concentration was associated with downstream effects on estrogen depletion or treatment-related toxicity. If so, these genetic and clinical characteristics could be useful for individualizing dosing of exemestane to ensure that all patients are receiving maximal benefit with minimal toxicity.
Title: Comprehensive assessment of the effect of genetic polymorphisms in drug metabolizing enzymes and transporters on tamoxifen activation to endoxifen

Hertz DL L, Danko W, Deal A, Walko CM M, Flockhart DA A, McLeod HL L, Ibrahim JG G and Irvin Jr WJ J. University of Michigan, Ann Arbor, MI; University of North Carolina, Chapel Hill, NC; Moffitt Cancer Center, Tampa, FL; Indiana University and Bon Secours Cancer Institute, Richmond, VA.

Body: Background: Tamoxifen is the most commonly prescribed hormonal drug for estrogen receptor positive breast cancer treatment. Tamoxifen itself has weak anti-estrogenic activity, but is bioactivated to the more potent inhibitor endoxifen. Recent data suggest inferior efficacy of tamoxifen treatment in patients who have low systemic endoxifen concentration. Genetic variability in drug metabolizing enzymes and transporters, particularly CYP2D6, are known to effect serum endoxifen concentration. The association of CYP2D6 genotype and endoxifen concentration is well established; however, there is a paucity of data regarding the effects of genetic variants in other drug metabolizing enzymes and transporters on endoxifen concentrations. The objective of our study was to comprehensively screen known, functionally consequential polymorphisms and copy number variations in genes of interest to detect additional pharmacogenetic predictors of endoxifen concentration during tamoxifen treatment.

Methods: This analysis includes patients prospectively enrolled on the Lineberger Comprehensive Cancer Center 0801 trial. Patients had received tamoxifen for a minimum of 4 months prior to enrollment and were not concurrently taking strong or moderate CYP2D6 inhibitors. Samples were collected at enrollment for measurement of steady state endoxifen level and collection of germline DNA. Genotyping was performed for CYP2D6 using the Amplichip® CYP450 test (Roche Diagnostics) and for other candidate genes (CYP2C9, CYP3A4, CYP3A5, ABCB1, SLCO1B1, SULT1A1, SULT1A2, and UGT2B7) using the iPLEX® ADME PGx Pro Panel (Agena Bioscience). Activity phenotype for each gene was inferred from genotype data based on known activity of variant alleles or copy numbers. Metabolite concentrations were measured via LC/MS-MS assay at Indiana University and square root transformed prior to analysis to improve normality. Linear regression models were used to evaluate the association of each gene individually with endoxifen concentration, assuming an additive pharmacogenetic effect, after adjustment for CYP2D6 phenotype (EM/UM, IM or PM).

Results: 304 Patients with steady-state endoxifen concentration and successful genotyping were included in the analysis. After transformation and adjustment, endoxifen concentration was significantly associated with carrying low-activity CYP2C9 variant alleles (*2, *3, *5, *6, *8, *11, *12) (p=0.016). Predicted endoxifen concentration based on CYP2C9 and CYP2D6 genotype can be found in

<table>
<thead>
<tr>
<th>CYP2C9 Phenotype</th>
<th>CYP2D6 EM/UM</th>
<th>CYP2D6 IM</th>
<th>CYP2D6 PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 WT/WT</td>
<td>9.71</td>
<td>6.55</td>
<td>3.41</td>
</tr>
<tr>
<td>CYP2C9 WT/Var</td>
<td>8.37</td>
<td>5.48</td>
<td>2.63</td>
</tr>
<tr>
<td>CYP2C9 Var/Var</td>
<td>7.13</td>
<td>4.48</td>
<td>1.96</td>
</tr>
</tbody>
</table>

Abbreviations: WT = wild-type, Var = Variant allele for CYP2C9

Conclusions: Polymorphisms in CYP2C9 and CYP2D6, but not other enzymes or transporters, contribute to variation in endoxifen exposure. If endoxifen exposure is validated to predict tamoxifen efficacy, personalized tamoxifen dosing algorithms should include CYP2C9, in addition to CYP2D6 and clinical factors, to improve efficacy and minimize side effects.
Body: Background: Luteinizing hormone-releasing hormone agonist + tamoxifen is standard postoperative adjuvant endocrine therapy for premenopausal patients with hormone receptor-positive breast cancer. Postoperative adjuvant endocrine therapy is now used for a longer period, and the longer-lasting leuprorelin acetate 6-month depot formulation (TAP-144-SR[6M]) is expected to increase patients' quality of life and decrease medical practitioners' burden.

Methods: The hormone dynamics, pharmacokinetics (PK), safety, and efficacy of TAP-144-SR(6M) were compared with those of the 3-month depot formulation (TAP-144-SR[3M]) in a 96-week, phase 3 open-label parallel-group comparison study in premenopausal breast cancer patients after surgery (ClinicalTrial.gov ID: NCT01546649). Inclusion criteria were estrogen receptor (ER) and/or progesterone receptor (PgR) positive; TNM classification of T1-T3, any N, M0; and premenopausal (menstruation confirmed within the previous 12 weeks or both follicle-stimulating hormone [FSH] <40 mIU/mL and estradiol [E₂] ≥10 pg/mL at enrollment). Patients were randomized to TAP-144-SR(6M) (6M group [6MG]) or TAP-144-SR(3M) (3M group [3MG]) based on number of axillary lymph node metastases, tumor size, age, ER/PgR status, chemotherapy or not, and study site. The primary endpoint was serum E₂ suppression rate based on the menopausal level (≤30 pg/mL) from 4 to 48 weeks after the first administration. Secondary endpoints were serum hormone dynamics, efficacy (disease-free survival [DFS] and distance DFS [DDFS]), PK and safety. The planned number of patients was 164 (82 in each group).

Results: A total of 180 patients were enrolled from Apr 2012 to Feb 2013 and 167 patients were randomized. We compared 83 patients in 6MG (age: mean 44.2; SD 4.90) and 84 patients in 3MG (44.0; 5.18). There were no significant differences in background factors between the groups. 6MG showed non-inferior suppression of serum E₂ levels to 3MG (See Table). Serum LH and FSH levels were also decreased. DFSs and DDFSs at 96 weeks after randomization were similar in both groups. A double-peak PK profile and sustainable release of the study drug for 24 weeks were found with 6MG. All-grade adverse events (AEs) occurred in 98.8% and 97.6% and grade 3 or higher AEs in 18.1% and 21.4% with 6MG and 3MG, respectively. There were no significant differences in lumbar spine bone mineral density change rates in both groups.

Table Serum E2 suppression rate based on the menopausal level (≤30 pg/mL) from 4 to 48 weeks after the first administration

<table>
<thead>
<tr>
<th></th>
<th>6MG (n = 83)</th>
<th>3MG (n = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum E2 suppression rate (%) (95% CI)</td>
<td>97.6 (91.565, 99.707)</td>
<td>96.4 (89.916, 99.257)</td>
</tr>
<tr>
<td>6MG – 3MG (95% CI)</td>
<td>1.2 (−5.241, 7.806)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Noninferiority margin of 10%.

Conclusion: This first clinical study of TAP-144-SR(6M) in premenopausal breast cancer patients showed clinically noninferior serum E₂ suppression levels to TAP-144-SR(3M), and no significant safety differences between the groups. TAP-144-SR(6M) was confirmed to have excellent usability in premenopausal breast cancer patients after surgery, and is considered valuable for the appropriate treatment of these patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-12-08

Title: Time course of changes in serum FSH, serum estradiol, and menstruation in premenopausal patients with breast cancer taking tamoxifen after completing chemotherapy: A report from the ASTRRA study

Kim HJ, Ahn SH, Nam SJ, Park SH, Ro JS, Im SA, Jung YS and Noh WC. Division of Breast and Endocrine, College of Medicine, University of Ulsan, Asan Medical Center; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; Yonsei University College of Medicine, Seoul, Republic of Korea; Center for Breast Cancer, National Cancer Center, Goyang, Republic of Korea; Seoul National University Hospital, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; Ajou University, School of Medicine, Suwon, Republic of Korea and Korea Cancer Center Hospital, Korea Institute of Radiological and Medical Sciences, Seoul, Republic of Korea.

Body: BACKGROUND
Ovarian suppression with tamoxifen after chemotherapy is a promising therapeutic approach, particularly in young, high-risk breast cancer patients. Assessment of restoration of ovarian function is important with respect to the initiation of ovarian suppression.

METHODS
In total, 1289 women who remained or resumed premenopausal status after chemotherapy were randomized to receive 5 years of tamoxifen or 5 years of tamoxifen plus 2 years of ovarian suppression. Prospectively collected hormone data were available for 24 months after completing chemotherapy for 267 breast cancer patients without ovarian suppression.

RESULTS
At 6 months, a premenopausal status was identified in 56.6%, 36%, and 16.2% of patients using serum FSH, E2, and with menstruation bleeding, respectively, and about 30% more women achieved ovarian restoration using all three parameters during the 24-month follow-up. Ovarian function restoration differed significantly according to age group (log-rank, \( P<0.001 \) for all definitions). At 6 months, the distribution of patients according to hormone levels was as follows: group 1 (FSH <30 mIU/ml, E2 >20 pg/ml), 28.0%; group 2 (FSH <30 mIU/ml, E2 \( \leq \) 20 pg/ml), 28.4%; group 3 (FSH \( \geq \) 30 mIU/ml, E2 >20 pg/ml), 8.0%; and group 4 (FSH \( \geq \) 30 mIU/ml, E2 \( \leq \) 20 pg/ml), 35.6%. During the 24-month follow-up, the prevalence of menstruation restoration was higher in group 1 (71.6%) than in the other three groups. Restoration of serum E2 and menstrual bleeding occurred in 44% and 33% of patients in group 2, respectively; the corresponding percentages in group 4 were 40.6% and 28.7% (\( P<0.001 \)).

CONCLUSIONS
Ovarian function should be monitored using serum FSH, serum E2, and menstruation history for at least 24 months after completing chemotherapy during tamoxifen treatment to establish eligibility for ovarian suppression.
Title: A novel commercial LC-MS/MS assay for tamoxifen (TAM) and its major metabolites


Body: The standard of care for women presenting with early stage- ER-positive breast cancer (BC) following “curative” surgery has been 5 years of TAM. Adjuvant treatment with TAM has changed the natural history of BC, producing a significant reduction in 5- and 10-year recurrence rates; however, because of its adverse effects, many women (approx. 40%) do not complete the recommended 5 years of treatment. Furthermore, since TAM is a pro-drug that needs to be converted to endoxifen to be effective, inter-individual variability in endogenous enzymatic activity (i.e., CYP2D6) can affect endoxifen exposure. Certain drugs (e.g., SSRIs) can also reduce endoxifen exposure by inhibiting CYP2D6.

It is thought that a reduction in endoxifen exposure reduces the efficacy of TAM treatment and increases recurrence risk. However, several recent studies contradict this hypothesis and suggest that a) there is more than one pathway to get to endoxifen, even in the presence of variant CYP2D6; and b) TAM may act through its other metabolites as well, not just endoxifen. Thus, a CYP2D6 genetic test may overly simplify our understanding of TAM metabolism and prompt clinicians to draw the wrong conclusions. As such, it would seem useful to develop an assay to directly measure each patient’s unique serum metabolite levels. The ability to quantitate all the major TAM metabolites would also allow researchers to assess which metabolite level(s) most closely correlates with both recurrence and toxicity, allowing individualized patient dosing. In this regard, results from the BIG1-98 study suggest that some metabolites may be more closely associated with adverse effects than others. This finding could be clinically useful when combined with outcomes data, as poor adherence to TAM may be an unrecognized reason for a number of recurrences that could potentially be avoided by therapeutic drug monitoring (TDM) using a sensitive and specific assay.

With this in mind we developed an HPLC-MS/MS method that quantitatively measures TAM and 6 of its major metabolites in a single assay. This high-throughput assay has been validated to CLIA ’88 standards and is run in a high-volume commercial CLIA certified laboratory. Although some of the metabolites had been measured previously by HPLC or LC-MS/MS, this is the first assay that measures all of the major metabolites, including the newly identified norendoxifen.

Results

Serum from 100 women taking TAM at 20 mg/d for > 6 months was tested using this assay, and observed ranges were calculated for this patient cohort. The observed ranges from the analysis are shown in Table 1, along with the lower limit of quantitation (LLOQ) for each analyte.

Table 1. Observed ranges and LLOQs for Tamoxifen and Metabolite

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LLOQ (ng/mL)</th>
<th>Observed Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endoxifen</td>
<td>0.4</td>
<td>0.93-43.19</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>1.5</td>
<td>12.5-233.1</td>
</tr>
<tr>
<td>N-Desmethyl Tamoxifen</td>
<td>1.5</td>
<td>3.0-374.0</td>
</tr>
<tr>
<td>4-Hydroxy Tamoxifen</td>
<td>0.2</td>
<td>0.24-5.05</td>
</tr>
<tr>
<td>N-Desmethyl 4'- Tamoxifen</td>
<td>0.4</td>
<td>1.17-19.95</td>
</tr>
<tr>
<td>4'-Hydroxy Tamoxifen</td>
<td>0.4</td>
<td>0.4-6.33</td>
</tr>
<tr>
<td>Norendoxifen</td>
<td>1.2</td>
<td>&lt;7.3</td>
</tr>
</tbody>
</table>

Conclusions
A novel commercial assay has been developed for TAM and its metabolites, which for the first time allows physicians to use a TDM approach for their TAM-treated patients.
Title: Comparison of the efficacy of tamoxifen and aromatase inhibitors on survival in adjuvant menopausal breast cancer


Body: Background

Meta-analyses of postmenopausal endocrine therapy and recent studies in premenopausal women suggest that aromatase inhibitors (AI) may be superior to tamoxifen (T) in preventing recurrence in early hormone receptor positive (HR+) breast cancer (BC), although there are recent concerns about the impact on overall survival (OS). The BC Cancer Agency adopted ASCO guidelines of an AI as part of adjuvant therapy for menopausal HR+ BC in 2003. Using our population based data, we sought to compare the 10 year survival outcomes for patients starting either T or AI following surgery for HR+BC.

Methods

Histopathologic and demographic data were collected for all menopausal patients referred to the BC Cancer Agency with a T1-2, node negative, HR+, HER2 negative BC diagnosed between 01/2003 and 12/2009. Patients with prior or synchronous contralateral BC were excluded. Data was cross-referenced to the provincial pharmacy database, which tracks hormone therapy. Significant factors affecting survival were identified using Cox proportional hazard model for OS and Fine and Gray’s (FG) model for BC specific and Cardiac Specific Survival (CSS) with causes other than event of interest defined as competing.

Results

We identified a cohort of 3421 cases with median follow up of 7.8 years (y) for T and 7.4 y for AI. Median age was 65y in both groups, and 8.5% received chemotherapy. 47.8% of tumors were T1c and 22.2% were T2; 15.8 % were grade 3. 10 year OS was 84.4% (95% Confidence Intervals [CI] 82.4%, 86.2%) and 82.7% (95% CI 79.4%, 85.6%) for T and AI cohorts, respectively, (p=0.02). BCSS did not differ between the groups (p=0.54). We categorized causes of death in each cohort as from BC (20.4% T, 20.6% AI), other cancers (25.4% T, 22.1% AI), cardiovascular-related (CVS) (25.8% T, 34.6% AI), thromboembolic (0.3% T, 0.7% AI %) and other (25.4%T, 19.1 % AI). Table 1 shows Univariate (U), multivariable (M), hazard ratios (Hz) and 95% CI.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>OS</th>
<th>BCSS</th>
<th>CSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>M (KM)</td>
<td>U</td>
</tr>
<tr>
<td>Tumour Size, continuous</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>1.282 (1.142,1.438)</td>
<td>1.882 (0.631,2.090)</td>
<td>1.272 (1.026, 1.577)</td>
</tr>
<tr>
<td>Age at dx,continuous</td>
<td>&lt;0.0001</td>
<td>0.019</td>
<td>0.2637</td>
</tr>
<tr>
<td></td>
<td>1.094 (1.080,1.108)</td>
<td>1.016 (0.988,1.044)</td>
<td>1.152 (1.122,1.183)</td>
</tr>
<tr>
<td>Grade , 3 vs 1</td>
<td>&lt;0.0001</td>
<td>0.0018</td>
<td>0.2993</td>
</tr>
<tr>
<td></td>
<td>1.341 (0.993,1.811)</td>
<td>2.973 (1.501,5.889)</td>
<td>1.051 (0.576,1.915)</td>
</tr>
<tr>
<td>LVI, Yes vs No</td>
<td>0.0004</td>
<td>0.0465</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>1.357 (1.005,1.833)</td>
<td>2.732 (1.637,4.558)</td>
<td>0.474, (0.213,1.054)</td>
</tr>
<tr>
<td>Chemo use, Yes or No</td>
<td>0.099</td>
<td>0.2122</td>
<td>0.0535</td>
</tr>
<tr>
<td></td>
<td>0.748 (0.474,1.180)</td>
<td>1.284 (0.618,2.670)</td>
<td>1.128 (0.264,4.824)</td>
</tr>
<tr>
<td>AI vs T</td>
<td>0.02</td>
<td>0.2927</td>
<td>0.5925</td>
</tr>
<tr>
<td></td>
<td>1.124 (1.142,1.438)</td>
<td>0.742 (0.443,1.241)</td>
<td>1.658 (1.116,2.463)</td>
</tr>
</tbody>
</table>
Conclusion
While trials show that AIs improve relapse free survival after menopausal HR+BC, their impact on BCSS has been minimal. By contrast they may contribute to CVS deaths, as suggested by our data. We plan to explore this observation further by examining baseline cardiac risk factors within our T and AI cohorts, and by exploring OS, BCSS, and CSS among patients switching to AI after starting T, to identify the optimal adjuvant hormone therapy strategy for menopausal women with HR+ early BC.
Title: Duration of ovarian function suppression for premenopausal women with hormone receptor-positive breast cancer: Retrospective study


Body: Background: Although tamoxifen (TAM) plus ovarian function suppression (OFS) is one of standard adjuvant treatments in premenopausal women with hormone receptor-positive breast cancer, the optimal duration of OFS has not been clearly established.

Patients and Methods: We retrospectively reviewed data of premenopausal patients with breast cancer, who received TAM and OFS (goserelin or leuprolelin) as adjuvant therapy between February 2004 and April 2015. The primary analysis was to compare disease-free survival (DFS) between patients who received OFS shorter than 3 years and those who received OFS longer than 3 years. The analyses were performed with Cox proportional hazards models and propensity score matching models.

Results: We analyzed 206 premenopausal patients with hormone receptor-positive breast cancer. Median follow-up time was 56 months. Median age was 42 years (range, 24-52 years). Twenty six per cent of the patients had positive axillary nodes and 30% had received neo-adjuvant or adjuvant chemotherapy. Median duration of OFS was 26 months. Duration of OFS was shorter than three years (OFS < 3y) in 74% patients, and longer than three years (OFS > 3y) in 26% patients. Patients with node-positive disease were more in OFS > 3y group than in OFS < 3y group, and more patients received chemotherapy in OFS > 3y group than in OFS < 3y group. 5-year disease-free survival (DFS) was 96.1%. DFS in patients aged ≤ 40 years and aged > 40 years were 91.8% and 99.0%, respectively (p=0.0223). Propensity score matching model showed that DFS was not significantly different between patients in OFS < 3y group and those in OFS > 3y group (97.4%, 91.6%; p=0.2406). In patients aged ≤ 40 years and/or those who received chemotherapy, 5-year DFS was 96.7% in OFS < 3y group, 90.1% in OFS > 3y group (p=0.3011).

Conclusions: Our data suggest that OFS < 3y is not inferior to OFS > 3y for premenopausal women with hormone receptor-positive breast cancer as adjuvant endocrine therapy. A randomized trial is needed to establish the optimal OFS duration for these patients.
Title: Hormone receptor expression level and nuclear grade associated with late recurrence in estrogen receptor-positive breast cancer patients


Body: Background: It is not known which population of estrogen receptor (ER)-positive breast cancer patients should continue endocrine treatment beyond 5 years to overcome late recurrences. The aim of this study was to examine a combination of nuclear grade (NG) and expression level of ER and progesterone receptor (PR) to predict late recurrences.

Methods: We assessed retrospectively 1677 consecutive ER-positive/HER2-negative patients who underwent surgical resection between 2004 and 2009. Patients with T2 or larger tumor and/or node-positive received pre- or postoperative chemotherapy following the international consensus panel from the St Gallen Conference, 2003. All patients had received adjuvant endocrine treatment. NG, ER and PR statuses were determined by immunohistochemistry on surgical specimen. We classified the patients into 3 groups as follows; ER-high (+++ or Allred score 7, 8)/PR-high (++ to +++ or 5-8) (n=212), ER-high / PR-low (- to + or 3-6) (n=208), and ER-low (+ to ++ or 3-6) / PR-any (n=255). We compared distant disease-free survival (DDFS) in each cohort based on the NG (1:low, and 2 or 3: high).

Results: A median follow-up period was 77.0 months. Four hundred sixty seven patients (27.8%) received neoadjuvant chemotherapy, 208 patients (12.4%) received adjuvant chemotherapy, and 1002 patients (59.8%) did not receive chemotherapy. Of the 467 patients with neoadjuvant chemotherapy, 65 patients (13.9%) had developed distant metastasis during study period (before 5 years in 51 (11.0%); and after 5 years in 14 (3.0%)). NG-low had significantly higher risk of late recurrence after 5 years than that of NG-high (p=0.005). According to hormonal receptor expression levels, in patients with NG-low, ER-low/PR-any had significantly higher overall DDFS rate than ER-high/PR-low (p=0.016). A similar trend was found before 5 years (p=0.077). However, ER-high/PR-high turned to have significantly high risk of recurrence after 5 years compared to ER-low/PR-any (p=0.024). Of the 208 patients with adjuvant chemotherapy, 16 patients (7.6%) had developed distant metastasis during study period (before 5 years in 5 (2.4%), and after 5 years in 11 (5.2%)). There was no association between a risk of recurrence and hormone receptor statuses at any study period. In the patients did not receive chemotherapy, ER-high/PR-high had a trend of higher DDFS rate than others before 5 years (p=0.067). Of the 1002 patients without chemotherapy, only 36 patients (3.6%) had developed distant metastasis during study period (before 5 years in 27 (2.7%), and after 5 years in 9 (0.9%)). There was no difference of late recurrence after 5 years among the patients regardless of ER and PR expression level and NG with only low recurrence rate (0.9%).

Conclusions: Our results demonstrated that, in ER-positive/HER2-negative patients who underwent neoadjuvant chemotherapy, NG-low/ER-high/PR-high should receive extend hormonal treatment over 5 years because of the high risk of late recurrence but compared with NG-high/ER-high might not need. Furthermore, patients with T1 and node-negative may not need extend hormonal treatment because of the extremely low risk of late recurrence regardless of NG and hormone receptor statuses.
What goes through your mind when you think about your medication? Thoughts about endocrine medication and treatment adherence

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Hormonal therapy in women with breast cancer is an important part of long-term treatment, conferring significant survival benefits. However, a substantial proportion of women are non-adherent to endocrine therapy. Patients' beliefs about their illness and subsequent thoughts about whether the treatment is appropriate for their condition are important factors in treatment adherence. We sought to investigate the thoughts that breast cancer patients have about their endocrine medication and how these thoughts are related to adherence to treatment. Methods: Women with breast cancer were recruited from the Army of Women research registry. Women were asked what thoughts go through their mind when they think about the endocrine medication they were taking. Open-ended responses were coded reliably into 9 independent categories. Adherence to endocrine therapy, using an adapted version of the Morisky Medication Adherence Scale, as well as demographic and medical information was collected. Analysis of covariance (ANCOVA) was used to investigate the relation of treatment-related thoughts with adherence. Results: 1371 women were included in this analysis, mean age 56 years; 76.4% married, 57% currently working full or part-time. Mean years since breast cancer diagnosis was 5.1 years, and most women had early stage breast cancer (87%). With regard to endocrine therapy, 36.3% of participants were taking Arimidex (anastrozole), 29.6% Nolvadex (tamoxifen), 21.5% Femara (letrozole), and 12.5% Aromasin (exemestane). Of the 1371 participants, 1058 responses were able to be coded into 1 of the 9 categories. The most frequent category of thoughts reported was concern about side effects (19%) followed by the drug preventing cancer recurrence (18%), worry about future effects (13%), hope the drug is working (13%), dislike taking the drug (9%), time remaining on treatment (9%), grateful that drug exists (8%), and the drug is saving my life (6%). Treatment-related thoughts were related significantly to self-reported adherence to endocrine medication, F(8, 1013) = 5.97, p < .001, partial η² = .05. Women who reported thinking that they don't like taking their medication were the least adherent to treatment (M = 7.01, 95%CI[6.60, 7.42]). This group reported significantly lower adherence than participants who reported feeling protected (M = 5.79, 95%CI[5.22, 6.37], p = .025), those who thought about their medication saving their lives (M = 5.88, 95%CI[5.40, 6.35], p = .014), preventing recurrence (M = 5.98, 95%CI[5.70, 6.26], p = .002), those who reported feeling grateful for treatment (M = 6.00, 95%CI[5.59, 6.42], p = .024), and those who thought about their hope that the treatment is working (M = 6.01, 95%CI[5.69, 6.34], p = .006). Women who were worried about the possible long-term effects of the treatment or were concerned about side effects were also significantly less adherent to treatment. Conclusions: The thoughts that patients with breast cancer have about their medication are significantly related to adherence to endocrine therapy. This simple open-ended question may be useful to assess patients' experiences of their medication and identify cognitions that can be targeted in interventions to improve adherence.
Title: Transcript analysis of PI3K and immune-related genes and gene signatures in the pre- and post-treatment samples from the window of opportunity study of anastrozole and anastrozole with pictilisib (GDC-0941) in patients with HR-positive early breast cancer (OPPORTUNE study)

Schmid P, Pinder SE E, Bundel N, Wheatley D, Macaskill J, Zammit C, Hu J, Price R, Shia A, Lim L, Parker P, Molinero L, Yu J, O’Brien C, Wilson T, Savage H, Derynck M, Lackner MR R, Amler L, Purushotham A, Thompson A and Gendreau S. Barts Cancer Institute, Queen Mary University London, London, United Kingdom; Kings College London, London, United Kingdom; Guys and St Thomas NHS Trust, Kings College London, London, United Kingdom; University Hospital of South Manchester, Manchester, United Kingdom; Royal Cornwall Hospital, Truro, United Kingdom; Ninewells Hospital Dundee, Dundee, United Kingdom; Brighton and Sussex University Hospital NHS Trust, Brighton, United Kingdom; Barts Health NHS Trust, London, United Kingdom; Kings College Hospital, London, United Kingdom; MD Anderson Cancer Centre, Houston, TX and Genentech, South San Francisco, California, South San Francisco, CA.

Background: The OPPORTUNE Study randomized postmenopausal patients (pts) to receive 2-week preoperative treatment with anastrozole (ANA) plus pictilisib (“ANA+PIC” arm) or ANA alone. Patients had newly diagnosed, operable, ER+, HER2- invasive breast cancer of ≥1 cm size. The primary outcome at interim analysis (n=70) revealed that the addition of PIC significantly increased the anti-proliferative response to ANA as measured by reduction in Ki67 immunohistochemistry (IHC). Multivariate analyses suggested benefit of PIC for patients with luminal B disease (Schmid et al. SABCS 2014).

Methods: RNA expression analysis of ~800 breast cancer-related genes was performed on patients analyzed at the interim analysis, including 14 (ANA) and 20 (ANA+PIC) patients with matched pre- and post- treatment paired tumour samples using the nCounter platform (NanoString). Differential expression of individual genes by arm was assessed using paired and moderated t-tests and statistical significance assessed through false discovery rate (FDR). Ingenuity Pathway Analysis (IPA) of differentially expressed transcripts identified pathways of relevance. Protein expression was analyzed by reverse protein array (RPPA) in pre- and post-treatment samples.

Results: In an unsupervised analysis, down-regulation of genes associated with ER signaling was observed in patients who received single-agent ANA and ANA+PIC, which included genes that regulate the cell cycle, cell death, survival, growth and proliferation and known ER target genes (e.g., PGR, GREB1). In addition, transcripts related to growth factor signaling pathway appeared to be specifically modulated in the ANA+PIC arm, possibly via the upregulation of the expression of RTK ligands. There were no clear changes in PI3K-related phosphoproteins (e.g., AKT, S6, 4E-BP1) in the post-treatment samples by RPPA. However, known PI3K-regulated genes, IRS2 and PIK3IP1, were upregulated in the post-treatment samples and a composite PI3K gene expression signature score (O’Brien et al. 2010) was reduced in both study arms following treatment. This PI3K signature was associated with pre-treatment luminal B status (n=27) and, consistent with this finding, the baseline PI3K gene signature score in the ANA arm, but not the ANA+PIC arm, was inversely associated with the decrease in post treatment Ki67. The tumor immune microenvironment was analyzed though the use of composite gene sets. In our initial observations, analysis of pre- and post-treatment samples showed that 2-week treatment with ANA resulted in a modest increase in transcripts associated with multiple immune signatures, which was further enhanced by the addition of PIC.

Conclusions: Gene expression analysis of pre- and post-treatment samples in the OPPORTUNE study demonstrates on-target inhibition of ER and PI3K signaling networks. The analysis of additional paired samples is in progress to further assess if 2-weeks of treatment with a regimen containing an AI in patients with early breast cancer impacts the tumor immune microenvironment.
Title: Neoadjuvant endocrine therapy for estrogen receptor (ER) positive breast cancer: Comprehensive systematic review and meta-analysis

Spring L, Gupta A, Reynolds KL L, Gadd MA A, Isakoff SJ J, Ellisen LW W, Moy B and Bardia A. Massachusetts General Hospital, Boston, MA and University of Texas Southwestern Medical Center, Dallas, TX.

Body: Background: ER positive tumors are generally highly responsive to endocrine treatment. However, the specific indications for neoadjuvant endocrine therapy, both as monotherapy and in combination with other therapies, in early breast cancer remain unclear. We conducted a comprehensive systematic review and meta-analysis to evaluate the impact of neoadjuvant endocrine therapy on response rate (RR), based on clinical response or imaging, and pathological complete response rate (pCR) for ER positive breast cancer.

Methods: Based on QUORUM guidelines, a librarian-led search of PubMed, Ovid, and EMBASE was performed to identify eligible trials published prior to May 15, 2015. Inclusion criteria were prospective, randomized neoadjuvant trials that had at least one arm with neoadjuvant endocrine therapy and reported RR. Pooled odds ratios (ORs), 95% confidence intervals (CI), and p values were estimated for endpoints using the fixed and random effects statistical model.

Results: We identified 477 citations initially with 277 remaining after duplications were removed. Twenty trials ultimately met inclusion criteria, with a total sample size of 3,493. The majority of studies (90%) focused on post-menopausal women and compared chemotherapy to endocrine therapy, different durations and types of endocrine therapies, or combinations with growth factor pathway inhibitors. Overall, as compared to chemotherapy, neoadjuvant endocrine therapy had a similar clinical RR (OR 0.93, CI: 0.43-2.02, p = 0.85) and radiological RR (OR 0.73, CI: 0.48-1.09, p = 0.12), but lower rate of toxicity (febrile neutropenia, mucositis). Aromatase inhibitors were associated with significantly higher clinical RR (OR 1.69, CI: 1.36-2.10, p = <0.01) and radiological RR (OR 1.49, CI: 1.18-1.89, p = <0.01), as compared to tamoxifen. Dual combination therapy with growth factor pathway inhibitors (n = 4) was also associated with higher radiological RR (OR 1.59, CI: 1.04-2.438, p = 0.03), but not clinical RR (OR 0.76, CI: 0.541-0.7, p = 0.11), as compared to endocrine therapy alone. The incidence of pCR in any arm was low overall (<10%) with resultant low numbers not suitable for inter-group comparisons.

Conclusion: Neoadjuvant endocrine therapy is associated with similar response rates as neoadjuvant chemotherapy but with lower toxicity, and neoadjuvant AIs are superior to tamoxifen for ER-positive tumors. Compared to endocrine monotherapy, dual combination therapy may have superior response rates by imaging, but the low number of trials limits strong conclusions. Additional studies and more predictive biomarkers are needed to personalize the optimal neoadjuvant endocrine combination for an individual patient with ER positive breast cancer.
Title: Fulvestrant plus anastrozole as neoadjuvant therapy in postmenopausal women with hormone receptor positive early breast cancer


Background: Aromatase inhibitors (AIs) are effective in reducing the risk of recurrence from breast cancer (BC) but 20% of patients (pts) with early BC still recur despite adjuvant AIs. Thus more effective endocrine therapies (HTs) are needed. In metastatic BC (MBC), combination of lower dose fulvestrant plus anastrozole improves survival compared to anastrozole alone. The 21-gene Recurrence Score® (RS; Oncotype DX®) has been validated to predict benefit from adding chemotherapy (CT) to HT where pts with a low score have little benefit from CT and derive a large benefit from HT. Ki-67 response to neo-adjuvant HT may predict adjuvant outcomes to HT. Postoperative Endocrine Prognostic Index (PEPI) and modified PEPI may further identify a subset of HT sensitive cancers that do not require adjuvant CT (PEPI 0 category). We conducted a single arm phase II trial to assess the efficacy of fulvestrant plus anastrozole as neoadjuvant HT in pts with operable BC.

Methods: Postmenopausal pts with stage II and III, ER/PR+, HER2 (-) BC with a RS<25 (performed on initial core bx) were included. Duration of neo-adjuvant HT was 4 months. Pts received anastrozole 1mg (PO) daily continuously from day 1 until surgery + fulvestrant (IM) 500mg on day 1, 14 and 28 of cycle 1, and on the last day of three subsequent 28 day cycles (total 6 doses of fulvestrant). At week 4, an optional core bx was repeated to assess change in Ki-67. Response assessments were made clinically every 4 wks. All pts had breast/axillary surgery after the 6th dose of fulvestrant. Ki-67, histologic grade, ER/PR status, and RS were assessed at baseline, core bx at 4 wks, and at definitive surgery. Primary end points were pathologic complete response (pCR) rate and change in Ki-67. Adjuvant CT was left to the discretion of treating physician.

Results: 42 pts were enrolled 7/2009 to 11/2014. Median age was 62. 32 (76%) patients had stage IIA, 7 (17%) had stage IIB and 3 (7%) had stage III disease. 14% had clinically node positive disease. The median RS was 12 (0-24). Median tumor size was 3.5cm. 21%, 74%, and 5% had grade 1, 2 and 3 tumors respectively. Mean ER expression was 95%. 16 (38%) pts had a clinical complete response (cCR), 13 (31%) had a clinical partial response (cPR) and 12 (29%) had stable disease. One pt had progression on therapy. There were no pCRs. Median baseline Ki-67 was 5% (1-36%). 94% of pts had decrease in Ki-67 from baseline to 4-week bx and 97% of pts had decrease in Ki-67 from baseline to surgery. Modified PEPI score at surgery was 0 in 53% of patients. 78% of pts did not receive adjuvant CT. At median follow up of 38 mos only 1 pt had a recurrence with 98% free of a recurrence. There were no grade 3 or grade 4 toxicities.

Conclusions: The neoadjuvant combination of anastrozole and fulvestrant in pts with RS<25 markedly improves Ki-67 response with more than half of pts achieving a modified PEPI score of 0 at surgery. At a relatively short median follow up, recurrence rate is very low. Given the efficacy and tolerability of anastrozole plus fulvestrant in MBC and now in the neo-adjuvant setting, an adjuvant trial of this combination is warranted in pts with ER+ BC.
Title: A phase II neoadjuvant trial of MK-2206, an AKT inhibitor, in combination with anastrozole for clinical stage 2 or 3 PIK3CA mutant estrogen receptor positive HER2 negative (ER+HER2-) breast cancer (BC)


Body: Background
Activating mutations in PIK3CA occur in approximately 40% ER+BC. MK-2206 (M), a pan-AKT inhibitor, induced apoptosis of ER+ BC under estrogen deprivation in preclinical studies. We conducted this neoadjuvant trial to determine the pathologic complete response (pCR) rate of M plus anastrozole (A) for PIK3CA mutant (Mut) ER+ BC.

Methods
This single arm open label study of M+A used a 2-stage Simon phase II design (stage 1, n=16; stage 2, n=13, alpha=0.10, power=0.90) to test whether pCR rate <1% (based on historical data with A alone), against the alternative that pCR rate ≥15% in PIK3CA Mut ER+ BC. At least 1 pCR in stage 1 was required to proceed to stage 2.

Eligible patients (pts) with clinical stage II or III ER+HER2- BC were pre-registered and proceeded to a research tumor biopsy for PIK3CA sequencing, followed by treatment with daily A monotherapy for 28 days (cycle 0). Pts with PIK3CA Mut BC were subsequently registered, underwent a second biopsy, and started M (150mg PO weekly) with daily A on cycle 1 day 1 (C1D1) for a maximum of four 28-day cycles followed by surgery. Goserelin was added for premenopausal pts. A tumor biopsy on C1D17, 17 days post the start of M, was performed. Those with C1D17 Ki67 >10% discontinued study treatment. pCR was defined as no invasive cancer in the breast and the lymph nodes. Tumor specimens collected at all timepoints are being analyzed for markers of proliferation, apoptosis, and PI3K pathway activity, gene expression microarray, intrinsic subtypes, and next generation sequencing of 83 genes.

Results
Of the 51 pts pre-registered, 35 pts did not register due to no PIK3CA mutation (n=22), inadequate specimen for testing (n=6), physician/pt decision (n=7). The remaining 16 pts (median age: 58, range: 40-77 years) received combination therapy. Three pts did not complete 4 cycles due to C1D17 Ki67 >10% (n=2) and intolerability (grade (Gr) 4 transaminase elevation in C1, n=1). Other severe toxicities possibly related to M included Gr 3 rash (25%) and pruritus (12.5%). Of the 13 pts completed study therapy and underwent surgery, all had residual disease in the breast and 7 also had positive nodes. Table 1 summarized changes in Ki67 during treatment.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>n</th>
<th>Absolute changes in Ki67 median (range)</th>
<th>Wilcoxon signed rank p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1D1 relative to pre-registration</td>
<td>11</td>
<td>-17.0% (-49.8 to 4.1%)</td>
<td>0.0020</td>
</tr>
<tr>
<td>C1D17 relative to pre-registration</td>
<td>14</td>
<td>-16.4% (-51.4 to 4.1%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>C1D17 relative to C1D1</td>
<td>12</td>
<td>-1.5% (-18.6 to 15.8%)</td>
<td>0.9697</td>
</tr>
<tr>
<td>C1D1, biopsy post 28 days of A alone; C1D17 biopsy post 17 days on combination therapy</td>
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Although Ki67 levels post A monotherapy (C1D1) or M+A (C1D17) were significantly lower than that of pre-registration samples, Ki67 did not differ between C1D17 and C1D1 samples. Other correlative studies are ongoing and results will be presented.

Conclusion
Despite the small sample size, biomarker analysis on serial biopsy specimens demonstrated that M+A is unlikely to be more effective than A alone in PIK3CA Mut ER+ BC. This trial demonstrated the feasibility of genomic sequencing for pt selection and the value of a small, well-designed proof-of-principle neoadjuvant trial for the evaluation of targeted agents.
Title: The relationship between the expression of FOXA1 and GATA3 and the efficacy of neoadjuvant endocrine therapy

Tanaka K, Tokunaga E, Inoue Y, Ueo H, Yamashita N, Sagara Y, Ohi Y, Taguchi K, Ohno S, Okano S, Kitao H, Oki E, Oda Y and Maehara Y. Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Graduate School of Medical Sciences, Fukuoka, Japan; Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; Sagara Hospital, Kagoshima, Japan; Sagara Hospital, Kagoshima, Japan; National Kyushu Cancer Centre, Fukuoka, Japan and National Kyushu Cancer Centre, Fukuoka, Japan.

Body: Background.
The estrogen receptor (ER)/ GATA3/ Forkhead box A1 (FOXA1) network is necessary for the ERα functional signature specific to luminal type breast cancers. High expression of FOXA1 indicates a good prognosis in ER-positive breast cancer. However, little is known about the association between the expression of FOXA1 and GATA3, and the efficacy of neoadjuvant endocrine therapy (NAE). This study investigated their predictive potential for NAE and the changes of their expression after NAE.

Methods.
The expression of ER, progesterone receptor (PgR), Ki67, FOXA1, and GATA3 were analyzed by immunohistochemistry in 66 patients with hormone receptor-positive/ human epidermal growth factor receptor 2 (HER2)-negative breast cancer who had been treated with NAE between March 2003 and December 2012 at Kyushu University Hospital, National Kyushu Cancer Center, and Sagara Hospital. The association between the expression of biological marker and the efficacy of NAE, and their expression changes after NAE were evaluated.

Results.
The median age of the patients was 60 years (range, 30–84 years). Pre- and post-menopausal patients were 24 (36.4%) and 42 (63.6%). Endocrine agents that were administered are as follows: aromatase inhibitors (AIs) for 42 patients (63.6%), luteinizing hormone-releasing hormone (LHRH) agonist plus AI for 10 patients (15.2%), LHRH agonist plus tamoxifen for 13 patients (19.7%). NAE yielded a partial response (PR) in 21 patients (31.8%) and stable disease (SD) in 45 patients (68.2%). Breast conserving surgery was performed in 56 patients (84.8%) and mastectomy was performed in 10 patients (15.2%). Preoperative Endocrine Prognostic Index (PEPI) score was 0 in 10 patients (15.2%) and 1 or greater (score 1 ≤) in 56 patients (84.8%). Pre-treatment FOXA1 expression was positively correlated with GATA3 (P = 0.0003) and PgR (P = 0.0138). Post-treatment Ki67 expression was significantly lower in tumors, which achieved PR compared with those with SD (P = 0.0007). The expression of PgR, Ki67, and FOXA1 was significantly lower in post-treatment tumors compared with those in pre-treatment samples (p < 0.0001, p < 0.0001 and p < 0.0001, respectively). The expression of PgR, Ki67, and FOXA1 was significantly reduced in both tumors with PR and those with SD (PR: P = 0.0004, P < 0.0001, and P = 0.0417, respectively; SD: P < 0.0001, P = 0.0001, and P < 0.0001, respectively). The expression of PgR, Ki67, and FOXA1 was significantly decreased in post-treatment tumors in both patients with the PEPI score 0 and those with score 1 ≤ (score 0: P = 0.0078, P = 0.0059, and P = 0.0098, respectively; score 1 ≤: P < 0.0001, P < 0.0001, and P = 0.0002, respectively). In tumors with PgR > 20%, the expression of Ki67 and FOXA1 were significantly lower in post-treatment tumors (P < 0.0001 and P < 0.0001, respectively).

Conclusions.
FOXA1 expression correlated with PgR expression, and was reduced significantly after NAE. These results suggest that blocking the effect of estrogen might reduce FOXA1 expression.
Concurrent gonadotropin-releasing hormone (GnRH) agonist administration with chemotherapy improves neoadjuvant chemotherapy responses in young premenopausal breast cancer patients


Background
Gonadotropin-releasing hormone (GnRH) agonist therapy for ovarian function preservation shows promising results. This study aimed to determine the oncologic efficacy of GnRH agonist treatment concurrent with chemotherapy in a neoadjuvant setting.

Patients and Methods
A retrospective analysis was performed on 332 cases of invasive breast cancer in patients who were <40 years old at diagnosis and received GnRH agonists concurrent with neoadjuvant chemotherapy (GnRH agonist group) or neoadjuvant chemotherapy alone (neochemotherapy-alone group) at Asan Medical Center from December 2010 to September 2014. Pathologic complete response rates (pCR) and Ki-67 changes were evaluated between the two groups. For hormone receptor (HR)-positive tumors, the clinical response and preoperative endocrine prognostic index (PEPI) score also were evaluated.

Results
The median age was 32 ± 3.9 and 36 ± 3.0 years old in the GnRH agonist group and neochemotherapy-alone group, respectively (P < .001). Adjusted for tumor size, grade, lymph node metastasis, HR status, and chemotherapy regimen, the GnRH agonist group exhibited a higher pCR rate with an odds ratio (OR) of 2.98 (95% CI, 1.37–6.34) and more decreased Ki-67 expression during treatment (P = 0.05) than the neochemotherapy-alone group. In HR-negative tumors, the GnRH agonist group showed a higher pCR rate (multivariate OR = 3.50; 95% CI, 1.37–8.95) and more decreased Ki-67 expression (P = 0.047). In HR-positive breast cancer, the pCR rate, change in Ki-67 index, and clinical response were higher and preoperative prognostic index (PEPI) scores were lower in the GnRH agonist group, but not significant between the two treatment groups.

Conclusion
Concurrent administration of GnRH agonists during neoadjuvant chemotherapy improved pCR rates and suppressed Ki-67 expression especially in HR-negative tumors.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-13-07

Title: An investigator-initiated registry trial of simple oral therapy for low risk breast cancer


Body: Background: Across multiple studies performed in several countries with widely instituted screening mammography programs at different intervals between exams, up to 19% of breast cancer identified is in patients whose disease would otherwise go undetected and not have caused any ill effect if left untreated. Recent advances in pathologic and multigene assays have demonstrated promise to better identify low risk breast cancer and appropriately tailor treatments. Nonetheless, most women who may have such low-risk, estrogen receptor expressing lesions continue to be offered only an aggressive treatment paradigm. This most commonly includes surgery and lymph node evaluation and, in the case of breast conservation, breast irradiation following surgery, with the option of endocrine therapy for 5-10 years.

Trial design: We propose a multi-center US registry study of post-menopausal, female breast cancer patients age 60 and older who will be managed 5 years with oral endocrine therapy for mammographically screen-detected, node-negative, unifocal invasive disease with low clinical grade, high estrogen/progesterone receptor expression, negative Her2 expression, Ki67 rate <20%, and low-risk multigene expression analysis with Mammaprint Breast Cancer Recurrence Assay. Target lesions will be confirmed with a pre-treatment bilateral breast MRI and imaged routinely with standard mammography or ultrasound at 3-month intervals during months 1-36 and at 6-month intervals during months 37-60 to assess for disease response. Enrolled patients will have an ECOG performance status of 0-2. Medication history will be documented at routine follow-up visits.

Our primary objective will be to determine the frequency of conversion from a low-toxicity approach with oral endocrine therapy to conventional care with surgery +/- radiation therapy as a result of progression of disease or patient/provider choice. Progression of disease will be quantified objectively as >20% growth of the target lesion as compared to baseline in imaging measurements. After 5 years of endocrine therapy sans disease progression, patients may elect to continue or stop treatment or convert to standard care.

Statistical methods: We will determine the conversion rate from oral therapy for any cause to conventional management (compliance). Compared to the most pessimistic assumed true-rate for compliance of 0.5, we predict >90% power to detect a decrease of 0.1 in outcomes with an alpha of 5% (corresponds to a 95% Confidence Interval). Using descriptive statistics, we will also quantify for disease responses and progression-free survival. Our sample size will be ample for multiple sub-analyses including measurement of differences emanating from tertiary care versus local oncologic management, advanced imaging outcomes (if performed on any subset of patients), effect of type of endocrine therapy type (SERM vs AI), and effect of age and/or comorbidity severity interaction.

Accrual: Clinic sites with large patient cohorts are now being selected nationwide to enroll and manage patients’ disease with endocrine treatment only. We will select up to 20 sites and enroll 300 patients with low-risk disease.
Title: A meta-analysis of clinical benefit rates for fulvestrant 500 mg versus alternative therapies for treatment of postmenopausal, estrogen receptor-positive advanced breast cancer

Robertson JFR FR, Zefei J, Di Leo A, Ohno S, Pritchard KI I, Ellis M, Bradbury I and Campbell C. Division of Breast Surgery, University of Nottingham, Nottingham, United Kingdom; 307 Hospital of Chinese People's Liberation Army, Beijing, China; Sandro Pitigliani Medical Oncology Unit, Hospital of Prato, Prato, Italy; Breast Oncology Centre, Cancer Institute Hospital, Tokyo, Japan; Sunnybrook Odette Cancer Centre and the University of Toronto, Toronto, Canada; Baylor College of Medicine, Houston, TX and Frontier Science, Inverness-shire, United Kingdom.

Background

Fulvestrant 500 mg has demonstrated improved efficacy vs fulvestrant 250 mg (time to progression [TTP]/progression free survival [PFS] and overall survival) in the treatment of postmenopausal women with advanced breast cancer (ABC). Few clinical trials have demonstrated a significant increase in clinical benefit rate (CBR) for one endocrine therapy (ET) over another for the treatment of ABC. This implies that TTP/PFS improvements have been achieved principally by prolonging time to developing acquired resistance. However, it would be beneficial to know whether CBR is also improved, indicating that more patients experience tumor remission. We performed a meta-analysis to determine if there was a difference in CBR between fulvestrant 500 mg and its comparators in randomized clinical trials (RCTs).

Methods

Five RCTs evaluating fulvestrant 500 mg were included: CONFIRM, China CONFIRM, FINDER1 and FINDER2 (vs fulvestrant 250 mg) as second-line ET and FIRST (vs anastrozole) as first-line ET. CBR was calculated as the proportion of patients experiencing a best objective response of stable disease for \( \geq 24 \) weeks, complete response or partial response. Peto method was used to calculate odds ratios (ORs), 95% confidence intervals (CIs) and \( p \) values. Separate fixed effect (FE) models were constructed for first- and second-line data combined, and for second-line only data. For each model Tarone's test for heterogeneity assessed the assumption of constant trial effect.

Results

Unadjusted ORs for CBR from CONFIRM, FINDER1 and FINDER2, adjusted OR for China CONFIRM (as reported in the trial publications), and combined FE models are shown (Table). The OR (95% CI) of the FE model for all trials indicated that CBR was higher with fulvestrant 500 mg than with comparator treatments (OR: 1.34 [1.12–1.61]; FE \( p=0.001 \); Tarone’s test \( p=0.91 \)). When assessing second-line ET only, the OR was similar to the overall combined analysis.

<table>
<thead>
<tr>
<th>ORs (95% CI) of CBR from individual trials and meta-analysis.</th>
<th>n</th>
<th>OR of CBR for fulvestrant 500 mg vs comparator (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First-line</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIRST</td>
<td>205</td>
<td>1.30 (0.72–2.38)</td>
</tr>
<tr>
<td><strong>Second-line</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONFIRM</td>
<td>736</td>
<td>1.28 (0.95–1.71)</td>
</tr>
<tr>
<td>China CONFIRM</td>
<td>221</td>
<td>1.37 (1.04–1.80)</td>
</tr>
<tr>
<td>FINDER1</td>
<td>92</td>
<td>1.20 (0.53–2.74)</td>
</tr>
<tr>
<td>FINDER2</td>
<td>93</td>
<td>1.96 (0.84–4.54)</td>
</tr>
<tr>
<td><strong>Fixed effects model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First- and second-line combined</td>
<td>1347</td>
<td>1.34 (1.12–1.61)</td>
</tr>
<tr>
<td>FE ( p=0.001 ); Tarone’s test ( p=0.91 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second-line only</td>
<td>1142</td>
<td>1.35 (1.11–1.63)</td>
</tr>
<tr>
<td>FE ( p=0.002 ); Tarone’s test ( p=0.81 )</td>
<td></td>
<td></td>
</tr>
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</table>
Conclusions
These data suggest that fulvestrant 500 mg is associated with a significant improvement in CBR of approximately 34% compared with comparator ETs (i.e. more tumors are placed into remission). This finding is consistent in both second- and first-line ET settings. We await the results of the Phase 3 FALCON first-line ET RCT to see if it supports this finding of increased CBR with fulvestrant 500 mg.
Title: Clinical predictors of benefit from fulvestrant in advanced breast cancer: A meta-analysis of randomized controlled trials

Niraula S, Pitz M, Gordon V, Grenier D, Amir E and Brandes L. CancerCare Manitoba and University of Manitoba, Winnipeg, MB, Canada and Princess Margaret Cancer Center and University of Toronto.

Body: Background: While fulvestrant is approved by the United Stated Food and Drug Administration as an alternate endocrine therapy for treatment of advanced breast cancer, data on its efficacy compared to other endocrine treatments are inconsistent. Clinical markers predictive of greater benefit from fulvestrant compared to the alternate endocrine agents have not been identified.

Methods: We searched the literature from inception to May, 2015 from MEDLINE, EMBASE, and major conference proceedings. We included randomized controlled trials that evaluated Fulvestrant compared to either tamoxifen or an AI. We collected the efficacy data reported as Time to Progression (TTP) or Progression Free Survival (PFS) on 7 distinct subgroup of patients from the RCTs defined by: age, time to cancer reoccurrence from primary diagnosis, presence of visceral metastasis, previous chemotherapy exposure, presence of measurable disease, hormone receptor status and, HER-2 status. Data on rates of occurrences of 9 most frequently reported adverse events were also collected from both arms of the studies. Data on both efficacy and toxicity were then weighted using generic inverse variance approach and pooled in a meta-analysis using RevMan 5.3 software.

Results: We identified 8 RCTs that fulfilled our criteria and involved 4,024 patients (2,032 on fulvestrant and 1,992 on control arms). TTP/PFS was the primary endpoint in 7 out of 8 RCTs and secondary endpoint in one. Compared to an AI or tamoxifen, there was a statistically significant improvement in TTP favoring fulvestrant in patients who had visceral metastasis [Hazards Ratio (HR) 0.86; 95% Confidence Interval (CI) 0.77 to 0.96, p<0.01], measurable disease [HR 0.74; 95% CI 0.58 to 0.93, p=0.01], and HER-2 overexpression [HR 0.43; 95% CI 0.27 to 0.70, p<0.001]. Similar effect sizes were observed in a sensitivity analysis excluding the trials of combinations of fulvestrant and AI in the experimental arm. Rates of occurrences of adverse events were similar between fulvestrant and other endocrine agents.

Conclusion: Patients with advanced breast cancer that have visceral disease, measurable disease, or HER-2 driven disease are likely to derive higher benefits from treatment with fulvestrant compared to tamoxifen or an AI. These results may have implications for selection of patients in the design of future clinical trials and to inform treatment decisions in clinical practice.
Title: A network meta-analysis of fulvestrant 500 mg versus alternative therapies for second-line treatment of postmenopausal, estrogen receptor-positive advanced breast cancer

Telford C, Jones N and Batson S. AstraZeneca Pharmaceuticals, Gaithersburg, MD and Abacus International, Bicester, Oxfordshire, United Kingdom.

Body: Background
A network meta-analysis (NMA) of randomized controlled trials compared overall survival (OS) and serious adverse events (SAEs) of fulvestrant 500 mg with alternative therapies for second-line treatment of postmenopausal, estrogen receptor-positive advanced breast cancer.

Methods
Data were identified by systematic literature review. Study level hazard ratios (HRs) were obtained by modeling survival data using Weibull distribution, based on statistical and visual fit. SAEs were calculated as rate of grade 3/4 adverse events per patient year. Fixed effect Bayesian NMA were conducted for both outcomes. The base case included the following comparators of interest: anastrozole 1 mg, letrozole 2.5 mg, fulvestrant 250 mg, exemestane 25 mg, everolimus 10 mg plus exemestane 25 mg. The first three comparators were included in a subgroup analysis of treatment post-antiestrogen (antiestrogen subgroup), the last three were included in a subgroup analysis of treatment post-aromatase inhibitor (aromatase inhibitor subgroup).

Results
Ten studies were included; all studies reported SAEs, 7 studies reported OS. In the base case, fulvestrant 500 mg improved OS versus all comparators and in the antiestrogen subgroup versus fulvestrant 250 mg (HR 0.74; 95% credible interval [CrI] 0.56, 1.00), anastrozole (HR 0.73; 95% CrI 0.52, 1.03), and letrozole (HR 0.69; 95% CrI 0.44, 1.09). In the aromatase inhibitor subgroup, fulvestrant 500 mg improved OS versus fulvestrant 250 mg (HR 0.89; 95% CrI 0.59, 1.33) and exemestane (HR 0.93; 95% CrI 0.58, 1.47); OS for fulvestrant 500 mg versus everolimus plus exemestane was similar (HR 1.02; 95% CrI 0.62, 1.69). Decreased SAE rates were seen for fulvestrant 500 mg versus letrozole, exemestane, and everolimus plus exemestane (13.22 versus 20.65, 46.63, 67.30 events, respectively) in base case and versus fulvestrant 250 mg and letrozole (14.88 versus 15.67, 23.41 events, respectively) in the antiestrogen subgroup. NMA in the aromatase inhibitor subgroup was not performed (lack of reporting SAEs).

Conclusions
This analysis suggests improved efficacy for fulvestrant 500 mg versus fulvestrant 250 mg and aromatase inhibitors, similar efficacy to everolimus plus exemestane, and decreased toxicity amongst a majority of the comparators. Fulvestrant 500 mg can be considered an efficacious and better tolerated alternative endocrine treatment in this setting.
**Title:** A phase 2 study evaluating orteronel, an inhibitor of androgen biosynthesis, in patients with androgen receptor (AR)-expressing metastatic breast cancer: Interim analysis

Yardley DA A, Peacock N, Young RR R, Silber A, Chung G, Webb CD D, Jones SF F, Shastry M, Midha R, DeBusk LM M, Hainsworth JD D and Burris HA A.  Sarah Cannon Research Institute/Tennessee Oncology, PLLC, Nashville, TN;  The Center for Cancer and Blood Disorders, Fort Worth, TX;  Yale School of Medicine, New Haven, CT;  Baptist Health Louisville, Louisville, KY and  Sarah Cannon Research Institute, Nashville, TN.

**Body:**

**Background:** The frequency of AR expression varies in the different breast cancer subtypes with 88%, 59%, and 32% expression reported in ER+, HER2+, and triple negative tumors, respectively. AR expression is associated with resistance to endocrine therapy in ER+ breast cancer. Androgen levels frequently increase following treatment with aromatase inhibitors suggesting a role for androgen synthesis inhibitors in ER+ breast cancer. AR signaling and expression are seen in triple negative breast cancer (TNBC), and a distinct AR TNBC subtype can be identified by gene expression profiling. AR expression in TNBC offers a potential therapeutic target. Preclinical and clinical studies demonstrated anti-androgen agent activity in breast cancer cell lines; preliminary clinical data suggests activity in TNBC. Orteronel is a novel, oral, selective, nonsteroidal inhibitor of 17, 20-lyase, a key enzyme in androgen biosynthesis that is being evaluated as endocrine therapy in various hormone-sensitive cancers. In this phase 2 study we are evaluating single agent orteronel in AR+ MBC.

**Methods:** Pts with AR expressing MBC (≥10% staining by central immunohistochemistry) were eligible. Pts were grouped into 2 cohorts for analysis: Cohort 1-TNBC and Cohort 2-ER+ (HER2 could be +/- in this cohort). Pts must have been previously treated with standard therapy for MBC (1-3 chemotherapy regimens for TNBC, 1-3 hormonal therapies + 1 chemotherapy for ER+ patients, ≥2 HER2-targeted regimens for HER2+ patients). A 6 pt lead-in for safety and tolerability of orteronel in AR+ female MBC pts was followed by open enrollment to either cohort. All pts received 300 mg orteronel PO BID over a 4 week cycle and underwent response assessment every 2 cycles. Treatment was continued until disease progression or unacceptable toxicity. The hypothesized response rate for Cohort 1 was 10% and 13% for Cohort 2. We present the results of a protocol-specified interim analysis of the ER+ MBC pts (Cohort 2).

**Results:** From 3/2014 to 4/2015, a total of 29 pts were enrolled on cohort 2. Median age was 65 years (range, 39-79); 90% ECOG ≤1; 90% HER2-/10% HER2+; median of 7 prior therapies (range 3-11). 93% had prior chemotherapy. Pts received a median of 2 cycles of orteronel treatment (range 1-4) and 3 pts (10%) are still on treatment. Of the 26 pts (90%) pts that have discontinued, 19 (66%) discontinued due to disease progression, 4 (14%) due to pt decision, 2 (7%) due to adverse event (AE), and 1 (3%) due to non-compliance. The most common treatment-related G 3/4 AEs were increased lipase [3 pts (10%)] and hypertension [2 pts (7%)]. There were no treatment-related SAEs or deaths on study. Three pts (10%) had stable disease as their best response. Further response evaluation is underway.

**Conclusions:** Orteronel monotherapy was well tolerated but appears to have limited single-agent activity in this heavily pre-treated ER+ MBC pt population. The full results from this interim analysis will be presented.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-14-05

Title: Phase 1 evaluation of the androgen receptor modulator CR1447 in patients with advanced breast cancer (SAKK 21/12)

Zweifel M, Thuerlimann B, Riniker S, Weder P, von Moos R, Pagani O, Bigler M, Rothgiesser KM M, Pilop C, Brauchli P, Tapia C, Schoenfeld W and Sessa C. Bern University Hospital, Bern, Switzerland; Breast Center, Kantonsspital St. Gallen, St. Gallen, Switzerland; Kantonsspital Graubünden, Chur, Switzerland; Istituto Oncologico della Svizzera Italiana, Bellinzona, Switzerland; SAKK, Swiss Group for Clinical Cancer Research Coordinating Center, Bern, Switzerland; Institute of Pathology, University of Bern, Bern, Switzerland and CURADIS GmbH, Erlangen, Germany.

Body: Background: CR1447 (4-OH-testosterone, 4-OHT), a steroidal small molecule, strongly binds to the androgen receptor (AR) and has aromatase inhibiting activity. Pre-clinical studies show that CR1447 given as an ointment is efficiently absorbed and has antiproliferative activity in both ER-positive and ER-negative/AR-positive breast cancer cells. Methods: CR1447 was administered topically on a daily basis to patients with ER-positive/HER2-negative or ER-negative/PR-positive/HER2-negative advanced breast cancer pretreated with several lines of therapy. One cycle was defined as 21 days of treatment. Disease evaluation was performed at 3 and 6 months in order to determine tumor response (i.e. complete/partial remission and stable disease) in 3 cohorts of 3 evaluable patients each plus 3 confirmatory patients (dose escalation 100, 200, 400 mg). Results: 14 patients have been treated for a total of 38 cycles. Two patients are still on treatment at the time of analysis. Two patients, one in cohort 1 and one in cohort 2, showed early tumor progression and were replaced. Related adverse events were all ≤ grade 2 and included fatigue, bone and joint pain, stiffness, dry skin and mouth, nausea, sweating, urinary tract infection, rash, headache and distress. No drug-related dose limiting toxicities (DLTs) were seen. Two patients (17%) achieved stable disease at 3 months. Pharmacokinetic analysis confirmed dose-dependent transdermal uptake of CR1447, resulting in sufficient plasma concentrations of 4-OHT. 4-OH-androstenedione, a key metabolite of 4-OHT, was undetectable in most of the plasma samples. Conclusions: CR1447 administered transdermally as an ointment is well tolerated and appears to have single-agent activity in heavily pretreated ER-positive/HER2-negative and ER-negative/PR-positive/HER2-negative breast cancer patients. The recommended phase II dose is 400 mg/day.
Title: Lapatinib reverses endocrine resistance in select patients with HER2 negative, hormone positive metastatic breast cancer


Body: Introduction: A significant proportion of breast cancer (BC) patients show primary or acquired resistance to endocrine therapy (ET). The cross-talk between estrogen receptor and other growth factor receptor (GFR) families is suggested to play a crucial role in the development of endocrine resistance and dual targeting of these pathways can potentially reverse endocrine resistance. Lapatinib (Lap) is an oral, dual inhibitor of EGFR/HER2 and preclinical studies have demonstrated combination of Lap and ET to be effective in setting of endocrine resistance. Aims: 1) To evaluate if Lap can restore efficacy of aromatase inhibitor (AI) or fulvestrant(F) in metastatic BC, 2) Study blood/tissue response biomarkers.

Methods: Eligible patients with HER2 negative (IHC and FISH) hormone positive metastatic BC who had progression of disease while on AI or Fulvestrant (+/-AI), or developed metastatic disease on adjuvant AI were enrolled on an IRB approved phase II study. Study treatment included continuation of same dose and schedule ET on which last progression was noted with the addition of Lapatinib 1500 mg PO/QD. Patients could have unlimited prior ET. The study end points included clinical benefit rate (CBR) at 16wks (CBR16), CBR at 24 wks (CBR24) and PFS. Correlative studies included blood markers (HER2/ECD, CTC, CTC HER2 phenotyping) and tumor next generation sequencing (NGS) (315 cancer-related genes, Foundation Medicine, Inc).

Results: Between 2009 and 2014, thirty-two patients were enrolled. Median age: 61.5 yrs, median number of prior endocrine regimens: 2 (range 1-4), 72% had visceral disease, 28% had bone only disease, 67% were considered to be responsive to the last ET. For 28 patients evaluable for efficacy the ET was AI =29%, F= 32%, AI+F=39%. The CBR16 was 25% (7/28) and the CBR24 was 14%(4/28). Median PFS for the entire cohort was 2.1 mths and the median PFS for patients with CB at 16 weeks was 7.4 mths. 12% of patients had Grade 3/4 AE (Diarrhea: 6%, Vomiting: 6%, Rash: 3%). Serum HER2/ECD was elevated in 43%, ≥1 CTC detected in 57%, HER2 positive CTC noted in 18%. HER2/ECD and HER2 positive CTC did not correlate with CBR16. Tumor NGS done on partial sample set (n=7, patients without early progression) demonstrate PI3K pathway activating mutations in 55% (4/7) of patients (PIK3CA mutations=3, AKT3 amplification & PTEN homozygous deletion=1). No ESR1 alterations were observed. All 4 patients with PIK3CA pathway mutations demonstrate CB at 16 wks, and 3/4 demonstrate CB at 24 wks.

Conclusion: Addition of Lap restored sensitivity to ET (AI/Fulvestrant) in 25% of patients with HER 2 negative BC. Response was independent of HER 2 positive CTC and HER2/ECD levels. Genomic profiling suggests a relationship between PIK3CA mutation and benefit from this approach. NGS of the entire study cohort is in process and will be reported. HER2-negative breast cancers harboring PIK3CA mutations may rely more on GFR/PI3K signaling than on estrogen for growth; thus, blocking GFR signaling with lap might restore hormonal sensitivity. A randomized study of lap + ET in selected patients with HER2 negative BC is warranted and biomarker results from this study may help identify subgroups that should be targeted in a larger study.
Title: Breast cancer specific survival in 38,568 patients with node negative hormone receptor positive invasive breast cancer and oncotype DX recurrence score results in the SEER database


Body: Introduction: The SEER Program of the National Cancer Institute (NCI) is an authoritative source of cancer incidence and survival statistics, collecting population-based data for ~28 percent of the US. As innovations in molecular testing are validated and recommended in guidelines, new genomic research models are needed to characterize their use and impact on patient outcomes in clinical practice. To that end, Genomic Health and SEER have collaborated to electronically supplement the SEER registries with Oncotype DX results. This first report characterizes breast cancer specific survival (BCSS) in node (N)- hormone receptor (HR)+ HER2- invasive breast cancer.

Methods: The prospectively-defined population biomarker-outcomes linkage had objectives, analysis cohort, standardized biomarker, and outcome defined prior to data linkage. Patients were eligible if N-, HR+, HER2- (by RT-PCR), had no prior malignancy, were 40-85 years of age, and were diagnosed between Jan 2004 (Oncotype DX available in Jan 2004) and Dec 2011 (SEER survival analysis complete through 2012). BCSS was defined as previously described and assessed rigorously (Howlader et al, JNCI 2010). Oncotype DX Recurrence Score results were provided to SEER as mandated by their registry operations. Analysis by treatment was interpreted in the context of the lack of randomization and well known limitations of registry reporting of chemotherapy (CT) treatment (Noone et al, Medical Care 2014).

Results: Among 169,158 eligible patients, 38,568 (23%) had a Recurrence Score (RS), increasing from 2% of 2004 diagnoses to 35% of 2011 diagnoses. Patients with RS had median age of 57 yr, were 99.4% female, 84% white, 29% grade 1 and 54% grade 2, 25% 1cm or less and 53% between 1 and 2cm. Median follow-up was 39 mo with 8,239 patients having >5 yrs follow-up. Among low risk RS <18 (N=21,023), intermediate risk RS 18-30 (N=14,494) and high risk RS ≥31 (N=3,051) patients, chemotherapy use was reported in 7%, 34%, and 69%, respectively. 5-year BCSS (with 95%CI) are provided in Table.

Continuous RS was significantly associated with BCSS unadjusted (p<0.001), and after adjusting for age, grade, and tumor size (p<0.001), and when stratified by treatment (p<0.001).

Conclusions: 5 yr BCSS outcomes are excellent (99.6%) in the over 21,000 patients with low RS disease. As expected, high RS disease is associated with lower 5 yr survival despite addition of chemotherapy. More rigorous ascertainment of treatment is planned to draw conclusions regarding chemotherapy treatment benefit. Analyses that examine test utilization as well as annual survival updates to obtain longer term event rates will be important to gain additional insights into patient subgroup outcomes that can be characterized by large population-based genomic studies.

<table>
<thead>
<tr>
<th></th>
<th>RS&lt;18</th>
<th>RS 18-30</th>
<th>RS≥31</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>21023</td>
<td>14494</td>
<td>3051</td>
</tr>
<tr>
<td>BCSS (95%CI)</td>
<td>99.6 (99.4-99.7)</td>
<td>98.6 (98.3-98.9)</td>
<td>95.6 (94.4-96.6)</td>
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<td>Analysis all pts</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No/Unknown CT</td>
<td>19554</td>
<td>9570</td>
<td>936</td>
</tr>
<tr>
<td>BCSS (95%CI)</td>
<td>99.6 (99.5-99.7)</td>
<td>98.6 (98.2-98.9)</td>
<td>94.0 (91.1-96.0)</td>
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<tr>
<td>Yes CT</td>
<td>1469</td>
<td>4924</td>
<td>2115</td>
</tr>
<tr>
<td>BCSS (95%CI)</td>
<td>99.3 (98.4-99.7)</td>
<td>98.6 (98.0-99.0)</td>
<td>96.4 (95.0-97.4)</td>
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</table>
Title: Indications for, and impact of oncotype DX on adjuvant treatment recommendations when third party funding is unavailable

Chin-Lenn L, Segelov E, De Boer R, Marx G, Hughes TM, McCarthy N, White S, Foo S, Rutovitz J, Della Fiorentina S, Jennens R, Antill Y, Tsoi D, Cronk M, Lombard J, Kiely BE E, Chirgwin J, Gorelik A and Mann GB. Royal Melbourne Hospital, Melbourne, Victoria, Australia; St Vincent's Hospital, Sydney, Victoria, Australia; Sydney Adventist Hospital, Australia; ICON Cancercare Wesley, Australia; Austin Health, Australia; Epworth Eastern Hospital, Australia; St Vincent's Private Hospital, Australia; Northern Haematology and Oncology Group, Australia; Macarthur Cancer Therapy Centre, Australia; Peter MacCallum Cancer Centre, Australia; Cabrini Health, Australia; University of Notre Dame Australia, Australia; Sunshine Coast Hospital and Health Services, Australia; Calvary Mater Newcastle, Australia; Eastern Health, Australia and Royal Women's Hospital, Australia.

Body: Background:
An Australian industry-funded decision impact study demonstrated that Oncotype Dx (ODX) changed treatment recommendations (TR) in 24% of hormone receptor+/HER2- patients. ODX is available in Australia, but is self-funded by patients (≈USD 4175), so its use is limited. We sought to evaluate the impact of self-funded ODX on TRs. A high proportion of TR changes would imply a benefit to the broader community if ODX testing were available to all appropriately selected patients.

Methods:
All Australian physicians who had ordered >5 ODX were invited to participate. Data collected included demographics, tumor characteristics, indication for ODX (confirm need for chemotherapy (CT), confirm omission of CT, or genuine equipoise). Pre- and post Recurrence Score (RS) TRs (CT recommended versus hormone therapy alone (HT)) were also collected. The primary endpoint was the frequency and predictors of TR change. Relationships between categorical variables were assessed using Chi2 test and logistic regression analysis determined factors associated with TR change post-ODX.

Results:
382 patients with median age 54 (range 31-76) were included. 18 physicians contributed a median of 17 (5-87) patients. Tumor characteristics were: T1 232 (61%); ≥T2 150 (39%); grade 1 49 (13%), grade 2 252 (66%) and grade 3 79 (21%) and Ki67>15% in 131/231 (49%). 257 (67%) were node negative (N0). Assay indications were: confirm need for CT in 36%, confirm omission of CT in 40% and genuine equipoise in 24%. RS was low in 55%, intermediate in 36% and high in 9%.

Of 355 patients with a TR recorded pre-ODX, 38% had TR change post-ODX. 109/168 patients (65%) recommended CT pre-ODX changed to HT alone, and this was more likely if lower grade (82.1% vs 50.8% p<0.001) and less likely if ER and/or PR≤10% (12% vs 25% p=0.03). 27/187 (14%) with pre-ODX TR for HT alone changed to CT, and this was more likely if ER and/or PR≤10% (27.6% vs 11.5% p=0.02) and if Ki67 >15% (27.5% vs 9.8% p=0.015). Overall, TR for CT decreased from 47% to 24%.

Influence of adverse prognostic factors (defined as ≥T2, grade 3, ER and/or PR <10%, nodal macrometastasis) on TR is tabulated. In 348 patients with complete data, TR changed in 31% (72/234) of N0 and 53% (60/114) of node positive (N+) patients.

<table>
<thead>
<tr>
<th>Number of &quot;adverse factors&quot;</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>N0 (n=234) Number of patients</td>
<td>90</td>
<td>116</td>
<td>24</td>
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</tr>
<tr>
<td>RS median (range)</td>
<td>16 (0-40)</td>
<td>17 (0-41)</td>
<td>26 (5-52)</td>
<td>31 (22-40)</td>
<td></td>
</tr>
<tr>
<td>TR change</td>
<td>19/90 (21%)</td>
<td>45/116 (39%)</td>
<td>8/24 (25%)</td>
<td>2/4</td>
<td></td>
</tr>
<tr>
<td>HT to CT</td>
<td>7/71 (10%)</td>
<td>10/61 (16%)</td>
<td>2/11 (18%)</td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>CT to HT</td>
<td>12/19 (63%)</td>
<td>35/55 (64%)</td>
<td>4/13 (31%)</td>
<td>1/2</td>
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<tr>
<td>N+ (n=114) Number of patients</td>
<td>22</td>
<td>42</td>
<td>41</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>RS median (range)</td>
<td>14 (5-34)</td>
<td>16 (0-38)</td>
<td>14 (0-32)</td>
<td>24 (12-50)</td>
<td>39</td>
</tr>
<tr>
<td>TR change</td>
<td>11/22 (50%)</td>
<td>21/42 (50%)</td>
<td>25/41 (42%)</td>
<td>3/8 (38%)</td>
<td>0/1</td>
</tr>
<tr>
<td>HT to CT</td>
<td>3/13 (23%)</td>
<td>3/14 (21%)</td>
<td>0/12</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Conclusions:
Patient self-funded ODX changed TRs in 38%. 65% who would have been recommended CT pre-ODX were spared CT post-ODX, suggesting that traditional histopathological indications for CT in ER+ patients has led to many receiving CT unnecessarily. Where the pre ODX TR was HT alone, only 14% changed to adding CT, suggesting that the indication was reassurance that CT could be omitted. Consideration could be made for third party funding in select patient groups.
Title: Bisphosphonate use and breast cancer recurrence risk in the QUILT cohort

Korde LA A, Doody DR R and Malone KE E.  University of Washington, Seattle, WA and  Fred Hutchinson Cancer Research Center, Seattle, WA.

Background: Women with breast cancer are often prescribed bisphosphonates (BP) for treatment or prevention of osteoporosis. Clinical trials designed to assess the effect of BP on risk of recurrence in women with breast cancer have produced conflicting results. Several randomized studies and a recent meta-analysis suggested that a protective effect of BP is limited to postmenopausal women. Current data have not definitively addressed the utility of BP in patients with ER-positive vs. ER-negative disease.

Methods: We examined use of BP for non-cancer therapy in the QUILT cohort study. The analysis included 1813 women diagnosed with invasive breast cancer at ages 45 to 79 in 1993-1999. Data collection was via medical record review, in-person interview and the Cancer Surveillance System, a population-based registry in the NCI SEER program. Cox proportional hazards regression methods were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for the association between BP use and outcome. Analyses were adjusted for age and year of diagnosis.

Results: Mean age at diagnosis of study participants was 64.2 years. Eighty-four percent were postmenopausal at diagnosis; 86% had estrogen receptor positive disease; 16.7% used a BP after diagnosis of breast cancer. Among women with any BP use, 91% had a diagnosis of osteopenia or osteoporosis. Mean duration of BP use after BC diagnosis was 35.2 months; 90% of BP users took alendronate. Ever use (2+ months) of BP was associated with a significant decreased risk of any breast cancer event (local recurrence, distant recurrence, or second primary breast cancer; HR 0.65; 95% CI 0.47-0.90). Within users, there was a trend toward lower risk with longer duration of use (p=0.096, see Table 1).

Association between BP use and any breast cancer event (by duration of use)

<table>
<thead>
<tr>
<th>Duration of bisphosphonate use</th>
<th>No event n (%)</th>
<th>Any event n (%)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None/Less than 1 mo</td>
<td>1070 (70.8)</td>
<td>441 (29.2)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>2 mo to &lt;1 yr</td>
<td>40 (76.9)</td>
<td>12 (23.1)</td>
<td>0.82 (0.46-1.45)</td>
</tr>
<tr>
<td>1 yr to &lt;3 yrs</td>
<td>89 (82.4)</td>
<td>19 (17.6)</td>
<td>0.76 (0.48-1.20)</td>
</tr>
<tr>
<td>3+ yrs</td>
<td>131 (92.3)</td>
<td>11 (7.7)</td>
<td>0.44 (0.24-0.81)</td>
</tr>
</tbody>
</table>

Though the number of events was smaller, similar effects were seen for each event type (distant recurrence: HR=0.62, 95% CI 0.37-1.06; local recurrence: HR=0.62, 95% CI 0.26-1.44; second primary breast cancer: HR= 0.75, 95% CI 0.47-1.18).

Stratification by menopausal status showed a decreased risk of recurrence with BP use for both pre or peri- and postmenopausal women (pre/perimenopausal: HR = 0.34, 95% CI 0.12-0.94; postmenopausal: HR=0.72, 95% CI 0.51-1.01). BP use was protective in both women with ER positive (HR=0.64, 95% CI 0.45-0.91) and ER negative (HR=0.46, 95% CI 0.16-1.27) disease.

Conclusion: In this study, we observed a decreased risk of locoregional recurrence, distant recurrence, and second primary breast cancer with BP use after a diagnosis of invasive breast cancer. This effect was seen in pre and postmenopausal women, and for both ER positive and ER negative disease. Women in this cohort were prescribed primarily oral BP for treatment or prevention of osteoporosis. Additional prospective studies of adjuvant BP use should focus on women with low bone density regardless of menopausal status, and should include patients with both ER-positive and ER-negative disease.
Title: Zoledronic acid combined with adjuvant tamoxifen with or without ovarian function suppression in premenopausal early breast cancer patients

El-Ibrashi MM, El-Sadda WM, Abdel-Halim II I and Elashri MS S. Mansoura University School of Medicine, Mansoura, Dakahlia, Egypt and Al-Ghad International Colleges for Health Sciences, Najran, Saudi Arabia.

Body: Background:
Bisphosphonates can delay the onset and reduce the risk of skeletal related events in patients with bone metastatic breast cancer; it can prevent and control cancer treatment-induced bone loss. Zoledronic acid (ZOL) plus adjuvant endocrine therapy significantly improved disease free survival. The aim of the study is to evaluate the benefits of ZOL combined with adjuvant therapy in premenopausal early breast cancer patients.

Patients and methods:
Patients were premenopausal females who had undergone primary surgery for stage I, II ER +ve and/or PR +ve breast cancer with < 10 positive lymph nodes. All 300 patients were scheduled for standard tamoxifen 20mg/day for five years plus goserelin 3.6 mg every 28 days and were randomized to ZOL 4mg every 6 months for 3 years (group A) and without ZOL (group B). The primary end points were toxicity and disease-free survival (DFS), while overall survival (OS) was the secondary end point.

Results:
Between April 2005 and March 2012, 300 patients were enrolled, the median follow up duration was 98.4 months (range 14-120 months), adding ZOL to endocrine therapy strongly suggests improved DFS versus endocrine therapy alone (90% versus 85% for an absolute increase of 5%). There were fewer disease recurrences in the ZOL group versus no ZOL group (12% vs. 16%) with the greatest reductions in the loco-regional recurrence (3% vs. 5%), distant metastasis (6% vs. 7%) and bone metastasis (3% vs. 5%).

Conclusion:
ZOL with adjuvant endocrine therapy were generally well tolerated with no reports of renal failure or osteonecrosis of the jaw. So, a twice yearly ZOL enhanced the efficacy of adjuvant endocrine treatment, and this benefit is maintained for long time.
Title: The expression of genes involved in cholesterol biosynthesis is heterogeneous in breast cancer and may predict sensitivity to statin treatment and clinical outcome

Kimbung S, Feldt M, Bosch A and Borgquist S. Lund University, Lund, Sweden.

Body: Background: Epidemiological evidence supports a protective role of cholesterol lowering drugs against breast cancer recurrence. Preclinical studies also support this role since statins can suppress breast cancer progression in various models. The response of breast cancer cells to statin treatment is however heterogeneous. We performed a comprehensive molecular characterisation of the anti-cancer mechanisms of statins in-vitro in view of identifying biomarkers of statin sensitivity in breast cancer.

Methods: Breast cancer cell lines were treated with atorvastatin and the effects on cell proliferation were determined. A panel of cell lines displaying a diverse range of sensitivity to atorvastatin treatment were selected for gene expression microarray profiling following treatment. Differentially expressed genes were identified and subjected to gene ontology analysis and links to outcome following breast cancer diagnosis were explored.

Results: Statin treatment elicited a more potent inhibition of proliferation in estrogen receptor (ER) negative cell lines. This was reflected on the whole genome transcriptional scale as the ER negative cells also displayed a more robust dysregulation of mRNA transcripts relative to the less-sensitive ER expressing cells. Down-regulation of DNA replication, regulation of cell cycle progression, and other proliferation-associated biological processes via mechanisms influencing the transcriptional activity of E2F was observed in the statin-sensitive cells. Importantly, cholesterol biosynthesis via the mevalonate pathway was upregulated in all cell lines following statin treatment. This effect was directly correlated with the pre-treatment expression levels of target genes involved in this biological process, suggesting a link with sensitivity to statin treatment. The expression of cholesterol biosynthesis genes was also found to be variable in primary breast tumors and a significant association was observed between decreased expression and an improved recurrence-free and overall survival, especially in ER positive tumors.

Conclusions: These data suggest that the normal feedback regulation of the mevalonate pathway induced by statins may be compromised in a subset of breast cancer cells and may be exploited to predict breast cancer prognosis and sensitivity to statin treatment.
Title: 10-year follow-up of adjuvant ovarian suppression in high risk premenopausal breast cancer

Recchia F, Candeloro G, Rosselli M, Bratta M and Rea S. Civilian Hospital, Avezzano, AQ, Italy; Civilian Hospital, Frasscati, RM, Italy and Department of Biotecnological and Applied Clinical Sciences, L'Aquila, AQ, Italy.

Body: Background: A large randomized study has shown the value of temporary ovarian suppression during the administration of chemotherapy in the treatment of high risk estrogen receptor positive (ER+) breast cancer (BC) (Pagani et al. N Engl J Med 2014). In another study ovarian suppression added to chemotherapy appeared to protect ovarian function (Moore et al. N Engl J Med 2014) and improve the expected outcome of estrogen receptor negative (ER-) BC. Nevertheless, both these studies have a median follow-up time limited to 68 months and 49 months, respectively. Here we present the data of a large non randomized phase II study of adjuvant ovarian suppression in ER+ and ER- breast cancer with a median follow-up of 120 months (range 90-220 months). The primary end point was the ovarian function preservation rate, secondary end points were disease-free survival (DFS) and overall survival (OS). Methods: Between 06-1997 and 06-2007, 200 premenopausal, high risk early BC patients entered the study. All patients received the LH-RH analogue before starting chemotherapy. Breast conserving and radical surgery were performed in 74% and 26% of patients, respectively. Systemic therapy was tailored to the biological characteristics of each patient, and followed by radiation therapy and hormonal therapy in ER+ tumors. Results: The median patient's age was 43 years (range 26-45). The mean number of positive axillary nodes was 3.2 (range 1-25). Seventy-one % of patients were ER+ and/or progesterone receptor positive (PGR+), 29% were ER - and PGR-. The median KI-67 was 30% (range 15%-100%). Twenty-one % of patients were c-ErbB-2 positive. After a median follow up of 120 months (range 90-220), normal menses returned in 90% of patients younger than 40 years and in 56% of patients older than 40 years. The 10 and 15-year DFS rate were 85.5% and 71%, respectively, while the 10 and 15-year OS rate were 91%, and 71%, respectively. The standard pattern of toxicity of chemotherapy was observed. Hot flashes and G1 osteopenia occurring after LH-RH analogue administration were temporary and subsided after the cessation of therapy. Conclusions: LH-RH administration, concurrent with chemotherapy is tolerable and effective. Five full term pregnancies were documented. A favourable impact on the expected DFS and OS was observed. ER- patients had late new primaries, but no recurrence after 5 years, while ER+ patients had disease recurrence even after 13 years.
Knowledge of oncotype Dx recurrence score increases confidence and concordance in adjuvant decisions of U.K. oncologists

Kiernan T, Olsson-Brown AC C, Innes H, Holcombe C, Thorp N, O'Hagan J, Wong H, Palmieri C and O'Reilly S. Clatterbridge Cancer Centre, Bebington, Wirral, United Kingdom and Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, Merseyside, United Kingdom.

Introduction
The addition of Oncotype Dx Recurrence Score (RS) to the assessment of patients with ER positive, HER2 negative, node negative breast cancer has led to a reduction in the use of adjuvant chemotherapy. However, crude measurement of this reduction masks a more complex scenario. Prior to the introduction of routine Oncotype DX testing by NHS England, we wished to explore in more detail the potential impact of the knowledge of the RS on the therapeutic discussion. This study analyses the impact of RS on the adjuvant therapy recommendations within a UK Cancer Centre. In particular, it examines how the degree of certainty the oncologist has about the best option changes with knowledge of RS and how this influences concordance of decision making between oncologists.

Methods
A panel of five breast oncologists reviewed 50 consecutive cases, collected from November 2012 until November 2014, across two hospitals. Oncologists allocated each case to one of four treatment categories: chemotherapy recommended (CRec), chemotherapy discussed with a bias towards recommended (CDis), chemotherapy discussed with a bias toward endocrine therapy alone (EDis) or endocrine therapy only advised (ERec). The cases were analysed blindly and in random order without and with RS. The degree to which knowledge of RS altered treatment recommendation was analyzed. Other outcomes included the proportion of patients who were scored ERec compared with any other outcome, the trend towards definitive recommendations, the impact of RS on concordant decision making and the degree to which outcome was stratified by RS result. Chi squared and Spearman's coefficient statistical tests were used in analysis.

Results
Knowledge of the RS altered the recommended treatment category in 66.7% of cases (p<0.001).

<table>
<thead>
<tr>
<th></th>
<th>CRec (n)</th>
<th>CDis (n)</th>
<th>EDis (n)</th>
<th>ERec (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without RS</td>
<td>2% (1)</td>
<td>40% (20)</td>
<td>52% (26)</td>
<td>6% (3)</td>
</tr>
<tr>
<td>With RS</td>
<td>12% (6)</td>
<td>16% (8)</td>
<td>26% (13)</td>
<td>46% (23)</td>
</tr>
</tbody>
</table>

Overall, RS correlated significantly with treatment recommendation. Oncologists were confident to recommend endocrine therapy alone in 46% of patients when RS was known compared with only 6% of patients without RS. Complete concordance between oncologists increased with the knowledge of RS from 14% to 64%.

Conclusion
Discussion of adjuvant chemotherapy with patients who have ER positive, HER2 negative, node negative breast cancer can be complex and, at times, confusing for the patient, leading to increased distress. This study shows that, in addition to the previously recognised reduction in overall use of chemotherapy, the knowledge of the RS increased the proportion of patients for whom the oncologist felt confident in making a firm treatment recommendation. An added benefit was to increase concordance between different oncologists compared to that achieved when relying on standard pathological features.
Body: Background: The optimal treatment strategy for patients with metastatic breast cancer (MBC) is currently unknown. Resistance to standard therapies, including anthracyclines and taxanes, limit the number of treatment options in many patients to a small number of non-cross resistant regimens. Rational combination approaches that are selected based upon genomic and proteomic analysis represents a possible advance that warrants extensive exploration.

Methods: Single center analysis of 77 consecutive metastatic breast cancer patients seen over a 12 month period (June 2014 through May 2015). All patients were referred for sequencing and the metastatic disease was rebiopsied. All samples were sent for standard pathologic, genomic (FoundationOne), and proteomic (TheraLink) analysis.

Results: Genomic and proteomic analysis yielded actionable targets in a majority of cases (89%). The most common pathways involved were the following: PI3K/Akt/mTOR (73%), MAPK (46%), ErbB (36%), FGFR (25%), and Jak/STAT (11%). Over 100 unique molecular aberrations were identified in 40 evaluable patients. Current outcomes are summarized in Table 1. The overall response rate was 45%, with another 43% of patients with stable disease. Average number of prior therapies was over 4, with a range of 1-11.

<table>
<thead>
<tr>
<th>ER+/HER2-</th>
<th>CR = 3</th>
<th>PR = 8</th>
<th>SD = 15</th>
<th>PD = 2</th>
<th>NE = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/HER2+</td>
<td>CR = 2</td>
<td>PR = 2</td>
<td>SD = 1</td>
<td>PD = 0</td>
<td>NE = 9</td>
</tr>
<tr>
<td>ER-/HER2+</td>
<td>CR = 0</td>
<td>PR = 1</td>
<td>SD = 1</td>
<td>PD = 1</td>
<td>NE = 2</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>CR = 0</td>
<td>PR = 2</td>
<td>SD = 0</td>
<td>PD = 2</td>
<td>NE = 6</td>
</tr>
</tbody>
</table>

Total evaluable patients = 40  Overall CR = 13%  Overall PR = 33%  Overall SD = 43%  Overall PD = 13%  Total Not Evaluable = 37 pts (48%)

Overall Response Rate (ORR) = 45%

CR = complete response  PR = partial response  SD = stable disease  PD = progressive disease  NE = not evaluable

Conclusion: Since current literature suggests that an overall response rate of approximately 10% or less is expected for patients that have received greater than 4 previous lines of therapy, the ORR seen in this analysis is quite remarkable. Most patients in this analysis were treated with FDA approved drugs off label, which provided additional challenges and was the primary reason that many patients were not evaluable. Patients were only evaluable if they received the recommended therapy and were measured for outcome. Our initial data provides growing evidence that it is critical to incorporate genomic and proteomic analysis (preferably as early as possible in the disease course) to allow for the best chance of disease response.
Table 1: Risk and timecourse of progression from DCIS to invasive cancer

<table>
<thead>
<tr>
<th></th>
<th>Progression risk [%], Point estimate (95% CI)</th>
<th>Mean progression time [years], Point estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases (n=1,733)</td>
<td>18.0 (13.6-22.5)</td>
<td>7.8 (5.7-12.5)</td>
</tr>
<tr>
<td>Age at diagnosis: 18-49</td>
<td>19.6 (12.5-26.7)</td>
<td>6.5 (4.1-14.7)</td>
</tr>
<tr>
<td>Age at diagnosis: 50-69</td>
<td>20.9 (11.4-30.4)</td>
<td>11.1 (6.8-31.0)</td>
</tr>
<tr>
<td>Age at diagnosis: 70+</td>
<td>12.5 (7.3-17.8)</td>
<td>4.7 (2.9-12.5)</td>
</tr>
<tr>
<td>Tumor grade: low/medium</td>
<td>22.8 (8.0-37.5)</td>
<td>11.5 (6.2-77.3)</td>
</tr>
<tr>
<td>Tumor grade: high</td>
<td>23.1 (13.5-32.7)</td>
<td>6.1 (3.7-16.6)</td>
</tr>
</tbody>
</table>
Title: Treatment and prognosis of DCIS during twenty years. A population-based register study from a Swedish cohort

Wadsten C, Heyman H, Holmqvist M, Ahlgren J, Lambe M, Sund M and Wärnberg F. Sundsvall Hospital, Sundsvall, Sweden; Uppsala University Hospital, Uppsala, Sweden; Regional Cancer Center, Uppsala University Hospital, Uppsala, Sweden; Faculty of Medicine and Health, Örebro University, Örebro, Sweden; Umeå University, Umeå, Sweden and Uppsala University, Uppsala, Sweden.

Body: The increasing incidence of ductal carcinoma in situ (DCIS) of the breast has been attributed to the wide adoption of mammography screening programmes. The aim of the present study was to analyse trends in incidence, treatment and outcome of DCIS over a 20-year time period in a Swedish health care region, with a source population of two million, based on systematically collected data in a regional Breast Cancer Quality Registry started in 1992.

All patients registered with a diagnosis of primary DCIS in the Breast Cancer Quality Registry in the Uppsala-Örebro healthcare region between 1992 and 2012 were included. The study period was divided into four time periods.

The registry contains information on tumour characteristics, treatment and follow-up data and is linked to the Swedish cancer registry, to which reporting of all newly diagnosed malignant tumours in Sweden is mandated. To verify the validity of the Breast Cancer Quality Registry, 300 women recorded with a diagnosis of DCIS were randomly selected and their medical records were collected to compare clinical data, treatment data and subsequent breast cancer events compared to registry data. The completeness and reliability of the registration of most key variables were overall good, 91-99%.

A total of 2,952 patients with DCIS were registered, of which eight were men. The proportion of DCIS to all diagnosed breast cancers was 9.5%, with no clear increase over time. The majority of the DCIS cases were detected by screening (68%). Tumour size increased over time; in 1992-1997 36.4% were larger than 15mm compared to 64.8% in 2008-2012. The frequency of mastectomy increased from 23.0% to 39.0% and the proportion of patients receiving adjuvant radiotherapy after breast-conserving surgery increased from 30.1% to 67.6%. Axillary lymph node clearance declined over time while the proportion of patients who underwent sentinel node biopsy increased from 1.4% in 1998-2002 to 33.9% in 2003-2007 and 54.9% in 2008-2012.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>693</td>
<td>628</td>
<td>835</td>
<td>796</td>
<td></td>
</tr>
<tr>
<td>DCIS size &gt; 15mm</td>
<td>252(36.4%)</td>
<td>257(40.9%)</td>
<td>339(53.8%)</td>
<td>511(64.2%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>161(23.2%)</td>
<td>150(23.9%)</td>
<td>323(38.7%)</td>
<td>313(39.3%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BCS</td>
<td>519(74.9%)</td>
<td>468(74.5%)</td>
<td>506(60.6%)</td>
<td>476(59.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BCS+RT</td>
<td>156/519(30.0%)</td>
<td>178/468(38.0%)</td>
<td>347/506(68.6%)</td>
<td>322/476(67.6%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

There was no statistical difference in the cumulative incidence of recurrent breast events over time or between different treatment modalities. The relative 5- and 10- year survival rates were 99.0% and 97.0% respectively with no clear trend over time.

In conclusion, while the proportion of DCIS did not increase over time between 1992 and 2012, there was a trend towards more intensified management to achieve local control. The increased tumor size over time could be secondary to a higher diagnostic activity, which clearly is manifested by the increased use of sentinel node biopsy.
Title: The 12-gene DCIS score assay: Impact on radiation treatment (XRT) recommendations and clinical utility

Manders JB B, Kuerer HM M, Smith BD D, McCluskey C, Farrar WB B, Frazier TG G, Li L, Leonard CE E, Carter DL L, Chawla S, Medeiros LE E, Guenther JM Michael, Castellini LE E, Buchholz DJ J, Mamounas EP P, Wapnir IL L, Horst KC C, Chagpar A, Evans SB B, Riker AI I, Vali FS S, Solin LJ J, Jablon L, Recht A, Sharma R, Lu R, Sing AP P, Hwang ES Shelley and White J. The Christ Hospital Health Network, Cincinnati, OH; University of Texas M.D. Anderson Cancer Center, Houston, TX; Ohio State University James Cancer Hospital, Columbus, OH; Bryn Mawr Hospital, Bryn Mawr, PA; Rocky Mountain Cancer Centers, Denver, CO; Rochester Regional Health System, Rochester, NY; Saint Elizabeth Medical Center, Inc., Edgewood, KY; UF Health Cancer Center at Orlando Health, Orlando, FL; Stanford University, Stanford Cancer Institute, Palo Alto, CA; Yale University, New Haven, CT; Advocate Christ Medical Center, Oak Lawn, IL; Albert Einstein Health Network, Philadelphia, PA; Beth Israel Deaconess Medical Center, Boston, MA; Genomic Health, Inc., Redwood City, CA and Duke University Medical Center, Durham, NC.

Body: Background: In the management of DCIS clinicians and patients (pts) must choose between the various options for breast conservation treatment based on an assessment of local recurrence (LR) risk. Traditional clinicopathologic (CP) factors such as age, size, grade, margin width or comedo necrosis, provide an average LR risk derived from clinical trials and population studies. The Oncotype DX® 12-gene assay for DCIS gives individual 10-yr LR risk estimates and has now been validated in two studies in a total of 893 pts. We report the 2nd study assessing the impact of the DCIS Score result on XRT recommendations. In addition, surveys assessing pt and physician confidence will provide insight into the overall clinical utility of the DCIS Score result. Baseline characteristics including the pre-assay LR risk and XRT recommendation are described here; final results on change in XRT recommendation from pre- to post-assay and distribution of the score across the CP factors will be presented.

Methods: 13 U.S. sites enrolled pts with DCIS from 3/2014-5/2015. 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, DCIS score, and pre/post XRT recommendation. Each pt had a surgeon and radiation oncologist complete study surveys. Pt surveys were also administered pre/post assay for decision conflict and the STAIT anxiety survey. The LR risk estimates and XRT recommendations were analyzed for all physicians as well as by specialty. Descriptive statistics summarized study variables. 95% Clopper-Pearson Exact CIs were calculated for percent change in XRT recommendation. McNemar’s test was used to determine if the proportion of pts had a significant change in XRT recommendation post assay. Paired t-tests were used to compare physician estimates of recurrence risk pre/post assay.

Results: Of the 121 pts enrolled, median age was 61y (34-83) and 80.2% were postmenopausal. Median size was 8mm and 40% were ≤ 5mm; 22.3% were grade 1, 51.2% grade 2, and 26.4% grade 3. Comedo necrosis was noted in 55.4% and 19% had multiple foci. Median margin width was 3mm and 47.1% had margins 1-3mm. ER and PR by IHC were positive in 88.4% and 75.2% of pts. Among the 242 MD risk assessments, mean 10-yr LR risk was 14.8% (range 4-50%) for any LR; 14.2% for surgeons and 15.3% for radiation oncologists. The pre-assay XRT recommendation was 70.2%; 68.6% for surgeons and 71.9% for radiation oncologists.

Conclusions: The role of new molecular tools such as the DCIS Score assay that provide individual risk estimates for LR on treatment decisions is evolving. The DCIS pts enrolled in the study reveal inclusion of baseline features like higher nuclear grade (26%), comedo necrosis (55%) and margin width of 1-3mm (47%) that have historically been associated with XRT use. This represents a continued broadening of the assay use from the predominantly lower risk DCIS cohort in the 1st validation study (E5194). The impact on XRT decisions is critical to establishing the clinical utility of the assay. The decision impact analysis, differences in use of the assay among surgeons and radiation oncologists and the impact on overall confidence with the treatment decision will be presented.
Title: Breast conserving surgery alone for ductal carcinoma in situ: Factors associated with increased risk of local recurrence

Mele A, Mehta P, Brook A, Recht A, Slanetz P and Sharma R. Beth Israel Deaconess Medical Center, Boston, MA.

Body: Background: There is ongoing debate regarding the added benefit of radiation therapy (RT) for patients with ductal carcinoma in situ (DCIS) believed to be at low risk for recurrence after wide local excision (WLE) alone since RT is costly and can cause significant adverse effects. In this retrospective study we aimed to identify clinicopathological characteristics associated with an increased risk for ipsilateral local recurrence (LR) in patients not undergoing RT.

Methods: All patients with DCIS treated with WLE alone at the Beth Israel Deaconess Medical Center, Boston between 2000 and 2010 were identified. We collected data on demographics, parity, personal or family history of breast cancer, exogenous hormone use, tobacco use, comorbidities, genetic mutation carrier status, imaging interval, and tumor-specific characteristics (size, margins, grade, architectural subtype, presence of necrosis, estrogen receptor status). We analyzed their effects on the risk of LR.

Results: The study cohort included 281 eligible patients (mean age at diagnosis 59 years, range 33-90). Median follow-up time was 8 years (range 0.11-16.59 years); 59 patients were excluded because they were not followed in our institution after undergoing WLE. LR occurred in 19 of 222 patients (9%), with a recurrence rate of 11.3 per 1000 person-years. The median time from excision to LR was 4.2 years (range, 0.8-11.7). The risk of recurrence was lower for the 64 patients with nuclear grade (NG) I tumors than for the 110 patients with a NG II or 20 patients NG III tumors (3%, 9%, and 20%, respectively, p for trend = 0.01). The mean margin width was 1.8 mm in patients experiencing LR, versus 2.5 mm in patients without LR (p=0.4). Patients who had used hormone replacement therapy or oral contraceptives (n=61) or patients with a history of tobacco use (n=41) had higher rates of LR than those who did not, although these did not reach statistical significance (15% versus 6%, p=0.06; and 17% versus 7%, p=0.07 respectively). There was no significant correlation between the use of tamoxifen or aromatase inhibitors and the risk of LR.

Conclusions: Our data indicate that higher nuclear grade, narrower margin width, use of exogenous hormones, and smoking history may be associated with an increased risk of LR. The evaluation of these factors may be helpful when considering whether to use adjuvant RT or not for patients with DCIS.
Title: Local recurrence rates after mastectomy undertaken for pure ductal carcinoma in situ

Timbrell SJ J, Himdani SA A, Shaw O, Morris J and Bunded NJ J. University Hospital South Manchester, Manchester, United Kingdom.

Body: Currently over 30% of patients with ductal carcinoma in situ (DCIS) are treated by mastectomy. Local recurrence rates (LRR) after simple mastectomy for DCIS have historically been reported as low\(^1\). Recent UK and US data has indicated 5-year local recurrence rates after mastectomy for DCIS are increasing\(^2\). Skin sparing mastectomy (SSM) offers a better cosmetic outcome however there is little work in the literature comparing local recurrence rates between simple and SSM for pure DCIS. Local recurrence rates may be higher after SSM compared to simple mastectomy\(^3\).

Aims:
1. To evaluate what local recurrence rates were after mastectomy performed for pure DCIS.
2. To compare LRR in simple against SSM.

Methods:
We undertook a retrospective analysis of all patients who underwent a mastectomy for pure DCIS at one breast unit between 2000-2010. Operation reports were reviewed and data collected on the type of mastectomy and reconstruction used. Pathology reports were reviewed and the histological type, grade, size of DCIS as well as the presence of micro-invasion, excision margin and molecular phenotype were recorded.

We excluded those patients who were having surgery for recurrent ipsilateral disease, gynaecomastia and risk reduction.

Results:
One hundred and ninety-nine patients had a mastectomy to treat pure DCIS between 2000-2010. Median follow up time was 65 (0-152) months.
102 patients had an SSM compared to 97 who had a simple mastectomy. The mean age was younger in the SSM group 53 as opposed to 61 (\(p = <0.01\), t-test).

Overall local recurrence rates were 3.1% at 5 years and 5.6% at 8 years.
Fiver-year contralateral recurrence rates were 4.2% and 8.5% at 8 years.
All of the recurrences occurred in the SSM group which had 8/102 local recurrences compared to 0/97 in the simple mastectomy group.

5 year recurrence rate following mastectomy for pure DCIS

<table>
<thead>
<tr>
<th></th>
<th>Simple (n=97)</th>
<th>SSM (n=102)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ipsilateral LRR</td>
<td>0%</td>
<td>5.9%</td>
<td>0.01</td>
</tr>
<tr>
<td>Contralateral RR</td>
<td>4.8%</td>
<td>3.2%</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\(^1\) LogRank test

All 8 of the recurrence were invasive ductal carcinoma with a median disease free survival time of 55 months.
Univariate analysis demonstrated that a young age and close margins predicted recurrence.

Univariate factors predicting recurrence

<table>
<thead>
<tr>
<th></th>
<th>Recurrence (n=8)</th>
<th>Non-Recurrence (n=191)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>48 (37-54)</td>
<td>57 (33-81)</td>
<td>0.01</td>
</tr>
<tr>
<td>Involved Margins (&lt; 2mm)</td>
<td>5</td>
<td>52</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Conclusion:
Our overall 5-year local recurrence rate was 3.1%. This is higher than historical data however in line with emerging UK and US data.
Five-year LRR was significantly higher after SSM 5.9% compared to simple mastectomy 0%. All recurrences were invasive disease, which represented a loss of control of disease.
Further work is needed to evaluate if there is a benefit of undertaking post reconstruction mammography to detect recurrences whilst still in situ disease. Our work corroborates with the literature highlighting the importance of achieving clear margins to prevent recurrence.
References:
Title: Prognostic value of method of detection in primary pure DCIS

Elshof LE E, Schaapveld M, Schmidt MK K, van Leeuwen FE E, Rutgers EJTh JTh and Wesseling J. Netherlands Cancer Institute, Amsterdam, Netherlands.

Body: Background
Population-based mammographic screening programs have led to a substantial increase in incidence of ductal carcinoma in situ (DCIS). We assessed whether the method of detection provides prognostic information among women with DCIS detected through the Dutch screening program (screen-detected DCIS) and those with DCIS not detected within the national screening program (non-screen-detected DCIS). This could have impact on the treatment strategy of screen-detected DCIS as compared to symptomatic DCIS.

Methods
We studied a population-based retrospective cohort comprising 7,106 women aged 49-76 years with primary pure DCIS, who were treated by mastectomy or breast conserving surgery with or without radiotherapy between 1989 and 2004 in the Netherlands. Risk of subsequent ipsilateral and contralateral invasive breast cancer and overall survival among women with screen-detected (n=4,905) and non-screen-detected (n=2,201) DCIS were compared using Cox regression, adjusting for treatment (time-dependent), age (time-scale), diagnosis period and follow-up duration. Because of gradual implementation of the screening program in the Netherlands, we defined two periods based on year of DCIS diagnosis: 1989-1998 (gradual implementation of screening) and 1999-2004 (full coverage of screening).

Results
With a median follow-up of 10.5 years (interquartile range 7.7-14.0 years) 366 ipsilateral (screen-detected DCIS n=234, non-screen-detected DCIS n=132) and 380 contralateral (screen-detected DCIS n=245, non-screen-detected DCIS n=135) invasive breast cancers were diagnosed, and 1,088 of 7,106 women died (screen-detected DCIS n=603, non-screen-detected DCIS n=485). From 1989 to 2004 the number of non-screen-detected DCIS remained stable (mean 140, range 110-187 per year), whereas the number of screen-detected primary pure DCIS increased from 8 in 1989 to 596 in 2004. Ipsilateral invasive breast cancer risk was lower for screen-detected DCIS compared to DCIS not detected within the national screening program, irrespective of DCIS treatment, period of diagnosis, and follow-up duration (adjusted hazard ratio [HR] 0.74, 95% confidence interval [CI] 0.59-0.92, p < 0.01). The prognostic value of method of detection was similar across categories of treatment, period of diagnosis, and follow-up duration. The risk of contralateral invasive breast cancer did not differ between screen-detected DCIS and non-screen-detected DCIS (adjusted HR 0.89, 95% CI 0.71-1.11, p = 0.3) and neither did all-cause mortality (adjusted HR 0.91, 95% CI 0.79-1.04, p = 0.2).

Conclusion
Women with primary pure DCIS detected through the Dutch screening program had lower risk of subsequent ipsilateral invasive breast cancer, irrespective of DCIS treatment, compared to women whose DCIS was not detected within the national screening program. However, the magnitude of this risk difference does not warrant a different treatment strategy of screen-detected DCIS as compared to non-screen-detected DCIS. Having a screen-detected DCIS was not associated with risk of subsequent contralateral invasive breast cancer and all-cause mortality.
Title: Abstract Withdrawn

Body:
Title: Study of boost radiotherapy's influence on local control in 646 pure ductal carcinoma in situ breast cancer with long-term follow-up

Cambra MJ José, Moreno F, Sanz X, Anglada L, Moià M, Reyes V, Arenas M, Pedro A, Ballester R, García V, Sanjosé S, Cusidó M, Jimenez C, Macià M, Solé JM María and Farrus B. Institut Oncològic del Vallés-IDC-HGC, Sant Cugat del Vallés, Barcelona, Spain; Institut Català d’Oncologia, Hospital de la Llobregat, Barcelona, Spain; Hospital de la Esperança. Parc de Salut Mar, Barcelona, Spain; Institut Català d’Oncologia, Girona, Spain; Hospital Universitari de la Vall de Hebrón, Barcelona, Spain; Hospital Universitari Sant Joan, Reus, Tarragona, Spain; Hospital Plató, Barcelona, Spain; Institut Català d’Oncologia, Badalona, Spain; Hospital Universitari Arnau de Vilanova, Lleida, Spain; Hospital Quirón, Barcelona, Spain; Consorci Sanitari de Terrassa, Terrassa, Barcelona, Spain and Hospital Clinic i Provincial, Barcelona, Spain.

Body: BACKGROUND: Boost radiotherapy (B-RT) improves outcome in patients (pts) with invasive breast cancer. Its use in patients with pure ductal carcinoma in situ (DCIS) is unclear. There are two ongoing randomize trials, results are expected in ten years. Twelve retrospective observational studies have been published since 2006, the recent meta-analysis, support boost in the presence of positive margins.

PATIENTS and METHODS: We analyse a retrospective women's cohort of 646 pure DCIS patients (pts) treated mainly in two Hospitals (n=518) from 1993 to 2014. The other ten Hospitals included 128 pts all 2005 long. Proportions were compared by boost status, using the chi-square tests. The impact of boost radiation on the development of local recurrence (LR) was determined using survival analyses. In the comparison of Kaplan-Meier (K-M) was used log-rank test.

RESULTS: B-RT subgroup is 394 pts (61%), noB-RT 252 (39%). Median follow-up (FU) is 8.8 years. High risk factors: young age, size, margin status and tamoxifen (TMX) show differences among B-RT (p<0.05). 46% were Estrogen Receptor positive (ER+), 30% B-RT and 16% noB-RT. 22% RE+ in B-RT take TMX vs 9.4 % RE+ noB-RT. Total LR 65 (10%). In situ LR 30 pts (4,8%) and Invasive (Inv) 35 (5,4%). By subgroup, LR in B-RT 47 (12%) vs. 18 (7%) in noB-RT. By subtype, In situ LR in B-RT 20 (5.1%) vs. noB-RT 10 (4%). Inv LR in B-RT 27 (6.9%) vs. noB-RT 8 (3.2%). In uni & multivariate analysis, tumour size, Re-Excision, and TMX, are significant LR risk factors (p<0.05). Boost total doses >16 Gy in the B-RT subgroup is a LR significant risk factor related to 10-16 Gy (p<0.05). TMX and Dose Boost are related (p<0.001). When Dose is introduced in multivariate analysis model, TMX lost signification. Contralateral local recurrence (CLR) in 29 pts (7%). Second tumours 9 pts (NSD between subgroups). Global disease free survival (DFS) is 80.5%, 77% in B-RT vs. 85% in noB-RT. Four pts have a LR combined with CLR; 2 pts have a LR and a second tumor; 1 pt with CLR and second tumour; 1 pt an Inv regional recurrence; 1 pt mixosarcoma in ipsilateral breast and lung metastases. Deaths: 3 pts (0.5%) after an Inv LR; 3 pts (0.5%) after Inv CR; 20 pts other causes; 10 pts lost their FU. Median FU in B-RT subgroup was 9y vs. 8.3y in noB-RT. The maximum FU according LR in B-RT is 20.6y vs. 17.4y in noB-RT. RL is not significant according to Boost (K-M p=0.398). Median LR in situ or inv depending of B-RT vs. noB-RT shows NSD (p=0.663).

CONCLUSIONS: In this large cohort retrospective study with long-term follow-up B-RT was associated with similar LR as noB-RT despite being used more frequently with higher risk disease. Dose boost >16 Gy has a protective effect. Tamoxifen and boost dose are related variables. Further evidence, based on ongoing randomized trials results is essential.
2015 San Antonio Breast Cancer Symposium

Publication Number: P5-17-09

Title: Biomarkers to distinguish hazardous from harmless ductal carcinoma in situ (DCIS) of the breast


Body: Background. The incidence of DCIS has increased since the introduction of population-based screening. This has not resulted in a decrease in invasive breast cancer incidence, implying overdiagnosis exists. All women with DCIS are still intensively treated, by surgery, radiotherapy, and/or hormonal treatment, although only a minority will develop a subsequent invasive breast cancer. As we cannot discriminate such hazardous from harmless DCIS lesions, accurate prognostic biomarkers are urgently needed. In the current study we aim to identify molecular markers for DCIS aggressiveness, using a large population-based cohort.

Patients and methods. We used a population-based, nation-wide cohort consisting of 10,090 women treated for primary DCIS between 1989 and 2004 with a median follow-up time of 10.7 years. Within this cohort, a case-control study was set up to analyse which markers are associated with progression to invasive breast cancer. Formalin-fixed paraffin embedded (FFPE) tissue blocks were retrieved from 1580 DCIS patients who were treated by breast conserving surgery without radiotherapy (316 DCIS patients with a subsequent ipsilateral invasive breast cancer (iIBC): i.e. the "cases"; and 1264 DCIS patients without subsequent invasive breast cancer: i.e. the "controls"). A first study using this population-based cohort will involve immunohistochemistry (IHC) on 200 "cases" and 500 "controls" for an 8-marker IHC panel (ER, PR, HER2, Ki67, p16, p53, COX-2, and Annexin A1). Molecular subtypes of the DCIS and invasive breast cancer lesions will be determined and intra-individual heterogeneity will be assessed. IHC marker expression will be both compared between "cases" and "controls" as well as between DCIS lesions and its subsequent invasive breast cancer. In a second study, DNA and RNA will be isolated from these specimens, using laser microdissection, and extensive molecular profiling will be performed.

Results. We have collected FFPE tissue blocks of 287 "cases" and 1149 "controls" (86% of requested material) from 56 participating hospitals. At present, the specimens of 223 "cases" (matched DCIS and iIBC specimen) and 103 "controls" have been centrally revised for extensive morphological characteristics. Only a small part (14%) of the specimens had to be excluded from the study population. IHC staining of the tissue specimens, using the 8-marker IHC panel is ongoing.

Conclusion. Within a nation-wide cohort of 10,090 patients diagnosed with primary DCIS, we were able to collect tissue material of a representative case-control series of 200 "cases" with subsequent invasive breast cancer and 500 invasive breast cancer-free "controls". This is the first time such a large unique, unbiased DCIS series, with long-term follow-up is analysed integrating clinical, histological, and immunohistochemical data. The results will be presented at SABCS 2015.
Title: Claudin -4 expression in carcinoma in situ and its association with local recurrence, clinical and immunohistochemistry characteristics


Body: Introduction: Claudins are tight junction molecules and have been associated to breast cancer prognosis. Claudin-low intrinsic subtype of invasive carcinoma was described recently and has been related to high grade carcinoma, low junction molecules expression and worse chemotherapy response. However, it is unknown whether Claudins expression could be associated to carcinoma in situ prognostic. The aim of this study was evaluated the Claudin – 4 expression in carcinoma in situ and its association with local recurrence, clinical and immunohistochemistry characteristics.

Methods: A tissue microarray (TMA) block was constructed, using region of interesting, with 137 pure carcinoma in situ paraffin blocks of patients treated in the Women’s Hospital Prof. Dr. José Aristodemo Pinotti – UNICAMP from 1999 to 2009. The TMA was submitted to immunohistochemistry analyze to: Claudin-4, beta-catenin, e-caderin, estrogen receptor (ER), progesterone receptor (PR), HER-2 and Ki-67. It was calculated Claudin-4 score based in percentage and intensity of expression and categorized in: Claudin-4 low and Claudin – 4 high. The clinical data, treatment data (surgery, radiotherapy and tamoxifen use), local recurrence data (date and type) and death of each patient were reviewed in the medical records. The statistical analyze used Kaplan-Meier curve and log-rank test to disease free survival; qui-square and Fisher test to compare others variables; significance level of 5 % was used.

Results: It was possible to evaluate Claudin-4 expression in 86 cases, 88.4% were Claudin-4 high and 11.6% Claudin-4 low. The follow up mean was 69 months and local recurrence rate was 10.5 %. There was no significant difference in local recurrence rate between Claudin-4 high and Claudin-4 low (10.0% x 10.5% , p=1.0). The disease free survival was similar between Claudin-4 low and Claudin-4 high (p=0.559). The Claudin-4 high was significantly more frequent in beta-catenin positive patients (p=0.048). There was no association significantly between Claudin-4 expression and: age (p=0.66), histology type (p=0.75), surgery (p=0.102), radiotherapy (p=0.29), tamoxifen use (p=0.432), ER (p=0.33), PR (p=1.0), HER-2 (p=0.23) and e-caderin (p=0.21).

Conclusion: Despite the Claudins are related to invasive carcinoma prognosis, our outcome did not show difference in local recurrence and disease free survival between Claudin-4 low and high in carcinoma in situ. The beta-catenin and claudin-4 expressions were significantly associated.
Title: Trends in incidence, patient characteristics, and management of lobular carcinoma in situ

Johnson AT T, Guo X, Nygaard RM M and Zera RT T. Hennepin County Medical Center, Minneapolis, MN.

Body: Introduction: Lobular carcinoma in situ (LCIS) is a rare lesion accounting for only 5% of total breast cancer diagnosis; however, the incidence has continued to increase, albeit at a slower rate than was seen in the 1980s and 1990s. Current National Comprehensive Cancer Network (NCCN) guidelines recommend surgical excision of the breast tissue containing LCIS due to risk of concomitant malignancy, but wide variations in treatment exist. Using the Surveillance, Epidemiology, and End Results (SEER) and National Cancer Database (NCDB) participant user files, an extensive survey of women with LCIS breast cancer was completed. Our aim was to identify patient and facility characteristics that are associated with aggressive treatment (mastectomy) or under-treatment (no surgical excision) following LCIS diagnosis.

Methods: Women with a diagnosis of LCIS from 1998 to 2011 within the SEER and NCDB databases were identified. Incidence data was gathered from SEER while all other characteristics (patient, facility, and treatment factors) were obtained from the NCDB. A logistic regression model was created to examine factors associated LCIS treatment modalities recorded in the NCDB.

Results: The incidence of LCIS increased from 3.85 to 4.46 / 100,000 women between 2000 and 2011. 62,923 female patients with LCIS were identified within the NCDB. The majority of women diagnosed with LCIS were between the ages of 40 and 59 years (66.8%, N=42,044), white (87.4%, N=55,022), and non-Hispanic (86.9%, N=54,679). Following a diagnosis of LCIS, most women underwent surgical excision (71.1%, N=44,731), with an additional 20.7% undergoing mastectomy (N=13,039) or opting for no surgery (8.0%, N=5,023). Logistic regression analysis demonstrated that age over 60 years was associated with no surgical intervention (OR 1.49, p<0.001), while age 40 to 59 were associated with aggressive therapy (OR 1.19, p<0.001). Economic factors associated with no surgical intervention following LCIS diagnosis include: no insurance (OR 1.24, p<0.001), government sponsored insurance (OR 1.48, p<0.001), live in areas with median income less than $63,000 per year (OR 0.744, p<0.001), and live in an area with higher rates of the population lacking high school education (OR 1.19, p<0.001). Patients undergoing aggressive treatment were more likely to be white (OR 1.57, p<0.001) and carry private insurance (OR 1.87, p<0.001). Patients receiving care at an academic center were more likely to receive aggressive treatment (OR 1.22, p<0.001). Living farther from treatment center was associated with increased odds of aggressive therapy, but not under-treatment (p<0.001 vs p=0.906, respectively).

Conclusion: Despite being a rare cancer, the incidence of LCIS continues to rise. LCIS is predominantly a cancer diagnosed in white, non-hispanic women. Advanced age, poverty, lack of insurance, low high school graduation rates were factors significantly associated with under-treatment of LCIS. Conversely, white women between 40-59 yo with private insurance and treated at a cancer center were more likely to undergo aggressive treatment.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-18-01

**Title:** A randomized clinical trial of postoperative adjuvant therapy for elderly breast cancer patients: Conditions of obtaining informed consent and reasons for declining participation

Sagara Y, Sawaki M, Taira N, Saito T, Kashiwaba M, Iwata H, Kobayashi K, Nakayama T, Bando H, Mizuno T, Yamamoto Y, Tsuneizumi M, Takahashi M, Yamaguchi M, Kawashima H, Takashima T, Uemura Y, Hozumi Y, Sagawa N, Mukai H and Ohashi Y. Hakuaikai Social Cooperation, Kagoshima, Japan; Aichi Cancer Center Hospital, Nagoya, Aichi, Japan; Saitama Red Cross Hospital, Saitama, Japan; Iwate Medical University, Morioka, Iwate, Japan; Okayama University Hospital, Okayama, Japan; Cancer Institute Hospital of The Japanese Foundation for Cancer Research, Tokyo, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; University of Tsukuba, Tsukuba, Ibaraki, Japan; Mie University Hospital, Tsu, Mie, Japan; Kumamoto University, Kumamoto, Japan; Shizuoka General Hospital, Shizuoka, Japan; Hokkaido Cancer Center, Sapporo, Hokkaido, Japan; Kurume General Hospital, Kurume, Fukuoka, Japan; Aomori City Hospital, Aomori, Japan; Osaka City University Graduate School of Medicine, Osaka, Japan; Tokyo University Hospital, Tokyo, Japan; Jichi Medical University Hospital, Shimotsuke, Tochigi, Japan; Kameda Medical Center, Kamogawa, Chiba, Japan; National Cancer Center Hospital East, Kashiwa, Chiba, Japan and Chuo University, Tokyo, Japan.

**Body:**

**Background:** There are few randomized clinical trials examining adjuvant treatment in elderly breast cancer patients. While obtaining informed consent is essential for participation in clinical studies, there is little information on the frequency of agreement to participate among elderly patients. Furthermore, elderly patients might have specific reasons to decline participation.

**Patients and Method:** The National Surgical Adjuvant Study of Breast Cancer 07 (N-SAS BC 07) is a randomized clinical trial in women over 70 years with HER2-positive primary breast cancer. The primary aim was to investigate the benefit of trastuzumab monotherapy compared with the combination of trastuzumab and chemotherapy. Key inclusion criteria were as follows: women between 70 and 80 years old with HER2-positive breast cancer; underwent curative operation; stage I to IIIA; with sufficient organ function. Patients were randomized to receive either trastuzumab plus chemotherapy or trastuzumab monotherapy. The primary endpoint was disease-free survival, and the secondary endpoints were overall survival, relapse-free survival, safety, health-related quality of life, and cost effectiveness (NCT01104935). It was not possible to predict the number of patients who would agree to participate. In order to comprehensively assess the effect of postoperative adjuvant therapy, we evaluated the reasons why eligible patients declined to participate. The patients were registered in a cohort study to prospectively evaluate the subsequent treatment options and prognosis (07-Cohort). This study examined the obtaining of informed consent for N-SAS BC 07 and the reasons for declining participation, and compared the clinicopathological backgrounds between the N-SAS BC 07 and 07-Cohort groups.

**Results:** 398 eligible patients have been recruited. Informed consent to participate in N-SAS BC 07 has been obtained from 275 patients (69%) and 123 patients (31%) who declined to participate in the RCT have been registered in the 07-Cohort. The common reasons to decline participation in the RCT were "cannot choose the treatment option (55%)", "refused chemotherapy (16%)", "wanted chemotherapy (9%)", "anxious about clinical studies (9%)" and "family opposition (8%)". The mean ages of the patients in N-SAS BC 07 and 07-Cohort were 73.9 and 74.6 years old, respectively. There were no differences in stage, surgical procedure, lymph node metastasis, or co-morbidities between the groups. ER-positive rate was higher in 07-Cohort group compared with N-SAS BC 07 group (53% vs. 37%, p=0.017, χ² test).

**Conclusion:** While we expected the number of registrants to be small, since N-SAS BC 07 investigated whether elderly patients with HER2-positive breast cancer should undergo chemotherapy, almost 70% of the patients accepted informed consent. The most common reason to decline participation in N-SAS BC 07 was "cannot choose the treatment option" and the majority refused chemotherapy. Furthermore, ER-positivity was higher in the 07-Cohort group, which suggested that ER expression in the patients with HER2-positive breast cancer might influence their decision to participate in the study or to choose the treatment option.
Title: The cohort multiple randomized controlled trial is a feasible and patient-acceptable design for pragmatic evaluation of multiple interventions, with potential for improved recruitment and generalisability: The UMBRELLA experience


Body: Introduction: The randomized controlled trial (RCT) is the gold standard for evaluation of effectiveness of new interventions. In oncology, however, RCTs are often complicated by logistic challenges, slow recruitment, limited generalisability, and strong patient's and doctor's preferences for new interventions. At the University Medical Center Utrecht (the Netherlands), we implemented an innovative alternative to the classic RCT: the cohort multiple randomized controlled trial (cmRCT). The cmRCT serves as a multi-trial facility and has the potential to improve recruitment and generalisability.

Methods: We initiated the 'Utrecht cohort for Multiple BREast cancer intervention studies and Long-term evaluation (UMBRELLA)' at the department of radiation oncology. UMBRELLA is a large prospective cohort of breast cancer patients, for whom we capture clinical data (patient and tumor characteristics, treatment, imaging, toxicity, recurrence and survival). Patients provide Patient Reported Outcome Measures (PROMs) on quality of life, pain, fatigue, anxiety and depression, physical activity and cosmetics. For each intervention to be tested, a subcohort of eligible patients is identified within UMBRELLA. From this subcohort, a random sample of patients is selected and offered the intervention (which they can accept or refuse). Patients from the subcohort, who are not randomly selected, receive standard care, serve as controls, and are not informed about the trial. Outcomes of patients offered the intervention are compared to those of patients receiving standard care. This process can be repeated (simultaneously) for multiple experimental interventions.

Results: Since October 2013, some 1000 patients have been enrolled in UMBRELLA. In order to make the design suitable for the clinical oncology practice, we developed a tailored informed consent procedure. First, we ask patients to enroll in the cohort and to provide PROMs. In addition, patients give broad consent to be either randomly selected to receive experimental interventions or to serve as control without further notice. In a second stage, at the initiation of a trial within the cohort, informed consent to receive the experimental intervention is sought in those randomly selected to receive the intervention. After completion of the trial, aggregated results are shared with all patients. Participation in UMBRELLA is 90%, and 90% of enrolled patients give broad consent for randomization. PROMs return rates vary from 70-90%. In September 2015, the first trial will be initiated, which aims to evaluate the impact of exercise programs on quality of life of inactive survivors. Preliminary inclusion and participation rates of this trial will be presented in December.

Conclusion: High participation rates, high PROM return rates and high levels of broad informed consent for randomisation within UMBRELLA indicate that this innovative design is feasible in the oncology practice, acceptable for patients and likely to provide generalisable results. Results of trials within UMBRELLA need to confirm whether cmRCT is indeed an acceptable alternative for classic pragmatic RCTs.
Title: Cooperation of clinically-relevant genomic alterations transforms mammary progenitor cells to metaplastic breast cancer


Body: Background: We have previously identified the MAPK phosphatase dual specificity phosphatase 4 (Dusp4) as frequently down-regulated in triple-negative and basal-like breast cancer (TNBC and BLBC, respectively). DUSP4 is deregulated in BLBCs by heterozygous and homozygous loss, as well as by promoter CpG methylation. In TNBC and BLBC, DUSP4 deregulation frequently co-occurs with MYC amplification and mutations in TP53. In addition, loss of DUSP4 promotes activation of ETS-1 and cJUN, transcription factors downstream of Ras/MAPK and JNK, while imparting resistance to chemotherapy and promoting tumor-initiating cells, suggesting DUSP4 is a tumor suppressor. Thus, we hypothesized that loss of Dusp4 in the mammary epithelium, together with MYC amplification and mutations in Trp53, would promote tumorigenesis.

Methods: We generated transgenic mice with a conditional floxed Dusp4 allele (Dusp4FLOX) and isolated mammary epithelial progenitor cells. The resulting cells were transduced with adenovirus to deliver either Cre recombinase to excise the Dusp4 locus (Dusp4NULL) or GFP control. CRISPR-mediated mutagenesis (or non-targeting CRISPR control) was used to disrupt exon 7 or 8 of Trp53 causing relevant p53 mutations and MYC was overexpressed (or LACZ control). Isogenic cell lines were assessed for tumorigenicity, ploidy, sensitivity to chemotherapy, proliferation, and cell signaling.

Results: The isolated parental (Dusp4FLOX) mammary cell line maintained proliferation through >50 passages without senescing and was highly cytokeratin (Ck) 5/14+ and moderately Ck 8/18+, suggestive of a bipotent progenitor-like lineage. As expected, p53 disruption, regardless of Dusp4 status, resulted in tetraploidy and loss of p21 expression. With concurrent Dusp4, p53 and MYC alterations, cells became increasingly resistant to chemotherapy and targeted agents, as measured with proliferation assays. In order to determine whether cells formed tumors in vivo, we implanted isogenic cell lines subcutaneously into nude mice. We found that only Dusp4NULL cells, together with p53 disruption and MYC overexpression (DPM) generated aggressive tumors (forming and ulcerating within 1-2 weeks), while the Dusp4FLOX counterparts (p53 disruption and MYC overexpression; PM) did not. Syngeneic transplantation revealed that DPM cells could also form tumors in an immune competent setting. Cells with Dusp4 loss and p53 mutation were also able to develop tumors without MYC amplification, but at a much slower rate.

Tumors derived from the DPM cells were primarily metaplastic or basal-like in nature, including intra-tumor heterogeneity (pockets of Ck5 and pockets of Ck8, with regions of Ck8+ keratin pearls).

Conclusions: We have developed a genetically relevant mouse model to study BLBC in immune competent mice for unique opportunities to understand the pathogenesis and therapy response in BLBC, including immunotherapy. These results support the hypothesis that DUSP4 loss promotes tumorigenesis in BLBC. An understanding of the mechanism whereby Dusp4 loss contributes to this process in vivo should provide a rational for novel therapeutic strategies to treat BLBCs with DUSP4 loss.
The genomics of response to neoadjuvant trastuzumab and chemotherapy in HER2-positive breast cancer – Results from the ACOSOG Z1041 (Alliance) trial

Lesurf R, Griffith O, Griffith M, Watson MA A, Hoog J, Ellis MJ J, Ota D, Suman VJ J, Meric-Bernstam F, Leitch AM Marilyn, Boughley JC C, Unzeitig G, Buzdar AU U, Hunt KK K and Mardis ER R. McDonnell Genome Institute, Washington University School of Medicine, St. Louis, MO; Baylor College of Medicine, Houston, TX; Duke University Medical Center, Durham, NC; Alliance Statistics and Data Center, Mayo Clinic, Rochester, MN; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas Southwestern Medical Center, Dallas, TX; Mayo Clinic, Rochester, MN and Doctors Hospital of Laredo, Laredo, TX.

Support: Alliance U10CA180821; Alliance Statistical Center grant U10CA180882; ACOSOG grant U10CA76001

HER2 gene amplification and its corresponding overexpression are present in approximately 12% of invasive breast cancers. While HER2-targeted agents (e.g. trastuzumab, pertuzumab, and lapatinib) are effective treatments, resistance remains a major cause of death from HER2-positive breast cancer. Mechanisms of resistance are poorly understood. Without a molecular understanding of these mechanisms, therapeutic advances will be delayed. We have generated molecular profiles of primary HER2-positive breast cancers treated on a neoadjuvant clinical trial, and compared features associated with response to treatment.

The American College of Surgeons Oncology Group (ACOSOG) Z1041 trial in HER2-positive breast cancer was designed to compare the pathologic complete response (pCR) rate of a regimen of paclitaxel and trastuzumab, followed by trastuzumab administered with fluorouracil, epirubicin, and cyclophosphamide (FEC-75) to a regimen of FEC-75 alone followed by paclitaxel and trastuzumab. The trial identified no difference in pCR rates between the regimens (Buzdar et al., The Lancet Oncology 2013). In supplement to the tissues obtained from 37 of the patients enrolled in the Z1041 trial, an additional 11 cases were obtained from a single institution study (201101961) of patients treated with neoadjuvant trastuzumab that had pre-treatment core biopsies suitable for genomic studies.

We have extracted genomic DNA from both pretreatment tumor biopsies and blood samples of these 48 patients and performed whole genome (WGS) and exome sequencing. Coincident with these efforts, we have extracted high quality RNA from 42 of the 48 biopsies, and have processed RNA-seq profiles of the tumors. Among patients in this cohort, 24 (50%) achieved a pCR. Because no difference was observed between arms of the Z1041 trial, patients with or without a pCR were directly compared without adjusting for treatment regimen.

On average, each tumor and normal sample pair were sequenced to a depth of 49.4x and 32.5x by WGS respectively. In total, 15,027 candidate somatic variants were identified in known genes, including 11,606 missense, 860 nonsense, and 418 frameshift insertions or deletions. Preliminary results identified mutations in HER2 that were associated with the failure to achieve pCR in several cases. Furthermore, tumors assigned to the HER2-enriched subtype by RNA-seq analysis were more likely to achieve a pCR (19 compared to 8) than tumors with genomic features indicative of either the luminal or basal-like subtypes (3 compared to 12); a significant difference in the proportion of cases that achieve pCR (Fisher's exact test p-value = 0.0032). The identification of these features suggests that it may be possible to predict, at the time of diagnosis, those patients who will not respond to the current standard of care for HER2-positive breast cancer.
Title: High prevalence and clonal heterogeneity of ESR1 mutations (mt) in circulating tumor DNA (ctDNA) from patients (pts) enrolled in FERGI, a randomized phase II study testing pictilisib (GDC-0941) in combination with fulvestrant (F) in pts that failed a prior aromatase inhibitor (AI)

Gendreau S, Spoerke J, Johnston S, Schmid P, Krop I, Qui J, Derynck M, Chan I, Walter K, Amler L, Hampton G and Lackner M. Genentech, South San Francisco, CA; Royal Marsden Hospital, London, United Kingdom; Barts Cancer Institute, Queen Mary University London, London, United Kingdom and Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Mutations in the ligand binding domain of the estrogen receptor gene (ESR1) have been associated with resistance to AI therapy in pts with ER+ breast cancer. To assess if ESR1 status has prognostic or predictive significance in the post-AI metastatic setting ESR1 mutation status was analyzed in circulating tumor DNA (ctDNA) from 168 pts enrolled on the FERGI study (NCT01437566; Krop et al., SABCS 2014).

Methods: Baseline and longitudinal mutational analysis for hotspot mutations in ESR1 (E380Q, S463P, V534E, P535H, L536R/H/P, L536Q, Y537N/S/C, D538G) and PIK3CA (C420R, E542K, E545K/G, Q546K, M1043I, H1047Y/R/L) was performed using droplet digital PCR (ddPCR) on ctDNA derived from plasma. Archival tissue was analyzed via RT-PCR and ddPCR.

Results: Baseline ctDNA analysis demonstrated a total of 62/156 (40%) and 57/153 (37%) pts with PIK3CA and ESR1 mutations, respectively. The most common ESR1 mutations are D538G, Y537S, and E380Q, representing 54%, 33% and 26% of the pts with a detectable ESR1 mutation at baseline, respectively. There was a numeric increase of ESR1 mutations in patients with LumA (41/99, 41%) vs LumB disease (14/44, 31%). PIK3CA mutations in asynchronously collected archival tissue were 85% concordant with plasma ctDNA mutations (sensitivity 78%, specificity 91%). PIK3CA mutations in baseline ctDNA showed a higher median allele frequency (AF) than ESR1 mutations (3.6% vs 0.46%), consistent with PIK3CA being an early event and ESR1 mutations occurring later in pts with recurrent disease. Of the pts with a detectable ESR1 mutation at baseline (n=57), 23 (40%) pts had multiple ESR1 mutations and 10 (18%) had ≥3 ESR1 mutations. The PFS outcomes for patients with and without ESR1 mutations detected at baseline are summarized below, indicating no obvious prognostic or predictive effect for combination of F with pictilisib compared with F in these underpowered subsets.

<table>
<thead>
<tr>
<th>Arm</th>
<th>ESR1 MT - mPFS (mo)</th>
<th>ESR1 WT - mPFS (mo)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F + placebo</td>
<td>5.4 (30 pts, 24 events)</td>
<td>3.7 (40 pts, 31 events)</td>
<td>1.06 (0.62, 1.81)</td>
</tr>
<tr>
<td>F+ pictilisib</td>
<td>5.8 (27 pts, 20 events)</td>
<td>6.7 (56 pts, 34 events)</td>
<td>1.36 (0.78, 2.38)</td>
</tr>
</tbody>
</table>

PIK3CA and ESR1 ctDNA analysis on serial plasma samples from 40 pts and the assessment of ESR1 mutation status in the patient's tumor sample by ddPCR is currently in progress and will be reported.

Conclusions: Mutations in ESR1 detected by ddPCR in patient plasma samples occur in nearly 40% of pts that failed a prior AI. The polyclonal nature of ESR1 mutations is consistent with the convergent evolution of multiple AI resistant subclones. While these conclusions should be interpreted with caution due to the relatively small sample size and post hoc nature of the analysis, this data does not support a prognostic or predictive PFS hypothesis for ESR1 mutations with F or in combination with pictilisib.
MBC immune related gene alterations (>0.5%) by MSK-IMPACT testing

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Overall N=641</th>
<th>HR+HER2- N=341</th>
<th>HR+/- HER2+ N=78</th>
<th>TNBC N=57</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL10 amp</td>
<td>25 (3.9%)</td>
<td>19 (5.6%)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CD79B amp</td>
<td>18 (2.8%)</td>
<td>6 (1.8%)</td>
<td>5 (6.4%)</td>
<td>0%</td>
</tr>
<tr>
<td>ICOSLG amp</td>
<td>6 (0.9%)</td>
<td>5 (1.5%)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CD274 (PD-L1) amp</td>
<td>4 (0.6%)</td>
<td>0%</td>
<td>1 (1.3%)</td>
<td>2 (3.5%)</td>
</tr>
<tr>
<td>CSF1R del</td>
<td>4 (0.6%)</td>
<td>0%</td>
<td>0%</td>
<td>2 (3.5%)</td>
</tr>
</tbody>
</table>

*165 with ER/PR/HER2 status unavailable, amp = amplification, del = deletion, HR=hormone receptor (ER and/or PR)
Title: Identifying genetic vulnerabilities in cancers driven by defects in DNA-damage response

Srihari S, Singla J, Wong L, Simpson PT T, Khanna KK and Ragan MA A. Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland, Australia; Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India; National University of Singapore, Singapore; The University of Queensland, Centre for Clinical Research and School of Medicine, Brisbane, Queensland, Australia and QIMR-Berghofer Medical Research Institute, Brisbane, Queensland, Australia.

Body: Although defects in cancer susceptibility genes within the DNA-damage response (DDR) machinery including BRCA1 and BRCA2 account for only 5-10% of all breast cancer cases, these defects are highly penetrant and significantly increase the risk of breast (60-80%) and also ovarian (35%) cancers [1]. Together with defects in the DNA-damage sensor ATM, apoptosis effector TP53, and PTEN and CDH1 with roles in regulation of DDR, these account for considerable proportions of sporadic breast (63%) and ovarian (85%) cancers. To compensate for these DDR defects and to avoid cell death triggered from a genomic catastrophe, cancer cells rewire their DDR network while also selecting (during clonal expansion) the optimal combination of oncogenic events. Deciphering these combinations of events would aid in mapping the vulnerabilities of cancer cells harbouring defects in DDR. While there have been several studies screening for essentiality of genes across DDR-deficient cell-lines, the essential genes so identified are either restricted only to these cell-line models or are not frequently (over)expressed in cancers. Here, we observe that oncogenic events that are mutually exclusive to DDR defects in large proportions of cancers constitute the (clonally) selected combinations that are amenable to cancer-cell survival, and therefore by systematically mining for these events, we infer vulnerability genes that if targeted in conjunction with DDR defects could induce a genomic catastrophe and trigger cancer-cell death.

Using data from DNA copy-number and mRNA-expression profiles we infer vulnerability genes that are mutually exclusive to defects in six DDR genes ATM, BRCA1, BRCA2, CDH1, PTEN and TP53 across four cancers (total 3980 samples) – breast (2029), prostate (623), ovarian (828) and uterine (500) from The Cancer Genome Atlas. Interestingly, across the four cancers these vulnerability genes form the most combinations with BRCA2 (59.02%), followed by CDH1 (24.59%), PTEN (8.20%) and TP53 (8.19%) at p<0.01 (1-hypergeometric test), whereas these show distinct patterns within the individual cancers: combinations dominated by CDH1 (90%) in breast, PTEN (78.38%) and BRCA2 (16.82%) in prostate, and BRCA1 (71.94%) and TP53 (16.21%) in ovarian cancers. Validation using GARP (Gene Activity Rank Profile)-score data from essentiality screens [2] from ten breast cancer cell lines (HCC1143, HCC1187, HCC1395, HCC1419, HCC1428, HCC1500, HCC1806, HCC1954, HCC38, MCF7) which harbour defects in at least one of the six DDR genes shows remarkable agreement between the GARP rankings and our inferred vulnerabilities. Our inferred genes are significantly enriched (p<0.0001 X^2 test) in the top quartile of the entire set of profiled (~16000) essential genes in these screens. Moreover, Kaplan-Meier analysis using survival data from 1000 breast cancer patients shows considerable overexpression of these genes (e.g. TLK2 in 37% luminal cases) which correlates significantly (TLK2: p<0.0006; Grade 3 hazard ratio 2.5) with poor prognosis. Experimental validation of these genes using single- and double knockout with DDR in breast cancer cell lines is currently underway.

2015 San Antonio Breast Cancer Symposium

Publication Number: PD6-06

Title: Somatic mutation patterns differentially affect survival in breast cancer molecular subtypes

Gyorffy B, Pongor L, Szabo A, Bottai G, Pusztai L and Santarpia L. MTA TTK Lendület Cancer Biomarker Research Group, Budapest, Hungary; Semmelweis University, Budapest, Hungary; Oncology Experimental Therapeutic Unit, Humanitas Clinical and Research Institute, Milano, Italy and Yale Cancer Center Genetics and Genomics Program, Yale University School of Medicine, New Haven, CT.

Body: Background: The prognostic effects of somatic gene mutations and correlated gene expression in breast cancer is argument of debate. In this study we analyzed the impact of specific mutations on gene expression and their relevance in the prognosis of breast cancer subtypes.

Materials and methods: Exome sequencing and RNA-seq data obtained from TCGA were analyzed. Data was processed using MuTect, MapSplice and RSEM. All together data from 757 patients (ER-/HER2- [n=143], HER2+ including ER positive and negative patients, [n=136], and ER+/HER2- [n=478]) were included. Univariate Receiver Operating Characteristic (ROC) analysis was performed for the top mutated genes (mutated in at least 5% of patients) using the ROC Bioconductor library in R to identify genes whose expression was significantly associated with a mutation. Then, the mean expression of the significant genes was designated as a metagene for each genotype. We assessed the correlation with survival for each metagene by Cox proportional hazards regression and by plotting Kaplan-Meier survival plots. A significance threshold of p<1E-04 was set for each gene to be considered in the survival analysis, and only the top 100 genes were used when there were more than 100 genes significant.

Results: In the overall population only few mutated genes including TP53 (HR=1.66), CDH1 (HR=0.61), AKT1 (HR=0.54), ATM (HR=1.76), NF1 (HR=0.58), KMT2D (HR=2.32), and UBR5 (HR=1.94) were significantly associated with survival. In ER-/HER2- mutant samples the PIK3CA (HR=2.79) and MAP3K1 (HR=2.98), and in HER2+ mutant samples the ARID1A (HR=0.26) and PIK3CA (HR=0.27) metagenes were associated with survival, respectively. Overall, using the combined metagene the majority of the significant mutated genes retained their prognostic power. Mutations of specific genes impacted their own expression and prognosis. The expression of TP53 (AUC=0.609, p=2.60E-06), and MAP3K1 (AUC=0.617, p=6.07E-03) was higher in samples with a mutation while the expression of CDH1 (AUC=0.684, p=2.72E-07), PTEN (AUC=0.687, p=1.47E-04), and BRCA1 (AUC=0.608, p=2.24E-02) was lower.

Conclusions: Our finding support that specific mutated genes may differentially impact prognosis in breast cancer subtypes. Further efforts are required to understand the biological and prognostic role of specific activating and inactivating mutations across molecular breast cancer subtypes.
Genomic sequencing in metastatic breast cancer patients to inform clinical practice at the University of North Carolina at Chapel Hill


Background: An increasing number of molecularly-targeted therapies for metastatic breast cancer (MBC) are clinically-available (approved and investigational). These anti-cancer agents target specific molecular abnormalities such as mutated, amplified, deleted, or rearranged genes. Reporting of unique tumor genetic alterations is not included in routine clinical/diagnostic panels. In MBC, knowledge of mutational status may foster efficient transitions in clinical care and trial enrollment at disease progression. We describe the development and implementation of a clinically-integrated genomic sequencing program and report how information regarding targetable genomic aberrations in MBC patients (pts) is used to improve clinical practice in an academic setting.

Methods: Genomic sequencing of investigative biomarkers was prospectively offered to pts with MBC. DNA libraries were prepared separately from a retrieved archival FFPE tumor sample and a matched normal sample from each pt. Relevant targets were enriched by custom Agilent SureSelect hybrid capture baits using standard protocols. Samples were sequenced on Illumina HiSeq 2000/2500 platforms. Mutational findings were reviewed by a molecular tumor board (MTB); variants identified to be potentially actionable underwent confirmatory testing in a CLIA-approved laboratory. Confirmed findings were inserted into the pt's EMR accessible by both the pt and the treating oncologist.

Results: Of the 725 MBC pts seen at UNC since 1/1/2012, 194 (27%) contributed samples for genomic sequencing. Of those whose tumors were sequenced, average age at MBC diagnosis was 54 (25 - 91); 73% were Caucasian, 16% African American. De novo MBC accounted for 39 (20%) sequenced pts. Of sequenced patients, sites of metastatic disease included bone only (7%), visceral only (46%), and both bone and visceral (47%). Approximately 1/3 of pts were consented for sequencing at time of initial MBC diagnosis, 1/4 after 1st line therapy for MBC, and the remaining at or beyond their 2nd line. In total, 131 (68%) pts have sequencing results available of which 43% of pts had reportable mutations deemed actionable by the MTB. Specific mutations and observed frequency by subtype are shown below. Pts (19%) whose tumors were sequenced were more commonly enrolled in a therapeutic clinical trial for MBC, a higher rate than seen in the non-sequenced group (7%) (p<0.001). To date, 27% of pts' tumors harbored an alteration that is an eligibility requirement for a molecularly-targeted therapeutic trial accruing pts at UNC.

Observed Mutation by Clinical Subtype

<table>
<thead>
<tr>
<th>Genes</th>
<th>Total # (56 pts)</th>
<th>HR+/HER2- (25 pts)</th>
<th>HER2+ (13 pts)</th>
<th>TNBC (18pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>15</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>TP53</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>CCND1</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>NF1</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>FGFR1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PTEN</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>KRAS</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MDM2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ROS1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TSC1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Other*</td>
<td>14</td>
<td>5</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>--------</td>
<td>----</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>TOTAL</td>
<td>73</td>
<td>28</td>
<td>17</td>
<td>28</td>
</tr>
</tbody>
</table>

*Mutations observed only once

Conclusion: Preemptive genomic sequencing can be integrated into the clinical and operational practice of a comprehensive cancer center. Currently this research tool and program provides valuable information that has the potential to foster both clinical trial eligibility and/or enrollment. With longer follow-up, we hope such an approach ultimately will improve patient outcomes.
2015 San Antonio Breast Cancer Symposium

**Title:** IMAGE: Individualized molecular analyses guide efforts in breast cancer with comprehensive genomic profiling of tissue and plasma tumor DNA


**Body:**

**Background:** Standard treatment options for patients with metastatic triple negative breast cancer (TNBC) are limited to chemotherapy. Molecular profiling of tumors may allow for novel treatment recommendations.

**Methods:** We initiated a prospective study designated IMAGE. Women with newly progressing metastatic TNBC who received at least one line of prior chemotherapy were eligible. New metastatic biopsies were obtained for molecular profiling at study entry. Archived metastatic biopsy specimens were allowed if patients had not commenced new systemic therapy. The specimens were reviewed by the study pathologist and stained for ER, PR, HER2, and androgen receptor (AR) by immunohistochemistry. Specimens underwent hybrid-capture based comprehensive genomic profiling (CGP) (Foundation Medicine Inc., Cambridge, MA). Clinical data and genomic profiling reports were reviewed by the GAITWAY (Genomic Alterations in Tumors with Actionable Yields) Molecular Profile Tumor Board. Recommendations were communicated to the treating oncologist and patients were followed for treatment decision and clinical outcomes. Peripheral blood was also analyzed by an investigational assay for circulating plasma tumor DNA (ptDNA) (Foundation Medicine Inc.) at study entry, and when obtainable, from serial blood draws at time of progression. The primary objective was to assess feasibility of completing the process from consent to GAITWAY recommendations within 28 days for at least 80% of patients.

**Results:** From September 2013 to April 2015, we enrolled 26 eligible women. Median age was 55 (range 25-67); patients identified as white 12 (46%), black 11 (42%), or other 3 (12%); median number of prior lines of treatment was 3; and 65.4% of patients had visceral disease. Twenty (77%) eligible patients received CGP of a metastatic site biopsy. Six patients did not undergo CGP due to either absence of a metastatic site amenable for biopsy or inadequate tissue for CGP. The study met the predefined statistical endpoint for futility and was closed after 20 patients had undergone CGP. Twelve (60%) evaluable patients received treatment recommendations within 28 days of study consent. Failure to meet this time frame was due to difficulties in accessing archival tumor tissue (N=5) and need for additional tissue for molecular analysis (N=3). Preliminary results demonstrate high concordance between mutations in metastatic biopsies and ptDNA in 15/17 patients.

<table>
<thead>
<tr>
<th>Enrolled in IMAGE</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful NGS</td>
<td>20</td>
</tr>
<tr>
<td>Potentially actionable mutation identified</td>
<td>15</td>
</tr>
<tr>
<td>GAITWAY recommended targeted therapy as possible next treatment</td>
<td>13</td>
</tr>
<tr>
<td>Received targeted therapy</td>
<td>4</td>
</tr>
</tbody>
</table>

**Conclusions:** CGP of patients with metastatic TNBC can provide additional information that may help direct treatment. However, difficulties in obtaining adequate tumor tissue may hinder this approach. Use of a well-validated ptDNA profiling assay could be an alternative to overcome these limitations.
Title: Interim analysis of multiplex gene panel testing for inherited susceptibility to breast cancer

Body: Background: Emerging evidence demonstrates the effectiveness of targeted gene sequencing panels as a practical method for the diagnosis of inherited susceptibility to breast cancer. Sequencing of multiple high and moderate risk genes simultaneously accelerates the discovery of deleterious mutations (DM) or variants of unknown significance (VUS). However, a consequence of Multiplex Gene Panel (MGP) testing is the discovery of unexpected DMs in high or moderate risk genes other than BRCA1 or BRCA2 (BRCA1/2). The overall clinical utility and incremental gain of information conferred by MGP testing in hereditary cancer risk assessment is still unknown.

Methods: We are conducting a multicenter prospective cohort study of patients undergoing cancer-risk assessment using a 25 gene sequencing panel, which includes APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMAD4, STK11, and TP53. Patients were recruited from August 2014 to June 2015 at three medical centers. Patients are enrolled if they meet standard criteria for genetic testing or are predicted to have ≥2.5% probability of inherited susceptibility to cancer calculated by validated risk prediction models. We present a planned interim analysis after enrolling 500 of 2000 total participants.

Results: HCP testing was performed for 332 patients referred for clinical suspicion of hereditary breast and ovarian cancer (HBOC). In this cohort, 96.7% were female (n=321) and the mean age was 50 years (standard deviation, SD=12.2); race/ethnicity was 43.1% Hispanic (n=143), 37% Non-Hispanic White (n=123), 4.2% Black (n=14), 10.5% Asian (n=35), and 1.8% other (n=6). Among this cohort, 37 tested positive for one deleterious mutation (DM) (11.1%: 95% confidence interval (CI), 8.2% to 15%) and 118 patients carried at least one variant of uncertain significance (VUS) (35.5%: 95% CI, 30.6% to 69%). Excluding BRCA1 or BRCA2, 14 patients (4.3%: 95% CI, 2.6% to 7.2%) have a DM in ATM (n=3), CHEK2 (n=2), MSH6 (n=1), MUTYH (n=3), PALB2 (n=1), PMS2 (n=1), RAD51C (n=2), and TP53 (n=2). In a patient with an unexpected PMS2 mutation, enhanced cancer surveillance based on Lynch Syndrome guidelines was recommended. Among 160 patients with a history of invasive breast cancer or breast DCIS, 19 patients carried a DM (11.8%: 95 CI, 7.7% to 17.8%).

Conclusion: In this multicenter prospective cohort study among a diverse group of participants undergoing 25-gene MGP testing, 11.1% of participants tested positive for a DM. Among participants testing negative for BRCA1 and BRCA2, MGP testing identified DMs in 4.3% of participants prompting clinically appropriate risk reduction recommendations and enhanced cancer surveillance. Ongoing recruitment and long-term follow-up are in progress.
Title: Multiplex Identification of genetic etiologies among women with bilateral breast cancer using a 25-gene hereditary cancer panel


Body: Background: Multiple primary cancers within an individual is one of the hallmarks of hereditary cancer predisposition. Technical advances in sequencing and identification of additional cancer susceptibility genes have led to multi-gene panel approaches to determine if patient cancers have a heritable cause. Simultaneous testing of multiple breast cancer associated genes to determine the prevalence, spectrum and combinations of mutations has not yet been evaluated in a large set of patients with two breast cancers.

Methods: Patients with two breast cancer diagnoses were identified from 80,748 consecutive cases that underwent a 25-gene hereditary cancer panel test at a commercial diagnostic laboratory. Patient clinical data were obtained by healthcare provider report on test requisition forms.

Results: Of 3,182 patients with two breast cancers, 13.3 % (n=424) had a pathogenic variant (PV) in at least one of 19 genes on the panel. PVs were most prevalent within BRCA1, representing 26.3% of the PVs, followed by BRCA2 (21.9%), CHEK2 (13.5%), PALB2 (11%) and ATM (11%). Fourteen patients had PVs in two different genes, including five with CHEK2/PALB2 in combination, which was the only combination observed more than once. PVs were more common among the 33.8% of patients with synchronous breast cancers compared to those with metachronous diagnoses (10.8% vs. 15%) and in the 58.4% who were first diagnosed younger than 50 years old (15.7% vs. 10%).

Conclusion: Multiplex testing in women with two primary breast cancers identifies a relatively high percentage with a PV, including those whose first diagnosis was older than 50. While still the most prevalent and clear cause, fewer than half of the PVs identified were in BRCA1 or BRCA2. The observation of 5 cases with double CHEK2/PALB2 PVs is statistically unexpected given their individual prevalence in other studies of high risk women with breast cancer, suggesting a possible synergistic or additive effect. This study adds to our understanding of susceptibility to multiple primary breast cancers, and reaffirms the importance of this circumstance as a prompt for genetic testing. Nonetheless, as 66% of cases had metachronous tumors, it would be preferable to identify those at risk with heritable cancer syndromes at their first diagnosis and enable subsequent prevention or early detection.
Title: Characterization of Li-Fraumeni syndrome diagnosed using a 25-gene hereditary cancer panel


Body: Background: Clinical diagnostic criteria for Li-Fraumeni syndrome (LFS) have evolved with increased utilization of TP53 germline testing and subsequent improved understanding of the diversity of the associated cancer phenotypes. However, data on LFS still suffer from ascertainment bias as patients are typically selected to undergo TP53 testing based on the presence of hallmark features of LFS. Analyzing TP53 mutation carriers identified from multi-gene panel testing, for which the diagnosis of LFS may not have been suspected or was included in a longer differential diagnosis, affords an opportunity to characterize additional TP53 carriers who might not otherwise have been ascertained.

Methods: Patients with a deleterious or suspected deleterious germline TP53 mutation were identified from 80,748 consecutive cases that underwent a 25-gene hereditary cancer panel test between September 2013 and March 2015 at a commercial diagnostic laboratory. Patient clinical data were obtained by healthcare provider report on test requisition forms. Each TP53 mutation carrier was evaluated to determine whether the National Comprehensive Cancer Network’s (NCCN) guidelines were met for TP53 testing.

Results: Eighty-one TP53 mutation carriers were identified and had a total of 115 cancers (0.1% overall prevalence). Among the 76 carriers with at least one cancer, the average age at first diagnosis was 42 years (range 11-76 years) and 24% were first diagnosed older than age 50 years. The most common first cancers were of the breast (n=45), ovary (n=9), and gastrointestinal tract (n=8). Fifty-two of the 75 (69%) women had breast cancer, 44% of which were first diagnosed at 35 years or younger, and 21% were first diagnosed at 50 years or older. Only 27 TP53 carriers met NCCN criteria for TP53 testing, 14 of whom only met based on having early-onset breast cancer. An additional 8 did not meet criteria themselves but had a first- or second-degree relative who did. Among the 28 individuals with more than one primary cancer, 21 (75%) developed their second primary at a site for which increased surveillance is recommended in LFS, but only 4 would have met NCCN criteria for TP53 testing at their first cancer diagnosis. The most common second cancers were of the breast (n=16), gastrointestinal tract (n=4), or kidney (n=2) and occurred an average of 11 years after the first cancer (range 0-36 years).

Conclusion: In this analysis, a large proportion of carriers would not have been identified as TP53 testing candidates based on NCCN guidelines. Our data are consistent with other studies demonstrating high second primary cancer risks in LFS, and highlight the value of multigene panel testing in identifying individuals who may be candidates for increased surveillance and/or cancer risk-reducing management options.
Exome based germline mutation detection in a panel of 372 cancer associated genes in BRCA1/2-negative familial breast cancer patients


Background:
Ten to 20% percent of all breast cancers occur in a familial context and in 20-30% of these cases a mutation in the BRCA1 or BRCA2, CHEK2 genes or, more rarely, in PALB2 can be found. The remaining cases remain routinely undiagnosed with regard to a possible genetic cause.

We have examined a cohort of undiagnosed probands using exome germline sequencing in order to identify other potential breast cancer predisposition genes.

Methods:
In total, 63 BRCA1/2-negative high risk familial BC cases (BRCAX) were considered for pair-end whole exome germline DNA sequencing on a HiSeq1500 (Illumina). High quality reads were mapped (BWA-MEM) to the reference genome (hg19) and variants were called according to GATK best practice guideline. The variants detected within the panel of 372 cancer associated genes were annotated with ANNOVAR. Synonymous variant as well as variants with MAF>1% were discarded. In a first phase protein truncating variants were validated using another NGS method. Subsequent validation of non-synonymous missense mutations and non-frameshift indels is planned. Patients signed a multilayered informed consent also covering disclosure or not of different types of incidental findings.

Results:
For each exome, the mean breath of coverage was about 96 % at 10X or more and the mean depth of coverage for targeted region was about 126X. In total, 357,070 SNPs (∼56,678 SNPs/sample) and 47,7801 INDELs (7,584 INDELs/sample) were called. Of them, 20,829 SNPs (331 SNPs/sample) and 16,071 INDELs (255 INDELs/sample) passed quality filter. In total, 445 variants were found in the publicly available cancer genes panel. Twenty-seven stop-gain/loss, frame-shift insertion/deletion and splice site variants were considered for validation with 454 Roch Junior, among which 22 variants in genes ABCC11, AFP, BARD1, BBS10, CD96, CYP1A1, Dnah11, ESCO2, EXO1, FANCI, FLCN, FLT4, HPS6, MYH8, NME8, PALB2, PDE11A, RECQL4, TTC8 were validated as true positive. Some of these genes have been found earlier to be associated with breast cancer and/or other cancer types. Functional prioritization of the remaining 416 non-synonymous and non-frameshift insertion/deletions was also done in-silico before further sequencing validation, which is ongoing. The mutations found are further clinically validated by examining other affected and non-affected family members and mining the literature. Some of the mutations in known cancer predisposing genes are considered for prudent application in clinical counseling. Genotype-phenotype correlations are being examined.

Conclusion:
Next-generation sequencing enabled us to detect variants with high/low penetrance in known cancer predisposing genes in >35% of BRCAX families, in addition to many novel variants in many other genes not yet tied to cancer predisposition occurring singly or in combination with known cancer gene mutations. Validated variants are further examined in the families for co-segregation with the disease and potential application in counseling.
Title: Multi-gene panel testing and the cancers identified in patients at risk for hereditary breast cancer

Kapoor NS S, Curcio LD D, Patrick M, Swisher J, West JD D and Banks K. Breastlink, Orange, CA; Breastlink, Laguna Hills, CA and Ambry Genetics, Aliso Viejo.

Body: Background: Next generation sequencing and broadened genetic testing guidelines have made it possible to perform multi-gene testing upfront for patients at risk for hereditary breast cancer. Breast surgeons and oncologists are ideally situated at the forefront of cancer treatment to initiate these tests since results can impact treatment decisions. This study evaluates the utility of multi-gene testing in a multidisciplinary breast practice.

Methods: Data was collected retrospectively from 500 consecutive patients who underwent multi-gene panel testing July 2013 – September 2014. Patients were evaluated at time of visit if they met criteria for genetic testing based on NCCN guidelines.

Results: Most patients had no prior genetic testing; 28.8% of patients had previous negative BRCA1 and BRCA2 (BRCA1/2) tests. All patients had a personal and/or family history of breast or ovarian cancer. All patients were evaluated with a multi-gene panel consisting of a minimum of 5 breast-cancer related genes (BRCA1, BRCA2, PTEN, TP53, and CDH1) and most (68.0%) had extended panel testing of up to 43 cancer-associated genes. Pathogenic mutations were identified in 32 (6.4%) patients. The majority of patients (79.0%) were not found to carry any mutations, while 16.2% had at least one genetic variant of uncertain significance. Of the patients with pathogenic mutations, 37.5% had a mutation in BRCA1/2 while most patients had mutations in non-BRCA1/2 genes.

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</tbody>
</table>

The majority of patients with mutations had a personal history of cancer including breast, ovarian, and thyroid cancer. There was no significant difference between age of breast cancer diagnosis and having a BRCA1/2 mutation compared to having a non-BRCA gene mutation. The majority of gene-positive patients with cancer had hormone-positive invasive ductal carcinoma (IDC) while only two patients had triple negative breast cancer. Compared to patients with BRCA1/2 mutations, patients with non-BRCA mutations were more likely to have a family history of non-breast or ovarian cancer (58.3% vs 90%, respectively, p=0.0735).

Conclusions: Multi-gene panel testing will identify more patients with risk of breast and ovarian cancer than routine BRCA1/2 testing alone, and may have an impact on screening for other cancers as well. Obtaining a thorough personal and family cancer history is necessary to provide optimal counseling and screening.
INTRODUCTION: The role of BRCA2 and BRCA1 in male breast cancer is well established, however, there is limited data regarding the role of other genes in male breast cancer. The aim of this study was to assess the clinical characteristics and genetic testing outcomes in men diagnosed with breast cancer undergoing multi-gene testing.

METHODS: Test results were reviewed for male breast cancer patients who underwent analysis of breast cancer-associated genes via multi-gene testing from March 2012 to March 2015. Panels consisted of 5-49 genes, depending on the test ordered. Clinical histories provided on test requisition forms were assessed. Statistical analysis was performed using the Wilcoxon Rank Test and the Fisher's exact test.

RESULTS: Approximately 12% (33/280) of men diagnosed with breast cancer were identified to carry a pathogenic mutation/likely pathogenic variant, ~2% (5/280) were identified to carry a moderate risk mutation (APC p.I1307K or CHEK2 p.I1577T), ~1% (2/280) were identified to carry a monoallelic MUTYH mutation, ~18% (51/280) had a variant of unknown significance but were otherwise negative, and ~67% (189/280) tested negative. The mutation rate was highest for BRCA2, at 7.3% (18/247 tested), followed by CHEK2, at 5.1% (10/196 tested). Of note, 3 men tested positive for multiple mutations: one man had BRCA2 and ATM mutations, one man had BRCA2 and TP53 mutations, and one man, with a clinical diagnosis of ataxia-telangiectasia, had two ATM mutations.

The average age at diagnosis for mutation-positive men (62.5 years) was not significantly different from negative men (59.6 years) (p=0.269). However, the average age of diagnosis for men with CHEK2 1100delC mutations (46.0 years) was significantly lower than the average age of diagnosis for men who were otherwise positive (p=0.012). Fourteen of the mutation-positive men were found to have multiple cancers; colon polyps, melanoma, and cancers of the bladder, prostate and pancreas were reported in more than one individual. Based on the family history provided, two men who tested positive for BRCA2 mutations would not otherwise have met NCCN criteria for testing. The majority of men in this cohort with a family history of male breast cancer (10/19) tested negative for a mutation. Five mutation-positive patients were found to have a family history of male breast cancer: three with CHEK2 mutations; one with a BRCA2 and a TP53 mutation; and one with two ATM mutations. Men with CHEK2 mutations were significantly more likely to have a family history of male breast cancer compared to other positive men (p=0.020) as were men with multiple mutations (p=0.008).

CONCLUSION: In this cohort of male breast cancer patients, multi-gene testing identified mutations in patients that would not previously have been identified with a single gene testing approach and allowed for identification of families with multiple mutations. Age at diagnosis and family history were not predictive of positive test results in general, although men with CHEK2 100delC mutations were diagnosed at a significantly younger age. Although 30% of this cohort was not tested for CHEK2, the CHEK2 mutation rate in the men tested was second in frequency to BRCA2, suggesting a role for CHEK2 testing in male breast cancer patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: PD7-07

Title: Abstract Withdrawn

Body:
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-01-01

Title: Impact of imaging surveillance on the risk of radiation induced malignancies in breast cancer survivors

Spera G, Gonzalez V, Meyer C, Fung H, Mackey JR R and Fresco R. Translational Research in Oncology (TRIO), Montevideo, Uruguay; Biostatistics, Translational Research in Oncology (TRIO), Edmonton, AB, Canada and University of Alberta, Edmonton, AB, Canada.

Body: Introduction: After curative treatment of breast cancer (BC), relevant clinical guidelines recommend against the use of imaging procedures other than yearly mammography for surveillance, based on the lack of survival benefit for intensive surveillance strategies. Nevertheless, use of non-recommended imaging tests occurs frequently in this context. Most BC surveillance studies have focused on the potential benefit of detection of early relapse, on financial burden, and risk of false positives with different follow-up regimens. No study has analyzed the risk of imaging radiation induced malignancies (IRIM) in BC survivors exposed to repeated body imaging during surveillance. We previously reported on the IRIM risk in the BC clinical trials setting (Fresco R. The Oncologist 2015). In this current study we report on this risk during surveillance in clinical practice in BC survivors.

Objective: To estimate IRIM risk in patients curatively treated for BC undergoing imaging tests during surveillance. Methodology: We defined 6 surveillance strategies with differing imaging requirements, from a non-imaging-intensive one (yearly mammography only) to intensive ones (mammography + CT, Bone scan, PET-CT and/or MUGA) (Table 1). For each strategy we calculated the imaging dose and excess lifetime attributable cancer risk (LAR) for a 60 year-old BC survivor, using NCI's Radiation Risk Assessment Tool (RadRat).

Results: Total effective imaging radiation dose received by a 60 year-old BC survivor during surveillance was 8.4 miliSieverts (mSv) when only yearly mammography is performed to 199.9 mSv when CT, MUGA and bone scan are added. Mean IRIM LAR ranges from 37.2/100,000 with the first strategy to 1,330/100,000 with the latter. Performing MUGA scans increased IRIM risk 31% compared to not performing it. The addition of any additional radiating imaging procedure to yearly mammography significantly increases LAR.

<table>
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</tr>
<tr>
<td>Yearly mammography + Chest/abdomen CT q6mo for 3y, then annually for 2y</td>
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<td>Yearly mammography + Chest/abdomen CT q6mo for 3y, then annually for 2y + Bone scan q12mo for 5y</td>
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<tr>
<td>Yearly mammography + Chest/abdomen CT q6mo for 3y, then annually for 2y + MUGA q6mo for 2y + Bone scan q12mo for 5y</td>
</tr>
<tr>
<td>Yearly mammography + PET-CT q6mo for 3y, then annually for 2y</td>
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</table>

Conclusions: A number of incremental second cancers could be derived from imaging performed during BC surveillance after curative treatment. Addition of non-recommended imaging for relapse detection increases IRIM risk compared to performing only
mammography. This, in addition to the lack of proven benefit in BC endpoints, emphasizes the need to follow recommendations for surveillance clinical guidelines, and forgo imaging studies other than annual mammography to detect relapses. Substituting MUGA with echocardiogram for cardiac assessment could also reduce IRIM risk.
Title: Fluorine-18-fluorodeoxyglucose positron emission tomography for the evaluation of response to therapy in bone-dominant metastatic breast cancer: Examination in patients enrolled on UPCC 17113


Body: Background: Response of bone-only (BO) and bone-dominant (BD) metastatic breast cancer (mBC) to therapy is difficult to assess by conventional imaging. UPCC 17113 is a single institution prospective cohort study evaluating FDG PET at early and conventional follow up intervals, 4 and 12 weeks respectively, in patients with hormone receptor (HR) positive mBC receiving endocrine therapy. The objective of this study is to assess the relationship between changes relative to baseline in standard uptake values (SUV) of specific bone lesions and progression free survival (PFS). We will also explore change in SUV as it relates to overall survival (OS) and skeletal related events (SRE). We present interim results.

Methods: Enrolled patients were ≥18 years with biopsy proven or documented clinically obvious HR-positive BD/BO mBC due to start new endocrine therapy. Any line endocrine therapy was allowed. FDG PET was performed at baseline, 4 and 12 weeks after initiation of new therapy. SUVmax for the 5 most metabolically active osseous lesions, excluding sites previously treated by radiation or surgery, were recorded at baseline, 4- and 12- week time-points. Average index lesion SUVmax (sum SUVmax/#lesions) and % change from baseline were calculated. Decline of ≥30% from baseline was defined as significant.

Results: As of 6/1/2015, 11 patients were enrolled. All patients have completed the 4-week scan and 8 have completed the 12-week scan. Five and 6 out of the 11 patients had BO and BD HR-positive mBC respectively. Detectable changes in SUV from baseline were noted in all patients at both 4 and 12 weeks, with a 37% overall decline in average index lesion SUVmax at 4 weeks. 8 of 11 patients had a ≥30% decline, in SUV at 4 weeks averaging 46%. Five of the 6 patients in this group who completed the 12-week scan had a sustained decline averaging 50% from the baseline. Of note, the average decline between 4 and 12 weeks in this group was only 8%. Despite having an overall decline from baseline of 44%, the sixth patient in this group had an increase between 4 and 12 weeks of 22%. Three of the 11 patients had a <30% decline at 4 weeks with an average decline of 12%. Of the two patients in this group with 12 week scans, both had average increase of 25% from 4 weeks to 12 weeks, and an average overall increase from baseline of 12%.

Conclusions: There are detectable changes in FDG SUV of osseous lesions at 4 and 12 weeks following initiation of endocrine therapy in patients with BO or BD HR positive mBC. Our interim results demonstrate the emergence of 3 groups of patients: (1) those who have a <30% decline at 4 weeks and increase of SUV at 12 weeks, (2) those who have a ≥30% decline in SUV at 4 weeks with sustained decline at 12 weeks, and (3) those who have a ≥30% reduction in SUV at 4 weeks but who do not have a sustained decline at 12 weeks. These interim results suggest that early FDG PET/CT may provide information on mBC response to endocrine therapy and insight into timing of response and progression. As more patients are enrolled and complete the studies, clearer patterns will emerge which will be correlated with PFS, OS and SRE.
Title: Trastuzumab-induced hypoxia changes in a HER2+ murine model of breast cancer

Sorace AG G, Quarles CC Chad and Yankeelov TE E. Vanderbilt University, Nashville, TN.

Body: Introduction: The primary goal of this study is to quantitatively map the changes in perfusion and hypoxia in response to trastuzumab treatment through imaging and immunohistochemical studies in a murine model of HER2+ breast cancer. Correlating quantitative imaging data with pathological validation of vessel architecture will identify the temporal changes in vascular function, which will enable optimizing the order and timing of multi-modal (i.e., trastuzumab, chemo- and radiation) therapy, leading to significantly improved anticancer response.

Experimental Design: Mice were implanted subcutaneously with BT474 breast cancer cells \((1 \times 10^7)\) and randomly assorted into groups: treated (10 mg/kg trastuzumab) or control (saline). After tumors reached ∼225 mm3, animals \((n = 20)\) were imaged with dynamic contrast enhanced-MRI (7.0 T MRI) before treatment (day 0), and 24 hours after each treatment (day 1 and day 4). Pharmacokinetic parameters, \(K_{trans}\) and \(v_e\), were extracted. Subgroups of animals were sacrificed for histology between days 0 through 7 \((n = 36)\). Tumor sections were paraffin-embedded and stained with CA-IX, CD31, α-SMA, Ki67 and H&E. Slides were scanned in high resolution \((20×)\) and quantitatively analyzed with Leica SCN400 software. Another cohort of animals \((n = 32)\) was utilized to identify longitudinal changes in functional vascular (Hoechst 33342) and hypoxia (pimonidazole) through immunofluorescence. Agents were injected prior to sacrifice between days 0 through 7 and tumors were immediately frozen for processing.

Results: Treated tumors exhibited a significant increase in \(K_{trans}\) \((p = 0.03)\) on day 4 compared to controls, indicative of heightened vessel perfusion and/or permeability. Additionally on day 4, treated tumors exhibited a significant increase in \(v_e\) \((p = 0.01)\), the extravascular extracellular volume fraction, indicating increased cell death. Significant decreases in Ki67 proliferation staining \((p = 0.02)\) and tumor volume at day 7 \((p = 0.04)\) in the treated group confirmed tumor response to trastuzumab. Immunohistochemical analyses revealed treated tumors have a significant decrease in CD31 microvessel density staining \((p = 0.01)\), with a simultaneous significant increase in α-SMA pericyte coverage staining \((p = 0.05)\) on day 4; thus, there was an overall increase in the "vessel maturation index" (ratio of α-SMA to CD31 staining) compared to controls \((p = 0.01)\). CA-IX staining demonstrated increased hypoxia in the control group compared to treated, showing significant increases on day 3 \((p = 0.03)\) and day 7 \((p = 0.002)\). Qualitative differences were noted between control and treated groups for both functional vasculature and pimonidazole hypoxia fluorescent staining.

Conclusion: Increased intratumoral vascular delivery \((K_{trans})\) with a simultaneous increase in vessel maturation (immunohistochemistry) are exhibited on day 4 post trastuzumab treatment. Additionally, decreased hypoxia is revealed in treated tumors compared to controls. Hypoxic tumor cells show resistance to both radiation and chemotherapy, therefore temporarily improving the tumor's functional vasculature and decreasing hypoxia during trastuzumab treatment has potential to enhance the effectiveness of these combination therapies.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-01-04

Title: Breast ultrasound and mammography and response to neoadjuvant lapatinib, trastuzumab and their combination in HER2 positive breast cancer: Results from Neo-ALTTO (BIG 1-06)


Body: Mammography (Mx) and breast ultrasound (US) are the most commonly used diagnostic imaging modalities to estimate primary tumor size at the time of diagnosis. However, there are uncertainties regarding their use in the context of neoadjuvant therapy to predict pathologic complete response (pCR) or event-free survival (EFS). In this study, we sought to determine the value of Mx and US in predicting outcomes in women with HER2-positive breast cancer treated within the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (Neo-ALTTO) trial.

METHODS
Neo-ALTTO enrolled 455 women with invasive HER2-positive breast cancer and compared rates of pCR to neoadjuvant lapatinib, trastuzumab, and their combination. Each anti-HER2 therapy was given alone for 6 weeks, followed by 12 weeks of the same therapy plus weekly paclitaxel prior to surgery. Mx and US were requested at baseline, week 6 and before surgery. Central imaging review was not pre-planned and two independent investigators (SDC and HAA Jr), blinded to assigned treatment and clinical outcomes, reviewed the measurements reported for each imaging modality and assigned the corresponding RECIST category of response. Responders were defined as patients who had either a partial or complete response (CR + PR). We evaluated the association between radiological response at week 6/surgery with both pCR and EFS.

RESULTS
A total of 340 (77%) and 267 (61%) pts had an evaluable US and Mx at weeks 6; and 309 (70%) and 248 (56%) pts had an evaluable US and Mx at the time of surgery. Early response (CR + PR) in the primary tumors was observed after 6 weeks of treatment in 32% pts by Mx and in 43% pts by US. pCR rates were twice as high for early responders than non-responders (week 6: 46% vs 23% by US, p <=0.0001; 41% versus 24% by Mx, p= 0.007). The positive predictive value of US and Mx at surgery were 57% and 53%, respectively; the negative predictive values were 72% and 81%. The results according to hormone receptor status were similar to those in the overall patient population. There was no significant relationship between response at ultrasound and/or at mammography at 6 weeks/surgery and EFS.

CONCLUSION
Our results show that both Mx and US are underused during neo-adjuvant treatment, and further recommendations regarding the use of both imaging modalities should be explored prospectively. US may be used to assess early response to preoperative treatment in patients with HER2 positive breast cancer receiving anti-HER2 therapies, whereas Mx appears to be more useful in detecting residual disease at the time of surgery.
Title: Radiological evaluation of neo-adjuvant endocrine therapy in hormone-receptor positive early breast cancer

Blok EJ J, Charehbili A, Kroep JR R, Seynaeve CM M, van de Velde CJH JH and Kuppen PJK JK. Leiden University Medical Center, Leiden, Netherlands; Leiden University Medical Center, Leiden, Netherlands and Erasmus Medical Center, Rotterdam, Netherlands.

Body: Recently, there is increased attention to neo-adjuvant endocrine therapy in breast cancer. Especially for selected tumour types and fragile elderly patients this might be a promising alternative to chemotherapy. Monitoring treatment effect during neo-adjuvant endocrine therapy is crucial to allow a timely switch to chemotherapy in case of a non-successful treatment. Most trials evaluating neo-adjuvant endocrine therapy use palpation as primary outcome and multiple radiological modalities as secondary outcomes. The aim of this study was to determine which evaluation corresponds best to pathological resection size after 6 months of neo-adjuvant endocrine therapy.

This analysis was conducted in the TEAM-IIA trial in which 102 patients with early breast cancer (>2 cm and >50% ER expression) were treated with neo-adjuvant exemestane. In total, 83 patients were treated for 6 months and 19 for 3 months. Therapy response evaluation was performed using repeated palpation (mostly by the same clinician), mammography, ultrasound and MRI. Only measurements within 2 months before surgery were considered. After surgery, the size of the remaining tumour was reported and used as reference. In total, 93 resection size measurements were available.

From the 93 patients for whom resection size was available, 69 patients were evaluated by palpation, 53 by ultrasound, 42 by mammography and 29 by MRI. Overall, palpation showed to be the most reliable predictor for resection size (correlation of 49%), followed by mammography (31%), ultrasound (14%) and MRI (1%). Mammography showed the smallest mean absolute error (MAE, 8.7 mm), followed by ultrasound (9.2 mm), palpation (11.4 mm) and MRI (12.3 mm). The low correlation of MRI with resection size was mostly due to a relative high number of radiological complete remissions (14%, n=4), of which only one was a true pathological complete response (pCR), while the other tumours were up to 80 mm at resection. Although of less influence on the correlation to resection size, false complete remissions were observed in all other modalities. Time to surgery was an important factor for all modalities. After correcting for non-predictive radiological complete responses and limiting the measurements to one month before surgery, all correlations increased significantly (mammography=72%, palpation=70%, ultrasound=58%, MRI=50%) with a concomitant decrease in mean absolute error. The low correlation of MRI with resection size was mostly due to non-visible measurements, interpreted as radiological complete remissions of which only one in four was a true pathological complete response (pCR) while the other tumours were 25, 65, and 80 mm at resection.

This is the first study to report on the reliability of radiological evaluation during neo-adjuvant endocrine therapy. In this study, mammography was the most reliable radiological method, with stronger correlation and small mean absolute error. Non-visible observations in neo-adjuvant endocrine therapy did not always reflect pCR. Hence, in the neo-adjuvant endocrine therapy setting, radiological complete responses should be interpreted carefully, especially in MRI, and use of other modalities or improved image processing methods may be considered.
Title: Screening mammography in women over age 75: Is it beneficial?

Cate SP P, Kohli MK K, Gillego A, Chadha M, Fulop T and Boolbol SK K. Mount Sinai Beth Israel Medical Center, NY, NY.

Body: Background:
In 2015 the U.S. Preventive Services Task Force (USPSTF) stated that there was insufficient evidence for the use of screening mammography in women aged 75 and older. This statement was based on the lack of randomized controlled trials demonstrating survival benefit in this population. As per the American College of Radiology, the acceptable cancer detection rate via screening mammography is at least 2.5 cases per 1000 examinations for an institution, with reported rates as high as 4.7 cases per 1000.

Aim:
In this study, we sought to examine our institution's practice of screening mammography for women 75 years and older. We aimed to determine the incidence of cancer detection in this age group through screening mammography.

Methods:
A search was performed to identify women aged 75 and above who underwent screening mammography at Mount Sinai Beth Israel Medical Center between January 1, 2013 and December 31, 2014. Patients classified as BIRADS 0 on initial screening were reclassified based on their subsequent diagnostic imaging, if performed. A chart review was performed for those patients who underwent breast biopsies to obtain their pathology results.

Results:
In this two year period, 2057 patients aged 75 and older underwent screening mammography. The majority of women in this age group had non-actionable results of their screening mammography, and were classified as BIRADS 1 or 2 (96%). There were a total of 49 patients who had BIRADS 3 final results (2.4%). Twenty-two patients had screening mammograms that were classified as BIRADS 4 (1.1%). Biopsies revealed 6 invasive ductal carcinomas, 4 cases of in situ carcinoma, 2 cases of duct ectasia, 2 intraductal papillomas, 3 fibrocystic biopsies, and 3 fibroadenomas. In total, 10 of 2057 patients were diagnosed with breast cancer (0.5%).

Mammography results from 2013-2014

<table>
<thead>
<tr>
<th>Final radiologic classification</th>
<th>Number of patients</th>
<th>Percent of population</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIRADS 1</td>
<td>829</td>
<td>40.3%</td>
</tr>
<tr>
<td>BIRADS 2</td>
<td>1147</td>
<td>55.7%</td>
</tr>
<tr>
<td>BIRADS 3</td>
<td>49</td>
<td>2.4%</td>
</tr>
<tr>
<td>BIRADS 4</td>
<td>22</td>
<td>1.1%</td>
</tr>
<tr>
<td>BIRADS 5</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>BIRADS 0</td>
<td>10</td>
<td>0.5%</td>
</tr>
<tr>
<td>Total screening mammograms</td>
<td>2057</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions:
In our institution, 98.4% of women aged 75 and older had screening mammography with benign results. Ten women in this group were found to have breast cancer. The breast cancer detection rate in this cohort was 4.9 per 1000 screening examinations, which is nearly double the cited recommendation put forth by the American College of Radiology. These results are certainly relevant when considering appropriateness of annual screening mammography in this age group.
Title: Increased interval cancers after the 2009 U.S. preventive services task force guidelines: A single-center, retrospective analysis

Townsend NT T, Everhart RM M, Bayliss EA A and Jaiswal K. University of Colorado, Aurora, CO; Denver Health Hospital Authority, Denver, CO and Institute for Health Research, Kaiser Permanente CO, Denver, CO.

Body: Background: In late 2009, the U.S. Preventive Services Task Force (USPSTF) increased the recommended time between screening mammography from one year to two years. We examined the effect of USPSTF recommendations in an integrated safety-net system whose patients often have intermittent access to care. The purpose of this study was to determine if changes in screening guidelines were associated with stage migration or changes in rate of interval cancers.

Methods: We conducted a retrospective cohort analysis of breast cancer patients diagnosed between 2005-2013 at one safety-net hospital. We abstracted stage at diagnosis, time intervals between screening and diagnostic imaging, as well as BIRADS classification from clinical and administrative billing data. We divided patients into two cohorts: Those diagnosed with breast cancer "pre-2010" (2005 – 2010) and "post-2010" (2011-2013). We compared stage of diagnosis between cohorts using Chi-Square tests. In a subset of patients for whom we had prior screening imaging information, we determined the rate of interval cancers. Interval cancers were defined as patients whose diagnosis occurred within 14 months (pre-2010) or within 26 months (post-2010) of normal screening mammography. Logistic regression was used to determine the unadjusted odds of interval cancer as a function of being post-2010 versus pre-2010.

Results: There were 521 unique, breast cancer patients between 2005-2013.

<table>
<thead>
<tr>
<th></th>
<th>Stage 0</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>249</td>
</tr>
<tr>
<td>41</td>
<td>81</td>
<td>61</td>
<td>42</td>
<td>24</td>
<td></td>
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<tr>
<td>2010-2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>272</td>
</tr>
<tr>
<td>46</td>
<td>79</td>
<td>82</td>
<td>44</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion: The USPSTF 2009 recommendation is associated with a statistically significant increase in interval cancers in a safety-net population, but we cannot conclude that this contributes to stage migration in this limited population. Investigation of larger groups is needed to further assess how USPSTF guidelines affect outcomes in underserved populations.
Body: Background
There is debate to what extend screen-detected cancers (SDC) differ in tumor characteristics and survival from tumors that are detected not trough screening. These can be divide into three groups. Firstly, tumors who manifest clinically in the period between two screens after a negative screening (interval cancers) within 12 months or, secondly, within 12-24 months. Thirdly, we identified tumors in patients with a positive screening, followed by a benign assessment in the hospital, who developed breast cancer 12-24 months after screening (IC-after-positive-screen). The aim of this study was to determine whether interval cancers and IC-after-positive-screen have worse tumor characteristics and survival compared to SDC. Regarding decision-making for more aggressive treatment, these data are essential.

Methods
All women (50-75) who underwent a screening by the Dutch National Screening Program, region North between 2004-2008 were selected and data were merged with the Netherlands Cancer Registry. SDC (diagnosed <12 months after positive screening), interval cancers diagnosed <12 months after negative screening (IC<12) or 12-24 months after negative screening (IC12-24), and IC-after-positive-screen were identified. Tumor characteristics of each group were compared to SDC using chi2. Differences in survival were analyzed with multivariable Cox regression, corrected for differences in tumor characteristics.

Results
In total 4,472 patients were included, 3,363 SDC, 501 IC<12m, 861 IC12-24m and 48 IC-after-positive-screen. Of all SDC, 14% were diagnosed as in situ cancers. A lower percentage of in situ cancers was diagnosed in IC<12m and IC12-24m (6% and 4%, respectively; p<0.001). In situ cancers were diagnosed in 15% of IC-after-positive-screen.

Compared to SDC, invasive IC<12m and IC12-24m were more often poorly differentiated (p<0.001), larger than 2 cm (p<0.001), and had more often positive lymph nodes (p<0.001) or metastasis (p<0.001; Table 1). Furthermore, invasive IC<12m and IC12-24m were less often of the ductal type (p=0.002) or hormone receptor positive (p<0.001), compared to SDC. IC-after-positive-screen were not statistically significant different from SDC for all these factors.

In total 608 (13%) women died. No difference in survival was found for IC<12m (HR=0.86, 95%CI=0.66-1.12) and IC-after-positive-screen (HR=1.40, 95%CI=0.58-3.39) compared to SDC. Women with an IC12-24m had a worse survival than SDC (HR=1.44, 95%CI=1.17-1.77).

Conclusions
IC<12m and IC12-24m had less favorable characteristics than SDC. IC-after-positive-screen had similar characteristics and have a similar prognosis as SDC. However, as the number of IC-after-positive-screen was small, this should be part of further research. Women with an IC12-24 had worse survival compared to SDC.

<table>
<thead>
<tr>
<th></th>
<th>SDC</th>
<th>IC&lt;12m</th>
<th>IC12-24m</th>
<th>IC-after-pos-screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly differentiated</td>
<td>20%</td>
<td>37%</td>
<td>42%</td>
<td>18%</td>
</tr>
<tr>
<td>Tumor size &gt;2cm</td>
<td>20%</td>
<td>47%</td>
<td>52%</td>
<td>17%</td>
</tr>
<tr>
<td>Positive lymph nodes</td>
<td>28%</td>
<td>52%</td>
<td>46%</td>
<td>38%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>1%</td>
<td>5%</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>Ductal type</td>
<td>81%</td>
<td>75%</td>
<td>76%</td>
<td>83%</td>
</tr>
<tr>
<td>Hormone receptor positive</td>
<td>85%</td>
<td>74%</td>
<td>69%</td>
<td>86%</td>
</tr>
</tbody>
</table>
**Title:** Screen detected and interval cancers; genomic analysis points to different molecular etiology?

Gorringe KL L, Hunter SM M, Byrne D, Devereux L, Rowley SM M, Elder K, Huynh R, Pridmore V, Hopper J, Kavanagh A, Mitchell G, Mann BG G, Fox SB B and Campbell IG G. Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia; Lifepool, East Melbourne, VIC, Australia; Royal Melbourne and Royal Womens Hospital, Parkville, VIC, Australia; BreastScreen Victoria, Melbourne, VIC, Australia and The University of Melbourne, Parkville, VIC, Australia.

**Body:** Breast cancers diagnosed after a negative mammogram but prior to the next screening episode are termed "interval cancers" and comprise as many as 25% of all cancers detected in women attending population-based screening programs. The high interval cancer rate is a major problem affecting the effectiveness of mammographic screening. It is unclear whether interval cancers represent a distinct biological entity compared to screen-detected cancers or whether their designation is simply an arbitrary outcome of screening timing. Using an Australian prospective population-based cohort of over 53,000 women (lifepool), 537 cases of breast carcinoma (in situ and invasive breast cancer) were identified, of which 293 had known screening status at time of diagnosis. Pathology reports, mammographic density data, germline DNA and tumor tissue were available for analysis. Screen and interval cases showed no significant differences in mammographic density or PR status but there were trends towards higher proportions of ER negative and HER2 positive cases in interval cancers (p<0.1). Interval cancers also had a younger age at diagnosis (p<0.01), increased tumor size (p<0.01) and higher grade (p<0.01). Copy number analysis was performed on a subset of invasive breast cancer cases using OncoScan MIP arrays. No difference in the overall number of copy number aberrations or fraction of the genome altered were observed, however specific differences were noted between interval and screen detected cases. These included copy number changes on chromosomes 8 and 11. Analysis of germline DNA was performed using a panel sequencing approach of known breast cancer genes as well as lower-penetrance SNPs. Pathogenic mutations in BRCA1, BRCA2, TP53 and PALB2 were identified in 1/13 interval cases (in BRCA2), 1/66 screen-detected cases and 8/74 cases with currently unknown screen/interval status. Screen detected cancers may thus have a reduced contribution from high-penetrance predisposing variants.
Digital versus screen-film mammography in population-based breast cancer screening: Performance indicators and tumor characteristics of screen-detected and interval cancers

De Munck L, De Bock GH H, Otter R, Reiding D, Broeders MJM JM, Willemse PHB HB and Siesling S. Netherlands Comprehensive Cancer Organisation, Utrecht, Netherlands; University of Groningen, University Medical Center Groningen, Groningen, Netherlands; National Cancer Screening Programme, Region North, Groningen, Netherlands and Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands.

Body: Purpose:
To evaluate whether the introduction of full-field digital mammography (FFDM) in screening has resulted in any changes in performance indicators compared to screen-film mammography (SFM), including tumor characteristics and incidence rates of interval cancers.

Materials and Methods
Data of the Dutch National Cancer Screening Programme, region North (2004-2010) were linked to the Netherlands Cancer Registry (N=902,868). Screening performance indicators (recall rate, detection rate, positive predictive value, sensitivity and specificity) and tumor characteristics of screen-detected cancers were compared between FFDM and SFM, as were incidence rates and tumor characteristics of interval cancers. Analyses were stratified by initial and subsequent examinations. Differences were compared using chi-square.

Results
Initial examinations: After initial examinations recall rates were 2.1% for SFM and 3.0% for FFDM (p<0.001). The positive predictive value was 25.6% for SFM and 19.9% for FFDM (p=0.002) and detection rates were similar. The detection rate for DCIS was 0.86 per 1000 women for SFM compared to 1.18 per 1000 women for FFDM (p=0.137). Similar percentages of low grade DCIS were found for SFM and FFDM (18% vs. 14%; p=0.860). No difference were found in tumor size, morphology, grade and nodal status of invasive screen-detected cancers.

Subsequent examinations: After subsequent examinations performance indicators were similar. Detection rates for DCIS were 0.74 per 1000 women for SFM versus 0.81 per 1000 women for FFDM (p=0.298). For invasive cancers, detection rates were 4.54 per 1000 women for SFM versus 4.33 per 1000 women for FFDM (p=0.210). The percentages of low grade DCIS were similar for SFM and FFDM (12% vs. 9%; p=0.524). Invasive cancers diagnosed with FFDM were more often of high grade (p=0.024) and ductal type (p=0.030).

No difference was found in the incidence rates of interval cancers for SFM and FFDM after initial examinations (2.69/1000 vs. 2.51/1000; p=0.787). The sensitivity after initial examinations was 66.1% for SFM and 69.1% for FFDM (p=0.657), specificity was 98.5% and 96.9% for SFM and FFDM, respectively (p<0.001). After subsequent examinations the incidence rates of interval cancer were 2.30/1000 for SFM and 2.41/1000 for FFDM (p=0.652), sensitivity was 69.7% for SFM and 66.7% for FFDM (p=0.232). Specificity was 99.4% SFM and 99.2% for FFDM (p<0.001). No differences were found in tumor size, morphology, grade or nodal status of interval cancers diagnosed after FFDM compared to SFM after initial or after subsequent examinations.

Conclusions
Compared to SFM, FFDM resulted in similar rates of screen-detected and interval cancers, indicating that FFDM performs as well as SFM in a breast cancer screening program, with more invasive cancers of high grade and ductal type found after subsequent screens. FFDM resulted in a higher recall rate and lower PPV. No signs of an increase in low-grade DCIS (which might connote possible overdiagnosis) were seen. Tumor characteristics of interval cancers were similar.
Title: Ductal carcinoma in situ and breast cancer screening in Japanese breast cancer registry

Iwamoto T, Kumamaru H, Miyata H, Tomotaki A, Niikura N, Kawai M, Anan K, Hayashi N, Masuda S, Tsugawa K, Aogi K, Ishida T, Masuoka H, Iijima K, Matsuoka J, Doihara H, Kinoshita T, Nakamura S and Tokuda Y. Okayama University Hospital; Tokyo University; Tokai University School of Medicine; Miyagi Cancer Center; Kitakyushu Municipal Medical Center; St. Luke's International Hospital; Nihon University School of Medicine; St. Marianna University School of Medicine; Shikoku Cancer Center; Tohoku University; Sapporo-Kotoni Breast Clinic; Cancer Institute Hospital; National Cancer Center Hospital and Showa University.

Body: Background: There is an increasing trend in the rate of breast cancer screening for women in all ages in Japan. While screening leads to early cancer detection and improved treatment outcome, it may lead to over-treatment of potentially benign tumors. Little is known about the biological differences between screen- and self-detected cancers.

Method: To compare the biological characteristics of breast cancers by the mode of detection, we used the data from Japanese Breast Cancer Registry (JBCR), a nation-wide registry of newly diagnosed breast cancer cases in Japan. We enrolled into the study cohort female patients who underwent surgical resection of their breast cancers during the period between January 1st 2004 and December 31st 2011, whose mode of cancer detection were recorded in the registry. We compared the clinico-pathological features of the tumors including histological classifications, estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status, and TNM stages between screen- and self-detected cases. Also, we assessed the yearly trend in the proportion of screen-detected cases over the study period.

Results: We identified 205,544 patients matching the inclusion criteria within the registry. In total, 31.8% (65,358 / 205,544) of cases were detected by screening. Cases detected by screening were more likely to have favorable prognostic features than those self-detected: (DCIS: screening 19.8% vs self-detection 4.1%, node negative: 77.0% vs 61.6% and ER positive: 82.0% vs 72.9%, respectively). On the contrary, self-detected tumors were more likely to have poor prognostic characteristic (HER2 positive: screening 11.7% vs self-detection 14.0%, T3 or T4: 2.1% vs 9.8, respectively). All these findings reached statistical significance (p-value < .001). Over the years, the proportion of breast cancers detected by screening among all cases increased from 21.7% in 2004 to 37.1% in 2011. During the same time period, among the all treated DCIS, the proportion of screen-detected DCIS increased from 41.5% to 66.0% and that of ER positive cancers also increased from 23.2% to 39.7%.

Interpretation: This study using JBCR demonstrated that DCIS tumors account for a substantial proportion of screen-detected cancers. The distributions of biological characteristics in screen-detected cancers differ from those observed in self-detected cancers. This may account in part for the favorable prognostics of screen-detected cases.
Title: Persistence of patient detected breast cancer in the mammography screening era: 1990-2013

Malmgren J, Atwood M and Kaplan H. HealthStat Consulting Inc, Seattle, WA and Swedish Cancer Institute, Seattle, WA.

Body: Background: It is estimated from most current National Health Interview Study data that 72% of women age 50-74 have had a screening mammogram in the past 2 years which is significantly less than the Healthy People 2020 goal of 81%. It is not known to what extent breast cancer (BC) is patient diagnosed vs. mammography diagnosed in the community.

Methods: We examined method of breast cancer diagnosis among a large institutional cohort of age 50-74 first primary breast cancer patients with no history of prior cancer identified between 1990 and 2013 (n=5736). Patients were all biopsy proven BC with diagnostic data abstracted from the charts and recorded in our breast cancer specific registry database. Chart recorded detection method was by patient presenting with a personally detected breast symptom (PtD) (n=1729), physician discovered physical findings (PhysD) (n=260) or by routine mammography in absence of physical complaints or findings (MammD) (n=3747). It is not known if patients participated in screening programs. Patients with unknown or other BC diagnosis method were excluded (n=91). BC staging was converted to AJCC 7 to remove inconsistency over time. Pearson chi square was used for bivariate comparisons and logistic regression for identification of characteristics associated with patient detection.

Results: Rate of patient detection of all stage BC decreased over time from 43% in 1990 to 27% in 2013 (chi square = 119.45, p<.001) (n= 5736). Rate of detection of invasive BC (excluding stage 0) decreased over time from 45% to 33% (chi square = 87.14, p <.001) (n=4690). Stage shift to lower stage disease occurred over time with the decrease in PtD BC and increase in MammD BC. Stage 0 BC increased from 4% to 24% (20%), stage I BC decreased from 57% to 44% (13%) and stage III BC decreased from 15% to 7% (8%) (chi square = 232.51, p <.001). Constant over time, stage II, III and IV breast cancer were most often patient detected (stage II 49%, stage III 70%, stage IV 81%, chi square = 1333.03, p<.001). In a logistic regression model adjusted for diagnosis year using PtD vs MamD BC as the outcome, non-white race, younger age (50-59) and all invasive breast cancer stages (not 0/DCIS) were significantly related to an increased risk of BC being diagnosed by the patient [non-white RR =1.32, 95% CI = 1.11, 1.59; age 50-59 RR = 1.43, 95% CI = 1.22, 1.69; stage I RR = 3.55, 95% CI = 2.73, 4.63; stage II RR = 15.42, 95% CI = 11.79, 20.17; Stage III RR = 38.61, 95% CI = 28.20, 52.87; Stage IV RR = 133.72, 95% CI = 61.89, 288.92].

Conclusions: Our results indicate improvement in mammography screening may be obtained by reaching out to women of non-white race and younger screening recommended age groups. Higher stage disease requiring extensive treatment for cure or diagnosed as incurable metastatic disease is significantly associated with patient detection. The number of higher stage patient detected cancers indicates a portion of the population may not be screened and are at risk for serious disease in an era when mammography screening is widely available.
Body: We are entering the era of precision medicine in which cancer screening, prevention and treatment will be tailored to each individual. The progress made in this field is due, in part, to advances in our understanding of cancer risk and tumor biology. The challenge before us is to harness this knowledge and apply it in the clinical setting. Breast cancer screening provides an excellent opportunity to test the value of precision medicine in the real world. In this report we describe the process of designing a model of personalized breast cancer screening.

Methods
Risk factors were selected that have the greatest impact, have been validated and can be measured across a population. A risk model was selected that is highly calibrated, has been validated in a large screening cohort and is easy to apply in a large population of women. An expert committee was convened that set risk thresholds for stratifying women into groups that will be recommended to undergo biennial, annual or every six month screening. Risk thresholds and screening schedules are in accordance with the United States Preventive Services Task Force breast cancer screening recommendations.

Results
Risk factors: Age, race/ethnicity, personal history of breast biopsies and benign breast disease, family history, breast density and breast cancer-associated genetic mutations and single nucleotide polymorphisms (SNPs) were chosen as the risk factors that will be used to determine breast cancer risk. Risk model: The Breast Cancer Surveillance Consortium risk model will be used to calculate a woman's 5-year risk and will be modified by a polygenic risk score based on 81 SNPs. Risk thresholds: Women will be recommended to undergo biennial screening mammography when they reach the age of 50 or have the risk of an average 50 year-old woman (1.3% 5-year risk). Women will be advised to undergo annual screening if they are at increased risk of developing an interval cancer (women in their forties with extremely dense breasts and women at increased risk of developing estrogen receptor negative breast cancer based on their SNPs). Women will be recommended to undergo annual mammography and annual MRI if they are found to be gene mutation positive, have the risk of a BRCA1 mutation carrier (6% 5-year risk) or have a history of mantle radiation.

Discussion
Selecting the appropriate risk factors and risk model and determining risk thresholds are key components of designing a personalized breast cancer screening model. Personalized screening may be the way forward, but this can only be determined within the setting of a randomized controlled trial. We will conduct such a trial to determine if personalized screening is as safe as, less morbid than, more preferred by women than and enables prevention when compared to annual screening. The WISDOM (Women Informed to Screen Depending on Measures of risk) study will compare risk-based screening to annual screening within the Athena Breast Health Network with support from the Patient-Centered Outcomes Research Institute. Our intent is that this trial will provide us with the data that we need to determine the safest and most effective way to screen women for breast cancer in the era of precision medicine.
Title: Bahcesehir mammography screening project (BMSP) is cost-effective in a developing country

Ozmen V, Cabiogsu N, Ozkan-Gurdal S, Ozaydin N, Kayhan A and Aribal E. Istanbul University, Istanbul Medical Faculty, Istanbul, Turkey; Faculty of Medicine, Namik Kemal University, Istanbul, Turkey; Faculty of Medicine, Marmara University, Istanbul, Turkey; Kanuni Sultan Suleyman Hospital, Istanbul, Turkey and Faculty of Medicine Marmara University, Istanbul, Turkey.

Body: Objective: This study aims to determine the cost-effectiveness of a population-based organized mammography screening in a developing country (Bahcesehir, Istanbul, Turkey).

Material and methods: Between 2009 and 2015, mammographies were obtained by 2-year intervals for women with ages 40-69 years (n=7167), living in Bahcesehir. Cost for BMSP included salaries paid for the staff, purchase and maintenance of diagnostic devices, and cost of diagnosis, treatment and follow-up of detected breast cancer patients. The incremental cost-effectiveness ratio (ICER) was calculated as the extra expense per extra "life year" saved by BMSP, when compared to breast cancer detected without screening (BCWS). ICER=CostBMSP-CostBCWS/Life-yearsBMSP – Life-yearsBCWS. Gross Domestic Product per capita in Turkey in 2014 was found (10.650 USD). According to WHO, ICER value below GDP per capita is defined cost-effective.

Results

Of 7167 women, 67 were diagnosed with breast cancer after the third screening round. Stages of BC patients diagnosed in BMSP and national breast cancer registry program (NBRP), expected 5 year survival rates and expected median survival life according to stages, and stage distribution of patients were shown in Table 1.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Expected 5-year Survival Rate</th>
<th>Expected Median Life (years)</th>
<th>Workload Loss (months)</th>
<th>National Breast Cancer Registry Program</th>
<th>Bahcesehir Mammography Screening Program</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>93%</td>
<td>25.89</td>
<td>2</td>
<td>4.9</td>
<td>19.4</td>
<td>14.5</td>
</tr>
<tr>
<td>Stage 1</td>
<td>88%</td>
<td>25.89</td>
<td>6</td>
<td>26.6</td>
<td>50.7</td>
<td>24.1</td>
</tr>
<tr>
<td>Stage 2</td>
<td>77.5%</td>
<td>13.60</td>
<td>6</td>
<td>44.9</td>
<td>20.9</td>
<td>-24</td>
</tr>
<tr>
<td>Stage 3</td>
<td>58%</td>
<td>6.36</td>
<td>9</td>
<td>20.8</td>
<td>7.5</td>
<td>-13.3</td>
</tr>
<tr>
<td>Stage 4</td>
<td>15%</td>
<td>1.83</td>
<td>12</td>
<td>2.8</td>
<td>1.5</td>
<td>-1.3</td>
</tr>
<tr>
<td>Median Expected Life time (years)</td>
<td></td>
<td></td>
<td></td>
<td>15.63</td>
<td>21.5</td>
<td>5.87</td>
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<tr>
<td>Total expected Life Time (years)</td>
<td></td>
<td></td>
<td></td>
<td>1047.43</td>
<td>1440.59</td>
<td>393.16</td>
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<tr>
<td>Average Work Loss (months)</td>
<td></td>
<td></td>
<td></td>
<td>6.6</td>
<td>5.54</td>
<td>-1.06</td>
</tr>
<tr>
<td>Total Work Loss (months)</td>
<td></td>
<td></td>
<td></td>
<td>441.93</td>
<td>371</td>
<td>-70.93</td>
</tr>
</tbody>
</table>

Median expected life time of patients in screening group was 5.87 years longer than patients in NBRP (21.50 years vs 15.63 years), and, totally 393 years were saved with BMSP. Additionally, 71 months of workload were saved with mammography screening (Table 2).
Table 2

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of population-based screening</td>
<td>0</td>
<td>937.500 USD</td>
<td>937.500 USD</td>
<td>609.296 USD</td>
<td>609.296 USD</td>
</tr>
<tr>
<td>Cost of diagnosis, treatment and follow-up</td>
<td>858.053 USD</td>
<td>696.114 USD</td>
<td>-167.939 USD</td>
<td>696.114 USD</td>
<td>-161.939 USD</td>
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<tr>
<td>Workload lost</td>
<td>185.832 USD</td>
<td>161.006 USD</td>
<td>-29.827 USD</td>
<td>166.006 USD</td>
<td>-29.287 USD</td>
</tr>
<tr>
<td>Total cost</td>
<td>1.043.885 USD</td>
<td>1.794.620 USD</td>
<td>745.734 USD</td>
<td>1.471.416 USD</td>
<td>418.070 USD</td>
</tr>
<tr>
<td>Total expected life time (years)</td>
<td>1047.43</td>
<td>1440.59</td>
<td>393.16</td>
<td>1440.59</td>
<td>393.16</td>
</tr>
<tr>
<td>Incremental loss effectiveness ratio (ICER)</td>
<td></td>
<td></td>
<td>1.897</td>
<td>1.014</td>
<td>1.062</td>
</tr>
</tbody>
</table>

The incremental cost-effectiveness ratio (ICER) was found 1.897 USD/year. Since ICER/year has been calculated to be less than PDG per capita (10.650 USD), BMSP definitely seems to be cost-effective.

Conclusion
Population-based mammography screening results in early detection of breast cancer, and seems to be cost-effective in Bahçesehir Mammography Screening Program in Turkey. Our findings also suggest that a nation wide organized population-based screening paid by National Social Security Institute in Turkey might be more cost-effective than BMSP.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-02-10

Title: Knowledge of the potential benefits and harms of breast cancer screening: A survey of participants and nurses

Shimada T, Takahashi M, Tsukisawa K, Shimizu Y, Tanaka M, Saito N, Takahashi K, Ishikawa N, Hashimoto M, Kinouchi K, Fujiwara M and Sato T. Hiraka General Hospital, Yokote, Akita, Japan; Kazuno Kosei Hospital, Kazuno, Akita, Japan; Kitaakita Municipal Hospital, Kitaakita, Akita, Japan; Noshiro Kousei Medical Center, Noshiro, Akita, Japan; Akita Kousei Medical Center, Akita, Japan; Yuri Kumiai General Hospital, Yurihonjo, Akita, Japan; Omagari Kousei Medical Center, Daisen, Akita, Japan and Ogachi Central Hospital, Yuzawa, Akita, Japan.

Body: Background:
It has been 10 years since a population-based mammography screening was implemented in Japan. Publicly funded mammography and breast examination are performed every two years on women who are over 40 years old. It is important that the subjects understand the benefits of breast cancer screening in terms of a reduced mortality from breast cancer; however, they must also be aware of the potential disadvantages, such as false-positive diagnoses and over diagnosis. It is unclear how well the Japanese people understand the benefits and harms of breast cancer screening. This study assessed the opinions of participants in Akita, Japan, asking them about the benefits and harms attributable to breast cancer screening. Their answers were compared with those given by a group of registered nurses, who had been asked the same questions.

Methods:
This study was carried out from August to December 2014 in Akita, Japan. A questionnaire survey, including questions on the expected benefits and harms of breast cancer screening was devised and given to women undergoing screening mammography and breast examination. The same survey was given to nurses working in eight hospitals in the Akita Kouseiren Hospital group.

Results:
The questionnaire survey was given to 1649 participants aged 29–96 years and 1905 registered nurses (including 89 men) aged 20–69 years. All the members of the first group experienced breast cancer screening, but 42% of the registered nurses had no screening experience. The questionnaire was returned by 1552 people (94.1%) and 1710 people (89.8%), respectively. There were many misunderstandings found in the answers to the questions. The common misconceptions were as follows: screening prevents or reduces the risk of development of breast cancer (86% of participants, 62% of registered nurses); screening reduces the mortality from breast cancer by more than 50% (69% of participants, 60% of registered nurses); and 10 years of regular screening for 50-year-old women will prevent 10 or more breast cancer deaths per 1000 women (62% of participants, 61% of registered nurses). However, there were some correct answers; approximately half of the people studied (46% of participants, 57% of registered nurses) knew that detecting a cancer early by screening did not always reduce mortality or lengthen life.

Conclusion:
In Japan, most people overestimated the benefit that can be expected from breast cancer screening and the number of women who would benefit was not well understood. These misunderstandings were seen in the group of registered nurses as well as in the participants. So far, breast cancer screening has been publicized with only positive messages, for example, “the earlier the better” and “the smaller the better.” Henceforth, screening should be publicized with more balanced messages, including figures for how many women will benefit and how many will be harmed. Individuals should then be able to use that information to make an informed choice about whether or not to undergo breast cancer screening.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-02-11

Title: When needles are needed

von Euler-Chelpin M, Vejborg I, LÁ¸nkholm A-V, Guleria S, Peterkin A and Lynge E. University of Copenhagen, Denmark; Diagnostic Imaging Centre, Copenhagen University Hospital, Rigshospitalet, Denmark and Region Zealand, Denmark.

Body: Background
Women with positive screening mammograms but negative at assessment (false-positive) are referred back to routine screening assuming that they have an average risk of breast cancer. However in a long-term follow-up of women with false-positive screening mammography we found that these women had in fact an excess risk of breast cancer later in life; RR=1.67 (von Euler-Chelpin et al, JNCI 2012). This finding has later been supported by Castells et al. (Cancer Epidemiol, 2013). Furthermore, we studied the breast cancer cases in false-positive women and found that 26% of the cases were misclassified and were in fact true positives. However, even correction for misclassification, the false-positive women in our study remained at an excess breast cancer risk. At the same time, it was clear that, in the later periods of our study, when more modern techniques were used, the rate of misclassification decreased (von Euler-Chelpin et al, Cancer Epidemiol., 2014).

Over time there have been changes both in the imaging and biopsy techniques. We used data from an organised screening programme in Denmark. Since its start in 1991, needle biopsies have been performed on all palpable solid lesions and on all uncertain, suspicious or malignant findings. In the very beginning fine needle aspiration cytology (FNAC) was used, later to be replaced by needle core biopsies in the majority of cases. Since 2002, suspicious micro-califications and impalpable mammographic findings not found by ultrasound have been examined using stereotactic needle core biopsy and later with vacuum-assisted breast biopsy as a replacement for surgical biopsy.

To have a needle breast biopsy taken is a psychological burden on the woman. To minimize the harms and maximize the benefits is at the core of developing medical care. We therefore mapped the use of needle biopsies over time in a long-standing, organised screening programme.

Method: We used data from the population-based screening mammography program in Copenhagen, Denmark. The programme has a database including personal records on demographic data, date of invitation, data of screening, and test results, 1998-2013. Data from the Mammography Register were linked to the Danish Pathology Register (for biopsy procedures).

Results: A total of 208,500 screens were included in the study. The rate of recall for assessment ranged between 1.7-3.2% of screened women with a mean of 2.4. The false-positive rate ranged between 1.0-2.5% with a mean of 1.6%. In 1998, 56% of all recalled women had a biopsy taken, which by 2013 had risen to 74% (using two-year smoothing). In proportion of all screens, the biopsy rate was 1.9% in 1998/99 which then fell to 0.7% in 2005/06 and the rose again 2.0% in 2012/13. In 1998, 59% of the biopsies were fine-needle aspiration cytology (FNAC) which by 2013 was only used in 11% of the cases, and replaced by core biopsies, ultrasound or stereotactic guided.

Conclusion
While the recall rate, as well as the false-positive rate, has been fairly stable during the study period the use of needle core biopsies has had a u-shaped development and has intensified in the later years.
Title: Abstract Withdrawn

Body:
Title: EGFR genomic alterations in 5,605 cases of refractory and metastatic breast cancer


Body: Background: Previous failed trials of anti-EGFR therapy (TBCRC001) in breast cancer have not directly assessed EGFR amplification instead relying on pathway activation surrogates. Previous studies have examined EGFR amplification primarily retrospectively for research purposes without being part of a standard diagnostic evaluation. We evaluated 5,606 clinically advanced breast cases for which comprehensive genomic profiling was performed in the course of clinical care to identify EGFR altered mBC cases.

Methods: DNA was extracted from 40 microns of FFPE sections from 5,605 mBC. Comprehensive genomic profiling (CGP) was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay of up to 236 or 315 genes to a mean coverage depth of >600X. The results were analyzed for base substitutions, short insertions and deletions, selected rearrangements, and copy number changes.

Results: 155 (2.7%) of 5,605 mBC featured EGFR alterations. 126 (10.6%) featured EGFR amplification, and 27 (2.2%) featured ERBB2 subs/indels, with no cases harboring both amplification and another GA of EGFR. These patient (pts) had a median age of 55 years (range 30 to 78). For EGFR altered cases, specimens utilized for CGP included 59 (38%) from the patient's primary BC and 93 (60%) from metastatic sites including liver (14%), lymph node (14%), lung (8%), soft tissue (6%), skin (5%) and with information not available for 3 cases. 75 (48%) mBC were submitted as IDC, 69 (45%) as breast carcinoma NOS, 5 (3 %) as metaplastic BC, and 7 (5%) as other mBC. For the 126 EGFR amplified breast patients, quantitative estimation of EGFR copy number ranged from 6 to 380 copies, with a median of 12 copies. A patient with heavily pretreated case and whose TNBC harbored 32 copies of EGFR was begun on erlotinib and has experienced a 20 months ongoing complete response to erlotinib, and will be presented as will other outcomes and biomarker characterization of these cases.

Conclusions: Given anecdotal evidence that mBC is responsive to anti-EGFR targeted therapies, prediction of benefit from such therapies may be linked to sensitive and specific detection of EGFR alteration in mBC cases. This study demonstrates a 2.7% EGFR alteration rate of mBC, with a striking median copy number of 12 for the subset of EGFR amplified cases. Given these findings along with anecdotal report of patient benefit, utilization of CGP in the course of clinical care or for clinical trials for mBC may help optimize treatment with targeted therapies for these patients including the direction of anti-EGFR monotherapy to EGFR altered patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-03-03

Title: The Q-CROC-3 project reveals novel genomic alterations in triple negative breast cancers in residual tumors after neoadjuvant chemotherapy

Basik M, Aguilar-Mahecha A, Lafleur J, Bareke E, Przybytkowski E, Alirezaie N, Discepola F, Légare S, Kovacina B, Lan C, Mihalcioiu CL L, Robidoux A, Marcus E, Roy J-A, Pelmus M, Aleynikova O, Nabavi S, Tonellato P and Majewski J. Lady Davis Institute, Segal Cancer Center, Montreal, QC, Canada; McGill University Hospital Center, Montreal, QC, Canada; Center for Medical Bioinformatics, Harvard Medical School, Boston, MA; McGill University and Genome Quebec Innovation Center, Montreal, QC, Canada; Hopital du Sacre Coeur, Montreal, QC, Canada; Centre Hospitalier de l 'Universite de Montreal, Montreal, QC, Canada; John H. Stroger Jr. Hospital, Chicago, IL and Jewish General Hospital, Montreal, QC, Canada.

Body: The prognosis of triple negative breast cancer that shows resistance and/or incomplete response to cytotoxic chemotherapy is poor. In order to understand the mechanisms of resistance to chemotherapy and the genomic evolution of TNBCs treated with chemotherapy, an international multi-center biopsy-driven clinical trial was created for the collection and study of drug-resistant primary and metastatic freshly frozen tumors (Q-CROC-03: NCT01276899). We consented 60 patients with operable TNBC undergoing neoadjuvant Anthracycline/Taxane-based chemotherapy for pre and post-treatment biopsies as well as collection of residual tumor at the time of surgery and serial blood sampling. In 12-15 patients, adequate residual tumor material was available for genomic studies, which included whole exome sequencing, array CGH, gene expression microarray profiling and RNAseq of paired tumors. Whole exome sequencing revealed clonal shifts as well as the relatively infrequent appearance of novel mutations in individual tumors, without any recurrently detected variants. Array CGH revealed a remarkable stability in the number of DNA copy number alterations with a few functional alterations enriched for in the residual tumor, including an amplicon involving the NFIB gene. Finally, gene expression profiling showed shifts towards the immune-modulatory and basal TNBC subtypes after chemotherapy as well as an increase in the expression of several targetable genes, including DUSP1, a dual specificity phosphatase. In the 4 cases of primary and matching metastatic tumors, the post-NAC residual tumor had acquired changes many of which persisted in the metastatic sites, indicating that the analysis of the residual tumors can provide a partial picture of genomic changes present in metastases but not in the primary tumor. In summary, the genomic characterization of residual post-NAC tumor tissue provides important information for the development of novel therapeutic strategies for drug-resistant TNBCs as well as a portrait of genomic evolution of TNBCs subjected to chemotherapy.
Amplification of the WHSC1L1 oncogene regulates expression and estrogen-independent activation of ER\(\alpha\) in SUM-44 breast cancer cells and is associated with ER\(\alpha\) over expression in breast cancer

Ethier SP P, Irish J, Mills J, Ivey B, Hardiam G, Wilson R, Domkowski A and Guest S. Medical University of South Carolina, Charleston, SC and Wayne State University School of Medicine, Detroit, MI.

**Body:** The 8p11-p12 amplicon occurs in approximately 15% of breast cancers and occurs almost exclusively in aggressive luminal B type tumors. Our lab and other labs have identified the WHSC1L1 oncogene as a driving oncogene from this region with potent transforming activity. In the present studies, we found that over expression of WHSC1L1 is linked to over expression of the ESR1 and ER\(\alpha\) protein in the SUM-44 breast cancer cell line, and also in primary human breast cancer specimens. Knockdown of WHSC1L1, and particularly the short isoform of WHSC1L1, had a dramatic effect on ESR1 mRNA and ER\(\alpha\) protein levels. SUM-44 cells do not require exogenous estrogen for continuous growth in vitro; however these cells are dependent on ER\(\alpha\) expression as determined from ESR1 knockdown experiments, and potent growth inhibition and ER\(\alpha\) degradation following exposure to the selective estrogen receptor degrader (SERD) fulvestrant. ChIP-Seq experiments utilizing ER\(\alpha\) antibodies demonstrated potent ER\(\alpha\) binding to chromatin in SUM-44 cells under estrogen-free conditions. ER\(\alpha\) bound to ERE and FOXA1 binding motifs under estrogen-free conditions and regulated expression of number of well-known estrogen responsive genes. Short term treatment with estradiol enhanced binding of ER\(\alpha\) to chromatin and influenced expression of many of the same genes expressed and to which ER\(\alpha\) was bound under estrogen-free conditions. Finally, knockdown of WHSC1L1 in SUM-44 cells resulted in loss of ER\(\alpha\) binding to chromatin under estrogen-free conditions; however treatment estradiol restored ER\(\alpha\) binding to chromatin at key estrogen-response elements and genes. These results indicate the SUM-44 cells are a good model for a subset of luminal B breast cancers that have the 8p11-p12 amplicon, over express the WHSC1L1 oncogene, and over express ER\(\alpha\) that is independent of estrogen for binding to chromatin and regulation of gene expression. Dependence on ER\(\alpha\) activity for growth and survival of breast cancer cells but independence of estradiol is a major cause of breast cancer mortality as such cells become non-responsive to current hormonally based therapies.
Cell-free DNA as molecular tool for monitoring disease progression and response to therapy in breast cancer patients

Liang DH H, Patel A, ENSOR JE E, Patel TA A, Chang JC C and Rodriguez AA A. Houston Methodist Hospital, Houston, TX and Houston Methodist Cancer Center, Houston, TX.

Body: Background: Identification of cancer-specific genes from breast cancer cells was instrumental in the advancement of targeted breast cancer therapy. However, with genomic heterogeneity within the breast cancer and evolution of cancer over time, genomic sequencing obtained from a single biopsy site may not capture the complete genomic profile. Thus, circulating cell-free DNA (cfDNA), isolated from plasma, is potentially a non-invasive source of identifying cancer-specific genomic alterations and may provide comprehensive genomic data throughout a patient's clinical course as they undergo anti-cancer therapy.

Method: We performed a retrospective chart review of 100 patients with stage 4 or high-risk stage 3 breast cancer who were tested for cfDNA genomic alterations. The most common actionable cancer specific genomic alterations were identified. In 23 patients who also had genomic analysis from tumor DNA (tDNA), an analysis using the Cohen's Kappa statistic was performed to determine the degree of agreement between genomic alterations found in tDNA and cfDNA. The proportion of patients with clinical disease progression between two cohorts determined by change in mutant allele frequency was compared using two-sided Fisher's exact test. Patients who received targeted therapy based on the identified genomic alteration were followed to determine response to therapy.

Results: In cfDNA of 100 breast cancer patients, the most commonly found cancer specific genomic alterations were TP53, PIK3CA, EGFR amplification, and ERBB2 amplification, with incidence rates 27%, 22%, 9%, and 7%, respectively. In tDNA of 23 patients, incidence rates were 65%, 26%, 9%, and 13%. PIK3CA and ERBB2 amplification demonstrated robust agreement between tDNA and cfDNA (Cohen's Kappa= 0.64 and 0.77, respectively). TP53 and EGFR amplification demonstrated poor agreement between tDNA and cfDNA (Cohen's Kappa= 0.18 and 0.33, respectively). There were 22 patients who had baseline and post-therapy mutant allele frequency measurements of TP53 and PIK3CA. Directional change of mutant allele frequency was closely associated with patient's response to therapy (p=0.0017). 8 out of 8 patients (100%) who had progression of disease had increase in mutant allele frequency. 10 out of 14 patients (71%) of patients who responded to therapy had decrease in mutant allele frequency. 6 patients who were found to have ERBB2 amplification were initiated on anti-HER2 cancer therapy. 5 of 6 patients (83%) had clinical response to therapy, while one patient had progression of disease. 3 patients who were found to have EGFR amplification (2 in cfDNA, 1 in tDNA) were initiated on anti-EGFR therapy. 2 of 3 patients (67%) had clinical response to therapy, while one patient had progression of disease.

Conclusion: There is no definite agreement between genomic alterations found in tDNA and those found in cfDNA. Whether this is due to tumor heterogeneity or tumor evolution over time with administration of anti-cancer treatment remains unknown. However, identification of selected cancer specific genomic alterations from cfDNA may be a non-invasive tool to monitor disease progression and response to breast cancer therapy.
Title: Circulating tumor DNA (ctDNA) for detection of molecular residual disease (MoRD) in breast cancer


Background
Metastatic breast cancer (BC) is an incurable condition and treated with palliative intent. Standard diagnostic imaging and serum markers have limited sensitivity and are not recommended in clinical practice. Micrometastatic disease in the bone marrow (DTC) and peripheral blood (CTCs) is a recognized prognostic marker but with limited clinical utility. The detection of asymptomatic disease using a sensitive, reproducible and robust blood-based molecular test, or molecular residual disease (MoRD) could potentially represent a tool with the capability to design early therapeutic interventions and improve outcome. Circulating tumor DNA (ctDNA) has the potential to reflect residual tumor burden with higher diagnostic accuracy. We performed a pilot study in patients with high-risk primary BC.

Methods
This is a prospective study of 30 patients with either locally advanced BC who had completed primary therapy (21 patients) and had no evidence of disease (NED), or were metastatic but treated with curative intent and currently NED or stable (9 patients). Plasma was analyzed for ctDNA either after completing neo-adjuvant therapy (NAT), for recurrence monitoring after surgery, or when there was a clinical suspicion of recurrence. Guardant360 ™ (Guardant Health) is a ctDNA next generation sequencing panel which produces a quantitative measurement of the mutant allele fraction for single nucleotide variants in 54 genes and copy number variants in 3 genes (panel was expanded to 68 genes in Feb ’15) using digital sequencing technology.

Results
Baseline ctDNA analysis was done for 30 patients and 25 (83%) had serial draws for a total of 76 samples. All patients were stage 3-4 except for two stage 2 patients. ctDNA or MoRD was detected in 17 (57%) of patients and in 39 (51%) of the samples. Of the 18 patients treated with NAT, 11 achieved pCR or had minimal residual disease. Of these 11, six had no ctDNA detected after surgery, 3 had mixed results of no ctDNA and low volume ctDNA alterations on different draws, and 2 had persistent mutations on 2 draws. Ten of these 11 patients remain NED with median follow up of 24 months, while the one patient who recurred had persistent low volume missense ctDNA alterations on serial draws, first detected 6 months before clinically evident recurrence. Of the 7 patients with significant residual disease (less than PR) after NAT, 6 had post-surgical ctDNA detected and 5 have recurred at a median of 13 months after surgery. Four of those patients had ctDNA tested prior to recurrence and all had alterations detected in the blood prior to clinical recurrence. Lastly, one HER2+ metastatic patient treated with curative intent with a subsequent negative PET scan and no ctDNA detected after HER2-targeted therapy progressed on CT 2 months later and repeat ctDNA revealed EGFR mutant allele fraction of 51% and ERBB2 amplification.

Conclusions
The evaluation of ctDNA in high-risk BC patients can identify MoRD and predict for clinical recurrence. Patients with no or low volume ctDNA after primary treatment remained NED longer than those with multiple or high volume alterations. Future studies will validate these early observations and aid in selecting patients for additional systemic therapy with the hope of improving outcome.
Title: Abstract Withdrawn

Body:
Title: Novel recurrent lncRNA fusions detected in breast cancer using RNA-Seq technology in a neoadjuvant setting

Agrawal V, Varadan V, Banerjee N, Miskimen K, Vadodkar A, Abu-Khalaf M, Sikov W, Harris L and Dimitrova N. Philips Research North America, Briarcliff Manor, NY; Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; Yale Comprehensive Cancer Center, New Haven, CT; Warren Alpert Medical School of Brown University, Providence, RI and Seidman Cancer Center, University Hospitals, Cleveland, OH.

Body: Background: Recent discoveries of recurrent and targetable gene fusions in breast cancer suggest the need to characterize the functional significance of such genomic aberrations within larger cohorts. We quantified fusion transcript expression in patient samples using RNASeq to identify recurrent gene fusion events in breast cancer as well as study the fusions post-brief exposure to mono-therapy.

Methods: We sequenced transcriptomes of core biopsy RNA from 130 breast tumors obtained from brief-exposure preoperative clinical trials BrUOG 211A/211B. HER2- patients were treated with brief exposure to bevacizumab (B) or nab-paclitaxel (nP) followed by treatment with B/nP/carboplatin while HER2+ patients received brief exposure to trastuzumab (T) or nP followed by T/nP/carboplatin. Paired-end sequencing on 75 baseline biopsies and 55 post-exposure biopsies using amplified total RNA yielded 55 million reads on average per sample. Fusion transcript abundance was evaluated using 2 pipelines, TopHat-Fusion and deFuse, due to their complementary strategies in fusion detection. We eliminated gene-pseudogene fusion pairs as likely false positives arising due to alignment artifacts. Fusions that met 1 or more of the following 3 criteria were considered high confidence:

i) Called by both deFuse and TopHat. ii) Called by deFuse with probability >95% iii) Called by TopHat with > 15 reads supporting the fusion.

Results: We identified high confidence gene fusions, detected by both TopHat and deFuse, in 73 of the 75 baseline biopsies with 16 fusions on average per sample. We looked for modulation of gene fusions upon brief exposure to therapy in 55 patients that had post exposure biopsy data and found that out of the 545 high confidence fusions detected across these patients, 62 (11.37%) of the fusions were found to be still present after the therapy exposure. For the recurrent fusion analyses, we considered the 75 baseline samples. We found a total of 1158 unique candidate fusions. Out of these, 116 (10%) were recurrent in more than 1 patient. After further filtering, we were able to narrow down to 9 (0.77%) fusions that were reliable since they were predicted by both the algorithms in different patients. 2 of these 9 fusions involved GAS5 as a partner gene. GAS5 have been found to have a role in apoptosis and its down-regulation has been associated with cell proliferation, which makes it a very interesting fusion candidate.

Conclusions: We find that gene fusions in breast cancer are highly heterogeneous but are enriched with cancer-related pathway genes. This is the first study to report 2 novel gene-lncRNA fusion transcripts: MDN1-GAS5 and GABRB3-GAS5. Both these fusions are called in the baseline & post-therapy for atleast 1 patient (different patients each). GAS5 has been found as participating in a fusion in B-cell lymphoma. We are currently in the process of validating the fusion calls using qRT-PCR. The heterogeneity of detected fusions suggests that multiple mechanisms could underlie the selective advantage of tumor cells expressing fusion transcripts. The brief-exposure preoperative paradigm provides a unique opportunity to evaluate modulation of fusion transcripts that can shed light on their functional importance.
Title: Genetic polymorphism and correlation with treatment induced cardiotoxicity and prognosis in HER2 amplified early breast cancer patients

Peddi PF F, Hurvitz SA A, Fasching PA A, Wang L, Cunningham J, Weinshilboum RM M, Liu D, Quinaux E, Fourmanoir H, Robert NJ J, Valero V, Crown J, Falkson C, Bruksky A, Pienkowski T, Eiermann W, Martin M, Bee V and Slamon DJ J. University of California, Los Angeles; University Hospital Erlangen; Mayo Clinic; IDDI; Hospital General Universitario Gregorio Marañón; University of Pittsburgh Medical Center; St Vincent's Hospital; Trio Oncology; US Oncology; MD Anderson; Univ of Alabama At Birmingham; Postgraduate Medical Center. European Health Center and Redcross Women Hosp.

Body: Introduction: Small studies have indicated a possible correlation between a HER2 gene polymorphism at codon 655 and trastuzumab-associated cardiotoxicity. Association between a synonymous coding variant rs7853758 within the SLC28A3 gene and anthracycline induced cardiotoxicity has also been reported. This study aimed to validate these correlations and assess for any relationship with prognosis.

Methods: Genomic DNA was isolated from 666 patients enrolled in a large trial of adjuvant chemotherapy in HER2 amplified early breast cancer (BCIRG 006). Genotyping was conducted using Sequenom MassARRAY System for HER2 G->A polymorphism at amino acid codon 655 (rs1136201) and variant rs7853758 (L461L) within the SLC28A3 gene.

Results: Of the 666 patients analyzed, 216 patients were treated with anthracycline based therapy, 226 with trastuzumab based therapy, and 224 with regimens containing both an anthracycline and trastuzumab. Compared with the overall results of the BCIRG006 study (N=3,222), in the subset of patients genotyped in this analysis, a less robust improvement in disease free survival (DFS) was observed for the trastuzumab arms than control arm (HR, 0.821). When stratified for prognostic features, the hazard ratio in favor of trastuzumab was consistent with that of the overall study (HR, 0.674). Samples from 662 patients were successfully genotyped for rs1136201. Of these, 424 (64%) were AA, 30 (4.5%) were GG, 208 (31%) were AG genotype. Samples from 665 patients were successfully genotyped for rs7853758. Of these, 19 (3%) were AA, 475 (71%) were GG, and 171 (26%) were AG genotype. There was no correlation seen between mean left ventricular ejection fraction (LVEF) and HER2 genotype at codon 655 in patients treated with trastuzumab. Of patients tested for the HER2 polymorphism, cardiac dysfunction [defined as > 10% decline in LVEF or clinical congestive heart failure (CHF)] developed in 16% of patients with AA, 17% of patients with GG and 20% of patients with AG. There was also no correlation between mean LVEF and variant rs7853758 in patients treated with anthracyclines. The percentage of patients who developed cardiac dysfunction was 13%, 17% and 21% in AA, GG, and AG genotypes respectively. No correlation between disease free survival and any of the genotypes was seen.

Conclusion: In the largest analysis to date to evaluate for relationship between cardiac toxicity and HER2 polymorphism, we did not find a correlation with rs1136201 HER2 polymorphism and trastuzumab induced cardiac toxicity. Our study also did not show a correlation between variant rs7853758 (L461L) and anthracycline induced cardiotoxicity. Neither polymorphism correlated with prognosis.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-03-10

Title: Genomic and transcriptomic heterogeneity in metaplastic breast carcinomas

Piscuoglio S, Ng CKY K Y, Cowell CF F, Mariani O, Martelotto L, Natrajan R, Lim RS S, Maher CA A, Vincent-Salomon A, Weigelt B and Reis-Filho JS S. Memorial Sloan Kettering Cancer Center, NY, NY; Institut Curie, Paris, France; The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom and The Genome Institute, Washington University School of Medicine, St Louis, MO.

Body: Introduction: Metaplastic breast carcinoma (MBC) is a rare form of triple-negative breast cancer (TNBC), accounting for approximately 0.2%-5% of all invasive breast cancers. These tumors are characterized by the presence of neoplastic cells displaying differentiation towards squamous epithelium or mesenchymal elements. MBCs are reported to have an aggressive clinical behavior, to exhibit a worse prognosis and to respond less frequently to conventional chemotherapy regimens than common forms of TNBCs. In this study, we sought to define whether morphologically distinct subgroups of MBCs would be underpinned by distinct gene expression or copy number profiles, and whether MBCs, akin to other special histologic types of TNBC (e.g. secretory carcinoma and adenoid cystic carcinoma), would be underpinned by a highly recurrent fusion gene.

Methods: RNA and DNA samples were extracted from microdissected frozen MBCs (5 squamous, 5 spindle and 7 chondroid) and subjected to gene expression profiling using the Illumina Human HT-12 v4 platform and gene copy number profiling using the Affymetrix Human SNP 6.0 arrays, respectively. Genes differentially expressed between MBC subtypes were identified using SAM, and functional annotation of these genes was performed using Ingenuity Pathway Analysis. Intrinsic molecular subtypes were determined using the PAM50 and claudin-low intrinsic gene lists. In addition, all cases were subjected to paired-end massively parallel RNA-sequencing (Illumina GAIIx). Putative expressed fusion transcripts were identified using a validated algorithm (i.e. ChimeraScan), and confirmed by means of RT-PCR.

Results: MBCs with spindle cell morphology were all classified as of claudin-low intrinsic subtype, whereas MBCs with chondroid or squamous cell metaplasia were classified as of normal breast-like, basal-like or claudin-low subtypes, suggesting that these morphologic subgroups are heterogeneous. Unsupervised analysis of microarray and RNA-sequencing gene expression data further demonstrated that MBCs with spindle cell differentiation displayed distinctive transcriptomic profiles, and formed clusters distinct from those enriched for MBCs with chondroid and squamous cell metaplasia. MBCs with spindle cell morphology preferentially expressed regulators of epithelial-to-mesenchymal transition including lower expression of E-cadherin and EpCAM. At the genomic level, MBC subtypes displayed patterns of gene copy number alterations similar to those of common forms of TNBCs from The Cancer Genome Atlas, and no significant differences were found among the distinct MBC subtypes. Nine in-frame fusion genes, TBL1XR1-PIK3CA, WAPL-CDHR1, MAP2K3-HMGCLL, PARG-BMS1, FN1-ICAM1, TNKS1BP1-SPARC, AAK1-ARNT2, MBTPS1-TCEANC2 and PSMA6-SHMT1 were identified and validated in the index cases, however none of these was found to be recurrent in the cases analyzed in this study.

Conclusion: MBC subtypes, despite harboring similar patterns of gene copy number alterations, display significant transcriptomic differences, which may account for their distinct histologic features. Our findings also demonstrate that unlike other histologic special types of TNBC, MBCs are not underpinned by a highly recurrent expressed fusion gene.
Title: Abstract Withdrawn
Title: Comprehensive genomic profiling of clinically advanced mucinous carcinoma of the breast


Body: Background: Mucinous carcinoma of the breast (mucBC) is generally associated with a favorable prognosis, but on occasion, may have an aggressive clinical course in which it is commonly refractory to cytotoxic chemotherapy. The low incidence of mucBC (~2% of breast cancers) precludes the development of consensus based guidelines for management of these relapsed/refractory cases. We performed hybrid-capture based comprehensive genomic profiling (CGP) to identify potential therapy targets not routinely searched for in clinical management of metastatic mucBC.

Methods: DNA was extracted from 40 microns of FFPE sections from 22 cases of stage IV mucBC. Comprehensive genomic profiling (CGP) was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >550X. The results were analyzed for all classes of genomic alterations (GA) including base substitutions, insertions and deletions, select rearrangements, and copy number changes. Clinically relevant genomic alterations (CRGA) were defined as those identifying anti-cancer drugs on the market or in registered clinical trials.

Results: The median age of the 22 mucBC patients was 57 years (range 32 to 79 years). Samples were from breast (11), lymph nodes (3), chest wall (2), liver (2), soft tissue (2), bone (1) and pleura (1). Three mucBC were grade 1, 17 were grade 2 and 2 were grade 3. Twenty-one (95%) mucBC were ER+, 19 (86%) were PR+ and 4 (18%) were HER2+ by IHC and/or FISH. There were 129 GA identified on the 22 mucBC (5.9 per tumor) including 51 CRGA with a mean of 2.3 per tumor. Amplifications of FGFR1 and ZNF703 were found in 8 out of 22 cases (36%) on the same amplicon. Other most frequently altered genes were TP53 (32%), CCND1 and FGF3/4/19 often co-amplified together (27%). ERBB2/HER2 alterations were found on 5 cases (23%) including amplifications on all 4 HER2+ cases by IHC and/or FISH, and ERBB2 substitution D769Y on one additional mucBC. CRGA were found on some other 20 genes included PIK3CA (5), BRCA1 (1), TSC2 (1), STK11 (1), AKT3 (1), and ESR1 (1).

Conclusions: The subset of relapsed/refractory mucBC presents a management challenge, but comprehensive genomic profiling offers avenues for benefit from targeted therapy. MucBC relative to breast cancer is predominantly ER+, enriched for FGFR1 amplification, 36% vs 11% from TCGA ER+ breast cancer (N=601) with Fisher's test p-value <0.005. Moreover, metastatic mucBC appears more often to have ERBB2/HER2 alterations (23%) than typical mucBC cured by local treatments. Comprehensive genomic profiling uncovers a variety of genomic targets in metastatic mucBC that could facilitate the introduction of targeted therapies for patients with this challenging disease.
The genomic landscape of PD-L1, PD-L2, Jak2 (PDJ) amplified triple negative breast carcinoma

Gawryletz CD D, Anderson KS S, Cunliffe HE E, Northfelt DW W, McCullough AE E, Lenkiewicz E, Malasi S, Pockaj BA A and Barrett MT T. Mayo Clinic Arizona Division of Hematology/Medical Oncology, Scottsdale, AR; Biodesign Institute Arizona State University, Tempe, AR; Mayo Clinic Arizona, Scottsdale, AR; Mayo Clinic Arizona Division of Surgery, Section of Surgical Oncology, Scottsdale, AR; Mayo Clinic Arizona, Scottsdale, AR and University of Otago, Dunedin School of Medicine, Dunedin, New Zealand.

Body: Introduction:
Triple negative breast carcinoma (TNBC) is a subtype of breast cancer with a paucity of therapeutic targets and a poor prognostic phenotype. In a retrospective cohort, we sought to determine the prevalence of the amplicon targeting the 9p24.1 locus, resulting in over-expression of PD-L1, PD-L2, and JAK2 (PDJ), and potentially actionable therapeutic targets. We then probed the genomic landscape of these PDJ positive tumors and identified co-occurring copy number aberrations, including focal amplifications and homozygous deletions. The presence of the PDJ amplicon and selected co-occurring aberrations provide a unique description of a clinically relevant subtype of TNBC.

Methods:
We evaluated fresh frozen and formalin-fixed paraffin embedded tumor samples from 64 patients with triple negative breast cancer whom underwent definitive surgical resection. Clinical annotation was available in 60 of the samples. Tumor populations (diploid, tetraploid, and aneuploidy) were sorted from each biopsy using DNA content flow cytometry. Each sorted sample was interrogated with oligonucleotide array comparative genomic hybridization (aCGH). All microarray slides were scanned using an Agilent 2565C DNA scanner and the images were analyzed with Agilent Feature Extraction version 10.7. The aCGH data was assessed with a series of QC metrics then analyzed using an aberration detection algorithm.

Results:
We detected a high level (log2ratio greater than or equal to 2) amplicon targeting 9p24.1 in 18 of 64 patients (28%) genomic profiles with triple negative breast carcinoma. In the PDJ positive population, we detected 8 of 18 patients (44%) with co-amplification targeting myc at 8q24, 3 of 18 patients (17%) with co-amplifications targeting EGFR at 7p11, 3 of 18 patients (17%) with co-amplifications targeting PIK3CA. These co-occurring genomic events in PDJ positive tumors may provide clinically actionable targets. Other selected amplifications detected included NOTCH3, KRAS, RUNX1, TUBAL3, FGFR2, AKT1, AKT2, YPEL2, PBXL7, KIT. We detected PTEN homozygous deletion in 2 of 18 patients (11%) PDJ positive tumors. Other homozygous deletions identified in the genomic landscape included FAT1, SOX3, Park2, TNFAIP3, GPC3, RB1, and CREBBP.

Conclusions:
In our retrospective analysis, the amplification of chromosome 9p24.1 involving PD-L1, PD-L2, and JAK2 is present in approximately 28% of triple negative breast cancer patients. The genomic landscape of these PDJ positive TNBCs include recurring high-level focal amplifications and targeted homozygous deletions of clinically relevant genes. The clinical implications of these data are under current investigation using model systems and are in early phase clinical trials. The efficacy of immune checkpoint inhibitors including nivolumab, pembrolizumab, and JAK2 inhibitors including ruxolitinib, in PDJ positive triple negative breast carcinoma is intriguing and remains to be elucidated.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-03-14

Title: Expression of genes for aromatase inhibitor targets to discriminate invasive lobular from invasive ductal carcinomas of the breast using LCM-procured cells to complement endocrine biomarkers

Sanders MAG G, Daniels MW W and Wittliff JL L. University of Louisville, Louisville, KY.

Body: Background: The BIG 1-98 trial and later the ABCSG 8 study reported that patients with invasive lobular carcinoma (ILC) exhibited better response to aromatase inhibitors (AIs) compared to those with invasive ductal carcinoma (IDC). Aromatase cytochrome P450 (CYP19) synthesizes estrogen from androgens and is the target of AIs. CYP19 substrates are generated by upstream enzymes including estrone sulfatase (SULT1E1) and 3b-hydroxysteroid dehydrogenase type 1 (HSD3B1). Enzymes of the aromatase pathway have been reported to be expressed in intact tissue biopsies of breast cancer. To learn more about the pathogenic mechanisms that may underlie the survival benefit of ILCs treated with AIs, we analyzed expression levels of key enzymes related to the aromatase pathway in ILC and IDC. Unlike previous studies, we determined gene expression levels directly on pure populations of carcinoma cells procured by laser capture microdissection, eliminating the contribution of non-cancerous cells.

Methods: Using an IRB-approved biorepository and database, total RNA was extracted from carcinoma cells of 247 de-identified biopsies to perform microarray analyses of 22,000 genes. Of the 247 samples, 16 were ILC, 13 were low grade IDC, 55 were intermediate grade IDC and 85 were high grade IDC, and 107 of these were hormone receptor positive. CYP19, HSD3B1 and SULT1E1 expression was directly detected in LCM-procured breast carcinoma cells of ILC and of IDC. Expression of other genes generally associated with the aromatase pathway, e.g., NADPH-cytochrome P450 reductase (POR), ATP-binding cassette gene (ABCG2), catechol-o-methyltransferase (COMT) and uridine-5’-diphosphate glucuronosyltransferase (UGT1A3 & UGT1A9) as well as HSD17B2 were assessed with LCM-procured cells. Estrogen receptor (ER) and progesterone receptor (PR) proteins were quantified by radio-ligand binding and EIA, and gene expression was validated by qPCR.

Results: Univariate Cox regression analyses indicated that ABCG2, HSD17B2 and UGTA3 independently predicted disease free and/or overall survival of breast carcinomas. We found that CYP19 expression in carcinoma cells, as well as SULT1E1, COMT, POR, HSD17B2 and UGT1A3 expression, decreased as either ER or PR protein increased. HSD3B1 appeared to be over-expressed in ILC compared to IDC, however this difference did not approach statistical significance, likely due to the small sample size. No differences were seen in expression levels of CYP19 and SULT1E1 between ILC and IDC.

Conclusions: An inverse relationship between CYP19 and ER and PR expression levels was observed and suggests that synthesis of estrogens by breast cancer cells in situ plays a significant role in defining tumor biology. Our results also indicate overexpression of HSD3B1 in ILC, although not statistically significant. This finding suggests that HSD3B1 may be a key contributor to the increased benefit of AI therapy seen in ILC. Collectively our results suggest a comprehensive study is warranted to ascertain the molecular basis for differences in expression of genes directing estrogen synthesis in situ in relationship to AI therapeutic responses of histologic subtypes of breast carcinomas.
Body: Background: Simvastatin is an HMG-CoA reductase inhibitor widely used to treat cardiovascular diseases. In retrospective studies, statin treatment has been associated with a modest decrease in overall cancer incidence, including breast cancer (10-15%). Simvastatin Inhibited the PI3K/Akt/mTOR pathway in a pilot window-of-opportunity trial in neoadjuvant breast cancer. Mutation in TP53 is correlated with elevated expression of genes regulating cholesterol biosynthesis and sensitivity to statin treatment in vitro. We hypothesized that specific mutations in TP53 would be associated with differential sensitivity to simvastatin.

Method: We generated MCF10A stably transduced cell lines over-expressing ten frequent TP53 missense point mutations. We assessed the impact of TP53 mutation on growth inhibition induced by simvastatin treatment (range of 1-30 µM for 96 hrs). In parallel, induction of apoptosis was measured by caspase3 reporter assay. Illumina 4-plexed 1x50bp RNA sequencing was performed on cells with R273H, G245S, R248Q, Y234C and wt TP53 before and after exposure to 2.5 µM simvastatin.

Results: We confirmed that mutation in TP53 was markedly associated with growth inhibition by simvastatin treatment in vitro. We now demonstrate that TP53 mutation in R273C, G245S and R273H are highly sensitive to simvastatin with IC_{50} values ≤ 2.1 µM. In contrast, TP53 mutations in Y234C and R248Q were approximately five-fold more resistant. The resistant mutations were similar to MCF10A overexpressing wild type TP53. Growth inhibition was correlated with induction of apoptosis, and could be rescued by addition of farnesylpyrpphosphate and geranylgeranylpyrophosphate. HMGCR upregulation was observed across all treated cell lines. RNA-seq analysis confirms down regulation of the PIK3CA, PIK3CB, and Akt in the sensitive cell lines, but paradoxical upregulation in the resistant cells. Further gene expression analysis will be presented.

Conclusion: Specific TP53 mutation status may impact sensitivity of breast cancer to simvastatin treatment.
Table 1. Representative Copy number gain regions in lung metastasis.

<table>
<thead>
<tr>
<th>Regions</th>
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<th>Start</th>
<th>End</th>
<th>Number of amplification genes</th>
<th>Known Oncogene</th>
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<td>1q41</td>
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<td>220374218</td>
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<td>167371489</td>
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<td>181432423</td>
<td>5</td>
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<td>3q28</td>
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<td>192445588</td>
<td>14</td>
<td>TP63</td>
</tr>
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<td>8p11.23</td>
<td>chr8</td>
<td>37553100</td>
<td>38962979</td>
<td>8</td>
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<td>chr8</td>
<td>85095252</td>
<td>85095252</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>8q23.1-23.3</td>
<td>chr8</td>
<td>106330946</td>
<td>113656012</td>
<td>15</td>
<td>EBAG9, ANGPT1</td>
</tr>
</tbody>
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Title: Genomic landscape of breast cancers from women of African ancestry across the diaspora

Olopade OI I, Odetunde A, Riester M, Yoshimatsu T, Labrot E, Ademola A, Sanni A, Okedere B, Mahan S, Nwosu I, Leary R, Ajani M, Johnson RS S, Sween E, Zheng Y, Clayton W, Khramtsova G, Oludara M, Omodele F, Benson O, Adeoye A, Morhason-Bello O, Ogundiran T, Babalola C, Popoola A, Morrissey M, Huo D, Falusi A, Winckler W, Obafunwa J, Papoutsakis D, Ojengbede O, Ibrahim N, Oluwasola O and Barretina J. The University of Chicago, Chicago, IL; Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Oyo, Nigeria; Novartis Institutes for BioMedical Research, Cambridge, MA; University of Ibadan, Ibadan, Oyo, Nigeria; Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria; University of Ibadan, Ibadan, Oyo, Nigeria; Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria; University of Ibadan, Ibadan, Oyo, Nigeria; Lagos State University, Ikeja, Lagos, Nigeria; The University of Chicago, Chicago, IL; Lagos State University Teaching Hospital, Ikeja, Lagos, Nigeria and University of Ibadan, Ibadan, Oyo, Nigeria.

Body: Objectives: Of all ethnic/racial groups, age-standardized mortality rate from breast cancer is highest for African American women in the US for reasons that remain understudied. The paucity of genomic studies of breast tumors across the African Diaspora further restricts our understanding of the biology of breast cancer in underserved populations. To gain a better understanding of the genomic landscape of breast cancer in women of African Ancestry, we have developed a cross continent translational research infrastructure to examine the spectrum of genetic alterations in breast tumors from West Africa compared to the spectrum of alterations observed in tumors from African-American and other women who are predominantly white in The Cancer Genome Atlas (TCGA) dataset.

Methods: Peripheral blood and breast cancer biopsy tissues were collected from 214 patients enrolled in the West Africa Breast Cancer Study (WABCS) at the University of Ibadan/University College Hospital (UI/UCH) and at Lagos State University Teaching Hospital (LASUTH). Blood DNA as well as breast cancer tissue DNA and RNA were extracted at the Novartis Institutes for Biomedical Research (NIBR), UI/UCH, and LASUTH using a modified protocol of PAXgene Tissue DNA and RNA extraction method. Whole-exome (WES) and transcriptome (RNA-seq) sequencing were performed on the Illumina HiSeq2000 platform at NIBR. Single Nucleotide Variants (SNVs) and insertions/deletions (indels) were called using MuTect and Pindel, while Copy Number Alterations (CNAs) were called using an in-house implementation of the ABSOLUTE method. Observed mutations were compared against those reported in the TCGA dataset. ER, PR and HER2 status were determined by immunohistochemistry (IHC) at UI/UCH, LASUTH and UChicago.

Results: WES data for 95 tumors have been analyzed thus far. Genes commonly mutated in breast cancer in TCGA are also mutated in WABCS but the mutational spectrum is vastly different. TP53 (64%), MYC (31%), and GATA3 (26%), showed significantly higher alteration frequencies in WABCS and African Americans. In contrast, PIK3CA (20%), CDH1 (2%), and MAP3K1 (2%) were less frequently mutated in women of African ancestry. In addition to the high proportion with TP53 mutations, the proportion with HER2 positive subtype of 42.1% and triple-negative subtype of 37.9% suggest that tumors with the most aggressive features are overrepresented in breast cancer patients in West Africa.

Conclusions: In the first study of its kind, high throughput genomic analysis of the largest cohort of women of African ancestry has uncovered alterations in cancer genes, some of which may be amenable to treatment with targeted therapies. Furthermore, we provide evidence that population differences in frequency and spectrum of mutations should drive the design and deployment of precision medicine initiatives. Only then can we develop innovative interventions to reduce the unacceptably high rates of mortality from breast cancer in underserved and under resourced populations.
Title: Outcome of FoundationOne testing in metastatic breast cancer therapy: A single center experience

Yuan Y and Mortimer JE E. City of Hope, Duarte, CA.

**Body:**

**Background:** Next generation sequencing (NGS) has made genomic, mutation-driven, cancer medicine feasible. However, few studies have reported the importance of NGS in identifying mutation-driven treatment or whether such therapies are effective in women with metastatic breast cancer (MBC). We reviewed the impact of NGS in women with MBC at our institution.

**Methods:** Among the medical oncologists who treat metastatic breast cancers at the City of Hope Comprehensive Cancer Center, we identified 21 patients with HER2 negative cancers, whose tumors were submitted for FoundationOne genomic profiling. We report the individual mutation profile, potential therapeutic options, and treatment outcome.

**Result:** Of the 21 patients (pts), 13 had triple negative breast cancer (TNBC) and 12 of 13 (92%) had actionable mutation(s). Mutation profiles have been heterogeneous. Loss or mutations of TP53 were the most common genomic alteration in TNBC (12 of 13). Four pts received targeted therapies (everolimus in 3, pazopanib in 1) and all experienced at least partial clinical response. An additional 3 pts were treated with directed therapy but are too early to evaluate, 4 pts were not treated because of disease progression or clinical deterioration, and 1 pt was lost to follow-up. All 8 pts with ER+ disease had actionable mutations identified and 7 (88%) received targeted therapy (pazopanib in 1, everolimus in 3, palbociclib in 3). All experienced at least partial clinical response; 1 was too ill for treatment. The most common genomic alterations are: PIK3CA mutation, PTEN loss, P53 mutation, FGFR mutation, and ZNF mutation.

**Conclusion:** Targeted genomic sequencing through FoundationOne can identify mutations that are amenable to treatment with agents that are not generally approved for use in breast cancer. In order to test the benefit of target-directed therapy, tumor should be submitted for NGS while patients have a good performance status.
Rs1008805 polymorphism in the CYP19A1 gene is related to the prognosis of stage I–II and operable stage III breast cancer


Body: Purpose Aromatase, encoded by CYP19A1 gene, is a rate-limiting enzyme in the conversion of androgens into estrogens. It has been demonstrated that genetic polymorphisms in CYP19A1 gene were significantly correlated with altered hormone levels in urine and serum, providing an explication for an elevated risk for progression in relation with increasing estrogen exposure. Consequently, it is biologically plausible that CYP19A1 gene polymorphisms may be related to the prognosis of breast cancer. Methods CYP19A1 gene rs1008805 polymorphism were genotyped on 406 Chinese Han women with stage I-II and operable stage III breast cancer. Associations were evaluated between CYP19A1 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 among the whole population with these three genotypes. However, postmenopausal women with GG variant had a poorer DFS when compared with those carrying AG or AA genotype (13.7 months versus 56.3 months; HR, 2.462; 95 % CI, 1.310-4.628; P = 0.004). And what's more, 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). Premenopausal women with GG variant had a marginally improved DFS when compared with those carrying AG or AA genotypes (87.0 months versus 48.7 months; HR, 0.544; 95% CI, 0.295-1.003; P = 0.051). However, this difference was not confirmed by multivariate analyses.

Conclusions The present study indicated that GG genotype of rs1008805 SNP in the first exon of CYP19A1 gene was significantly related to a worse DFS in postmenopausal women with early breast cancer. This founding is novel, if confirmed, CYP19A1 rs1008805 genotypes may turn to be a prognostic biomarker for early breast cancer.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-03-20

Title: Gene expression profile of triple negative breast cancer in patients highlight biomarkers involved in cell metabolism


Body: Introduction: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype, lacking expression of estrogen, progesterone and HER2 receptors. Evidence suggests that TNBC present more frequently in young African-American and Hispanic women, with lower socioeconomic status, hormonal environment and obesity. To this date, there are no studies that have compared TNBC versus nonTNBC in a homogeneous population. Comparison of these groups may help to identify genes that are key hallmarks in TNBC patients.

Objective: To analyze the expression profile of TNBC versus nonTNBC in a homogeneous population from northwestern Mexico, in order to find distinctive biological pathways characteristics to TNBC.

Methods: A prospective study was conducted involving a total of 50 patients (25 TNBC and 25 nonTNBC) undergoing neoadjuvant chemotherapy (NAC) for breast cancer. Both groups were similar in mean age at diagnosis (51 vs 47 years), mean of glucose levels (107 mg/dl vs 104 mg/dl) and BMI (28 vs 29) for TNBC and nonTNBC respectively. Core biopsies were obtained for histological diagnosis and gene expression, total RNA was isolated and expression profiling performed. Some of the differentially expressed genes were selected and validated by qPCR

Results: Seventy percentage of the population presented BMI > 25. We identified a genomic profile expression composed of 40 genes associated with TNBC phenotype. Out of 40 genes, 9 were overexpressed (FOXC1, PRKX/PRKY, UGT8, BCL11A, HMGA1, LPIN1, FAM171A1, HAPLN3, y ANKRD11) and 31 under-expressed. Interestingly, some of these genes were previously associated to breast cancer. HMGA1, PRKX and LPIN1 participate in the insulin metabolism, UGT8 in the sphingolipids metabolism, while two others are transcription factors associated with metastasis and poor prognosis (FOXC1) and tumor growth (BCL11A).

Conclusions: To our knowledge this is the first study in Latin American woman reporting a genomic signature for TNBC strongly associated with aberrant metabolism itself, such as seen in obesity. Understanding cell metabolism may help to clarify the mechanism for tumor development and progression in TNBC patients.
Title: Single cell RNA sequencing reveals key expression signatures of primary breast cancer cells and immune infiltrates

Lee H-B, Eum HH, Chung W, Lee H-O, Lee K-M, Kim K-T, Moon H-G, Noh D-Y, Han W and Park W-Y. Seoul National University College of Medicine, Seoul, Republic of Korea; Samsung Genome Institute, Samsung Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea and Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea.

Body: Introduction: Cancers display intratumoral heterogeneity which interferes with the precise analyses of the tumor entity, and which may affect therapeutic outcomes of targeted treatments. The aim of this study was to evaluate the feasibility of single cell RNA sequencing on primary breast cancer cells and to demonstrate the key gene expression signatures and transcriptome heterogeneity of breast cancer subtypes.

Methods: We performed RNA sequencing on 246 individual cells from 4 primary breast tumors and 2 metastatic lymph nodes from 4 patients, using C1 Single-Cell Auto Prep System (Fluidigm, South San Francisco, CA). RNA sequencing reads were aligned to the human genome reference (hg19) using the 2-pass default mode of STAR_2.4.0d, and gene expression was quantified by RSEM v1.2.18 as the sum of isoform expression.

Results: Pathologic characteristics of the 4 patients is summarized in Table 1.

Table 1. Pathologic characteristics of patients

<table>
<thead>
<tr>
<th></th>
<th>BC01</th>
<th>BC02</th>
<th>BC03</th>
<th>BC04</th>
</tr>
</thead>
<tbody>
<tr>
<td>pathologic stage</td>
<td>pT1N0 (IA)</td>
<td>pT2N0 (IIA)</td>
<td>pT1N3 (IIIC)</td>
<td>pT2N1 (IIB)</td>
</tr>
<tr>
<td>immunohistochemistry</td>
<td></td>
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</tr>
<tr>
<td>estrogen receptor</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>progesterone receptor</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>HER2 (FISH)</td>
<td>2+/3 (negative)</td>
<td>3+/3 (positive)</td>
<td>1+/3 (negative)</td>
<td>3+/3 (positive)</td>
</tr>
<tr>
<td>No. of single cells</td>
<td>21</td>
<td>49</td>
<td>47(50)</td>
<td>28(51)</td>
</tr>
<tr>
<td>tumor (lymph node)</td>
<td></td>
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</tbody>
</table>

HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridization

To distinguish tumor cells, we chose epithelial cell adhesion molecule (EpCAM) as an archetypal epithelial tumor marker and performed gene signature enrichment analysis for the EpCAM-positive cells. A total of 673 genes with enrichment score > |0.5| were selected and used as an "epithelial breast cancer" signature. Consensus clustering with the epithelial breast cancer signature separated 246 cells into 145 epithelial tumor and 101 non-tumor cell groups. Gene expression profiling in the tumor cells revealed many co-regulated genes in the estrogen receptor (ER)-positive tumor at a single cell resolution, which represent previously known ER-associated genes including MYC, BCL-2, and GATA3. Individual tumor cells from the two human epidermal growth factor receptor 2 (HER2)-amplified tumors demonstrated drastically different degree of HER2 signaling pathway activation, indicating the necessity of molecular subtyping for the identification of HER2-activated tumors. TNBC tumor cells showed an overall upregulation of activator protein 1 transcriptional pathway and strong activation of epithelial-mesenchymal transition signatures in a small sub-population. Immune cells comprised all the non-epithelial population, with mostly T lymphocytes in the primary tumor samples and B lymphocytes in the lymph nodes. The tumor-infiltrating T cells expressed an activated phenotype and many cytotoxic components. Altogether, the single cell RNA sequencing revealed the true identity of the tumor cells and the tumor-associated immune cells.

Conclusion: Single cell RNA sequencing of breast cancer was used to reveal the true gene expression characteristics of the tumor cells and tumor-associated non-tumor compartments. The results showed key gene expression signatures of specific tumor subtypes and a wide range of transcriptome heterogeneity which is shaped by the tumor and microenvironments.
Identification of ESR1 splice variants associated with prognosis in estrogen receptor positive breast cancer

Lee H-B, Han W, Ko S, Kim M-S, Lim S, Lee K-M, Kang YJ, Han JH, Kim Y, Yoo T-K, Moon H-G, Noh D-Y, Kim S and Han W. Seoul National University College of Medicine, Seoul, Republic of Korea; Bioinformatics Institute, Seoul National University, Seoul, Republic of Korea; Seoul National University, Seoul, Republic of Korea; Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul, Republic of Korea; Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea and Interdisciplinary Program in Bioinformatics, and Bioinformatics Institute, Seoul National University, Seoul, Republic of Korea.

Body: Background: Splice variants play a major role in carcinogenesis and disease progression. It is well known that androgen receptor splice variants are associated with resistance to prostate cancer treatment. Estrogen receptor (ER)-positive breast cancers constitute about 70% of all breast cancers and have better prognosis compared to ER-negative cancers. However, there are ER-positive breast cancers that acquire resistance to anti-estrogen therapy, and 12-55% of those tumors were shown to possess ESR1 mutations. The aim of this study was to identify common splice variants in the ESR1 gene and investigate their association with disease outcome.

Methods: Whole transcriptome sequencing was performed on breast cancer specimens from 120 invasive breast cancer patients who underwent operation at Seoul National University Hospital (SNUH) and data from SNUH, GEO, and The Cancer Genome Atlas (TCGA) was used for normal breast tissue sequencing. Exon-exon junctions were identified on aligned RNA sequencing data and was used to construct exon graphs. Splice variant candidates were selected from exon graphs and were merged according to variant subtypes of samples. Subtypes were accessed differentially in relation to how frequent the junctions appear in tumor samples and common exon skipping types with frequent junctions were identified. TCGA RNA sequencing data was then used to search for the common exon skipping subtypes detected from SNUH RNA sequencing data.

Results: Of the 120 tumor samples, 50 were clinically ER-positive by immunohistochemistry. Among exon paths logically possible, 125 paths were not observed in normal breast tissues. Exon 4-5 junction was the most commonly observed junction in the tumor samples. In a search for exon skipping type that results in missing ligand-binding domain of ER, three exon skipping types were identified. Exon skipping with exon 5-10 junction (type 1), exon 9-12 junction (type 2), and exon 10-12 (type 3) was seen in 4 (8%), 4 (8%), and 10 (20%) ER-positive samples, respectively. Retrospective medical chart review of the 18 patients showed recurrence in 4 (100%), 2 (50%), and 4 (40%) patients with type 1, 2, and 3 exon skipping, respectively. Evaluation of TCGA RNA sequencing data of 872 ER-positive samples suggested exon 4-5 junction as the most common junction. A search for exon skipping types in TCGA revealed 1 (0.1%), 9 (1.0%), and 454 (52.1%) samples with type 1, 2, and 3 exon skipping, respectively. However, none of the patients with type 1 or 2 had metastasis or had expired. Of the 454 patients with type 3 exon skipping, 54 patients had died, constituting 61.4% of 88 mortalities in the whole ER-positive population.

Conclusion: Certain splice variants of ESR1 gene yields exon skipping subtypes commonly observed in the ER-positive breast cancer. Estrogen receptor-positive breast cancer with these exon skipping types resulting in a missing ligand-binding domain of ER may be associated with poorer disease outcome. Further investigation is warranted to validate the role of ESR1 exon skipping subtypes in the disease progression of breast cancer.
Aging is the number one risk factor for breast cancer development. Increasing evidence suggests the potential of mammary stem cells (MaSCs) and their progenitors to generate certain types of breast cancers through neoplastic transformation. Our previous study has shown increased percentage of MaSC-enriched basal cell population (Lin-CD49f^high^CD24med) and increased MaSC frequency during aging in murine models. On the other hand, a recent study from another group showed an increased frequency of CD49f^high^ cells in the human luminal population (CD227^+^) during aging, indicating possible aberrant expression of CD49f in the aged luminal cells. However, how these age-related luminal cells with basal markers are generated and how they contribute to potential breast cancer development remains unknown. Here we apply bioinformatics analysis on Next Generation Whole Transcriptome Sequencing data of MaSC-enriched basal cell population (Lin-CD49f^high^CD24med) and luminal progenitor-enriched cell population (Lin-CD49f^low^CD24^high^) of both young (4 to 6 months) and old (26 to 31 months) mouse mammary gland to test the hypothesis that age-associated increase of basal cell population and MaSCs may be due to the gain of basal cell markers and features by luminal cells. By Gene Set Enrichment Analysis (GSEA) we found a significant loss of basal cell and basal mammosphere signatures and a significant enrichment of luminal cell and luminal mammosphere signature in the old basal cell population and mammospheres in comparison with the young basal cell population and mammospheres. The core enrichment luminal genes from GSEA are able to cluster the old MaSC-enriched basal cell population as well as mammospheres closer to the cluster of luminal population than to the young basal population. These analyses indicate that aging may be associated with an expansion of aberrant MaSCs with both basal and luminal markers in mice, which may be the precursors of certain types of breast cancer. We are now studying the potential function of the basal-like luminal cells in the aged basal population.
Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific lncRNAs of triple-negative breast cancer

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Background: Triple-negative breast cancer (TNBC) is a highly heterogeneous group of cancers with no effective therapeutic targets hitherto. Thus molecular subtyping is necessary to better identify molecular-based therapies. While some classifiers have been established, no one has integrated the expression profiles of long-noncoding RNAs (lncRNAs) into such subtyping criterions. Considering the emerging important role of lncRNAs in gene regulation and other cellular processes, a novel classification integrating the transcriptome profiles of both messenger RNA (mRNA) and lncRNA would help us better understand the heterogeneity of TNBC and treat patients accordingly.

Methods: Using human transcriptome microarray, we retrieved the transcriptome profiles of 165 consecutive TNBC samples. We used k-means clustering to classify the samples based on the most differentially expressed genes (standard deviation>0.65). Empirical cumulative distribution function was analyzed to determine the optimal number of subtypes. Then the new classifier was compared with the Lehmann/Pietenpol system, and survival analyses were performed to compare the recurrence-free survival (RFS) in different subtypes. Gene Ontology (GO) and pathway analyses were applied to determine the main function of the subtype-specific genes and pathways. We conducted co-expression network analysis to identify interactions between lncRNAs and mRNAs, and to predict possible functions of subtype specific lncRNAs.

Results: All 165 TNBC tumors were classified into four distinct clusters, each displaying unique GOs and pathways. These include an immunomodulatory (IM) subtype, a luminal androgen receptor (LAR) subtype, a mesenchymal-like (MES) subtype and a basal-like and immune suppressed (BLIS) subtype, accounting for 17.0%, 17.6%, 33.3%, and 32.2% of the patients, respectively. The IM subtype had unique GOs and pathways involving immune cell process. The LAR subtype was highly enriched in hormonally regulated pathways. Enriched pathways in the MES subtype included ECM-receptor interaction, focal adhesion, and processes linked to growth factor signaling pathways. The BLIS subtype was characterized by downregulation of immune response gene and activation of cell cycle and DNA repair, and patients in this subtype experienced worse RFS compared to other subtypes (log-rank test, P=0.045), which was in concordance with the highly proliferative and immune-suppressed nature of these tumors. When analyzing the distribution of the Lehmann/Pietenpol subtypes in our classification system, we found that the two classification systems were significantly correlated (P=0.039). However, our novel classification was more concise and significantly connected with survival outcome. Subtype-specific lncRNAs were identified and their possible functions were predicted using co-expression network analysis.

Conclusions: We developed a novel TNBC classification system integrating the expression profiles of both mRNAs and lncRNAs, and determined subtype-specific lncRNAs that are potential biomarkers and targets of TNBC. If further validated in larger population, our novel classification system could facilitate patient counseling and individualize treatment of TNBC.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-04-05

Title: Genotype-phenotype classification of triple negative breast cancers (TNBC) in women of African descent using the PAM50 NanoString platform and genomic data

Olayiwola OA A, Ogundiran TO O, Hardeman A, Yoshimatsu TF F, Clayton W, Adeoye A, Ademola A, Ajani MA A, Khramtsova G, Grushko TA A, Huo D, Zheng Y, Parker J, Perou C and Olopade OI I. University College Hospital, Ibadan, Nigeria; University College Hospital, Ibadan, Nigeria; Center for Clinical Cancer Genetics and Global Health, The University of Chicago, Chicago, IL; The University of Chicago, Chicago, IL and University of North Carolina at Chapel Hill, Chapel Hill, NC.

Body: Introduction: TNBC has the highest mortality rate amongst all other breast cancer types due to its complex tumor heterogeneity and lack of well-defined molecular targets. It is known that women of African descent are two to three times more likely to develop TNBC compared to women of European ancestry, yet wide-scale genomic studies of African and African American breast tumors are limited. To elucidate genotypes and molecular subtypes associated with the most aggressive forms of breast cancer, we used the PAM50 NanoString platform to reclassify Nigerian (NG), African American (AA) and Caucasian (CA) tumors previously annotated by Immunohistochemistry (IHC), and correlated our findings to their germline genotype data obtained using high-throughput technologies.

Methods: RNAs were isolated from formalin-fixed, paraffin embedded (FFPE) tumor tissues using the High Pure Paraffin Kit (Roche) following manufacturer's protocol, and assayed on NanoString nCounter Analysis System using a custom Nano110 (PAM50 + claudin-low & VEGF signatures) probe set. Intrinsic subtyping and gene-expression data were evaluated using R statistical software. All study samples were previously annotated and subtyped by the ER/PR/HER2 IHC classifier. Genotypes were obtained from next generation sequencing or Illumina Human2.5M BeadChip platform using germline DNA from more than 2000 breast cancer cases and 2000 controls were studied.

Results: To date, Intrinsic molecular subtyping by Nano110 has been completed on 69 NG, 81 AA and 74 CA tumors. Concordance between IHC and PAM50 was 59%, which is adequate and comparable to previous studies. Basal-like subtype was overrepresented and accounted for nearly 30% of NG and AA cases, compared to 17% in CA cases. HER2-enriched subtype was overrepresented only in NG cases (9%). The proportion with Luminal A tumors were 44% NG, 56% AA and 68% CA, respectively.

Conclusions: PAM50 NanoString assay is reliable and high-throughput for molecular subtyping breast cancer using RNA extracted from FFPE tumors. Ongoing work will correlate PAM50 intrinsic subtypes to genotype data.
Transcriptome analysis reveals possible therapeutic targets in a non-immunogenic, mesenchymal-type triple negative breast cancer, resistant to anti-EGFR/cytotoxics-based neoadjuvant treatment: A pilot study

Radosevic-Robin N, Ponelle F, Chabaud V, Rouzaire P-O, Privat M, Vidal V, Bignon Y-J and Penault-Llorca F. ERTICa Research Group, University of Auvergne EA4677, Jean Perrin Cancer Center, Clermont-Ferrand, France; Helixio, Hybrigenics Group, Saint-Beuzire, France and Gabriel Montpied University Hospital Center, Clermont-Ferrand, France.

Background: Triple negative breast cancer (TNBC) patients (pts) left with a residual tumor (RT) after neoadjuvant treatment (NAT) have a poor prognosis, often developing fatal metastases within first 3 years post-NAT. We investigated TNBCs which showed no response or progression under the pioneer anti-EGFR/cytotoxics-based NAT (PMID 24827135), in order to see whether tumor gene expression (GE), associated with a powerful response-predictive biomarker, the amount of tumor-infiltrating lymphocytes (TIL) can help identifying that highly resistant TNBC category and its possible therapeutic targets.

Methods: The study included 27 TNBCs treated with panitumumab combined to standard fluorouracil-epirubicin-cyclophosphamide and docetaxel. Response was evaluated according to the Chevallier (Ch) classification (PMID 8338056). TIL amount was reported as the % of pre-NAT tumor stroma occupied by them (PMID 25214542). EGFR protein expression was assessed by immunohistochemistry and reported as a histoscore (% of positive cells x signal intensity as 0, 1, 2 or 3). Pre-NAT tumor RNA was hybridized on Agilent's Human SurePrint microarrays. Data were extracted and analyzed by Feature Extraction 10.7, Genespring GX 12.0 and TNBCtype softwares and Man-Whitney test.

Results: The responses were: Ch-1 (pathologic complete response): 14 pts, Ch-3 (partial response): 8 pts, Ch-4 (no response / progression): 5 pts. The Ch-4 group showed significantly lower TIL level as compared to Ch-1 (5±4 vs 60±31, p=0.0014) and a trend of lower TIL in comparison to Ch-3 (5±4 vs 32±27, p=0.053). With regards to the TNBCtype-determined molecular classification, the only remarkable difference was the predominant mesenchymal subtype in Ch-4 (4/5 cases) while Ch-1 and Ch-3 contained a mixture of 6 TNBCtype subtypes. Interestingly, ERBB1 (EGFR) GE was significantly lower in Ch-4 compared to Ch-3 (p<0.01), which was also reflected by EGFR protein expression scores (36±49 vs 153±93, p=0.029). In addition, 34 selected genes, known for their role in immunity/inflammation (among which IL1,6,8,10, CXCL1,2,10,12, CD274/PDL1 etc) were all underexpressed in Ch-4 in comparison to Ch-3 or Ch-1. Among 189 genes significantly overexpressed (p<0.05) in Ch-4 compared to Ch-3, we observed NPNT (nephronectin), OVGP1 (oviductal glycoprotein 1/mucin 9), TTLL7, UQCRB, RAPGEF5, POLE, TNFAIP8L1, ZNF124, CLUL1, NRG2, ANKS1B, MYB, IL17RB, SOX10 and BCL2, which have been reported to have a role in tumor progression, metastasis, hypoxia-activated angiogenesis, Ras-pathway activation and prevention of apoptosis.

Conclusion: In this pilot study, the TNBC highly resistant to NAT were mostly of the mesenchymal molecular subtype, with low EGFR gene/protein expression and immunologically "calm". Some genes overexpressed in this group worth further investigating as therapeutic targets, since the anti-EGFR or immunotherapy approaches are likely ineffective for those tumors.
Title: Profiling alternative polyadenylation in triple-negative breast cancer based on large-scale data

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Body: BACKGROUND: Triple-negative breast cancer (TNBC) accounts for 15% to 20% of breast cancers. It has an aggressive clinical course and high tendency for both early relapse and distant metastasis. TNBC is also a highly heterogeneous disease with many pathological, immunohistochemical, and gene expression features. Understanding the characteristics of TNBC subtypes at the multi-omics level would be profoundly useful and molecular portraits may provide a rationale for therapeutic regimens. Alternative polyadenylation (APA) has recently gained attention as a major player influencing the dynamics of gene regulation. In this study, the APA landscape of TNBC was profiled, the signaling pathways involved in APA dynamics in TNBC molecular subtypes were characterized and the clinical relevance was explored.

MATERIALS AND METHODS: A novel bioinformatics algorithm based on Bayesian analysis for de novo identification of dynamic APAs from transcriptome microarray data was here developed. APA portraits were characterized in surgical specimens collected from 154 patients diagnosed with histologically proven TNBC in Fudan University Shanghai Cancer Center (Shanghai, China). Gene set enrichment analysis (GSEA) and consensus clustering were then performed to analyze and re-define TNBC subtypes at the APA level and to identify actionable pathways.

RESULTS: Here, 631 genes were found to present dynamic APA changes in TNBC, with 54% shortening tandem 3′UTR and 29% lengthening tandem 3′UTR. Genes undergoing 3′UTR shortening were enriched for cell cycle, DNA conformation change, and the immune system, but those undergoing 3′UTR lengthening were enriched for cell adhesion and cell locomotion. GSEA results indicated co-enrichment in pathways with the changes in the length of the 3′UTR and in mRNA expression of Basal-like 1 (BL1) and Immunomodulatory (IM) subtypes. Changes in mRNA levels and 3′UTR length within the same TNBC subtype occurred in different genes, but they all targeted the characteristic pathways of the respective subtype. In BL1 tumors, the co-enriched pathways were cell cycle, cell cycle mitotic, RNA metabolism, and mRNA metabolism, but signaling pathways were co-enriched in adaptive immune system and class I MHC mediated antigen processing presentation in IM subtype. Four distinct TNBC subtypes were distinguished by the length of the 3′UTR and confirmed. This is consistent with TNBC classification based on the mRNA profiles (P < 0.001). The four subtypes differed in age (P = 0.029), CK5/6 (P = 0.001), and CK14 (P < 0.001). However, no difference in relapse-free survival was observed between APA subtypes with 15 months of median follow-up time (log-rank test, P = 0.286). The correlation analysis indicated three different types of relationships between 3′UTR length and the level of gene expression, including positive (63%), negative (26%), and no correlation (11%). The various correlation modalities may explain the additional subtyping information provided by the length of 3′UTR, which is independent of mRNA profiles.

CONCLUSION: The dynamic APA landscape was characterized in TNBC, and data suggest that APA events are specific to tumor subtype and could serve as novel biomarkers in the future.
Expression profile of long non-coding RNAs characterizes different subtypes of breast cancer

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Body: Long non-coding RNAs (lncRNAs) are RNA molecules longer than 200 nucleotides that are not translated into proteins, but regulate the transcription of genes involved in different cellular processes, including differentiation, cancer initiation and progression. The relevance of several lncRNAs in the transcriptional and post-transcriptional field is now well established. Their roles have been found to vary from forming complexes needed for gene transcription to having critical roles in the cytoplasm where they regulate protein localization, mRNA translation and stability. Microarray analysis of normal breast of parous and nulliparous postmenopausal women revealed that lncRNAs are up-regulated in the parous breast [Int. J. Cancer: 131, 1059-1070, 2012]. Subsequently, RNA sequencing was performed to a subset of these samples to understand the role of lncRNAs in the regulation of transcription and their potential function in pregnancy's effect in reducing the lifetime risk of developing breast cancer. In this work, RNA sequencing of healthy postmenopausal breast tissue biopsies from 8 parous and 8 nulliparous women using Illumina platform was performed. The sequencing results show that there are 42 lncRNAs differentially expressed between parous and nulliparous breast tissue. After analysis of these 42 lncRNAs using bioinformatics and thermodynamic filters, 10 lncRNAs were selected to be tested in vitro. Using RT-qPCR, we have demonstrated that the expression profile of at least 10 lncRNAs is characteristic of each epithelial breast cancer cell subtype (luminal, triple negative and cells that overexpress HER2). Moreover, these lncRNAs are differentially expressed in breast tumors when compared to its adjacent normal tissue. Overexpression and knock out experiments of several of these lncRNAs are underway to prove their relevance in breast differentiation and cancer initiation. The results in cancer/normal immortalized breast cell lines and tumor/adjacent normal breast tissue point to the potential of these lncRNAs as targets, not only for therapeutic approaches against breast cancer, but also as preventive measures for nulliparous women.

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Title: Cellular and molecular determinants of breast cancer sensitivity to all-trans retinoic acid: Identification of a gene expression fingerprint predicting responsiveness


Body: All-trans retinoic acid (ATRA) and derived natural as well as synthetic retinoids are promising agents in the treatment and chemoprevention of various types of neoplasia, including mammary tumors. ATRA is an important component of the therapeutic schemes used for the treatment of a rare form of Acute Myelogenous Leukemia known as Acute Promyelocytic Leukemia. A rational use of the paradigmatic retinoid, ATRA, in a heterogeneous disease, like breast cancer, requires the definition of the cellular and molecular determinants of sensitivity to the agent. The major aim of the study was the definition of a predictive gene expression fingerprint that can be used for the selection of patients who may benefit from treatment protocols containing ATRA. To this purpose, we selected 45 breast cancer cell lines characterized for the constitutive whole genome gene expression profiles. The sensitivity of 30 cell lines (training set) to the anti-proliferative action of ATRA was defined after challenge with increasing concentrations of the retinoid for 3, 6 and 9 days. This analysis established that Luminal and ER-positive cell lines are enriched within the ATRA sensitive group. In contrast, cell lines characterized by a Basal-like phenotype, according to the PAM50 gene expression signature, are generally refractory to the growth inhibitory action of ATRA. The sensitivity of Luminal-A and Luminal-B and the general refractoriness of Basal-like tumors to ATRA was validated in short-term tissue slice cultures of surgical breast cancer specimens. The training set was used to define a gene-expression fingerprint consisting of approximately 50 genes significantly associated with ATRA sensitivity. The fingerprint was generated by reprocessing the RNA sequencing data contained in the CCLE (Cancer Cell line Encyclopedia) of the Broad Institute and it was built from approximately 60,000 coding and non-coding loci. The approach involved the use of general linear models (machine learning algorithm). The identified gene-expression fingerprint was subsequently used to successfully predict ATRA sensitivity in a test set consisting of the remaining 15 cell lines. As a first step towards the use of the fingerprint for the stratification of patients, we evaluated the proportion of predicted ATRA sensitive breast tumors in the TCGA dataset. In accordance with the cell line and primary tumor data, approximately 30% of the Luminal tumors present with a high similarity score to the identified gene expression fingerprint associated with ATRA sensitivity. In contrast, only 5% of the Basal-like or Triple-negative mammary tumors are characterized by the same high similarity score. Curiously, the ATRA sensitivity signature seems to be tumor context independent, as it correctly identifies the 20 Acute Promyelocytic Leukemia patients present in the 198 Acute Myelogenous Leukemia patients present in the TCGA dataset.
Title: Clinicopathological and molecular characteristics of pleomorphic invasive lobular carcinoma of breast


Body: Background: Pleomorphic invasive lobular carcinoma (PILC) is described as a distinct morphological variant of invasive lobular carcinoma (ILC) but its clinical behavior is not well characterized. PILCs have loss of E-cadherin similar to ILCs but have distinct morphological features like nuclear contour irregularity, a single prominent nucleolus, increased hyperchromasia and more frequent mitoses. In addition, some studies have reported that PILCs have acquired further molecular alterations such as gain of HER2/neu, amplification of c-myc and loss of p53. To the best of our knowledge there have been no studies evaluating Phosphoinositide 3 kinase/Akt/mammalian (or mechanistic) target of rapamycin (PI3K/Akt/mTOR) pathway in PILC. We hypothesize that there is increased activation of PI3K/Akt/mTOR pathway in PILC compared to ILC. Activation of the PI3K/Akt/mTOR pathway was evaluated by quantifying protein expression of phosphatase and tensin homolog (PTEN) and phosphorylated-S6 kinase1 (p-S6K1). PTEN is a negative regulator of the PI3K pathway and its loss/decreased expression (by mutation or allelic imbalance) activates downstream signaling. Loss (or decrease) of PTEN expression has been reported to be associated with PI3K pathway activation in more than 50% of ER+ breast tumors. Since PI3K pathway can be activated by other mechanisms in addition to PTEN loss, we hypothesized that evaluation of pS6K1 may predict activation of this pathway more than PTEN protein expression alone.

Methods: We conducted a retrospective translational study at the University of Arizona Cancer Center. Our Pathology database was searched to identify PILCs from 2012-2014. Two investigators reviewed the pathology reports independently and abstracted clinicopathological data. Formalin-fixed paraffin embedded (FFPE) primary PILCs were stained for PTEN and pS6K1 expression. Expression of PTEN and pS6K1 was quantified by long score methodology as low (< 10), moderate (11-50) or high (≥ 50) expression.

Results: We identified 19 patients with PILC. All tumors were either moderately (n=10) or poorly differentiated (n=9). Estrogen receptor (ER) was positive in all, progesterone receptor (PR) was positive in 11 (52%) and HER2 was negative in all tumors. Proliferation index (Ki67) was elevated in all tumors (median 32%, range 20-70%). Lymph nodes were involved with metastatic carcinoma in 7 patients (negative in 9 and unknown in 3). The 21-gene recurrence score assay (Oncotype Dx) was performed in 10 patients and demonstrated higher scores (median 23, range 6-36) with the majority being in the intermediate or high range (8/10). Expression of PTEN and p-S6K1 was quantified on 10 FFPE tumor tissues. PTEN expression was high in all while pS6K1 was high in 8 and low in 2 tumors.

Conclusion: PILCs are a biologically distinct group of ILC. Clinicopathological characteristics suggest they would have a more clinically aggressive behavior (higher grade, high proliferative index and 21 gene recurrence score). In addition, our results indicate that PI3k/Akt/mTOR pathway in activated in majority of these tumors and that PTEN is not the key regulator of this pathway. Genomic profiling is currently underway to further analyze other causes of pathway activation.
The foundation one assay influences clinical decision making in metastatic breast cancer

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Background: Tumor DNA sequencing is now readily available in metastatic breast cancer (MBC). The purpose of this study was to determine the effect of molecular testing on clinical decision-making in MBC at an academic cancer center.

Methods: We obtained the Foundation One (FO) tests that were requested from Duke Cancer Institute breast oncologists between 12/2013 and 4/2015. We examined the following: ER/PR/HER2 status, histology, biopsy site and time, #months (mons) from time tissue was obtained to testing, #lines of prior therapy prior to tissue sampling and FO testing, #mons from initial diagnosis/MBC to FO. The following variables from the FO test were abstracted: #genomic alterations, #rx with potential benefit, #clinical trials available, #variations of unknown significance (VUS). Physicians were retrospectively surveyed regarding influence of FO results on clinical treatment decisions and on clinical trial consideration.

Results: To date, 58 specimens have been sent for FO testing. From the time of FO testing, the mean # of mons since initial diagnosis (dx) was 84.4(7-435) and the mean # of mons since the dx of MBC was 31.4(1-140). Pts with triple negative breast cancer (TNBC) were more likely to have FO ordered within 1 year of MBC diagnosis (OR=2.93, p=0.048). On average, pts had received 3.78 lines of rx for MBC (0-10) at the time FO was sent. The timing of tissue acquisition for FO testing was bimodal (45% had a new bx for the assay whereas 55% had the FO test on archival bx). 50% of un-resulted (unsuccessful) FO assays were from archival tissue with a mean #mons since the archival tissue was obtained of 42 mons (18-74). To date, 56% of resulted samples were from archival tissue with a mean #mons since the archival tissue was obtained of 19.6(2-75).

Per the FO report: the mean #genomic alterations per pt = 6.21 (1-16); the mean #VUS per pt = 11.5 (3-30); the mean #mutation-directed rx per pt= 3.4 (0-15), the mean #mutation-directed clinical trials per pt= 9.33 (0-20). Genomic alterations occurring in ≥ 10% patients included: TP53 (48%), CCND1 (27%), FGF4 (27%), FGF19 (27%), FGFR1 (25%), PIK3CA (25%), FGF3 (25%), MYC (25%), ZNF703 (21%), ESR1 (19%), MCL1 (15%), CDH1 (13%), ERBB2 (10%), EMSY (10%), MYST3 (10%). 36 pts had invasive ductal carcinoma (IDC), 6 pts had inflammatory breast cancer (IBC), and 6 had invasive lobular carcinoma (ILC). No genomic alterations were associated with a sub-type of MBC with the exception of ESR1 mutations in ER+ IDC (100%) and CDH1 mutations in ILC (67%). Pts found to have ESR1 mutations had on average 63 mons(10-200) of endocrine therapy at the time of tissue sampling.

When the breast cancer medical oncology physicians were retrospectively surveyed, 42% FO assays influenced clinical treatment decisions and 14% resulted in clinical trial enrollment.

Conclusions: FO utilization is variable based on MBC sub-type and the timing of tissue collection is bimodal. ESR1 mutations were associated with history of prolonged endocrine rx treatment in ER+ IDC and CDH1 mutations were associated with ILC. FO assays frequently influenced clinical treatment decisions but did not result in a high number of pts enrolled on clinical trials. We will update our dataset with additional FO assays and clinicopathologic variables.
**Title:** Immunoprofile of TIL, genetics of Fc receptor gama and apoptosis activation in patients with Her2-positive breast cancer – Pilot study

Stanek L, Tesarova P, Vocka M, Musil Z, Kasparova K and Petruzelka L. First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic and Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic.

**Body:** Possible markers helping us to detect molecular-immunologically patients with Her2 positivity are Fc-gamma receptors (FcR). FcR binds to CH3 section of Fc trastuzumab fragment and is the key factor for activation of cell lysis via immune system cells (NK cells, macrophages). Tumors, where the H131R mutation decreased the effect of NK cells and macrophages, show a higher percentage of tumor cells entering apoptosis and activation of tumor infiltrating lymphocytes (TIL) (CD8+), which can result in tumor volume reduction and inhibition and thus extend overall survival.

In the pilot study included 50 patients with histologically verified breast cancer treated in the Department of Oncology (First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague) participated in our pilot study. 40 patients were with a median age of 49.4 years (14 postmenopausal [46.7%] and 16 premenopausal [53.3%]) with detected HER-2 FISH amplification (histologically 18 patients with HER-2 subtype which means without hormone receptor positivity and 12 patients with luminal B subtype and thus hormone receptor positive). All patients were without evidence of distal metastases (6 in stage I, 13 in stage II and 11 in stage III). A control group was formed of 10 patients with a median age of 49.2 years (4 postmenopausal [40.0%] and 6 premenopausal [60.0%]) and without HER-2 amplification (histologically 7 patients with luminal A subtype, 2 patients with luminal B subtype and 1 patient with basal-like subtype). All patients of the control group were without evidence of distal metastases (2 in stage I, 6 in stage II and 2 in stage III). All patients signed the written informed consent.

Our aim was to carry out the H131R mutation analysis using the StripAssay (ViennaLab, Austrian) method in correlation with clinical data, to quantify cells entering apoptosis using molecular method TUNEL (DeadEnd, Fluorometric TUNEL System, USA) and to immunohistochemically determine the expression and ratio of CD8 (Clone C8/144B) to CD4 (Clone 4B12) (Dako, Denmark) in a tumor.

Results showed that in Her2-positive patients responding badly to biological treatment according to the clinical data the H131R mutation in the FcR gene was detected in 34/40 (85%) cases (26 heterozygotes / 8 homozygotes). Kaplan-Meier analysis of patients with the H131R mutation (FcR mutation / activation of apoptosis / CD8 positivity) showed significant differences in patients’ survival from the generalization of a tumor – 28 vs 83 months. In patients with longer survival molecular analysis TUNEL confirmed a large number of cells entering apoptosis (20-30% in the field of vision) and activated antitumor immunity (CD8+ positivity) in more than 60% of a tumor and concurrently no expression (CD4-).

Our study showed that in Her2-positive patients with detected H131R mutation in the FcR gene the function of NK cells and macrophages in tumor inhibition was decreased (blocked). However, in some patients the apoptotic pathway was activated and the process of programmed cell death was induced. Increased expression of CD8 was a positive prognostic factor, with a longer survival of these patients.

Dedication: PRVOUK-P-27/LF1/1., MZ 00064203.
Title: The role of precision medicine in "real-life" management of breast cancer patients: A survey assessing the current use and attitudes towards tumor molecular sequencing in clinical practice


Body: Background: Personalized medicine is a rising paradigm in cancer care. The identification of pathways involved in carcinogenesis along with the development of targeted therapies has revolutionized cancer treatment. There is increasing availability of technologies that can interrogate the genomic landscape of the tumor; however, it is still uncertain whether such platforms are used in clinical practice.

Methods: We conducted a 28-item survey to investigate the current use of tumor molecular sequencing in the management of breast cancer patients. A link to the online survey was communicated via various platforms such as the European Society for Medical Oncology (ESMO) and European School of Oncology (ESO) newsletter, and via a dedicated mailing by the Breast International Group (BIG) and other academic groups. Descriptive statistical analysis and Fisher's exact tests were applied to explore potential association between the demographic characteristics and responses.

Results: A total of 211 physicians from 35 countries participated to the study between the 9 March and 3 June 2015, with 92% fully completed questionnaires. The mean age of the participants was 45 years (range 27-77). The majority of responders were medical oncologists (88%), practicing in Europe (69%) and working in academic institutions (66%). 62% (130/211) of participants had never requested tumor molecular sequencing for breast cancer patients. Working in academic institutions and having more time allocated to research were associated with the use of tumor molecular sequencing (p = 0.007 and 0.009, respectively). For the 81 participants that used tumor molecular sequencing in the past (Table 1), there was a significant association between accessibility and frequency of use (p=0.02). 92% (181/211) of participants claimed that they would probably use tumor molecular sequencing more often if it was more accessible. Lack of funding and lack of access to the technology were the main reasons for poor endorsement. 89% of participants believe that tumor molecular sequencing will play a major role in the management of breast cancer patients in the future. Current weak evidence and poor access to matched targeted therapy are the main concerns against a wider use of these platforms in clinical practice.

Table 1. Summary of replies from the 81 participants that used tumor molecular sequencing for breast cancer patients

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In what percentage of your breast cancer patients has tumor molecular sequencing been performed at least once?</td>
<td>≤5%</td>
<td>55 (68%)</td>
</tr>
<tr>
<td></td>
<td>&gt;5%</td>
<td>26 (32%)</td>
</tr>
<tr>
<td>How often do the results lead to enrollment in a clinical trial?</td>
<td>≤10%</td>
<td>53 (65%)</td>
</tr>
<tr>
<td></td>
<td>&gt;10%</td>
<td>28 (35%)</td>
</tr>
<tr>
<td>How confident are you in interpreting tumor sequencing results?</td>
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<td>Somewhat/Highly</td>
<td>64 (21%)</td>
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<tr>
<td>Do you consider molecular sequencing platforms accessible?</td>
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</tr>
<tr>
<td></td>
<td>Somewhat/Highly</td>
<td>36 (45%)</td>
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</table>

Conclusion: Our survey indicates that molecular sequencing platforms are sometimes used, albeit not widely in guiding management of breast cancer patients. Poor accessibility may contribute to the low frequency of use, but lack of evidence and poor access to matched targeted therapy are also major concerns.
Title: Integrating whole genome sequencing data with RNAseq, pathway analysis, and quantitative proteomics to determine prognosis after standard adjuvant treatment with trastuzumab and chemotherapy in primary breast cancer patients

Benz SC C, Rabizadeh S, Cecchi F, Beckman MW W, Brucker SY Y, Hartmann A, Golovato J, Hembrough T, Janni W, Rack B, Sanborn JZ Zachary, Schneeweiss A, Vaske CJ J, Soon-Shiong P and Fasching PA A. NantOmics, LLC, Santa Cruz, CA; NantOmics, LLC, Culver city, CA; NantOmics, LLC, Rockville, MD; University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany; University Hospital Tübingen, Tübingen, Germany; University Hospital Ulm, Ulm, Germany; Ludwigs-Maximilians University, Munich, Germany; University Hospital Heidelberg, National Center for Tumor Diseases, Heidelberg, Germany; CSS Institute of Molecular Medicine, Culver City, CA and Institute of Pathology, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany.

Body: Background: Despite improvements in the treatment of HER2+ breast cancer (BC), almost all patients (pts) progress in the metastatic setting. Three examples of resistance mechanisms are: PI3K mutations, lack of ADCC, or low expression of HER2. We recently showed that among 237 pts who had HER2 amplifications, 49% had normal or low levels of HER2 RNA. In addition, quantification of HER2 protein by selected reaction monitoring mass spectrometry (SRM-MS) accurately predicted HER2 expression status compared with IHC (3+)/ISH (≥2.0). Here we report a comprehensive panomic approach that integrates whole genome sequencing (WGS), RNASeq, quantitative proteomics, and pathway analysis to determine associations between tumor molecular profiles and prognosis among HER2+ pts.

Methods: Matched tumor-normal samples (FFPE tumors and blood) were obtained from 58 pts with HER2+ BC who had received standard adjuvant chemotherapy and trastuzumab. Pts were divided into 2 groups: those who had no recurrence after 5 years and those who had developed metastases. The HER2 status of each pt was previously determined using IHC/FISH. Samples underwent WGS and RNASeq according to NantOmics CLIA-approved assay specifications. WGS data were processed using Contraster; RNASeq data confirmed the presence of gene mutations and was used to identify mutational and transcript abundance. PARADIGM was used to reveal associations between gene mutations and pathway levels. SRM-MS was used for proteomics analysis of a panel of 53 proteins. Tumor areas from FFPE tissue sections were analyzed after laser microdissection. Absolute protein quantitation was accomplished through simultaneous detection of endogenous target and synthetic labeled heavy peptide identical to analytical targets. Genetic alterations in germline and tumor DNA were compared in pts with vs without recurrence.

Results: There was no statistically significant difference in the mean concentration of HER2 in the tumors of pts with vs without recurrence: 2.34 fmol/µL vs 2.56 fmol/µL. Other analyzed proteins did not appear to be associated with recurrence; however, expected correlations between pt and tumor characteristics and protein expression were found. With regard to clinically relevant mutations, we found one germline BRCA2 mutation in a pt with no family history of this mutation. The most commonly found somatic mutations were in TP53 (11 pts), AMBRA1 (11 pts), MORC4 (10 pts), SETD2 (8 pts), CDC27 (6 pts), BCLAF1 (5 pts), ZNF479 (4 pts), PIK3CA (3 pts), PIK3R1 (3 pts), RUNX1 (3 pts), and GATA3 (3 pts).

Conclusion: Whereas HER2 expression status was predictive of OS and PFS in pts treated with trastuzumab (Nuciforo et al. Mol Onc. 2015), in this small exploratory study of HER2+ BC pts, HER2 expression status was not predictive of recurrence. To better understand the molecular mechanisms driving recurrence beyond HER2 status alone, genomic sequencing may define a signature of recurrence after anti-HER2 therapy.
Title: Identification of HER2 positive breast cancer subgroups with combined whole genome sequencing and transcriptomic analyses

Vincent-Salomon A, Ferrari A, Pivot X, MacGrogan G, Arnould L, Treilleux I, Romieu G, Sertier A-S, Thomas E, Tonon L, Boyault S, Letexier V, Paupote I, Birnbaum D, Saintigny P and Viari A. Institut Curie, Paris, France; Fondation Synergie Lyon Cancer, Lyon, France; Centre Hospitalier Universitaire, Besançon, France; Institut Bergonie, Bordeaux, France; Centre Georges François Leclerc, Dijon, France; Centre Leon Berard, Lyon, France; Institut de Cancérologie de Montpellier, Montpellier, France; Institut National du Cancer, Boulogne-Billancourt, France; Centre de Cancérologie de Marseille-INSERM, Marseille, France and INRIA - équipe Erable, Grenoble, France.

Body: Background: HER2-positive (HER2+) breast cancer (BC) represent 15% of all BCs and their natural history has been improved with anti-HER2 targeted therapies. However, the benefit of these therapies varies widely among patients. Deciphering the genomic heterogeneity of HER2+ BC may provide a basis to understand patients’ outcome. As part of the ICGC Breast Cancer Working Group effort, we completed a comprehensive molecular characterization of a set of HER2+ tumors to gain insight into the heterogeneity of this type of BC.

Material and Methods: A total of 64 HER2+ primary invasive carcinomas were prospectively collected in the context of the PHARE trial. Grade, ER, PR, and HER2 status were assessed through a pathological central review. Whole genome sequencing (WGS) of paired tumor and normal tissue was performed as well as array-based genome-wide expression profiles from the same set of tumor samples. Sequencing data was analyzed for CNAs and the fraction of the genome altered (FGA), single nucleotide variations (SNVs), small indels, and structural variants (SVs). RNA expression-based subgroups were identified by selecting most variable transcripts and validated in the HER2+ BCs from the METABRIC dataset.

Results: Analysis of gene expression profiles allowed the identification of 4 groups (A, B, C, D) that were validated in the METABRIC dataset. ER-positive (ER+) tumors were clustered in groups A & B and ER- tumors in groups C & D. Besides the ERBB2 amplicon, 17q chromosome harbored other previously reported amplified regions, occurring more frequently in group A. A total of 24,203 somatic SVs were detected based on clipped or abnormal reads mapping, with a median of 327 per sample (range 132-952). The majority (18,058; 75%) of these variants were intra-chromosomal and composed of 7,438 (41%) inversions, 5,889 (33%) deletions and 4,731 (26%) duplications. Intra-chromosomal SVs were more frequent on chromosome 17 (with a median of 30.5 SVs) than on all other chromosomes (median 6.0; P < 10^-10). No association was observed between the number of SVs on chromosome 17 and ER status. Interestingly, RNA groups A & C displayed more intra-chromosomal SVs on chromosome 17 than groups B & D (A vs. BCD, P = 1.10-2 and AC vs. BD, P = 2.10-3). A total of 554,553 somatic variations, including 549,002 SNVs and 5,551 small indels were detected. The median number of mutations was 5,464 (range 1,546-99,103) and C>>T transition (40%) was the most frequent mutation. As expected, the most recurrent mutations were identified in TP53 & PIK3CA. Interestingly, group A was devoid of TP53 mutation, while all tumors in group D were TP53 mutant. HER2 mutations were observed independently of the level of HER2 amplification in 4/64 tumors (6%). Other mutations included PDE4DIP (9%), JAK2 (6%), KMT2C (6%) and KMT2D (6%).

Conclusion: Our study demonstrates the existence of genomic heterogeneity of the HER2+ breast carcinomas group. In particular, two groups were defined among ER+ tumors, with significantly different genomic alterations. Further research is needed to elucidate the potential relationship between these results and the outcome of patients treated with HER2-targeted therapies. Funding: Institut National du Cancer, France.
Title: Intrinsic subtype and gene expression changes between primary and metastatic breast cancer

Prat A, Martínez de Dueñas E, Galván P, García S, Burgués O, Paré L, Antolín S, Martinello R, Blanca S, Adamo B, Guerrero Á, Muñoz M, Nuciforo P, Vidal M, Pérez RM, Chacón JI, Caballero R, Gascón P, Carrasco E, Rojo F, Perou CM, Cortés J, Adamo V, Albanell J and Lluch A. Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Hospital Clínic -- IDIBAPS, Barcelona, Spain; Hospital Provincial de Castellón, Castellón, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; Hospital Clínico San Cecilio de Granada, Granada, Spain; Instituto Valenciano de Oncología, Valencia, Spain; Hospital Universitario Quirón de Madrid, Madrid, Spain; Hospital Virgen de la Salud, Toledo, Spain; GEICAM, Spanish Breast Cancer Group, Madrid, Spain; Fundación Jiménez Diaz, Madrid, Spain; University of North Carolina, Chapel Hill, NC; University of Messina, Messina, Italy and Hospital del Mar, Barcelona, Spain.

Body: Background: A better understanding of the biological changes occurring during metastatic progression of breast cancer is needed to identify new biomarkers, targets and novel treatment strategies. Here, we compared the intrinsic type and the expression of a gene panel across a large dataset of paired primary and metastatic tissues.

Methods: Expression profiling of 105 breast cancer-related genes was performed on 254 (127 pairs) formalin-fixed paraffin-embedded tumor tissues using the nCounter platform. Tumor samples were obtained from 3 independent sources (ConvertHER trial [BCRT 2014] and two in-house datasets). Tumors were classified into each intrinsic subtype using the research-based PAM50 classifier (Parker et al. J Clin Oncol 2009). Chi-square tests were performed to determine the differences in the distribution of variables. Paired two-class Significance of Microarrays (SAM) was performed to determine the genes differentially expressed between paired primary and metastatic tissues. In vitro stable transfection of FGFR4-GFP was performed on Luminal B MCF7 cell line. RNA was purified on control vs. transfected cell lines. 7-AAD cell viability was performed following estrogen deprivation for 6 days.

Results: Subtype distribution in primary vs. metastatic disease was 39.0% vs. 26.8% for Luminal A (p=0.012), 26.0% vs. 35.0% for Luminal B (p=0.322), 11.4% vs. 20.3% for HER2-enriched (p=0.115) and 10.6% vs. 13.0% for Basal-like tumors (p=0.843). The rate of subtype conversion was 7.7% in Basal-like, 23.1% in HER2-enriched, 30.0% in Luminal B and 54.3% in Luminal A disease. The majority of subtype conversions in Luminal A disease were to Luminal B (72.0%) and HER2-enriched (24.0%). Overall, 13.2% of primary Luminal A/B tumors progressed to a HER2-E subtype despite 70% of them being clinically HER2-negative. In a paired analysis using all samples, 10- and 12- genes were found up- and down-regulated in metastatic tissues (False Discovery Rate [FDR] <5%). The up-regulated gene list in metastatic disease was composed of FGFR4 (top gene) and proliferation genes (CDC6, CCNB1, CEP55). The down-regulated gene list in metastatic disease was enriched for luminal-related genes (ESR1, PGR, NAT1 and MAPT). A similar paired analysis within Luminal A, Luminal B, HER2-enriched and Basal-like disease revealed 22, 8, 7 and 0 differentially expressed genes (FDR<5%), respectively. Finally, MCF7 cell line transfected with FGFR4 showed a relative increase in the HER2-enriched profile compared with transfected control. In vitro, MCF7-FGFR4 cells showed estrogen independent growth compared to transfected controls.

Conclusions: Metastatic tissues are relatively more proliferative and less luminal compared to primary tumors. This is especially relevant in primary Luminal A disease. In contrast, metastatic tissues from Basal-like primary disease remain largely unchanged. In luminal disease, a significant increase in the HER2-enriched profile is observed in metastatic disease despite most tumors being clinically HER2-negative. A potential driver of the HER2-enriched profile and estrogen independence in clinically HER2-negative metastatic tissues might be FGFR4.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-05-03

Title: Genomic diversity of ductal carcinoma in situ (DCIS) as a driver of invasion and metastasis

King LM M, Marks JR R, Hall AH H, Temko D, Graham TA A, Mardis ER R, Maley CC C and Hwang E. Duke University, Durham, NC; Duke University, Durham, NC; Evolution and Cancer Laboratory, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom; Washington University School of Medicine, St. Louis, MO and School of Life Sciences, Arizona State University, Tempe, AZ.

Body: Background: Recent evidence indicates that breast cancers may have high levels of heterogeneity. Based on principles of tumor evolution, we are investigating whether DCIS with higher levels of intra-tumoral genetic heterogeneity are more likely to progress to invasive and/or metastatic disease.

Methods: Cases (DCIS with co-existing invasive or metastatic cancer) and controls (pure DCIS) are identified from Duke Pathology archives. From cases and controls, we are analyzing two areas of DCIS separated by >1cm with germ line DNA from the same subject to measure allele frequencies of somatic mutations and copy number variation (CNV). Small amounts of FFPE derived DNA are made into NGS libraries for full exome sequencing and hybridization to a 4.2 million element SNP array. Comparison of allele frequencies and CNV is made between specimens from the same cancer to assess heterogeneity.

Results: We identified a series of pure DCIS (controls for this study) and generated high coverage sequencing data from 20ng of FFPE DNA from 12 samples (4 subjects, germline + 2 DCIS containing areas from each subject) as proof of principle. We compared the occurrence of deletions, insertions and SNP’s after a 20X coverage filtration. From these data, we identified between 50 to greater than 600 sequence changes in these DCIS compared to normal DNA. Present/absent calls and allele frequencies indicate both significant and variable degrees of heterogeneity even in these pure DCIS samples. Additional data is now being gathered and analyzed based on this established technical approach.

Conclusion: We have demonstrated the feasibility of acquiring high quality NGS data from archival DCIS specimens allowing us to test the hypothesis that genetic heterogeneity is a driving force in breast cancer progression. The degree and nature of this heterogeneity will be assessed in a panel of pure DCIS and DCIS co-existing with invasive and/or metastatic cancer. We are now generating and analyzing these data for Symposium presentation.
**Title:** Stratification of breast carcinomas with double-equivocal HER2 status

Marchio C, Trisolini E, Maletta F, Annaratone L, Scalzo MS, Mascali D, Verdun di Cantogno L, Medico E and Sapino A.
University of Turin, Turin, Italy; Pathology Unit, Azienda Ospedaliera Città Della Salute e Della Scienza di Torino, Turin, Italy and Candiolo Research Institute, University of Turin, Candiolo, Italy.

**Body:** BACKGROUND AND RATIONALE: Immunohistochemistry (IHC) and in situ hybridization (ISH) represent the cornerstone of HER2 evaluation in breast cancer (BC). We have demonstrated that up to 13% of BCs with equivocal HER2 expression (score 2+ in IHC) harbor an equivocal HER2 gene status (HER2/CEP17 <2 and mean HER2 copy number between 4 and 6), leading to the definition of the double-equivocal category. When we assessed HER2 gene levels in these cases by a PCR-based method we observed copy gain in 25%. When we tested HER2 protein levels by an in situ quantitative assay, HER2 levels ranged from those of score 0/ISH- to those observed in score 2+/ISH+ BCs. These data suggest that, rather than exploring alternative methods to assess HER2 status, a complementary functional approach may be beneficial. Our aim was to stratify double-equivocal BCs using global transcriptomics.

METHODS & RESULTS: We retrieved a series of 27 formalin fixed paraffin embedded double-equivocal BCs and two control groups matched for oestrogen receptor and histological grade: 22 HER2- (IHC score 0/ISH-) and 22 HER2+ (IHC score 3+/ISH+) BCs. RNA was extracted following microdissection to enrich for tumor cell content >80% and subjected to whole-Genome DASL (cDNA-mediated Annealing, Selection, extension and Ligation; Illumina).

We first identified genes with differential expression in HER2+ versus HER2- BCs based on T-test significance (p<0.01) and on mean gene expression variations higher than +/- 2-fold. To best characterize the signature we performed a cluster analysis with the GEDAS software using the "Fuzzy Self-organizing Maps" algorithm and the cosine distance to generate the clusters. The classifier (24 genes) tested on double-equivocal cases led to a separation in "HER2+ like" and "HER2- like" BCs. More in details, three main clusters emerged, showing a significantly different distribution of cases with distinct HER2 status (p<0.0001, Chi square). Cluster A included all HER2+ and 5 double-equivocal cases and was associated to high expression of genes that pertain to the HER2 amplicon. Cluster B, composed of 9 HER2- and 12 double-equivocal cases, showed low expression of HER2 and HER2 amplicon-related genes and high levels of expression of TPRG1, NOVA1, AGTR1, SEZ6L, MAPT, GSTM1, SORCS1, DSCR6, NPY1R genes. Cluster C, formed by 13 HER2- and 10 double-equivocal cases, showed low expression of HER2 and HER2 amplicon-related genes but a non-homogeneous expression of the other genes of the classifier.

The expression of best performing genes of the classifier (TPRG1, NOVA1, AGTR1) was investigated in The Cancer Genome Atlas (TCGA) dataset of breast cancer (Breast Invasive Carcinoma, TCGA provisional from cbioporal.org) and a striking mutually exclusive pattern with HER2 expression was observed.

CONCLUSION: By resolving HER2 double-equivocal BCs into HER2 likely-positive or likely-negative, this approach may pave the way to an informed therapeutic decision in this controversial category of patients.
Title: Long-range expression analysis reveals new luminal subgroups associated with different patient outcomes

Mankovich AR R and Dimitrova N. Philips Research, Briarcliff Manor, NY.

Body: Breast cancer subtyping using gene expression is well established in breast cancer research and gaining traction in the clinical setting. While it is known that there are large chromosomal regions affected by copy number polymorphisms, histone modifications, and other spanning alterations, it is not clear whether expression patterns regulate such regional changes. We present a method to integrate any type of expression data - here, we analyze mRNA, lincRNA, and mRNA and lincRNA together - and quantify long-range expression patterns affecting large regions of the genome.

TCGA alignment and gene expression RNA-Seq data for breast cancer were generated at the Carolina Center for Genome Sciences, UNC at Chapel Hill. We examined 715 samples which each had at least partial data for ER/PR/HER2 status and complete data for PAM50 subtype assignment. Our method defines long-range expression within a window of a particular length (e.g. 100 Kb, 1 Mb). We take the mean weighted expression values for all genes that fall within each window and concatenate these windows to obtain larger chromosome-wide patterns. The final chromosome-wide vectors are joined to represent long-range expression patterns across the whole genome. We retain the top 10% most varying windows. Then, we apply hierarchical clustering, perform survival analysis, and evaluate enrichment of clinically meaningful subtypes using hypergeometric test.

Hierarchical clustering across each analysis revealed clear separation of all PAM50-classified breast cancer subtypes at 1 Megabase resolution in the available data set. Interestingly, clustering of samples (n = 715) using 215 bins revealed distinct subgroups at each level of analysis - mRNA, lincRNA, and mRNA plus lincRNA. At these levels, three clusters contained significant enrichment for Her2-amplified (mRNA, p=1.5E-35; lincRNA, p=1.8E-26; mRNA + lincRNA, p=1.4E-33), Normal-like (mRNA, p=8.9E-82; lincRNA, p=1E-71; mRNA + lincRNA, p=1.6E-77), and Basal-like (mRNA, p=9.2E-67; lincRNA, p=6.9E-93; mRNA + lincRNA, p=8.2E-72) breast cancer. In view of the association of these mRNA clusters with PAM50 classifications, it is surprising that less than 10% of the genes in the analysis were overlapped (42 of the 465 intersected with 1734 genes in the original PAM50 study). The Luminal clusters exhibited a more diverse clustering pattern; however, the lincRNA and combined analyses were capable of delineating Luminal A from Luminal B and into several subclusters. These subclusters, interestingly, differed in overall survival, particularly amongst the Luminal B subgroups in the lincRNA analysis (about a 2 fold 5-year OS delta, p = 0.127).

Hierarchical clustering relying on long-range expression regions at 1 Megabase resolution produces clusters that are enriched with well-known clinically relevant subtypes. A surprising finding is the capability for this method to reveal existing PAM50 subtypes across non-coding, intergenic regions. Of special interest is the demarcation of Luminals into different survival profiles using this method. To date, this is the first study to our knowledge that attempts to analyze and reveal existing and novel breast cancer subtypes across large regions of the genome and in long intergenic non-coding regions.
Incidence and molecular phenotype of multifocal invasive breast carcinomas; A UK multi institutional series


Introduction: Historically, multiple synchronous breast cancers are defined as multifocal (MF) when they occur in the same quadrant of the breast, and multicentric (MC) they are in different quadrants; a number of authors continue to use this distinction. Multifocality has been reported to be an independent prognostic factor for survival and local recurrence. The molecular implications of MF and MC versus univocal breast cancers remain to be defined. We sought to investigate the incidence and molecular basis for this phenomenon.

Materials and methods: Following a systematic review of the literature, breast cancer excisions reported by three specialist centers between 2005 and 2014 were investigated (n=4409 cases). Within this cohort, cases identified radiologically and confirmed histologically as mutifocal/multicentric were identified. Data on age at presentation, histological features and molecular profile were collected and compared between MF/MC breast cancer and the unifocal disease. Chi square test was used to compare categorical groups for ER, PR, HER2 and student t-test for mean age comparison. A p-values of ≤ 0.05 was considered significant.

Results: 446 cases (10.12%) were reported as MF/MC invasive breast cancer. Most cases were treated by mastectomy. The majority of these were early breast cancer (Stage 1, 59.1% pT1(m), 72.2% pN0-1). Compared with unifocal breast cancer, patients with multifocal breast cancer were significantly younger (56.6 vs 59 years old, p=0.004) and more likely to have lymph node metastasis on presentation (50% vs 32%, p=0.001). There were differences, some significant, in the molecular profiles of unifocal cancers vs the largest focus of multifocals (ER positive: 80% vs 84% p=0.239, PR positive: 72% vs 76% p=0.301, HER2+ negative: 89% vs 76% p=0.002).

Discussion: A significant proportion (one tenth) of breast cancer presented as multifocal disease. Differences in the immunohistochemical profile, in particular HER2 status, between MF and unifocal breast carcinomas are identified. Both the literature review and analysis of our available cases demonstrated a paucity of data on the incidence, degree of intratumor heterogeneity of multifocal breast cancers and its appropriate management. There are important questions unanswered about the molecular classification of multifocal breast cancer. Future genomic testing of those cases may highlight more pronounced differences. The findings form basis of a biomarker driven trial in set up comparing conservative surgery and mastectomy (MIAMI).
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-05-07

Title: Improving personalized management of primary breast cancer: Mammaprint® risk stratification and blueprint® molecular subtyping


Body: Background:
Historically, breast cancer (BC) patients were offered cytotoxic or endocrine therapy based on factors such as tumor size, stage, and immunohistochemistry (IHC) markers for estrogen receptor (ER) and HER2-positivity. In 2010 the College of American Pathologists revised the breast cancer guidelines on endocrine therapy (ET) to include a lower threshold of ER positivity by immunohistochemistry, changing the definition from 10% to 1% [Hammond et al]. As a result, although a larger number of patients are offered ET, not all may benefit from this expanded definition of ER positivity if their disease is not truly estrogen driven. More recently, sensitive gene profiling assays, such as Blueprint®, can determine intrinsic molecular subtype which may be more sensitive in predicting which patients will benefit from ET. Additionally, Mammaprint® provides risk stratification which can aid in determining which patients could benefit from neoadjuvant therapy.

Methods
This is an observational analysis of 60 patients with stage I-IV BC. Tissue analysis for ER, PR and HER2 status were determined by IHC/FISH. mRNA expression profiles of 80 genes for Blueprint® (Agendia) analysis provided molecular subtyping: luminal, basal or her2. Moreover, Mammaprint® (Agendia) analysis of 70 genes subdivided patients into low risk or high risk providing further stratification for Luminal-type.

Results
By IHC staining, 48% of patients were ER+/HER2-, 10% were ER+/HER2+, 8.3% were ER-/HER2+, and the remaining patients (20%) were triple negative (TN) BC. By comparison, molecular profiling classified 21% as luminal A, 18% luminal B, 11.6% Her2 and 35% basal subtype. The 35 ER+ patients were heterogeneous by subtype: 13 were classified as molecular luminal A, 16 were luminal B, 4 were reclassified as HER2 and 2 were basal-like (one of whom had 40% ER positivity). Of the ER+ patients whose IHC quantitative staining was known, 29% with low positivity (less than 10%) were reclassified as basal subtype. Of the 5 patients who are ER+/HER2+, 2 were luminal B and 3 were of the HER2-subtype. Two patients who were TN were reclassified as luminal B, and an ER-/HER2+ was classified as a basal subtype. One patient with ER+/HER2- disease had evidence of both HER2 and luminal B subtype. Of the patients who received neo-adjuvant therapy, pCR was obtained in 33% of luminal, 60% of HER2 and 50% of basal-type patients.

Conclusions
Blueprint® and Mammaprint® Molecular profiling are useful diagnostic tools which further characterize tumors to predict risk of recurrence and response to treatment. About one third of ER+ patients with low positivity (less than 10%) were reclassified as basal subtype, suggesting that there is a proportion of patients who are exposed to the morbidity of hormonal therapy with little therapeutic benefit. Additionally, the test is predictive of pCR, with the highest rates in the basal and Her2 subtypes, thus enabling clinicians to predict and improve clinical outcomes through more personalized treatment decisions.
**Title:** Integrating whole exome sequencing data with RNAseq and quantitative proteomics to better inform clinical treatment decisions in patients with metastatic triple negative breast cancer


**Body:** Background: The use of next-generation sequencing has significantly advanced personalized medicine for patients (pts) with breast cancer. Despite this technological advancement, there remains the challenge of understanding how and if tumor heterogeneity can confound molecular analysis and treatment decisions. It has been shown that the expression of ER, PR, and HER2 can vary widely within different areas of the same tumor and between matched primary and metastatic lesions. The "Intensive Trial of OMics in Cancer"-001 (ITOMIC-001; NCT01957514) enrolls pts with metastatic TNBC who are platinum-naive and scheduled to receive cisplatin. Multiple biopsies of up to 7 metastatic sites are performed prior to cisplatin and repeated upon completion of cisplatin and following subsequent therapies. A subset of specimens is chosen for DNA sequencing, RNA sequencing, and quantitative proteomics. We explored the discordance of genomic and proteomic alterations for intrapatient and temporal heterogeneity in pts with TNBC, and the potential benefit of panomic analysis to better inform treatment decisions.

Methods: Between 7 and 107 tumor samples/biopsy specimens were obtained from each pt from 1-23 different time points. Blood samples were collected for matched tumor-normal genomic analysis. DNA sequencing data were processed using Contraster; RNASeq data confirmed the presence of gene mutations and was used to identify mutational and transcript abundance. PARADIGM was used to determine associations between gene mutations and signaling pathways. Selected reaction monitoring-mass spectrometry (SRM-MS) was used for proteomics analysis.

Results: Almost all pts had loss of TP53 (common in TNBC), and 5 pts had germline BRCA1/2 events, some exhibiting a signature of mutations corresponding to a mismatch repair defect in ≥1 pt. FGFR1/2/3 mutations/amplifications occurred in 5 pts. Three of 12 pts (25%) achieved partial responses after receiving treatments (post cisplatin) based on the molecular profile of their tumor: 1 pt with two FGFR2 activating mutations treated with ponatinib, 1 with a germline BRCA2 mutation treated with veliparib, and 1 with highly expressed Gpnmb treated with an antibody drug conjugate against Gpnmb. Tumor samples showed increased mutational and rearrangement burdens over time but shared mutational characteristics that were unique to each pt. Through the shared alterations across time points for 3 pts, it was possible to reconstruct the clonal history and heterogeneity of the tumors as various therapeutic approaches were attempted.

Conclusions: Here we show in TNBC, intrapatient and temporal heterogeneity that may lead to a lack of response to identified targeted therapies. Tumor samples taken over time from the same pt become enriched for more complex genomic structures post therapy but share mutational characteristics, indicating the presence of recurrent tumor populations. This study enabled us to reconstruct the clonal history and heterogeneity of tumors across space (metastatic vs primary at t=0) and time, illustrating the need for comprehensive molecular analysis and combination/multi-targeted therapeutics for optimal treatment in TNBC.
Subtyping of triple negative breast cancer by a novel immunohistochemistry panel: Assessing the correlation between subtypes and clinical outcomes

National University of Ireland, Galway, Ireland and Galway University Hospital, Galway, Ireland.

**Body:** Background:
Triple negative breast cancer (TNBC) is characterised by a lack of expression of oestrogen receptor and progesterone receptor and lack of HER2 overexpression. Using gene expression profiling, TNBC can be subtyped into six subtypes: BL1,BL2,IM,M,MSL,LAR. However, gene profiling is not yet routine in clinical practice. Also, despite the heterogeneity of TNBC, standard of care remains combination chemotherapy without targeted therapy. It is necessary to identify TNBC subtypes with differing responses to chemotherapy. In chemoresistant TNBCs, identifying alternative therapeutic targets will facilitate improved treatment strategies and outcomes.

**Aims:**
1. To develop and validate an IHC panel to facilitate subtyping of TNBC
2. To determine the prognostic impact of TNBC subtypes by assessing clinical features, RFS and OS rates
3. To determine the predictive impact of TNBC subtypes by assessing the sensitivity of TNBC subtypes to differing chemotherapy regimens
4. Ultimately to identify new molecular targets to individualise treatment strategies for TNBC

**Methods:**
In order to identify TNBC subtypes on FFPE tissue, an 8-protein IHC protein panel has been developed based on genes enriched in the 6 TNBC subtypes.

**IHC Protein Panel**

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<td>MMP2</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>RAD21</td>
<td>++</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IL2R</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+/-</td>
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</tr>
</tbody>
</table>

A tissue microarray has been constructed of 301 TNBCs diagnosed from 1999–2014. To date, 196 cases have been stained and scored for androgen receptor (AR) and 199 cases for Bcl2. A database has been constructed incorporating clinical data with pathological and outcome data.

**Results:**

<table>
<thead>
<tr>
<th>n=301</th>
<th>AR+</th>
<th>Bcl2</th>
<th>Ki67 &gt;10%</th>
<th>p53+</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>301</td>
<td>20</td>
<td>114</td>
<td>121</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
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<td>57</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>116</td>
<td>61</td>
</tr>
</tbody>
</table>
On initial observation, AR+ TNBCs were diagnosed at an older age than other TNBCs (65 v 55), were less likely to have BRCA mutations, more likely to be metastatic at diagnosis and were associated with longer median OS (44.5 mos). 21% of AR+ cases were Bcl2+. 45% of AR positive cases had Ki67 <10%.

Bcl2+ TNBCs had low rates of metastatic disease at diagnosis and had the longest median time to progression (34 mos). TNBCs with high Ki67 had the shortest median overall survival (31.5 mos).

We propose that at the time of presentation, the entire IHC panel will be stained and scored. Statistical analysis will assess the association of TNBC subtypes on clinicopathological features as well as the impact of subtype on chemotherapy response. Final analysis will also include duration of treatment response, DFS and OS. Our aim is that this study will prognostically and predictively subtype TNBCs so that clinical decision making, therapeutic strategies and patient outcomes can be improved.

<table>
<thead>
<tr>
<th>Family History</th>
<th>Median 35-40%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Age</strong></td>
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</tr>
<tr>
<td><strong>Range</strong></td>
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<table>
<thead>
<tr>
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<tr>
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<td>11 - 2 3 2</td>
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<tr>
<td>BRCA2</td>
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</tr>
<tr>
<td>NACT</td>
<td>61 1 14 13 11</td>
</tr>
<tr>
<td>CR</td>
<td>22 - 1 1 -</td>
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<tr>
<td>PR</td>
<td>33 1 11 11 8</td>
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<td>SD</td>
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<td>POD</td>
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<table>
<thead>
<tr>
<th>Mets at Dx</th>
<th>Median 35-40%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>9 2 2 2 2</td>
</tr>
<tr>
<td><strong>%</strong></td>
<td>3 10 1.75 1.65 1.75</td>
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</table>

<table>
<thead>
<tr>
<th>Recurrence</th>
<th>Median 35-40%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
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</tr>
<tr>
<td><strong>%</strong></td>
<td>22 20 24 22 24</td>
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<tr>
<td>Median TTP</td>
<td>20.5 30.5 34 26 25</td>
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<tr>
<td>Range</td>
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</tr>
<tr>
<td>Median OS</td>
<td>39 44.5 34 26 25</td>
</tr>
<tr>
<td>Range</td>
<td>1-326 1-136 2-326 1-326 1-163</td>
</tr>
</tbody>
</table>
Purpose
The clinical significance of progesterone receptor (PgR) expression in estrogen receptor (ER)-negative breast cancer is controversial. Here we systemically investigated the clinicopathologic features, molecular essence, and endocrine responsiveness of ER-/PgR+/HER2- phenotype.

Methods
Four study cohorts were included. The first and second cohorts were from the Surveillance, Epidemiology, and End Results database (n=67,932) and Fudan University Shanghai Cancer Center (n=2,338), respectively, for clinicopathologic and survival analysis. The third and fourth cohorts were from two independent publicly available microarray datasets including 837 operable cases and 483 cases undergoing neoadjuvant chemotherapy, respectively, for clinicopathologic and gene-expression analysis. Characterized genes defining subgroups within the ER-/PgR+/HER2- phenotype were determined and further validated.

Results
Clinicopathologic features and survival outcomes of the ER-/PgR+ phenotype fell in between the ER+/PgR+ and ER-/PgR- phenotypes but were more similar to ER-/PgR-. Among the ER-/PgR+ phenotype, 30% (95% confidence interval [CI] 18%-44%) were luminal-like and 58% (95% CI 44%-72%) were basal-like. We further refined the characterized genes for subtypes within the ER-/PgR+ phenotype and developed an immunohistochemistry-based method that could determine the molecular essence of ER-/PgR+ using three markers, TFF1, CK5, and EGFR. Either PAM50-defined or immunohistochemistry-defined basal-like ER-/PgR+ cases have lower endocrine therapy sensitivity score compared with luminal-like ER-/PgR+ cases (Mann-Whitney test P-values<0.0001). Further analysis showed that basal-like ER-/PgR+/HER2- cases had as low endocrine therapy sensitivity scores as those in triple-negative cases (Mann-Whitney test P=0.80). Immunohistochemistry-defined basal-like ER-/PgR+ cases might not benefit from adjuvant endocrine therapy (log-rank P=0.61 for sufficient versus insufficient endocrine therapy).

Conclusions
The majority of ER-/PgR+/HER2- phenotype are basal-like and associated with lower endocrine therapy sensitivity score. Measurement of immunohistochemical TFF1, KRT5, and EGFR helps to identify subgroups within the ER-/PgR+/HER2- phenotype, and the basal-like subgroup may reduce the use of ineffective endocrine therapy. Additional studies need to validate our findings.
Title: DEPArray™ enables recovery of pure tumor cells from heterogeneous fine needle aspirates for routine downstream NGS analysis


Body: Introduction: We have previously shown reliability in isolating pure populations of cells from complex tissues using the DEPArray™. Fine Needle Aspiration (FNA) is a quick and simple procedure often performed to make a diagnosis or rule out conditions such as cancer. Although FNA is also used to assess response to treatment, the procedure is often deemed insufficient in yield and purity of tumor cells. Here we provide preliminary results showing 100% efficiency in recovering pure tumor cell populations from FNA samples of patients affected by Metastatic Breast Cancer and known to have low tumor burden (<20%) prior to using the DEPArray™ platform.

Method: FNA paraffin embedded sections (50 microns thickness) from metastases originating from breast (n=3) primary tumors were evaluated. Each FFPE curl was processed to yield single cells followed by DEPArray™ sorting based on cytokeratin (Ker), vimentin (Vim) and nuclear staining. The recovered cell populations were directly lysed in the collection tube prior to PCR-based target enrichment for next generation sequencing using Ion AmpliSeq™ CHPv2.

Results: DEPArray™ analysis allowed identification of 3 well separated cell populations, including tumor (Ker+/Vim-), stromal (Vim+/Ker) and putative EMT (Ker+/Vim+) cells. Overall, only 21% (4.3% to 42.7% range) of the total (mean of 6335) cells analyzed were of tumor (KER+/Vim-) origin. Groups of pure cells (mean 105 cells, range 15-200) for each population were recovered for sequence analysis. In one breast cancer FNA sample, we observed TP53 LoH but only in the recovered tumor (KER+) cells and not in the unsorted, stromal (VIM+), or EMT (KER+/VIM+) populations. In addition, a PIK3CA missense somatic heterozygous variant was identified in both the tumor and putative EMT populations but not in stromal cells, confirming this as a somatic mutation.

Conclusion: DEPArray™ allows resolution of two main limitations associated with FNA samples obtained for genomic analysis: too few target cells and unwanted admixture of normal cells. DEPArray™ allows for phenotypic distinction between the sorted cells prior to recovery; thus, enabling sequence analysis that is suitable for detecting genomic aberrations such as CNVs and LoH, which cannot be evaluated as precisely in an unsorted sample. Clearly, the DEPArray™ platform brings precision to detection, quantification and recovery of pure target cells that are suitable for subsequent downstream molecular analysis that can improve cancer diagnosis and personalized treatment strategies for breast cancer patients.

Yao R, Pan B, Sun Q, Zhou Y, Mao F, Lin Y, Guan J, Wang X, Zhang Y, Zhang X, Shen S, Zhong Y, Xu Y, Shi J, Zhu Q, Cai F and Liang Z.  Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China;  Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China;  Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China and  Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

Background: The heterogeneous nature of the mucinous breast cancer (MBC), with its subtypes of pure (PMBC) and mixed carcinoma (MMBC), calls for more precise individualized prognosis assessment. PMBC showed favorable prognosis in both Chinese and Caucasian women, with nodal status and TNM stage as the prognostic predictors [PMID: 18026874, 22451233]. However, few studies had investigated tumor biology and prognosis of MMBC in Chinese population, especially with respect to the different co-existing cancer components.

Methods: From January 1983 to December 2014, 197 consecutive MBC patients, including 117 PMBC and 80 MMBC, received breast cancer surgery in Peking Union Medical College Hospital. The clinicopathological characteristics, treatment choice, disease-free survival (DFS) and overall survival (OS) were compared both between PMBC vs MMBC, and among subgroups of MMBC according to the mixed entities, including 24 women with ductal carcinoma in situ (DCIS) and 45 with IDC. Univariate and Cox multivariate analyses were performed to identify the prognostic factors.

Results: The 197 MBC comprised 1.9% of contemporary 10,192 breast cancer (BC). Compared to PMBC, MMBC had significantly more lymph node metastasis (p=0.038), Her2 positivity (p=0.036), high Ki-67 index (defined as >20%, p=0.026) and anti-Her2 targeted therapy (p=0.006). All these differences remained significant when the comparison were performed among PMBC, MBC+DCIS and MBC+IDC, and additional significant difference were identified in tumor size (p=0.036), pTNM stage (p=0.003) and chemotherapy (p=0.003). However, no significant difference was found in DFS or OS between any two subtypes/subgroups of MBC, including PMBC, MMBC, MBC+DCIS and MBC+IDC. [Table 1]

<table>
<thead>
<tr>
<th>Survival</th>
<th>PMBC (N=117, Median, range, and Mean±SD)</th>
<th>MBC+DCIS (N=24, Median, range, and Mean±SD)</th>
<th>MBC+IDC (N=45, Median, range, and Mean±SD)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS (months)</td>
<td>43 (1-233), 52.7±45.2</td>
<td>27 (1-84), 34.3±25.3</td>
<td>26 (1-113), 33.1±26.6</td>
<td>0.187</td>
</tr>
<tr>
<td>OS (months)</td>
<td>46 (1-312), 56.9±51.8</td>
<td>27 (1-84), 34.4±25.3</td>
<td>26 (1-113), 34.8±28.7</td>
<td>0.628</td>
</tr>
</tbody>
</table>

§ Kaplan-Meier survival curves would be displayed in the poster

High Ki-67 index (p=0.046) appeared to be the significant DFS related prognostic factor for PMBC, whereas estrogen receptor (ER) status (univariate p=0.000, multivariate p=0.062) and immunophenotype (luminal, her2, or triple-negative, univariate p=0.000, multivariate p=0.079) might be the potential DFS predictors for MMBC. None of the above-mentioned clinicopathological factors could serve as OS predictors for MBC.

Conclusion: This population-based study showed that there were significant difference in nodal status, Ki-67, Her2 positivity and targeted therapy between PMBC and MMBC, and furthermore in tumor size, stage and chemotherapy among PMBC and subgroups of MMBC such as MBC+DCIS and MBC+IDC. However, survival outcomes were similar between these clinical entities and subgroups, suggesting the intra-tumoral heterogeneity might not interfere with survival outcomes of MBC in Chinese woman. High Ki-67 index was identified as the significant DFS related prognostic factor for PMBC, whereas ER status and immunophenotype as the potential DFS predictors for MMBC.
Title: The changing paradigm of hereditary cancer testing: Comparison of tests in 497 women with breast cancer evaluated at an NCI designated cancer center


Body: The Hereditary Cancer Program at Vanderbilt-Ingram Cancer Center (VICC) was established in mid-2012 and provides cancer genetic services to patients and family members who are at risk for family cancer syndromes. During this time, the testing paradigm has markedly shifted from testing a small number of genes to a larger multi-gene set.

HYPOTHESIS AND METHODS:
We hypothesized that multi-gene testing would identify a higher rate of pathogenic mutations in breast cancer patients than the standard BRCA1/2 testing paradigm. To test this notion, we examined the records of 641 women with breast cancer seen in our clinic from July 2012 through Dec 2014 and tabulated the test outcome in women tested for BRCA1/2 only and women who had multi-gene panels. Patient characteristics were compared between the two groups.

RESULTS: Excluding 17 (3%) women with a known familial mutation and the 127 (20%) women who did not proceed with testing, 497 women had usual Sanger BRCA1/2 (189; 38%) or a multi-gene NGS testing (308; 62%). 40 (13%) women were found to have a pathogenic mutation using the multi-gene panel compared to 13 (6%) women who had a restricted BRCA1/2 sequencing (P=0.035, Fishers Exact Test). The 13 women with Sanger BRCA1/2 were younger at diagnosis (40.6 vs 48 yrs) and more likely to have triple negative (TN) disease (38% vs 18%) compared to the 40 women diagnosed with multi-gene panels. TN disease was not confined to BRCA1 carriers, however, as 3 of 7 TN patients had a mutation in BRCA2, PALB2, and ATM, respectively. Thus, 29% (2/7) of our triple negative patients would not have been identified without multi-gene panels. In addition to BRCA1 (6; 15%) and BRCA2 (5; 12.5%), 6 women had mutations in ATM (15%), 7 in CHEK2 (17.5%), 6 in MUTYH (15%), 4 in PALB2 (10%), 2 in TP53 (5%), and 1 each in FANCC, PMS2, RAD51D and XRCC2 (2.5% each). 105 patients (35%) who did not have a deleterious mutation on a multi-gene panel were found to have one or more variants of uncertain significance (VUS) compared to 4 patients who underwent BRCA1/2 testing alone (4/189; 2%). There was a significant difference between providers when ordering hereditary cancer testing, with MD or NP ordering panel testing at a greater rate compared to Genetic Counselors (72% vs 53%; P< 0.0001).

CONCLUSION: We have examined the outcomes of genetic tests for 497 women with breast cancer during a time of great change in the approach to testing. Our study supports the paradigm that multi-gene panels will identify additional pathogenic mutations in genes other than BRCA1/2, which could increase clinical efficiency and improve patient outcomes. However, our study also found a high VUS rate in this group of patients, which will require additional clinical time to track for potential changes in pathogenicity. Future studies will focus on potential differences in management for patients found to have alterations on multi-gene tests.
**Title:** Germline CDH1 mutations in lobular carcinoma in situ

Reyes SA A, Sakr RA A, Schizas M, Towers R, Park AY Y, Ng CKY KY, Weigelt B, Reis-Filho JS S and King TA A. Memorial Sloan-Kettering Cancer Center, NY, NY and Memorial Sloan-Kettering Cancer Center, NY, NY.

**BACKGROUND:** Germline CDH1 mutations are responsible for the increased risk of both gastric cancer and invasive lobular breast cancer (ILC) in families with hereditary diffuse gastric cancer syndrome; yet germline CDH1 mutations in women with ILC without a family history (FH) of gastric cancer are rare. Lobular carcinoma in situ (LCIS) is both a risk factor and non-obligate precursor of ILC and recent data suggest that germline CDH1 mutations may be present in up to 8% of patients with bilateral LCIS +/- ILC; raising questions about the role of genetic testing in this context. The purpose of this study was to determine the frequency of germline CDH1 mutations in a large prospectively followed cohort of patients with pathologically confirmed bilateral LCIS.

**METHODS:** Patients with a biopsy proven history of LCIS, entering surveillance or presenting for surgery (prophylactic or therapeutic mastectomy), between 2005 and 2013 were prospectively identified and enrolled in IRB approved protocols at Memorial Sloan-Kettering Cancer Center for the collection of tissue and/or germline DNA (IRB 01-135, 99-030). All biopsies were reviewed to confirm LCIS and mastectomy specimens were subject to extensive sampling of all quadrants. Cases with confirmed bilateral LCIS were chosen for the primary analysis. Cases where bilateral mastectomy tissue sampling confirmed only unilateral LCIS were included for comparison. Germline DNA was anonymized and analyzed for CDH1 mutations using targeted capture sequencing with baits for all exons of CDH1 on HiSeq2000. Germline single nucleotide variants were called using GATK HaplotypeCaller and insertions/deletions by Varscan and Scalpel. Mutations were manually inspected using the Integrative Genomics Viewer (IGV). Clinical data were abstracted prior to anonymization.

**RESULTS:** Germline DNA was available for 114 patients; 78 underwent bilateral mastectomy for breast cancer (BC), 8 chose prophylactic mastectomy and 28 patients with biopsy proven bilateral LCIS were identified in surveillance. Following mastectomy, tissue sampling confirmed bilateral LCIS in 67/86 (78%) patients, and ruled out bilateral LCIS in 19 patients; yielding 95 patients with bilateral LCIS for the primary analysis. Median age at LCIS diagnosis for bilateral and unilateral cases respectively was 48yrs (range 36-70) and 44 yrs (range 38-63). One patient with bilateral LCIS also reported a FH of gastric cancer. Pathogenic germline CDH1 mutations (D72N (missense) and E35* (nonsense)) were identified in 2/95 (2%) patients with bilateral LCIS, one of whom also had invasive breast cancer (ILC). A germline CDH1 mutation was not identified in the patient with bilateral LCIS and a FH of gastric cancer, nor were CDH1 mutations identified among the 19 patients with unilateral LCIS.

**CONCLUSIONS:** In this cohort of 95 patients with pathologically documented bilateral LCIS +/- BC, the overall frequency of CDH1 germline mutations was 2%; considerably lower than previously reported. To our knowledge this is the largest series to address this question and these findings do not support germline testing for CDH1 mutations in women with bilateral LCIS.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-06-03

Title: Novel hereditary breast cancer gene mutations: Should there be greater concern regarding ovarian cancer risk?

Nassikas N, Wilbur JS S, Laprise JK K and Legare RD D. Women and Infants' Hospital - The Breast Health Center, Providence, RI.

Body: INTRODUCTION: As the use of multi-gene breast cancer panel testing increases the phenotype of the included genes continues to evolve. PALB2 and NBN have a well characterized association with breast cancer and are included on many breast cancer gene panels. Germline PALB2 and NBN mutations have been identified in a small percentage of ovarian carcinoma cases with one study reporting a non-significant twofold increase in carriers of the PALB2 mutations amongst ovarian cancer patients (P=0.4). Similar to BRCA1 and BRCA2 both genes are members of the Fanconi Anemia pathway therefore a potential increased risk for both breast and ovarian cancer could be anticipated. However at present overall the current literature on the association with ovarian cancer is sparse. Here we present the cases of three hereditary breast and ovarian cancer families found to carry pathogenic mutations within the PALB2 and NBN genes. In all three families the proband was diagnosed with ovarian or fallopian tube cancer and carries a pathogenic PALB2 or NBN mutation. Our PALB2 family carries the well-known French Canadian founder mutation, c.2323C>T, and includes the proband who was diagnosed with a stage II-C, grade 3, fallopian tube carcinoma at age 58, her heterozygote sister with ovary cancer at 68 and her mother with ovary cancer at 43 who was not able to be tested. This PALB2 proband has 5 sisters, 7 brothers and 41 nieces and nephews with only one sister diagnosed with breast cancer at 69 who also is PALB2 positive and a maternal grandmother with breast cancer at 48 who was unable to be tested. The NBN Slavic founder mutation, c.657_661del, was discovered in a 66 year old woman with stage IV, high-grade serous ovarian cancer having a mother with breast cancer at 91 and a maternal aunt with ovary cancer at 59 who have not yet been tested. Lastly, our patient from Laos with a diagnosis of a stage II-C endometrioid adenocarcinoma of the ovary diagnosed at 38 years old was found to carry the deleterious NBN mutation, c.1550dupA. She is not aware of any cancer family history and reports a large family including five brothers, four sisters and multiple aunts and uncles on both side of the family. CONCLUSION: These case studies suggest a link between PALB2 and NBN, two known breast cancer susceptibility genes, and hereditary ovarian cancer risk. These observations suggest that further data are needed to accurately evaluate ovarian cancer risk within novel hereditary breast cancer genes now commonly tested as part of multiplex panel analysis.
Title: PALB2 mutations in breast cancer patients of multiple ethnic region in northwest China

Li YT, Zhang MS, Wang XW and Ou JH. Cancer Hospital, Xinjiang Medical University, Urumqi, Xinjiang, China.

Body: Background: Germline mutations in PALB2 gene make a small contribution to the heritable breast cancer susceptibility, a new report about women with mutations in the PALB2 gene were more than nine times as likely to develop breast cancer. The aim of this study is to understand the status of PALB2 mutations among patients with hereditary predisposition to breast cancer in multiple-ethnic regions of China.

Methods: 112 patients with hereditary predisposition to breast cancer were enrolled in Xinjiang region of China. we sequenced the coding sequences and flanking intronic regions of PALB2 gene from all subject's genomic DNA samples by direct sequencing.

Results: A total of 3 deleterious mutations were identified in 112 breast cancer patients. The prevalence of PALB2 germline mutation was about 2.7% (3/112) in Xinjiang region of China. Except the three deleterious mutations, we identified seven missense variants in 10 patients, by using the prediction Softwares SIFT and PolyPhen, four missense variants might be disease associated in 5 patients. 2 of the 3 patients with deleterious mutation and 2 of the 5 patients presented putative deleterious missense mutations were triple negative breast cancer.

Conclusion: PALB2 mutations account for a small, but not negligible, proportion of patients with hereditary predisposition to breast cancer in Xinjing region of China, and PALB2 mutation might be associated with triple negative breast cancer.
Title: Breast cancer risk with mutations in PALB2 in Mexico

Sanchez-Forgach ER R, Sanchez-Basurto C, Alvarado D, Astudillo H and Perez ME E. Mastologica Lomas. Mexico City Breast Center, Mexico City, Mexico and Hospital Angeles Mexico, Mexico City, Mexico.

Body: PALB2 (Partner and Localizer of BRCA2), was originally identified as a BRCA2 interacting protein that is crucial for key BRCA2 genome caretaker functions; it was later shown to interact with BRCA1 as well, it encodes a protein that may function in tumor suppression, and binds to and colocalizes with the breast cancer 2 early onset protein (BRCA2) in nuclear foci which allows intranuclear localization and accumulation of BRCA2. The PALB2 gene is located on the short (p) arm of chromosome 16 at position 12.2, from base pairs 23-603-161 to 23-641-356.

Biallelic germline loss-of-function mutations in PLAB2 (also known as FANCN) cause Fanconi’s anemia, in which monoallelic loss-of-function mutations are associated with an increased risk of breast and pancreatitis cancer. Previous studies of familial breast cancer have yielded estimated risk in association with loss-of-function mutations in PALB2 that are two to four times as high as the risk among non-mutations carriers.

PALB2 loss-of-function mutations have now been observed in persons from many countries and are found in 0.6 to 3.9% of families with history of breast cancer, depending on the population.

To obtain more precise estimation of cancer risk associated with loss-of-function mutations in PALB2 in our center, we collected data on mutation carriers and their relatives.

The goal of our study was to present the first identifying PALB2 mutations in Mexico.

Our results included three patients with PALB2 mutations, two of whom with breast cancer, but it is important to specify that all of them are relatives.

As a clinical genetic testing for breast cancer evaluating increasing risks, it includes other genes in addition to BRCA1 and BRCA2, because it is important to have robust risk estimates for women who carry loss-of-function mutations in genes such as PALB2.

The results suggest that no single set of penetrance estimates applies to all PALB2 mutation carriers; the risk of breast cancer associated with PALB2 will also depend on genotypes at other modifying loci or on other familial factors.

Loss-of-function mutations in PALB2 are an important cause of hereditary breast cancer, with respect of these factors: the frequency of cancer-predisposing mutations and the risk associated with them.

It is important to conduct studies to meet patients carrying mutations in PALB2, so it is important to monitor not only the patients but their families, in the process of getting to know the risk for developing cancer and improve the prognosis for the future.

We have a few cases, however, we are the first center that works to identify mutations PALB2 in Mexico and continue our work in the process of improving the quality of life of our patients and future generations.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-06-06

Title: Comprehensive analysis of BRCA (BRCAm) and other germline mutations (GRm) with a clinicopathological association in breast cancer: An Indian study


Body: Background: BRCAm and other GRm testing using next generation sequencing (NGS) in early diagnosed and/or metastatic breast cancer (BC) helps in the identification of both unambiguously defined deleterious mutations and sequence variants of unknown clinical significance (VUS). The early detection of these mutations in the proband and the family members help in risk stratification and instituting effective monitoring, surveillance and disease management strategies.

Methods: Out of total 200 patients diagnosed with BC (April 2013-15) 77 unrelated individuals were consented to be profiled by NGS on MiSeq platform using TruSight Cancer panel (consisting of 94 genes including 13 genes highly associated with risk of inherited breast and/or ovarian cancer) in an IRB-approved prospective study in a CLIA compliant laboratory. Paired end sequencing was done with an average coverage of > 450X. Data was processed using STRAND software and interpreted using "Strand Omics" platform. The paired tumor samples were analysed for pathological stage, histological type and hormonal status. Results: GRm were detected in 61 cases (79%). Among all mutations detected, BRCA1/2 were found in 51% (31% in BRCA1, 20% in BRCA2) of cases. BRCA1 was found to be co-mutated with BRCA2 in 2 cases. Out of 37 deleterious mutations in BRCA1/2 genes only 10 were reported to be pathogenic (6 in BRCA1 and 4 in BRCA2) and rest were VUS. Mutation frequencies were higher among high grade IDC with HER2-ve tumors including TNBC (53%, p<0.05) with an early onset of the disease. TNBC with BRCAm were found to have no/incomplete pCR on conventional TAC regimen, subsequently started with platinum therapy and the outcome being monitored. Interestingly, 4 BRCA1 mutations including 3 non-sense and 1 frameshift mutation were found in two unrelated individuals suggesting them to be founder mutations in Indian population. The other GRm frequency (alone/ co-mutated with BRCA) was also found to be significantly high (49%) and include BRIP1, CHEK2, ERCC2, CDH1, SDHB, APC, MSH6, TP53, PALB2 and RAD51C. Stratification based on age of diagnosis(dx) showed a detection rate significantly higher in the age group of 25-50 yrs (74%) as compared to the 50-75 yrs (26%). Also a strong association of GRm status with the family history(Hx) of BC in 1st or 2nd degree relatives was indicated.

<table>
<thead>
<tr>
<th>Gene</th>
<th>n</th>
<th>%</th>
<th>Age at dx (yrs)</th>
<th>Family Hx (Yes=Y, No=N, Unknown=UK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>19</td>
<td>31</td>
<td>25-50 (n=23) 50-75(n=8)</td>
<td>Y(n=13) N(n=3) UK(n=3)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>12</td>
<td>20</td>
<td>25-50(n=21) 50-75(n=9)</td>
<td>Y(n=8) N(n=2) UK(n=2)</td>
</tr>
<tr>
<td>PALB2</td>
<td>1</td>
<td>1.7</td>
<td>&gt;50</td>
<td>Y</td>
</tr>
<tr>
<td>CHEK2</td>
<td>5</td>
<td>8.8</td>
<td>25-50 (n=4) 50-75(n=1)</td>
<td>Y</td>
</tr>
<tr>
<td>ATM</td>
<td>6</td>
<td>10.5</td>
<td>25-50 (n=4) 50-75(n=2)</td>
<td>Y(n=5) N(n=1)</td>
</tr>
<tr>
<td>RAD51C</td>
<td>1</td>
<td>1.7</td>
<td>&lt;50</td>
<td>Y</td>
</tr>
</tbody>
</table>

Table 1: Correlation of GRm with Dx and Hx

Conclusions: Our study in a small cohort clearly highlighted the significance of germline testing and classifying the variant in larger cohort of BC patients with a strong family Hx of cancer particularly in BRCA1/2 positive families, and in women <50yrs for early detection and risk assessment. The study also indicates BRCAm to be an important contributor to the etiology of high grade HER2-/TNBC in Indian patients. Expanded testing of this subtype is warranted to impact management of the disease with PARP inhibitors and/or platinum therapy.
Title: Multigene profiling to identify clinically relevant actionable mutations in breast cancer: An Indian study


Body: Background: Numerous chemotherapeutic agents are available against breast cancer (BC), but a vast majority of patients diagnosed with this disease still develop treatment resistance and eventually succumb to disease. It remains an unmet need to identify specific molecular defects against which targeted therapy are available for improving clinical outcomes in BC. Our study aims to identify frequent hotspot mutations in BCs and determine their clinical impact.

Methods: 200 women with BC (early diagnosed and/or metastatic) aged 26-75 yrs (median age 50.5yrs) diagnosed at HCG from April 2013-15 were consented to be profiled by targeted deep sequencing for hotspot mutations in 48 cancer-related genes using Illumina’s TSCAP panel and MiSeq technology in an IRB-approved prospective study in a CLIA compliant laboratory. All the cases had pathology review for stage, histological type, hormonal status and Ki67. The average coverage across 220 hot spots was greater than 1000X. Data was processed using Strand Avadis NGS™. Mutations identified in the tumor were assessed for 'actionability' i.e. response to therapy and impact on prognosis. Results: Somatic variants were detected in 75% of cases with direct impact on therapy or prognosis. Genetic aberrations were identified in PI3K/AKT/ mTOR signalling pathway in substantial fraction (27%) of breast cancer cases, out of which 17% had PIK3CA activating mutations, 13 and 5 cases had PTEN and AKT deletions or truncating mutations respectively. Aberration in this pathway was more prevalent in HR+ve (53%) and HER2-ve including TNBC (61%) than in HR+/HER2+ve tumors (10.6%) of IDC histology. However, no correlation was found with stage and Ki67 index of the tumor. Notably 80% of BC cases presented with liver metastasis at the time of diagnosis were detected with PIK3CA mutation indicating its role as a surrogate marker of organ specific metastasis. PIK3CA was found to be co mutated with p53 in 16 cases (9%) of which 4 cases showed npCR post NACT. Also disruptive and non-disruptive mutations in TP53 alone were found in 25% of BC, varying widely among different histologies. A follow up of few cases showed shorter PFS and poor outcome in resected BC treated with NACT indicating its robust prognostic value in NACT setting. Furthermore, two patients were detected with cKIT mutations indicating sensitivity to imatinib and therefore enrolled on a clinical trial. The other variants were found in RB1(n=8),Her2 (n=2),FGFR amplification(n=1), KRAS(n=2),NRAS(n=3)CDH1(n=1),FBXW7(n=2) and EGFR(n=1).All these variants detected indicated resistance to conventional therapy and suggested sensitivity to available targeted therapy, either approved or in clinical trials. The response and outcome are being monitored in about 20 (10%) patients who have been enrolled in clinical trials and receiving mutation specific targeted therapy. Conclusions: This study confirms the utility of multigene profiling in early diagnosed and advanced BC patients, to stratify them on their molecular profile who could potentially benefit from targeted therapy. Prospective studies and randomized clinical trials are ongoing to confirm the independent prognostic and therapeutic value of the mutations in a larger cohort of Indian population.
Title: Targeted next generation sequencing of advanced breast cancers identifies potentially actionable alterations and variants of standard biomarkers in the majority of patients

Estrada MV V, Warner J, Rioth M, Balko JM M, Rexer B and Sanders ME E. Vanderbilt University Medical Center, Nashville, TN.

Body: Background: Molecular tumor profiling is increasingly important in the management of oncologic patients. Targeted next-generation sequencing (T-NGS), using formalin fixed paraffin embedded (FFPE) clinical samples, allows for molecular characterization of genes with known or potential therapeutic and prognostic importance in cancer. Actionable alterations include those with on- or off-label therapeutic implications, those that might be biomarkers of response to clinical trial agents, or those which are non-indicators for response.

Design: We correlated information on patients with metastatic or refractory locoregional recurrence of breast cancer (BC) with potentially actionable genetic alterations detected by a commercially available T-NGS assay, which sequences the coding regions of 315 genes and introns of 28 genes involved in rearrangements selected for their demonstrated role in solid malignancy. We developed an informatics pipeline to capture test results in real-time and store them for subsequent research analysis. Results were analyzed by clinical subtype (estrogen receptor positive [ER+]; HER-2 amplified [HER2+]; triple-negative [TN]) for actionable alterations, most frequently altered genes/pathways and variants of standard biomarkers not detected by routine studies.

Results: Between 11/2013 and 4/2015, 141 FFPE samples from 139 patients were tested by T-NGS. At least one potentially actionable genetic alteration was identified in 98% of patients (median 5.5 alterations/tumor [range 0-18]). 64% had alterations predicting sensitivity to approved BC therapies and 10% to approved therapies in other tumor types. An additional 1231 variants of uncertain significance (VUS) (median 9 per tumor [range 0-28]) were identified. The most frequently altered genes were TP53 (64%), PIK3CA (37%) and MYC (24%). Genes involved in cell cycle, DNA damage and PIK3CA/mTOR pathways were highly altered among all receptor subtypes. The RAS/MAPK pathway was more commonly altered in ER+ (28%) vs. HER2+ (13%) and TN (17%). CCND1 amplifications were found in 16% (57% ER+, 30% HER2+, 13% TN) and FGFR1 amplifications in 13% (61% ER+, 22% HER2, 17% TN). Co-amplification of 8p and 11q (including FGFR1/ZNF703 and CCND1/FGF3/FGF4/FGF19) was found in 28% of patients (ER+ 21%, HER2+ 25%, TN 7.5%). The combination of PIK3CA mutation and MAP3K1/MAP2K4 alteration occurred in 12% of patients (82% ER+, 18% TN). 4 ESR1 mutations and 2 amplifications (all in ER+) as well as 4 HER2 mutations (1 ER+ and 3 TN) were also identified. The number of patients receiving genotype-directed treatments informed by T-NGS results and patient outcome after genotype-directed treatment will be subsequently presented.

Conclusion: Mutation profiling using T-NGS identified potentially actionable alterations in a majority of advanced BC patients, providing novel yet rational therapeutic options and facilitating clinical trial enrollment. T-NGS results will be used to guide therapy in increasing numbers of BC patients.
Title: Genetic heterogeneity revealed by targeted exome sequencing in advanced triple-negative breast cancer

Pannuti A, Filipovic A, Hicks C, Lefkowitz E, Ptacek T, Miele L and Stebbing J. Louisiana State University Health Sciences Center; Imperial College London and UAB.

Body: Introduction: Clinical decisions in oncology are increasingly guided by molecular genetics. Next-generation sequencing (NGS) provides a platform for identifying potential therapeutic targets and clinically relevant genetic markers for personalized therapy.

Methodology: We performed sequencing with the SmartGen 421 NGS gene panel, on relapsed triple negative breast cancer (TNBC) specimens (n = 11) by obtaining a biopsy from the most recent site of progressive disease. Custom HaloplexTM reagents were used to capture the regions of interest by hybridization to probes corresponding to target regions. Targets were amplified to enrich DNA libraries. Libraries were sequenced on an Ion Torrent Personal Genome Machine, and sequences compared to the reference genome GRCh37/hg19. The coding regions and +/-5 base pairs of the introns of 421 genes were sequenced. Analytic sensitivity for SNP calls was 94.1% (69.2%-99.7%, at 95% CI), specificity was 100%, for introns and deletions sensitivity was 78.9% (62.2%-89.9%, 95% CI) and specificity was 99.994%. Results: A total of 96 genes contained genetic variants, with each case harbouring 4 to 15 variants. No two cases had identical mutation profiles, indicating significant genetic heterogeneity. We tabulated a) all the genetic variants and b) potentially actionable variants based on predicted effects on protein structure and conservation of mutated amino acids, using COSMIC. The others are previously unreported, de novo mutations of unknown functional significance. Not surprisingly, n = 8/11 tumors contained TP53 mutations, 7 of which previously described. Genes involved in DNA repair and cell stress responses were found frequently mutated (e.g., ATM, ATR, CHEK1, CHEK2, MLH1, MUTYH, PMS1, PMS2, XPC). Interestingly, 5 mutations in the PI3K-AKT-TOR pathway were identified, including two previously described PIK3CA mutations historically linked to estrogen receptor positive disease. We did not observe PTEN mutations often linked to basal-like breast cancer. Among identified genetic variants, n = 23/96 affected genes encoding kinases (receptor and non-receptor tyrosine kinases, serine/threonine kinases, lipid kinases), including 4 involved in DNA damage response. Developmental genes in the Notch, Wnt and Hedgehog pathways were also mutated. Our results emphasize molecular heterogeneity of TNBC and suggest a variety of possible mechanisms for treatment resistance. We must also acknowledge limitation of this study, which include: lack of information on transcript expression levels of the mutated genes, the limited set of genes examined (n = 421/case), incomplete knowledge of functional and structural effects of genetic variants on each target gene and the contextual effect of coexisting variants, and the possibility that rare cellular clones may be below the limit of detection. Conclusions: We demonstrate that targeted sequencing using NGS is cost-effective, reliable and feasible for clinical use. Multiparamenter molecular profiling (DNA, RNA, protein, metabolism) will eventually provide more complete pictures of individual tumors for truly personalized treatment.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-07-04

Title: Distinct repertoires of somatic mutations affecting driver genes in mucinous and neuroendocrine carcinomas of the breast

Piscuoglio S, Ng CKY K Y, Marchio C, Eberle CA A, Guerini-Rocco E, Mariani O, Vincent-Salomon A, Reis-Filho JS S and Weigelt B. Memorial Sloan Kettering Cancer Center, NY, NY; School of Pathology, University of Milan, Milan, Italy and Institut Curie, Paris, France.

Body: Introduction: Mucinous carcinoma of the breast (MCB) is a rare histologic type, which accounts for approximately 2% of all invasive breast cancers (IBCs) and is characterized by clusters of tumor cells floating in large amounts of extracellular mucin. MCB comprises two main subtypes based on architectural and cytologic features: mucinous A (paucicellular) and mucinous B (hypercellular). Although MCBs are low-grade ER-positive/HER2-negative tumors of luminal molecular subtype, these cancers lack concurrent losses of 16q and gains of 1q, the hallmark genetic features of low-grade ER+/HER2- breast cancers, and have low levels of genetic instability. Neuroendocrine carcinoma of the breast (NCB) accounts for 2% - 5% of IBCs and displays morphologic features similar to those of neuroendocrine tumors of other organs. Previous transcriptomic analyses have revealed that NCBs and mucinous B, but not mucinous A, breast cancers display similar gene expression profiles. The aims of this study were to determine whether MCBs and NCBs share a similar repertoire of somatic genetic alterations and if these aberrations are distinct from those reported in common forms of ER+/HER2- IBCs.

Material and methods: DNA extracted from microdissected MCBs (n=7 mucinous A, n=6 mucinous B), NCBs (n=14) and adjacent normal tissues were subjected to massively parallel sequencing targeting all exons of 254 genes most frequently mutated in IBC or related to DNA repair. Somatic point mutations were identified using MuTect and somatic insertions and deletions (indels) were defined using Strelka and Varscan2. We retrieved mutations in the same 254 genes in common forms of ER+/HER2- IBC (n=252) from The Cancer Genome Atlas (TCGA).

Results: The most frequently mutated genes in MCBs were GATA3 (31% of cases, 4/13, all frame-shift indels), followed by KMT2C (MLL3) and MAP3K1 (both 23%). GATA3 and KMT2C (29%) were the most frequently mutated genes in mucinous A cancers, whereas MAP3K1 (33%) was the most frequently mutated gene in mucinous B cancers. ARID1A mutations were found in three of 14 (21%) NCBs, of which 2 were truncating mutations. A comparative analysis of the repertoire of somatic mutations found in mucinous A, mucinous B and NCBs did not reveal any statistically significant differences. As compared to common forms of ER+/HER2- IBCs, MCBs were found to have a significantly lower frequency of PIK3CA (8% vs 42%, p=0.02) mutations, which was particularly evident in mucinous A cancers (0% vs 42%, p=0.04). NCBs displayed significantly higher frequencies of somatic mutations affecting ARID1A (21% vs 2%, respectively, p=0.006), FOXA1 (14% vs 2%, respectively, p<0.05) and a lower frequency of PIK3CA somatic mutations (14% vs 42%, respectively, p<0.05) than common forms of ER+/HER2- IBCs. Conclusion: The frequency of mutations affecting bona fide breast cancer genes differed among mucinous A, mucinous B and NCBs. The repertoire of somatic mutations found in MCBs and NCBs differed from that of common forms of ER+/HER2- IBCs, in particular by the low frequency of somatic mutations affecting PIK3CA.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-07-05

Title: Mutational spectrum and tumor response in metastatic breast cancer


Body: Background: While several comprehensive genomic sequencing tests are clinically available for breast cancer (BC), little is known about the spectrum of findings reported in the general population and clinical utility of findings for patients (pts). Here we report tumor sequencing from the METAMORPH study, a comprehensive genomic testing approach in pts with metastatic (met) BC.

Methods: Pts with either known or suspected BC mets consented to and clinically underwent concurrent diagnostic and research tumor biopsies (bx). FFPE specimens were profiled via Illumina TruSeq Cancer Panel next generation sequencing platform covering 212 amplicons in 47 cancer genes. Pathology, treatment and outcome data were prospectively collected and tracked. Aside from Her2-directed treatment, therapy was not mutation (mut)-matched.

Results: 64 pts enrolled between 11/2013 – 05/2015. Of these, 48 had bx successfully sequenced (75%). Of those without sequencing, 5 had negative/insufficient tissue, 2 had insufficient DNA, remainder no bx/pending. Median age of those sequenced was 56 (range 31-78); 81% Caucasian, 17% African American. 25% (12 pts) presented with de novo stage IV disease. Of those with recurrence (n=36), 83% had prior adjuvant chemotherapy; 81% hormone receptor positive (HR+) had prior endocrine therapy. Median # prior lines of therapy for met disease was 2 (IQR 0 – 8). Tumor characteristics, including mut analyses, are shown in Table 1. # muts did not differ significantly by subtype (p=0.22). Frequency of TP53 and PIK3CA hotspot muts was nearly identical to TCGA. Median # muts was 1 for pts with both de novo mets and recurrence (p=0.79). # of muts was not associated with time to recurrence (p=0.80). Excluding pts found to have TP53 mut only or ERBB2 alterations in known Her2+ disease, 42% of pts were identified as having at least one potentially actionable alteration (PIK3CA mut, AKT1 mut or EGFR amplification). Median time to treatment failure (TTF) on subsequent therapy was 4.1 months for overall group, and 4.1, 6.2, and 1.6 months for HR+/Her2-, any Her2+ and TN, respectively, adjusted for line of therapy (p=0.03). After adjustment for # lines of prior met therapy, TTF was 4.7 vs. 4.1 months for pts with any mut vs. none (p=0.89); 5.7 vs 4.1 months for PIK3CA+ vs. not (p=0.94); 3.3 vs. 6.5 months for TP53+ vs. not (p=0.03).

Conclusion: Pts with met BC have frequent and potentially actionable muts. While overall # of muts did not affect response, tumors with TP53 muts had shorter response to subsequent therapy in this cohort. Additional data are needed to determine the clinical utility of mut testing in met BC, for both standard and mut-matched therapy.

<table>
<thead>
<tr>
<th>Receptor concordant with primary</th>
<th>Total (n=48)</th>
<th>HR+/Her2- (n=28)</th>
<th>Any HER2+ (n=7)</th>
<th>TN (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td># Mutations Median (Range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (0-4)</td>
<td>1 (0-3)</td>
<td>1 (1-2)</td>
<td>1 (0-4)</td>
</tr>
<tr>
<td>1</td>
<td>14 (29%)</td>
<td>10 (36%)</td>
<td>0</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>2</td>
<td>18 (38%)</td>
<td>11 (39%)</td>
<td>4 (57%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>3+</td>
<td>13 (27%)</td>
<td>5 (18%)</td>
<td>3 (43%)</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>Prevalent Mutations (&gt;20%)</td>
<td>TP53 (38%), PIK3CA (35%)</td>
<td>PIK3CA (50%), TP53 (25%)</td>
<td>TP53 (60%), ERBB2amp (86%)</td>
<td>TP53 (62%), PIK3CA (23%)</td>
</tr>
<tr>
<td>Other Alterations (#)</td>
<td>ATM (1), KIT (1), PDGFRA (1), PTEN (1), RB1 (1), SMAD4 (1), SMO (1), STK11 (1)</td>
<td>AKT1 (1), ATM VUS (1), ERBB2 (1), PTEN (1), SMAD4 VUS (1), SMO VUS (1)</td>
<td>ERBB2 (1), STK11 (1)</td>
<td>EGFR amp (2), KIT amp (1), PDGFRA amp (1), RB1 VUS (1)</td>
</tr>
</tbody>
</table>
Title: Clinicopathologic characterization and comprehensive genomic profiling (CGP) of advanced breast cancer patients with fibroblast growth factor receptor (FGFR) alterations


Body: BACKGROUND: FGFR family members are infrequently mutated but are frequently overexpressed in breast cancer and often accompanied by increased, or altered, expression of FGF ligands. In this retrospective study, we reviewed a large series of FGFR altered breast cancer cases that received comprehensive genomic profiling (CGP) in the course of clinical care.

MATERIAL AND METHODS: CGP was performed on hybridization-captures, adaptor ligation-based libraries using DNA extracted from 40 µm formalin-fixed paraffin-embedded (FFPE) section cut at 10 µm performed in a CLIA-certified lab (Foundation Medicine, Inc.). The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin-stained slides, and all samples forwarded for DNA extraction contained a minimum of 20% of DNA derived from tumor cells. The FoundationOne test sequences the full coding regions of up to 315 cancer-related genes, and up to 28 genes that are frequently altered in cancer to detect all classes of genomic alterations including base substitutions, indels, copy-number alterations (CNA), and fusions/rearrangements. The average depth of coverage is greater than 600X. The genomic profiles of 2,617 patients with diverse advanced malignancies who were evaluated at Cancer Treatment Centers of America between 12/24/12 and 03/11/15 were reviewed. 176 FGFR alterations (7.8%) were detected, of which 76 (43.5%) were found in breast cancer cases out of 434 (16.5%). The study was carried out in accordance with WIRB Institutional Review Board.

RESULTS: A total of 76 female breast cancer patients, having a median age 50 (range, 28-69), with FGFR alterations were reviewed. All patients had metastatic/relapsed advanced breast cancer. 54 patients were Estrogen Receptor-positive (70%), and 15 were HER2+ (20%). 6 patients had gBRCA deleterious mutations. 84% of the samples (n=67) tissue block were analyzed, and the anatomic sites represented by the samples were 24 breast primary tumor (31%), 15 liver (19%), 10 lymph nodes (13%), and other sites (37%). The median number of chemotherapies cycles was 4 (range, 1-12), and the median time to metastasis was 31 months (range, 0-175). At the time of this report, 31 patients (40%) were deceased. 79 FGFR gene alterations were identified in 76 patients, including FGFR1 (65), FGFR2 (6), FGFR3 (2), and FGFR4 (4), with all but 7 of these being amplifications. The most co-existent altered gene was TP53 (66%), and other altered genes included PIK3CA (37%), MYC (28%), FGFR3/4/19 (17%), CCND1 (17%), and CCNE1 (16%). The subset of co-amplified FGFR3/4/19 and FGFR amplified patients were all (7) ER+ except for 1 patient.

CONCLUSIONS: FGFR genomic alterations in breast cancer patients are predominantly amplifications and are most commonly observed in ER+ patients. Further review of treatment history will be performed to evaluate the hypothesis that alterations of FGFR is a modifier of response to endocrine therapy, and co-amplified FGFR3/4/19 and FGFR breast cancer cases may be a distinct clinic-pathologic entity. Any patients in this series initiated on anti-FGFR targeted therapy will also be reported.
Title: Clinical significance of ESR1 mutations using droplet digital polymerase chain reaction assay in 325 breast cancer samples

Takeshita T, Yamamoto Y, Yamamoto-Ibusuki M, Inao T, Sueta A, Fujiwara S and Iwase H. Kumamoto University, Graduate School of Medical Science, Kumamoto, Japan and Kumamoto University Hospital, Kumamoto, Japan.

Purpose: We aimed to develop a droplet digital Polymerase Chain Reaction (ddPCR)-based method for the sensitive detection of estrogen receptor (ER) α (ESR1) mutations in the primary and recurrent/metastatic tumor tissues of breast cancer.

Experimental Design: We studied a total of 325 tumor specimens (270 primary breast cancer specimens and 55 ER-positive recurrent/metastatic tumor specimens). Because the recurrent/metastatic tumor specimens had much inflammatory and stromal cells, we captured only tumor cells using laser microdissection. We investigated the quantification of rare ESR1 mutations, four representative types, Y537S, Y537N, Y537C, and D538G in extracted genomic DNA using ddPCR system that simultaneously performed thousands of PCRs on a nanoliter scale.

Results: In 270 primary breast cancer samples, we analyzed each ESR1 alteration percentage in each subtype. ESR1 Y537C tended to be higher in hormone receptor-positive (HR+)/ human epidermal growth factor receptor 2-negative (HER2-) group (P = 0.06) and higher percentage of ESR1 D538G was statistically significant in HR+/HER2- group (P = 0.027), compared with HER2+ group. There was no statistically different in each ESR1 alteration percentage between HR+/HER2- group and HR-/HER2- group. Whether each ESR1 alteration was dichotomized as positive or not, we used the percentage which HER2+ group and HR-/HER2- were not identified, as a cutoff point. ESR1 mutations occurred in 7 samples (2.5%) out of 270 primary samples, but ESR1 mutations occurred in 11 samples (20%) out of 55 metastatic/recurrent breast cancer samples.

Table 1 Patients characteristics of 11 metastatic ER-positive breast cancer cases with ESR1 mutations

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>HER2 status</th>
<th>Ki67 LI</th>
<th>ER HS</th>
<th>PgR HS</th>
<th>Biopsy site</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>72</td>
<td></td>
<td>10</td>
<td>205</td>
<td>1</td>
<td>Lymph node</td>
<td>Y537C 1610 A&gt;G only</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td></td>
<td>5</td>
<td>182</td>
<td>18</td>
<td>Lymph node</td>
<td>Y537S 1610 A&gt;C Y537N 1609 T&gt;A D538G 1613 A&gt;G</td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td></td>
<td>20</td>
<td>110</td>
<td>5</td>
<td>Skin</td>
<td>Y537C 1610 A&gt;G only</td>
</tr>
<tr>
<td>18</td>
<td>63</td>
<td></td>
<td>10</td>
<td>162</td>
<td>130</td>
<td>Lymph node</td>
<td>D538G 1613 A&gt;G only</td>
</tr>
<tr>
<td>33</td>
<td>54</td>
<td></td>
<td>4</td>
<td>170</td>
<td>15</td>
<td>Lymph node</td>
<td>Y537S 1610 A&gt;C Y537N 1609 T&gt;A D538G 1613 A&gt;G</td>
</tr>
<tr>
<td>42</td>
<td>68</td>
<td></td>
<td>5</td>
<td>169</td>
<td>50</td>
<td>Skin</td>
<td>Y537C 1610 A&gt;G only</td>
</tr>
<tr>
<td>44</td>
<td>66</td>
<td></td>
<td>24</td>
<td>270</td>
<td>159</td>
<td>Lymph node</td>
<td>Y537N 1609 T&gt;A only</td>
</tr>
<tr>
<td>46</td>
<td>73</td>
<td></td>
<td>20</td>
<td>224</td>
<td>110</td>
<td>Lymph node</td>
<td>Y537N 1609 T&gt;A and D538G 1613 A&gt;G</td>
</tr>
<tr>
<td>49</td>
<td>52</td>
<td></td>
<td>20</td>
<td>275</td>
<td>138</td>
<td>Skin</td>
<td>Y537S 1610 A&gt;C and Y537C 1610 A&gt;G</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td></td>
<td>10</td>
<td>174</td>
<td>0</td>
<td>Lymph node</td>
<td>Y537S 1610 A&gt;C only</td>
</tr>
<tr>
<td>51</td>
<td>40</td>
<td></td>
<td>10</td>
<td>189</td>
<td>5</td>
<td>IBTR</td>
<td>Y537S 1610 A&gt;C only</td>
</tr>
</tbody>
</table>

Abbreviations: HER2, human epidermal growth factor receptor 2; LI, labeling index; ER, estrogen receptor; HS, histoscore; PgR, progesteron receptor; ET, endocrine therapy; IBTR, ipsilateral breast tumor recurrence; SD, stable disease; PD, progressive disease; MPA, medroxyprogesterone acetate

Two biopsies were performed in 8 women, in which four women had primary and recurrent/metastatic samples. Four out of these 8 women acquired ESR1 mutation, whereas no ESR1 mutation could be identified at first biopsy.

Conclusions: We demonstrated the sensitive detection and accurate quantification of low frequency ESR1 mutations in 270 primary breast cancer samples and 55 recurrent/metastatic samples using ddPCR assay. This technique could prove a useful method for the precise detection of ESR1 mutations in endocrine therapy resistant cases.
Body: Background: AR mutations have been described as a mechanism of resistance to AR antagonists in prostate cancer. There are limited data regarding the presence of AR mutations in breast cancer. We aim to describe the presence of tumor AR mutations in a cohort of patients (pts) with breast cancer who are candidates for targeted cancer therapy, who underwent tumor genomic profiling as part of a clinical trial at MSK (NCT01775072).

Methods: MSK-IMPACT is a targeted tumor sequencing assay capable of detecting mutations and other critical genetic aberrations in 410 cancer genes; these data are available through an institutional database. Following IRB approval and using an electronic medical record, we examined the subset of pts in this database with breast cancer for the presence of AR mutations and performed a chart review for clinicopathologic features and outcomes. Statistics are descriptive.

Results: As of 03JUN2015, 628 of 4,379 samples that underwent MSK-IMPACT testing since 2012 were from invasive breast cancers. 6 of 628 (1%) harbored AR mutations (Table 1), none of which contribute to a reported or predicted functional alteration.

Patient/tumor characteristics are shown in Table 2. Five out of 6 patients recurred between 7.4 and 117.4 months (mo) from the date of initial diagnosis. One patient is without disease recurrence at 3.9mo from diagnosis at date of last contact, 7.9mo ago.

Table 1

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Type</th>
<th>Allele Frequency</th>
<th>Functional Impact According to MutationAssessor</th>
</tr>
</thead>
<tbody>
<tr>
<td>R31H</td>
<td>Missense</td>
<td>0.21</td>
<td>Low</td>
</tr>
<tr>
<td>R856C</td>
<td>Missense</td>
<td>0.31</td>
<td>Medium</td>
</tr>
<tr>
<td>A430T</td>
<td>Missense</td>
<td>0.12</td>
<td>Neutral</td>
</tr>
<tr>
<td>G409R</td>
<td>Missense</td>
<td>0.18</td>
<td>Medium</td>
</tr>
<tr>
<td>Q445P</td>
<td>Missense</td>
<td>0.47</td>
<td>Medium</td>
</tr>
<tr>
<td>Q73L</td>
<td>Missense</td>
<td>0.25</td>
<td>Neutral</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Stage at Diagnosis</th>
<th>Histology</th>
<th>ER%/PR%/HER2</th>
<th>AR%</th>
<th>Sites of MBC</th>
<th>Site of IMPACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>R31H</td>
<td>T1N0</td>
<td>Lobular</td>
<td>90/20/1+</td>
<td>-</td>
<td>Skin</td>
<td>Skin</td>
</tr>
<tr>
<td>R856C</td>
<td>T3N3</td>
<td>Ductal</td>
<td>20/0/1+</td>
<td>0</td>
<td>Chest wall, LN</td>
<td>LN</td>
</tr>
<tr>
<td>A430T</td>
<td>T2N1</td>
<td>Ductal</td>
<td>95/0/-</td>
<td>-</td>
<td>Bone, liver</td>
<td>Breast</td>
</tr>
<tr>
<td>G409R</td>
<td>T2N0</td>
<td>Ductal</td>
<td>90/80/FISH 1.3</td>
<td>-</td>
<td>Bone, liver</td>
<td>Liver</td>
</tr>
<tr>
<td>Q445P</td>
<td>T3N3</td>
<td>Ductal</td>
<td>99/50/1+</td>
<td>-</td>
<td>LN</td>
<td>Breast/LN</td>
</tr>
<tr>
<td>Q73L</td>
<td>T3N0</td>
<td>Malignant Phyllodes</td>
<td>-</td>
<td>-</td>
<td>N/A</td>
<td>Breast</td>
</tr>
</tbody>
</table>

MBC metastatic breast cancer; LN lymph node; N/A not applicable
Conclusions: AR mutations were uncommon in this dataset of 628 breast cancers. Similarly, TCGA sequencing data has revealed only 12 invasive breast cancers with AR mutations (2.2% of 973 samples). The functional significance of these mutations has not been demonstrated. As tissue from therapeutic studies using AR antagonists for the treatment of breast cancer become available, mutations or amplification in AR may be more readily identified as a potential mechanism of resistance to AR-targeted therapy.

Acknowledgments: We gratefully acknowledge the members of the Molecular Diagnostics Service in the Department of Pathology and the Marie-Josée and Henry R. Kravis Center for Molecular Oncology.
Somatic mutations in A kinase anchoring proteins (AKAPs) in metastatic tumors - A potential characteristic of breast cancer metastasis

Kjällquist U, Erlandsson R, Alkodsi A, Tobin N, Karlsson E, Hatschek T, Hartman J, Linnarsson S and Bergh J. Karolinska Institutet, Stockholm, Sweden; Karolinska Institute, Sweden; University of Helsinki, Biomedicum, Finland and Radiumhemmet – Karolinska Oncology at Karolinska University Hospital, Stockholm, Sweden.

Body: Introduction
Genomic heterogeneity in primary solid tumors has been extensively studied using deep sequencing technologies during the last decade. The heterogeneity of cancer tumors is today a well-established concept partly reflected in the low number of genes being recurrently mutated in over 10% of the tumors. However, most available data relates to the primary breast cancer tumors and little has been described about the mutational profiles of the metastatic lesions and their relation to its original malignant cell population. Prospective and retrospective studies have demonstrated that altered receptor status in the metastatic lesion occurs at high rates during cancer progression and is additionally affected by adjuvant therapy with major implications for management of the metastatic disease. Here, we report the exome sequences of paired primary and metastatic lesions from ten breast cancer patients.

Results
We found a marked heterogeneity of somatic mutations as well as chromosomal aberrations in the metastatic lesions. A number of mutated genes were enriched in the metastases including, significantly, members of the A-kinase anchoring protein family (AKAPs), p < 0.02; Fisher's exact test. The enrichment of AKAP mutations in metastatic lesions was confirmed in an independent cohort containing 20 patients with paired primary and metastatic lesions, which showed the same mutational pattern. In total, 14 nonsynonymous mutations were found in ten of the fourteen AKAP family members. Out of the totally 30 patients examined, ten (30%) carried one or more mutations in AKAP genes either in primary tumor, metastasis, or both. In seven of these ten patients, the AKAP mutation was found uniquely in the metastatic lesion.

Several copy number variations (CNV), mostly deletions in regions containing AKAP genes were detected. For example, the down-regulation of AKAP12 is often associated with promoter hypermethylation or loss of its locus 6q24-25.2 and has been associated with tumor progression and metastasis. In our data deletion of the AKAP12 locus is present in six out of twenty patients.

Discussion
AKAPs are members of a protein family acting as anchors for Protein Kinase A (PKA) by specifically associate PKA regulatory subunits to cellular organelles and direct its active signal transduction spatially and temporally. Several of the AKAP members have been associated to cancer development and metastatic spread, mostly based on differential expression and effects on migration in in vitro assays but both polymorphisms and somatic mutations have been reported in human tumors. Our findings indicate that in metastatic lesions, the primary tumor genome is extensively transformed, with enrichment of mutations in a distinct set of genes. Together, these findings suggest the involvement of AKAPs in the metastatic process and provide a potential avenue for targeted therapy directed at metastatic breast cancer. Molecular and genetic characterization of the metastatic lesions is not only important in the clinical setting but should also provide the means to reveal genetic patterns specific for the disseminated malignancy.
Title: Cytogenetic analysis of squamous cell carcinoma of the breast reveals inter- and intra-tumoral heterogeneity


Body: Background: Squamous cell carcinoma (SCC) of the breast is a rare and generally aggressive disease constituting less than 0.1 % of all breast carcinomas. Although they have distinct morphological features, the origin and cytogenetic profiles of SCCs are still not well understood. In this study, five cases of SCC of the breast, three of which had an SCC component and an invasive or noninvasive ductal carcinoma of no special type (NST) component, were analyzed to evaluate their cytogenetic inter- and intra-tumoral heterogeneity.

Methods: Using a pathological database of approximately 3,000 patients with breast cancer, five patients with SCC were identified. Their medical records were retrospectively reviewed to obtain clinicopathological, radiological, therapeutic and prognostic information. The area largely consisting of the SCC component was macro-dissected from five 10 µm-thick sections and tumor DNA was extracted using the QI Amp DNA Mini Kit (Qiagen). Three cases contained a component of invasive or noninvasive ductal carcinoma of NST as well as an SCC component, and tumor DNA from NST components were also extracted as described above. Tumor DNA from each case were used for array comparative genomic hybridization (aCGH) analysis using a high-density oligonucleotide microarray (Agilent® SurePrint G3 8x60k microarray) and the cytogenetic profiles of SCC components were compared with each other and in three of the five cases with their paired NST components.

Results: Sufficient amounts of DNA were obtained for aCGH analysis with an average of 0.78 µg (0.39–1.35 µg). The quality of the aCGH was acceptable, as judged by the mean derivative log ratio spread (DLRSpread) of 0.45 (0.20–0.55), which estimates the log ratio noise by calculating the spread of log ratio differences between consecutive probes along all chromosomes. The cytogenetic profiles of SCC components showed large inter-tumoral heterogeneity with between 2 and 160 copy number alterations per case and no common copy number alterations were found among cases. Meanwhile, cytogenetic profiles were almost identical between paired SCC and NST components. However, in one case, a large number of copy number aberrant (CNA) regions were detected in the SCC component compared with the NST component and all aberrations in the NST component were also present in the SCC component, which implies that the SCC component originated from the NST component. There were no common SCC-component-specific aberrations in the three cases with NST components.

Conclusion: These results demonstrate the degree of cytogenetic inter- and intra-tumoral heterogeneity in SCC of the breast. The comparison of cytogenetic profiles in one case indicated that the SCC component originated from the NST component.
Title: On the origin of T>C transition mutations in breast cancer

Choi M, Radovich M, Brown C and Clare SE E. Northwestern University Feinberg School of Medicine, Chicago, IL; Indiana University School of Medicine, Indianapolis, IN and Expression Analysis, Durham, NC.

Body: Background: Mutations in cancer driver genes are thought to occur early in tumorigenesis. Examination of the sequence context enables the inference of causality. For example, C>A transversions in lung malignancies are ascribed to tobacco smoke. A large proportion of clonal and subclonal mutations in breast cancer are attributed to the APOBEC cytidine deaminases. However, 26% of clonal mutations in cancer genes in the breast and 15% of subclonal mutations are T>C transitions [Sci Transl Med. 2015;7:283ra54]. The purpose of this study was to determine if the T>C transitions are likely an early event in breast carcinogenesis and look for clues to the mechanism by which they are produced.

Methods: Histologically normal tissue adjacent to a cancer provides a window into the early steps of oncogenesis. BAM files of exome sequencing data from 11 matched trios of tumor, adjacent normal tissue and blood (leukocyte DNA) from the TCGA (The Cancer Genome Atlas) breast cancer dataset were downloaded from the UCSC cgHUB repository. Mutations were called using the MuTect software (Broad Institute, Cambridge, MA). As a validation cohort, data was downloaded from 29 additional specimens that had been added to TCGA subsequent to the initial data set, and analyzed as above. RNA-Seq data from the initial adjacent normal was downloaded from the TCGA data portal. The epithelia from 20 frozen tissue cores from healthy premenopausal donors to the Susan G. Komen Tissue Bank were microdissected and the RNA isolated. RNA-sequencing was carried out using the Life Technologies SOLiD Platform. RPKM gene expression values from TCGA and sequencing of the Komen normal tissues were merged, quantile normalized, and batch effect corrected. Normalization and differential gene expression was performed using Partek Genomics Suite.

Results: C>T and T>C transition mutations make up the majority of the mutations in histologically normal tissue adjacent to breast cancer. Displaying mutations in their trinucleotide context, i.e., by the sequence context immediately 5’ and 3’ to the mutated base revealed that T>C mutations most frequently occur in the 5’-ATG-3’, 5’-CTG-3’ and 5’-ATA-3’ contexts. Analysis of the RNA-Seq data discovered 1821 genes to be differentially expressed (FDR<5%). Relevant to the questions being addressed in this study, the expression of Nitric Oxide Synthase 3, an enzyme that synthesizes nitric oxide from L-arginine, is 4-times greater in adjacent normal (p= 4.55E-03). [Of note, published data reveals no significant contamination of adjacent normal with tumor in the TCGA breast data.]

Conclusions: NOS3 expression is enhanced in certain inflammatory environments as well as by estrogen. Nitric oxide induces DNA damage; one mechanism is through the N_2O_3 pathway. Reaction of DNA with N_2O_3 leads to the deamination adenine to form hypoxanthine. Thymine-DNA glycosylase (TDG) initiates base excision repair (BER). Talhaoui et al [Nucleic Acids Res. 2014;42:6300-13] have recently shown that TDG excises T when it is paired with hypoxanthine. Downstream in this aberrant BER pathway the abasic site is repaired to a C resulting in the T>C transition. The excision of the T is efficient only in a specific nucleotide context, which is 5’-TpG-3’, as was observed in our study.
Title: Abstract Withdrawn
Title: Inverse relationships between high somatic copy number load and immune phenotypes in breast cancer

Bense RD D, van der Vegt B, de Vries EGE GE, van Vugt MATM ATM, Schröder CP P and Fehrmann RSN SN. University of Groningen, University Medical Center Groningen, Groningen, Netherlands and University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Body: Introduction
Currently, there is no clear biomarker to predict which breast cancer patient may benefit from immunotherapy. High somatic point mutational load is thought to lead to immune activation and CD8+ T cell activation and infiltration. In addition, pre-existing CD8+ T cells distinctly located at the invasive tumor margin are associated with expression of the PD-1/PD-L1 immune inhibitory axis and may predict response to PD-1 targeted therapy. However, breast tumors are considered copy number-driven rather than mutational-driven cancers. Therefore, in order to identify which breast cancer patients may potentially benefit from immunotherapy, we investigated relations between copy number load and CD8+ T cell abundance, immune pathways and immune activation scores in a large set of publically available breast cancer expression profiles.

Methods
Functional genomic mRNA-profiling (Fehrmann et al., Nat Genet, 2015) was used to capture the downstream effect of somatic copy number alterations on gene expression levels. This method allowed using gene expression profiles to quantify somatic copy number load occurring in tumor samples in a univariate measurement called the copy number load index (CNL-index). Immune activation scores were calculated according to two known gene immune signatures (Teschendorff et al., Genome Biol, 2007 and Desmedt et al., Clin Cancer Res, 2008). Cibersort (Newman et al., Nat Methods, 2015) was applied to estimate CD8+ T cell abundance. Publicly available gene expression profiles of 7,270 primary breast cancer samples were used. The relation between CNL-index, immune activation scores and disease-free survival (DFS), defined as time from diagnosis until distant metastasis development, was assessed by multivariate cox-regression analysis including age, ER and HER2 status, tumor size, lymph node involvement, tumor grade and treatment regimen. Gene set enrichment analysis (GSEA) was applied to assess relations between immune pathways and CNL-index.

Results
In primary breast cancer, low CNL-index was found in 1,796 samples (24.7%) versus 5,474 samples with a high CNL-index (75.3%). Higher CNL-index was correlated with shorter DFS (HR 2.51, \(P = 1.6 \times 10^{-4}\)), whereas higher immune activation scores were associated with prolonged DFS (HR 0.199, \(P = 0.003\) and HR 0.347, \(P = 0.017\)). CD8+ T cell abundance was negatively correlated with the CNL-index (Spearman \(R = -0.14, P = 8.11 \times 10^{-34}\)). GSEA showed enrichment of immune pathways amongst genes that negatively correlated with CNL-index. Subset analysis in 1,555 TNBC samples showed low CNL-index in 287 samples (18.5%) versus high CNL-index in 1,268 samples (81.5%). Similar to the entire breast cancer set, CD8+ T cell abundance and CNL-index were negatively correlated in TNBC (Spearman \(R = -0.11, P = 4.11 \times 10^{-05}\)). Immune pathway enrichment in genes in TNBC also negatively correlated to CNL-index.

Conclusion
High CNL-index (i.e. high copy number load) is inversely related to immunoactivation, immune pathways and CD8+ T cell abundance. This implies that in breast cancer, tumors with low CNL-index may be intrinsically sensitive to immune modulation, which warrants further confirmation in prospective trials.

This research was supported by Dutch Cancer Society Grants RUG 2010-4739 and RUG 2013-5960.
Title: New insights on HER2 amplification from the constitutional and somatic standpoints: Results from the ICGC and SIGNAL/Phare studies

Vincent-Salomon A, Ferrari A, Pivot X, Macrogan G, Arnould L, Treilleux I, Romieux G, Sertier A-S, Thomas E, Tonon L, Boyault S, Kielbassa J, Letexier V, Pauporte I, Birbaum D, Saintigny P, Cox D and Viari A. Curie Institute, Paris, France; Leon Berard Cancer Center, Lyon, France; University Hospital Minjoz, Besançon, France; Cancer Center, Bordeaux, France; GF Leclerc Cancer Center, Dijon, France; Val d’Autel Cancer Center, Montpellier, France; INCa, Paris, France and Inserm - Paoli Calmette Cancer Center, Marseille, France.

Body: Background: HER2-positive (HER2+) breast cancers are defined by the amplification and/or overexpression of the human epidermal growth factor receptor (HER2/ERBB2) gene on chromosome region 17q12. Although anti-HER2 targeted therapies have greatly improved treatment of HER2+ breast cancer, the magnitude of benefit varies widely between patients. Deciphering the genomic and genetic heterogeneity of HER2+ breast cancer may provide a basis to better understand their natural history, opening new avenues of treatment.

Methods: As part of the ICGC Breast Cancer Working Group effort, we combined whole genome sequencing and transcriptomic analyses of 64 HER2+ primary invasive carcinomas, and a genome wide association study (GWAS) of over 9.836 breast cancer patients in the prospective SIGNAL/PHARE cohort (NCT00381901 – RECF1098).

Results: Using WGS data we precisely delineate the ERBB2 amplicon as a 106 kb region involving six genes and show that the amplification mechanism was consistent with breakage-fusion-bridge (BFB) cycles. Four RNA expression-based groups were identified, displaying specific genomic alterations in terms of amplification, rearrangements and mutations. On other hand, GWAS analyses failed to identify any constitutional variants associated with HER2 amplification.

Discussion: By combining whole genome sequencing and expression analysis, we provide evidence showing that HER2+ tumours display considerably more molecular heterogeneity than previously reported. These results are reinforced with the lack of association between any genetic variants and HER2 amplification from GWAS analyses. Taken as a whole, these results suggest that HER2+ breast cancers do not represent per se a homogeneous subtype, but are distributed along the whole breast cancer spectrum, from ER-positive luminal to ER-negative basal phenotype. Genome alterations present in HER2+ tumors are in accordance with these phenotypes, and it is likely that the HER2 amplification is a secondary event in the course of tumorigenesis, not favored by any particular constitutional or somatic genetic variants.
Title: Developing *in vitro* models of ductal carcinoma *in situ* from primary tissue

McAuliffe PF, Brown DD, Oesterreich S, Lee AV, Johnson RR, McGuire KP, Davidson NE, Brusky AM and Dabbs DJ. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

**Body:** BACKGROUND: Because there are currently no reliable predictors for progression of ductal carcinoma *in situ* (DCIS) to invasive disease, nearly all patients receive aggressive therapy, leading to over-treatment in many cases. Few *in vitro* models for studying DCIS progression have been developed. We report here the successful culture and expansion of primary DCIS from surgical specimens using a conditional reprogramming protocol.

**MATERIALS AND METHODS:** From 2/2014 to 4/2015, patients with percutaneous core needle biopsy demonstrating DCIS were enrolled in a tissue banking protocol after informed consent was received. Under supervision of the surgical pathologist, fresh tissue measuring between 5-15 mm in length was taken from lumpectomy or mastectomy specimens. Tissue was divided such that half was mechanically and enzymatically dissociated and then cultured in medium conditioned by irradiated mouse fibroblasts and supplemented with rho-associated protein kinase (ROCK) inhibitor, and the second half, known as the "mirror image" remained as part of the clinical specimen.

**RESULTS:** Of 49 consented patients, mean age was 59 ± 10 years. 7 were excluded due to final pathology not consistent with DCIS: 4 upstaged to invasive ductal cancer, 2 had microinvasion and 1 showed pleomorphic lobular carcinoma *in situ*. Of the remaining 33 cases of DCIS, 70% (n=23) were nuclear grade 2 and 27% (n=9) were nuclear grade 3 respectively. 91% (n=30) were ER-positive, with H-score ranging between 4 and 300. 19 (58%) were expanded in cell culture for up to two months in culture, and 14 were frozen immediately after mechanical dissociation for future growth. The 19 cell cultures could be cryopreserved and expanded. The cultures are almost exclusively composed of cytokeratin 8- and EpCAM-positive luminal cells and cytokeratin 14-, cytokeratin 5-, and p63-positive basal mammary epithelial cells, suggesting maintenance of heterogeneity *in vitro*. Furthermore, as assessed by luminal and basal marker expression, these cells retain their cellular identities both in the "conditionally reprogrammed" proliferative state and when conditioned media and ROCK inhibitor were withdrawn. When grown to 100% confluency, the cultures appear to organize into luminal and basal layers as well as luminal compartments surrounded by basal cells.

**CONCLUSION:** Primary cultures of DCIS derived directly from patient tissues may serve as *in vitro* models for the study of DCIS.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-08-03

Title: Functional detection and inhibition of the targetable oncogenic kinome of chemotherapy-treated triple-negative breast cancer cells

Pan B, Olow A, Sun Q, Mori M, Lee PRE, Hartog M, Wang C, Wolf D, Yau C, van’t Veer L and Coppé J-P. Peking Union Medical College Hospital, Beijing, China; Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA and Showa University, Tokyo, Japan.

Body: Background: Triple-negative breast cancer (TNBC) accounts for 10-15% of all breast cancer cases. A major area of innovation in TNBC is identifying potential treatment targets, especially in TNBC cells which survive chemotherapy. Our previous study showed that TNBC cells displayed deregulated kinase-dependent signaling cascades, and uniquely divergent phospho-circuits could be distinguished between TNBC vs non-TNBC cell lines [2014 SABCS abstract 1672, poster P2-05-09]. We further hypothesized that specific dysfunctional phospho-signaling network played a key role in the early adaptive changes in DNA damage response of TNBC cells exposed to DNA damaging chemotherapeutic agents.

Methods: TNBC cell lines MDA-MB-231 and MDA-MB-436 were treated with 5-fluorouracil (5-Fu), carboplatin and doxorubicin at their respective half maximal inhibitory concentrations (IC50s). MiSeq gene sequencing of the untreated vs treated TNBC cells was performed to investigate whether exposure to chemotherapy agents for 3-day’s duration would induce additional adaptive genetic mutation. Apoptosis and cell-cycle distribution of the untreated and treated TNBC cells were analyzed with flow cytometry. The functional phospho-signature of each TNBC cell sample was analyzed using a high throughput experimental platform that monitors the level of activity of myriad kinases at once. This technique used over 450 phospho-sensing probes, including over 150 controls in an aqueous-based assay to simultaneously and directly measure the phospho-catalytic activity of phosphorylating enzymes in cell lysates. The kinome activities of the untreated vs treated TNBC cell lines were compared respectively, and the most significantly deranged and functionally altered phospho-signaling cascades and their related kinases were identified as the early adaptive changes of the survived TNBC cells after the 3-day exposure to DNA damage chemotherapies.

Results: Using the two TNBC cell lines treated with the three chemotherapies, we made 8 cell line samples, including 6 treated and 2 untreated as the control. MiSeq gene sequencing showed no significant additional adaptive genetic mutations in the treated TNBC cells after the 3-day short-term exposure to 5-Fu, carboplatin and doxorubicin. 36 phospho-signatures were generated and validated for repeatability and robustness. The kinase activity signature of each TNBC sample was analyzed and compared to each other using unsupervised hierarchical clustering. The phospho-sensing assay revealed that phospho-signaling cascades related to CHK1/2 and IKK kinases were differentially altered in the untreated vs treated TNBC cell lines, which, when respectively inhibited by AZD7762 and IKK16, successfully increased growth inhibition and cell death of TNBCs.

Conclusions: We identified specific phospho-fingerprints of the early adaption of TNBC cell lines and combinatorial targeted therapies that improve treatment outcome. Our next goal is to identify specific phosphorylation cascades in a broader range of cell lines and tumor tissues, to explore the actionable, kinase-dependent mechanisms critical to the DNA damage-induced adaptive reprogramming of TNBCs and early changes driving drug-resistance.
Abstract Withdrawn
Title: Protein tyrosine kinase activity and miRNA expression profiling reveals differences according to progesterone-receptor-status in HER-2 negative breast cancer

Tahiri A, Satheesh SV V, de Wijn R, Lders T, Aure MR R, Quigley DA A, Bukholm IR R, Hurtado A, Kristensen VN N and Geisler J. Akershus University Hospital, Lrenskog, Oslo, Norway; Institute of Clinical Medicine, University of Oslo, Oslo, Norway; Centre for Molecular Medicine Norway (NCMM), Oslo, Norway; Pamgene International B.V., ’s -Hertogenbosch, Netherlands; Institute for Cancer Research, Oslo University Hospital Radiumhospitalet, Oslo, Norway; Helen Diller Family Comprehensive Cancer Center, San Fransisco, CA; Akershus University Hospital, Lrenskog, Norway and Akershus University Hospital, Lrenskog, Norway.

Body: Background: Kinases are considered as promising source of biomarkers for diagnostic, prognostic and therapeutic purposes in cancer patients. We assessed tyrosine kinase activity in 39 primary breast cancer samples that were all hormone receptor positive (ER+ and/or PR+) with differential HER-2 status, using microarray technology. Methods: Pamchip® peptide microarrays were used to measure the activity of protein tyrosine kinases in 32 breast cancer samples. The breast cancer cell lines MCF-7, BT474 and ZR75-1, was studied for kinase activity, both untreated and treated with estradiol. Results: Results showed differences in phosphorylation amongst breast cancer samples. A total of 37 peptide kinases were highly phosphorylated in a group of breast cancer samples representing 33 protein tyrosine kinases involved in cancer pathways and immunological responses. In vitro studies with breast cancer cell lines exhibited the same phosphorylation profiles, but increased phosphorylation was only observed in one cell line, ZR75-1. Eliminating HER-2 positive samples, we obtained differences in phosphorylation profiles based on PR-status only. Samples lacking PR-expression exhibited higher kinase activity of downstream kinases compared to PR-positive samples. Similar results were obtained with miRNA expression profiles of 31 breast cancer samples. Five miRNAs were identified to be significantly differentially expressed (p < 0.05) between PR-negative and PR-positive samples. This effect was even stronger when eliminating HER-2 positive samples, with 13 miRNAs exhibiting significant differential expression based on PR-status. Conclusion: Although our data are based on a small dataset, the lack of PR expression seems to have a profound effect on tyrosine kinase activity and miRNA expression in HER-2 negative breast cancers without any effect on gene expression. This indicates that regulatory and functional molecules might exhibit phenotypical features of cancer that cannot be explained by gene expression alone.
Title: Neoadjuvant tamoxifen therapy synchronizes ERα binding and gene expression profiles


Body: Background: The majority of breast cancer patients are diagnosed with ERα-positive breast cancer. Most ERα-positive patients are treated with adjuvant endocrine therapy — typically tamoxifen or aromatase inhibitors — to block cellular proliferation. Although these treatments are considered successful, resistance is common. Notably, cross-resistance between the two types of therapies is not always observed suggesting molecular heterogeneity and underlining the need for development of personalized treatments. The Anastrozole, Fulvestrant or Tamoxifen Exposure — Response in molecular profile study (AFTER study, NCT00738777) aims to investigate prospectively whether short-term treatment can induce molecular changes indicative of pre-operative therapy response. Study Design: ERα-positive breast cancer patients are included in this open-label multicenter study. Post-menopausal patients are randomized between tamoxifen, anastrozole and fulvestrant and pre-menopausal and male patients receive tamoxifen. Treatment occurs during the pre-operative window between diagnosis and surgery (4±2 weeks). Clinical characteristics collected are ERα/PR and HER2 status as well as lymph-node status. The primary endpoint is the decrease in tumor cell proliferation, as assessed by Ki67 gene expression and published cell proliferation gene expression signatures. All data are collected from both pre- and post-treatment samples. Additionally, we will compare the changes induced by treatment in gene expression, ERα/DNA binding interactions, DNA copy number, endoxifen and estradiol levels. Results: Among 67 patients currently enrolled, we examined the data from the subset of 28 tamoxifen treated patients. ERα and PR levels did not differ significantly between pre- and post-treatment. All tumors were HER2-negative. Proliferation examined by Ki67 (IHC and gene expression, MKI67) was significantly lower in post-treatment samples (P < 0.01). A significant association was identified with the change in gene expression proliferation signature score and change in MKI67 (rho = 0.7, P < 0.001). We identified two samples, which changed from MammaPrint (MP) low-risk to high-risk among 17 pairs with data. One sample's score was on the cutoff for high-risk definition. Interestingly, the second sample also had an increase in Ki67 gene expression and proliferation gene signature score in the post-treatment sample. Overall, ERα/DNA binding interaction regions overlapped significantly more among post-treatment samples as compared to pre-treatment samples (P <0.001). There were 3 samples that increased in MKI67 gene expression after drug exposure. Among these, only the MP low- to high-risk sample had an increase in proliferation gene signature and decrease in ERα/DNA binding interactions. Conclusions: Pre-treatment samples were more variable for both proliferation gene expression signatures and ERα/DNA binding interactions indicating the underlying molecular heterogeneity of the group prior to therapy. This inter-tumor heterogeneity appears to have been lowered by exposure to tamoxifen. Interestingly, not all samples were uniform in their response to tamoxifen exposure as measured by Ki67 and MP scores suggesting samples taken after treatment exposure may be useful for predictive biomarker discovery.
Title: Association between phenotype of triple negative breast cancer cell lines and sensitivity against eribulin mesylate in vitro

Bräutigam K, Mitzlaff K, Uebel L, Steinert G, Köster F, Polack S, Rody A and Liedtke C. University Hospital Schleswig-Holstein, Luebeck, Germany and Eisai GmbH, Frankfurt/Main, Germany.

Body:

Introduction:
Diagnosis of triple negative breast cancer (TNBC) is associated with adverse prognosis particularly in case of chemotherapy resistance. TNBC is a heterogeneous entity and seems to consist of at least six distinct molecular subtypes (Lehman subtypes) with distinct chemotherapy sensitivity. The cytotoxic agent eribulin induces tumor cell apoptosis through depolymerization of the cell spindle apparatus. Based on clinical data it has recently been suggested that TNBC is particularly sensitive against eribulin. The goal of this analysis was to compare (i) TNBC vs. non TNBC lines and (ii) cell lines of distinct TNBC subtypes with regard to eribulin sensitivity in vitro.

Methods:
17 established breast cancer cell lines comprising both TNBC (4 basal-like 1/2; 1 mesenchymal; 3 mesenchymal stem cell; 1 interleukin; 2 luminal AR; 1 unclassified) and non-TNBC (n=5) phenotypes were cultured and subjected to cell viability assay (MTT test), migration experiment (scratch assay), apoptosis analysis (Western Blot experiment for PARP cleavage) and quantitative RT-PCR analysis (for GABRP gene expression) after exposure to eribulin or control. Furthermore, gene expression of 8 genes known to induce malignant transformation (MMP7, ELF5, YBX1, RARRES1, PRNP, SOX 10, EGFR and GABRP) was analyzed via quantitative RT-PCR analysis in the triple negative cell line MDA-MB 231 after exposure to eribulin or control.

Results:
The effect of eribulin on the cell viability varied to a lesser extent among the TNBC compared to the non-TNBC cell lines though we could not observe a significant difference between both groups. Mentionable the TNBC cell line DU 4475 representing the interleukin phenotype displayed a significant stronger resistance to eribulin compared to all other phenotypes. A decelerated migration could be observed in the TNBC cell line MDA-MB 231 after exposure to the IC50 concentration of eribulin compared to non-treated cells. Induction of apoptosis by eribulin treatment was verified by PARP cleavage in various TNBC cell lines. GABRP known to be overexpressed especially in basal like TNBC showed a slight increase in gene expression after exposure to eribulin in various phenotypes of TNBC - most prominent in MDA-MB 231. Additionally, upregulation of ELF5 and downregulation of YBX1 and PRNP, and, to a lesser extent, of MMP7 and SOX 10 gene expression could be investigated in MDA-MB 231 after eribulin treatment.

Conclusion:
We did not observe a significant association with regard to eribulin sensitivity between TNBC and non-TNBC. Chemotherapy sensitivity varied to a lesser extent among TNBC cell lines compared to non-TNBC cell lines. Eribulin inhibits cell proliferation and migration, induces apoptosis in TNBC, and influences gene expression of overexpressed genes in TNBC known to participate in and induce malignant transformation. Though the current work did not explicitly specify one phenotype of TNBC for eribulin treatment regarding chemotherapy sensitivity, we identified possible target genes influenced by eribulin treatment, e.g. GABRP, and therefore need further investigation for a potential treatment approach combining eribulin with e.g. GABRP inhibitor.
**Title:** Expression and role of ING3 gene in breast cancer

Kalender ME, Cakir M, Ergun S, Oztuzcu S, Cengiz B, Ulasli M, Sevinc A, and Camci C. Gaziantep University Medical Faculty, Gaziantep, Turkey; Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; Gaziantep University, Gaziantep, Turkey; Gaziantep University, Gaziantep, Turkey; Gaziantep University, Gaziantep, Turkey; Gaziantep University, Gaziantep, Turkey; Faculty of Medicine, Gaziantep University, Gaziantep, Turkey; Gaziantep University, Gaziantep, Turkey; Faculty of Medicine, Gaziantep University, Gaziantep, Turkey.

**Body:** Background: Inhibitor of growth (ING) tumor suppressor gene family has been discovered over the past decade and five different genes have been identified from ING1 to ING5. They have some functions like cell transcription regulation, cell cycle control, DNA repair and apoptosis. Because of the fact that ING3 gene expression has not been studied in breast cancer so far, we aimed to determine whether there was a relationship between ING3 gene expression and breast cancer prognostic factors.

Methods: 46 female breast cancer patients in different stages were enrolled to our study. ING3 gene expressions obtained from tumoral and healthy breast tissue samples of patients were evaluated together with pathological and histological parameters.

Results: The median age of the patients was 49 years. ING3 expression rate has been significantly higher in the tumor tissue compared to normal tissue and was statistically significant (p=0.001). In estrogen receptor (ER) and progesterone receptor (PR) positive patients, gene expression ratio was significantly higher than negative ones (p<0.001 and p<0.001, respectively). ING3 expression in tumor tissues of the patients with advanced disease (stage 3-4) was detected higher than ones with early stage disease and it was found to be statistically significant at the border (p=0.048). Moreover, there were no significant changes when ING3 gene expressions were compared with c-erbB2 (Receptor tyrosine-protein kinase erbB-2) status and tumor grade (p> 0.05).

Conclusions: This study was the first study on ING3 gene expression in breast cancer. ING3 gene expression has been shown to be associated with the receptor positivity and advanced stage disease. Further studies should be conducted on the prognostic significance of ING3 gene in breast cancer.
Title: IPC-366 cell line, a canine inflammatory breast cancer (IBC), as a good model for in vitro studies on human IBC research


Body: Inflammatory breast cancer (IBC) is an aggressive type of cancer with poor survival in women. Canine IBC is clinically and histopathologically very similar to human IBC and has been proposed as a good surrogate model for study the human disease. Recently a triple negative canine IBC epithelial cell line, IPC-366 with many characteristics of the human IBC cell line SUM149, has been established. The aim of this study was to validate IPC-366 as a good model for IBC research in terms of stem cell markers expression by flow cytometry, protein production by western blot and their capacity to form tumors in vivo in SCID mice in adherent (2D) an non-adherent (3D, mammospheres) culture conditions. Our results revealed that the canine IBC cell line IPC-366 is capable of forming long-term mammospheres with a grape-like morphology. IPC-366 2D and 3D exhibited fast growth in vivo having differences in histology tumor sections. Stem cell marker expressions showed that IPC-366 in adherent and non-adherent conditions has mesenchymal-like characteristics that could be due to its aggressive and angiogenic phenotype. Epithelial-to-mesenchymal transition (EMT) markers expression, such as E-cadherin and N-cadherin, was higher in 2D than in 3D cultures, revealing that the loss of their expression is an important characteristic for forming mammospheres. In spite of this, scanning electron microscopy showed that mammospheres are formed mainly by cohesive cells and flattered cells resembling endothelial cells (attributable to vasculogenic mimicry phenomenon). These results were consistent with those found in SUM149 under the same conditions. This work is of significance because there are currently very few cell lines to study human IBC. As such, we believe that the IPC-366 cell line provides a useful vehicle to conduct basic tumor biology studies on IBC and aggressive metastatic cancer in general, but also it will be helpful for the development of potential therapeutic agents and for future interspecies comparative new therapeutic strategies against IBC/IMC.
Title: Mutational signatures impact the breast cancer transcriptome and distinguish mitotic from immune response pathways

Martens JWM WM, Smid M, Rodríguez-González G, Sieuwerts AM M, Prager-Van der Smissen WJC JC, Van Der Vlugt - Daane M, Van Galen A, Nik-Zainal S, Staaf J, Brinkman AB B, Van de Vijver MJ J, Richardson AL L, Berentsen K, Caldas C, Butler A, Martin S, Davies HD D, Debets R, Meijer-Van Gelder ME E, Van Deurzen CHM HM, Ramakrishna MR R, Ringné R, Viari A, Birney E, Børresen-Dale A-L, Stunnenberg HG G, Stratton M and Foekens JA A. Erasmus MC, Rotterdam, Netherlands; Wellcome Trust Sanger Institute, Hinxton, United Kingdom; Lund University, Lund, Sweden; Radboud University Nijmegen, Nijmegen, Netherlands; Academic Medical Center Amsterdam, Amsterdam, Netherlands; Dana-Farber Cancer Institute, Boston, MA; University of Cambridge, Cambridge, United Kingdom; Synergie Lyon Cancer, Lyon, France; European Bioinformatics Institute, Hinxton, United Kingdom and University of Oslo, Oslo, Norway.

Body: A comprehensive whole genome analysis of a large breast cancer cohort of 560 cases (Nik-Zainal et al, submitted 2015) reports novel and existing DNA substitution and rearrangement signatures next a comprehensive list of events driving the breast cancer cell to its malignant potency. In the current study, we linked the observed genetic diversity to the breast cancer transcriptome for 260 cases for which whole genome and whole transcriptome data were both available. Cluster analysis of the global gene expression showed the familiar view of a coherent basal-like and a heterogeneous luminal subgroup. New and previously reported\(^1\) subtype-specific aberrations with concordant expression changes were found in TP53, PIK3CA, PTEN, CCND1, CDH1 and GATA3, and mutations in PIK3CA, PTEN, AKT1 and AKT2 were mutually exclusive confirming they are active in the same pathway in breast cancer. Integrating the identified DNA substitutions signatures with the transcriptome, we observed that the total number of substitutions in a cancer, irrespective of substitution type, was positively associated with cell cycle regulated gene expression and with adverse outcome.

In addition and more remarkably, we observed that the number substitution of two substitution signatures\(^2\) particularly associated with immune-response specific gene expression, with increased amount of tumor infiltrating lymphocytes and with a better outcome. These two signatures comprised 1) mutations of the APOBEC-type (predominant C>G in a TCN context), and 2) mutations which lacks specific features but which are strongly associated with genetic and epigenetic inactivating aberrations in BRCA1 and BRCA2.

Thus, while earlier reports\(^3-5\) imply that the sheer number of driver events triggers an immune-response, we refine this statement by observing that substitutions of a particular type are much very effective in doing so explaining the superior outcome of cancer having these particular types of substitutions. This result also implies that purposefully augmenting T-cell reactivity against amino-acid substitutions resulting from either of these two DNA substitution types could potentially improve immunotherapies in breast cancer.

Title: Identification of tumors with an indolent disease course: MammaPrint ultralow signature validation in a retrospective analysis of a Swedish randomized tamoxifen trial

Esserman LJ, Thompson CK, Yau C, van 't Veer LJ, Borowsky AD, Tobin NP, Nordenskjöld B, Fornander T, Stål O, Benz CC and Lindström LS. University of California, San Francisco; Buck Institute for Research on Aging; University of California, Davis; Karolinska Institutet and University Hospital, Stockholm, Sweden and Linköping University, Linköping, Sweden.

Body: Background Better tools are needed to identify breast cancer patients at very low risk of dying from their disease. We applied a previously specified 'Ultralow risk' threshold for the FDA-cleared MammaPrint 70-gene expression score to predict long-term absence of breast cancer-specific mortality in a Swedish randomized trial of breast cancer patients treated with tamoxifen (Tam) versus not and followed for more than 25 years.

Methods Between 1976-1990 the Stockholm Tamoxifen (STO) trial enrolled and randomized node negative breast cancer patients with tumor size less than 30 mm to receive 2 years of Tam versus not, without regard to hormone receptor status. In the Tam-treated arm, patients without relapse at 2 years were further randomized to receive 3 additional years of Tam versus no additional endocrine therapy. From the original STO randomized trial cohort, about half (778 cases) had remaining formalin-fixed paraffin-embedded primary tumor blocks for additional tumor characterization and MammaPrint analysis. In this validation dataset, which now has >25 years follow-up, breast cancer-specific survival was assessed between MammaPrint scored 'Ultralow risk', 'Low risk' or 'High risk' categories by Kaplan-Meier analyses and multivariate Cox proportional hazard modeling, adjusting for treatment, age, period of diagnosis, tumor size, grade, receptor (ER, PR, HER2) and Ki-67 status.

Results: In this unscreened patient population MammaPrint scored 15% of patients as 'Ultra-low risk', 43% as 'Low risk' and 42% as 'High risk'. At 20 years, a statistically significant difference in survival between risk categories was seen for all patients (log rank, P=0.0001), the Tam treated arm (log rank, P=0.0088) and the untreated arm (log rank, P=0.014). Ultra-low risk patients have significantly lower risk of disease-specific death relative to Low and High risk patients in our multivariate Cox analysis. Among Ultra-low risk patients there were no deaths out to 15 years in the Tam-treated arm; by 20 years, disease-specific survival in Tam-treated and untreated arms were 94% and 90%. The majority of Tam-treated patients received only 2 years of treatment, with ~35% going on to receive 5 years of treatment. All Ultra-low risk cases were HR+HER2-. 78% were luminal A by PAM50; but only 31% of luminal A were Ultra-low risk. Invasive ductal (no-special-type) carcinomas were the most frequent, but lobular, tubular, invasive papillary and invasive cribriform subtypes were enriched and mucinous types were absent.

Conclusions: Node-negative, T<3cm breast cancer cases designated as 'Ultralow risk' by the 70-gene MammaPrint assay have minimal risk of metastatic death within the first 15 years if given a short (2+ year) course of adjuvant Tam. While a small but very late (>15 y) metastatic recurrence risk exists if left untreated, 'Ultralow risk' status could identify women for whom lumpectomy alone and a short course of adjuvant endocrine therapy is sufficient. Given the high frequency (~50%) of such 'Ultralow risk' tumors detected in modern screening cohorts (e.g. MINDACT), a substantial fraction of newly diagnosed women could benefit from more targeted and less aggressive local and adjuvant therapy.
Analysis of the International tamoxifen pharmacogenomics consortium (ITPC) dataset shows that genotyping DNA derived from tumor does not introduce CYP2D6 genotyping error or mask an association with tamoxifen efficacy

Kidwell KM M, Hertz DL L, Leyland-Jones B, Regan MM M, Dowsett M and Rae JM M. University of Michigan, Ann Arbor, MI; Avera Cancer Institute, Sioux Falls, SD; Dana-Farber Cancer Institute, Boston, MA and The Royal Marsden Hospital, London, United Kingdom.

Background: The anti-estrogen tamoxifen is metabolized into the more potent anti-estrogen, endoxifen, primarily by polymorphic CYP2D6. An association between CYP2D6 genotype and tamoxifen efficacy was assessed in a meta-analysis of 4,973 breast cancer patients by the ITPC. A subgroup analysis in 1,996 estrogen receptor (ER)-positive postmenopausal patients receiving 20 mg/day tamoxifen for 5 years (criterion 1) found CYP2D6 poor metabolizer genotype was associated with worse invasive disease free survival (IDFS; hazard ratio (HR) 1.25, 95% confidence interval (CI) 1.06-1.47, P=0.009). This meta-analysis did not include data from two prospective trials (Anastrozole, Tamoxifen, Alone or In Combination (ATAC) and Breast Intergroup (BIG) 1-98) both of which meet the patient criteria but failed to replicate the ITPC findings. Some ITPC investigators criticized ATAC and BIG 1-98 for genotyping CYP2D6 from tumor-derived DNA, positing this leads to genotyping errors detected by Hardy-Weinberg Equilibrium (HWE). However, ITPC analyses did not exclude tumor-derived CYP2D6 genotypes or report HWE. Therefore, we re-analyzed the ITPC data to investigate claims that tumor-derived genotyping causes HWE departure and masks the association between CYP2D6 genotype and tamoxifen efficacy.

Methods: The ITPC dataset was filtered to patients fulfilling criterion 1. HWE for CYP2D6*4 was analyzed in Caucasian patients (n=1,619) by study, by DNA source (tumor or blood), and in the entire subgroup. The ITPC meta-analysis was rerun stratified by DNA source using the same specifications (patients, phenotype, genotype, statistical model, etc.) as in ITPC.

Results: Significant HWE deviation for CYP2D6*4, the most common variant (MAF =0.2), was not observed in any study genotyped from tumor but was observed in one study genotyped from blood. Combining studies led to significant HWE deviations in studies genotyped from both blood and tumor, and for the entire subcohort (Table 1). Associations between CYP2D6 genotype and IDFS stratified by DNA source yielded similar, non-statistically significant, results (blood: n=933, HR=1.19, 95% CI 0.91-1.57, P=0.21; tumor: n=997, HR=1.19, 95% CI 0.99-1.44, P=0.07).

Conclusions: HWE deviations for CYP2D6*4 are not uniformly or exclusively found in studies using tumor DNA, but can occur as a statistical consequence of combining genotypes from heterogeneous populations like in the multi-institution BIG 1-98 and ATAC studies. Re-analysis of the ITPC dataset stratified by DNA source refutes the hypothesis that genotyping tumor DNA masks a pharmacogenetic association. These findings reaffirm the validity of the BIG 1-98 and ATAC analyses and support inclusion of these studies in the ITPC meta-analysis to rigorously assess the association between CYP2D6 genotype and tamoxifen efficacy.

CYP2D6*4 HWE Test in Caucasian Patients from Each ITPC Study, by DNA Source, and Combined

<table>
<thead>
<tr>
<th>DNA Source</th>
<th>Study Number</th>
<th>N</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>2</td>
<td>70</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>53</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>13</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>464</td>
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</tr>
<tr>
<td></td>
<td>9</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td>10</td>
<td>222</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>825</td>
<td>0.0004</td>
</tr>
<tr>
<td>Tumor</td>
<td>5</td>
<td>197</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>217</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>380</td>
<td>0.04</td>
</tr>
<tr>
<td>Combined</td>
<td>Total</td>
<td>794</td>
<td>0.0037</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
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<td>--------</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1619</td>
<td>0.00006</td>
</tr>
</tbody>
</table>
Title: Time-dependent nomogram for risk of locoregional recurrence in early breast cancer patients: 10 year extension

Witteveen A, Vliegen IMH MH, Sonke GS S, Klaase JM M, IJzerman MJ J and Siesling S. MIRA institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Netherlands; Netherlands Comprehensive Cancer Centre Organisation (IKNL), Utrecht, Netherlands; Center for Healthcare Operations Improvement & Research, University of Twente, Enschede, Netherlands; Netherlands Cancer Institute (NKI), Amsterdam, Netherlands and Medical Spectrum Twente (MST), Enschede, Netherlands.

Body: Background
The objective of this study was to extend the recently developed and validated time-dependent logistic regression model and web-based nomogram. This nomogram is suitable for the annual long term risk prediction of locoregional recurrence (LRR) in individual breast cancer patients and clinical decision support with regard to the follow-up.

Methods
Women first diagnosed with early breast cancer between 2003-2006 in all Dutch hospitals were selected from the Netherlands Cancer Registry with five year of recurrence follow-up (n=37,230). Of the year 2003 follow-up was retrieved for ten years. In the first five years following primary breast cancer treatment 3.7% of the selected patients developed a LRR as a first event, in ten years 6.2%. Risk factors were determined using logistic regression and the risks were calculated per year, conditional on not being diagnosed with recurrence in the previous year. Discrimination and calibration were assessed. Bootstrapping was used for internal validation. Data on primary tumors diagnosed between 2007-2008 in 43 Dutch hospitals was used for external validation of the performance of the nomogram (n=12,308).

Results
The final model included the variables grade, size, multifocality, and nodal involvement of the primary tumor, and whether patients were treated with radio-, chemo- or hormone therapy. Model predictions were well calibrated. Estimates in the validation cohort did not differ significantly from the index cohort. The results were incorporated in a web-based nomogram. In 0.7% of the patients, the risk of LRR between year 5-10 was higher than the average risk of all patients in the first five years. All of these patients were aged below 50, had a tumour size larger than 2 cm, non-negative hormone status, received radiotherapy, but no hormone therapy and 19% developed a recurrence during ten years.

Conclusion/discussion
This validated and time-dependent nomogram for the prediction of annual LRR risks over ten years is simple to use and shows a good predictive ability in the Dutch population. It can be used as an instrument to identify patients with a low or high risk of LRR who might benefit from a less or more intensive and longer follow-up after breast cancer and to aid clinical decision-making for personalized follow-up.
Title: Differential classification of dense tissue and risk of breast cancer by clinical breast density measures

Vachon CM, Scott CG, Ma L, Mahmoudzadeh AP, Jensen MR, Whaley DH, Wu FF, Malkov S, Hruska CB, Norman AD, Heine J, Shepherd J, Pankratz VS, Kerlikowske K and Brandt KR. Mayo Clinic, Rochester, MN; University of California San Francisco, San Francisco, CA; Moffitt Cancer Center, Tampa, FL and University of New Mexico School of Medicine, Albuquerque, NM.

Body: Background: Increased breast density decreases mammographic sensitivity and is an established breast cancer (BC) risk factor. For these reasons, 22 US state governments mandate that women be informed if they have dense breasts and the corresponding implications for masking and risk. Determining the appropriate density measure for the clinical setting, then, is important. We compare the classification of breast density by two commercially available volumetric breast density measures for full field digital mammography (FFDM), Volpara™ and Quantra™, and the radiologists' BI-RADS clinical density. We also assess and compare the corresponding associations of these measures with BC risk based on mammograms prior to the time of BC diagnosis.

Methods: We examined 1911 cases and 4170 age-matched controls from two BC case-control studies nested within large FFDM screening mammography practices. All participants had clinical risk factors and raw or “for processing” screening mammograms from Selenia-Hologic machines at least 6 months (average 2.2 years) prior to the cancer diagnosis (or corresponding date for controls). We retrieved the clinical BI-RADS density (defined in the BI-RADS, 4th edition: almost entirely fatty breasts (<25% glandular), scattered fibroglandular densities (25-50% glandular), heterogeneously dense (51-75% glandular) and extremely dense (>75% glandular)) and measured BI-RADS-like density, volumetric percent density (VPD) and dense volume (DV) from the Volpara™ and Quantra™ programs. Agreement between BI-RADS measures was assessed with weighted kappa (κ) statistics. BC associations were evaluated using conditional logistic regression, adjusting for age and body mass index. Odds ratios (OR), C-statistics (C) and 95% Confidence Intervals (95% CI) were estimated.

Results: Agreement of Volpara™ and Quantra™ BI-RADS-like and clinical BI-RADS density was moderate: clinical and Volpara™ (κ = 0.57, 95% CI 0.55-0.59); clinical and Quantra™ (κ = 0.46, 95% CI 0.44-0.47). We found differences of up to 14% in dense breast classification (heterogeneously and extremely dense categories), with Volpara™ defining 51% of women having dense breasts, Quantra™ 37%, and clinical BI-RADS 43%. The clinical and automated measures showed similar BC associations; OR's for extremely dense vs. scattered fibroglandular densities were 1.8 (95% CI 1.5-2.2), 1.9 (95% CI 1.5-2.5) and 2.3 (95% CI 1.9-2.8) for Volpara™, Quantra™ and clinical BIRADS. Clinical BI-RADS density showed better discrimination of BC status (C = 0.60; 95% CI: 0.58 – 0.61), than Volpara™ (C = 0.58; 95% CI: 0.56 – 0.59, p=0.03) and Quantra™ (C = 0.56; 95% CI: 0.54 – 0.58; p<0.001) BIRADS-like measures. Quintiles of both VPD and DV were positively associated with BC: OR = 1.3 per quintile for VPD and OR = 1.2 DV for Volpara™; OR = 1.2 per quintile for VPD and 1.1 DV for Quantra™. ORs for VPD were significantly stronger than those seen with DV for both Volpara™ (P = 0.002), and Quantra™ (P<0.001).

Conclusion: Automated and clinical assessments of breast density are positively associated with BC risk but differ in the proportion of women classified with dense breasts. This could have a significant impact on practice patterns, women's anxiety, and healthcare costs.
Publication Number: P6-09-05

Title: No evidence of association between mammographic breast density and risk of breast cancer in women with atypical hyperplasia


Body: Background

Women with atypical hyperplasia (AH) are at an approximately four-fold increased risk of subsequent breast cancer (BC). Mammographic breast density (MBD) is a well-established risk factor for BC, but its contribution to BC risk in women with AH remains an open question. We previously reported no association between MBD [measured by Wolfe's parenchymal pattern (PP)] and BC risk in a cohort of 147 women with AH. Here, we present results in an expanded cohort of 459 women diagnosed with AH between 1985 and 2001.

Methods

The Mayo Clinic Benign Breast Disease Cohort includes 13,485 women who had benign core and/or excisional biopsy 1967-2001. Biopsy tissues were reviewed by our study pathologist to determine presence of AH. MBD was available from clinical records starting in 1985, coded as PP (the standard for 1985-1996) or BI-RADS (1997-2001) density criteria. The original four-level PP (N1-fatty, P1-ductal prominence <25% of breast, P2-ductal prominence >25%, DY-dysplasia) and BI-RADS (fatty, scattered densities, heterogeneously dense, extremely dense) measures were re-categorized as low, moderate or high MBD by combining the middle two categories for each. BC events and clinical information were obtained by questionnaires, medical records and the Mayo Clinic Tumor Registry. Women were followed from benign biopsy to date of BC, death or last contact. Standardized incidence ratios (SIRs) were generated overall and within subgroups defined by density measure (PP vs. BI-RADS), number of atypical foci, and BMI by dividing the observed number of BCs by population-based expected values. Cox regression was used to estimate MBD hazard ratios after adjustment for demographic and clinical variables.

Results

Of the 551 women diagnosed with AH between 1985 and 2001, 459 (83%) had MBD data within 1 year prior to biopsy. Of these, 68 (15%) had low, 221 (48%) had moderate, and 170 (37%) had high MBD, respectively. Over a median follow-up of 11.7 years, 80 BCs were observed. SIRs for breast cancer did not differ significantly across density categories, overall or within any subgroups examined (see Table). Cox regression adjusting for age, BMI and density measure (PP vs. BI-RADS) also failed to identify an association with MBD (p=0.55).

<table>
<thead>
<tr>
<th></th>
<th>Low MBD</th>
<th>Moderate MBD</th>
<th>High MBD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N / BCs</td>
<td>SIR (95% CI)</td>
<td>N / BCs</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>68/12</td>
<td>3.5 (1.8,6.1)</td>
<td>221/39</td>
</tr>
<tr>
<td><strong>MBD Measure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>59/11</td>
<td>3.6 (1.8,6.5)</td>
<td>85/15</td>
</tr>
<tr>
<td>BI-RADS</td>
<td>9/1</td>
<td>2.7 (0.1,14.7)</td>
<td>136/24</td>
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<tr>
<td><strong>No. Atypical Foci</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47/6</td>
<td>2.3 (0.9,5.1)</td>
<td>123/18</td>
</tr>
<tr>
<td>2</td>
<td>14/4</td>
<td>7.5 (2.0,19.1)</td>
<td>58/13</td>
</tr>
<tr>
<td>3+</td>
<td>7/2</td>
<td>6.7 (0.8,24.0)</td>
<td>40/8</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>25/2</td>
<td>1.5 (0.2,5.4)</td>
<td>75/18</td>
</tr>
<tr>
<td>25-29</td>
<td>19/5</td>
<td>4.9 (1.6,11.3)</td>
<td>68/9</td>
</tr>
<tr>
<td>30+</td>
<td>23/5</td>
<td>4.8 (1.6,11.2)</td>
<td>76/12</td>
</tr>
</tbody>
</table>
SIRs compare observed number of BCs to expected using Iowa SEER data. Analyses account for the effects of age and calendar period. P-value is test of heterogeneity in SIRs across columns.

Conclusions
We found no evidence of an association between MBD and subsequent BC in women with AH.
**Title:** Effects of reproductive risk factors for ductal carcinoma in situ, invasive ductal carcinoma, and invasive ductal carcinoma with ductal carcinoma in situ on clinical outcomes

Lee J, Lee J and Oh M. College of Medicine, Dong-A University, Busan, Republic of Korea; Haeundae Paik Hospital, College of Medicine, Inje University, Clinical Trial Cancer, Busan, Republic of Korea and College of Medicine, Inje University, Clinical Trial Cancer, Kimhae, Republic of Korea.

**Body:** Background: It has been well investigated with regards to reproductive risk factors of invasive ductal carcinoma (IDC), yet it is still controversial if these factors could be applicable for either ductal carcinoma in situ (DCIS) patients or invasive ductal carcinoma with ductal carcinoma (DCIS-IDC) patients. We aimed to investigate effects of reproductive risk factors on clinical outcomes of IDC, DCIS, and DCIS-IDC patients who received proper treatments. **Method:** A total of 37,049 patients of IDC, and DCIS who were registered in the web-based breast cancer registration program of the Korean Breast Cancer Society (KBCS), was assessed with a retrospective design. All patients were classified into three categories: 1) patients with pure DCIS, 2) patients with IDC with DCIS (DCIS-IDC), and 3) patients with pure IDC that is less than 1 cm without lymph node metastasis; overall survival (OS) and breast cancer specific survival (BCSS) of each group in response to parity, age at first birth (AFB), breast feeding, time interval between AFB and diagnosis of breast cancer, time interval between menarche and AFB were analyzed via the multivariate Cox regression analysis was performed. **Results:** The high parity (≥4) considerably elevated the hazard ratio (HR) of OS in three groups (DCIS; [HR], 1.52; 95% confidence interval [CI], 0.615-3.778; P<.0001; IDC; HR, 1.43; 95% CI, 0.885-2.314; P<.0001, and DCIS-IDC; [HR], 1.44; 95% confidence interval [CI], 0.452-4.594; P<.005) yet the parity influenced on the BCSS differently in the IDC group and the DCIS-IDC group. Meanwhile, in the DCIS-IDC group, the HR was elevated in which patients gave birth to 4 children whilst the HR of BCSS was lowered if they gave birth to either 1-2 children or more than 5 children. The AFB significantly reduced the HR of OS in the DCIS group and IDC group compared to nulliparous patients. In patients who breast-feed, the HRs of OS and BCSS were shown to be significantly elevated in the IDC group and DCIS group (HR of OS in IDC, 1.486; 95% CI, 1.212-1.821; P=.0001 and HR of OS in DCIS, 2.036; 95% CI, 1.267-2.271; P=.0033; and HR of BCSS in IDC, 1.474; 95% CI, 1.111-1.957; P=.0072, HR of BCSS in DCIS, 3.362; 95% CI, 1.18-9.56; P=0.023)(Table 3). Considering the time interval between AFB and age at diagnosis, the HR of OS was considerably elevated in all three groups (HR of DCIS, 1.067; 95% CI, 1.050-1.085; P<.0001, HR of IDC, 1.041; 95% CI, 1.034-1.049; P<.0001, HR of DCIS-IDC, 1.031; 95% CI, 1.008-1.054; P=0.008) yet the HR of BCSS was only influenced in the IDC group (HR, 1.027; 95% CI, 1.016-1.037; P<.0001). When it comes to the time interval between menarche and AFB, the HR of OS was significantly elevated in the group of DCIS (HR, 1.053; 95% CI, 1.007-1.100; P<.0219). **Conclusion:** The breast feeding history and age gap between AFB and diagnosis were found to be breast cancer risk factors that might be different from generally accepted trends, reducing occurrence of breast cancers. Taken altogether, we were able to demonstrate that known reproductive risk factors, such as AFB and parity, influence on clinical outcomes differently amongst the IDC group, the DCIS group, and the DCIS-IDC group.
Title: Reproductive factors and hormone receptor status among very young (<35 years) breast cancer patients

Jia X, Liu G, Shen Z and Shao Z. Key Laboratory of Breast Cancer in Shanghai, Fudan University Shanghai Cancer Center, Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: The prognosis for breast cancer occurs in young women is usually poor. The impact of different reproductive factors on disease characteristics is still largely unknown.

Patients and methods: We analyzed 261 patients aged ≤35 years old who were treated at the Cancer Hospital of Fudan University, Shanghai, China. The relationships between certain reproductive factors (age at menarche, parity, number of children, breastfeeding, history of abortion, age at first full-term pregnancy and oral contraceptive (OC) use) and disease characteristics were evaluated.

Results: Compared with patients who experienced fewer full-term pregnancies (<2 times), the patients with more full-term pregnancies (≥2 times) exhibited higher percentage of ER-positive tumors (61.5%) (P=0.015)

Table1. Impact of full-term pregnancy on disease characteristics

<table>
<thead>
<tr>
<th>Number of full-term pregnancies</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤25</td>
<td>2(33.3)</td>
<td>4(66.7)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0.037</td>
</tr>
<tr>
<td>26-30</td>
<td>9(16.1)</td>
<td>45(80.4)</td>
<td>2(3.5)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>31-35</td>
<td>10(6.3)</td>
<td>124(78.5)</td>
<td>23(14.6)</td>
<td>1(0.6)</td>
<td></td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.583</td>
</tr>
<tr>
<td>Positive</td>
<td>10(7.5)</td>
<td>109(82)</td>
<td>13(9.8)</td>
<td>1(0.7)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6(9.1)</td>
<td>50(75.8)</td>
<td>10(15.1)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.826</td>
</tr>
<tr>
<td>I</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>17(10)</td>
<td>135(79)</td>
<td>18(10.5)</td>
<td>1(0.5)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4(8.2)</td>
<td>38(77.6)</td>
<td>7(14.2)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.222</td>
</tr>
<tr>
<td>I</td>
<td>7(13.7)</td>
<td>39(76.5)</td>
<td>5(9.8)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>13(8.9)</td>
<td>116(79.5)</td>
<td>17(11.6)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1(4.8)</td>
<td>16(76.2)</td>
<td>3(14.3)</td>
<td>1(4.7)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0(0)</td>
<td>2(100)</td>
<td>0(0)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>ER status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Positive</td>
<td>17(14.5)</td>
<td>83(70.9)</td>
<td>16(13.7)</td>
<td>1(0.9)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>4(3.9)</td>
<td>90(87.4)</td>
<td>9(8.7)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>PR status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.207</td>
</tr>
<tr>
<td>Positive</td>
<td>14(11.6)</td>
<td>89(73.6)</td>
<td>17(14)</td>
<td>1(0.8)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7(7.1)</td>
<td>84(84.8)</td>
<td>8(8.1)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>HER2/neu status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.401</td>
</tr>
<tr>
<td>Positive</td>
<td>5(8.5)</td>
<td>47(79.7)</td>
<td>6(10.2)</td>
<td>1(1.6)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>16(9.9)</td>
<td>126(78.3)</td>
<td>19(11.8)</td>
<td>0(0)</td>
<td></td>
</tr>
</tbody>
</table>
...and patients whose age of menarche was ≥15 years exhibited a greater chance of PR-positive tumors (64.8%) (P=0.036) compared with those whose age of menarche was <15 years old. Additionally, patients who had taken OCs were more likely to present with late-stage tumors (IIstage or later) (87.5%) (P=0.002) than patients who had never taken OCs. Conclusions: Our study provides evidence that women with more full-term pregnancies and later age at menarche are more possible to exhibit hormone receptor-positive tumors. Additionally, patients who have taken OCs are more likely to present with advanced disease.
Title: Exposure to multiple sources of polycyclic aromatic hydrocarbon and breast cancer incidence

White AJ J, Bradshaw PT T, Herring AH H, Teitelbaum SL L, Beyea J, Stellman SD D, Steck SE E, Mordukhovich I, Eng SM M, Engel LS S, Conway K, Hatch M, Neugut AI I, Santella RM M and Gammon MD D. University of North Carolina at Chapel Hill; Ichan School of Medicine at Mt. Sinai; Consulting in the Public’s Interest; Columbia University; University of South Carolina and National Cancer Institute.

Body: Background. Previous epidemiologic studies, including our own, have consistently linked long-term exposure to single-source polycyclic aromatic hydrocarbons (PAHs) to increased breast cancer incidence. It is unclear whether single sources, specific groups, or all PAH sources should be targeted for breast cancer risk reduction. This study considers the impact on breast cancer incidence from multiple PAH exposure sources in a single model, which better reflects exposure to these complex mixtures.

Methods. In a population-based case-control study conducted on Long Island, New York (N=1,508 breast cancer cases/1,556 controls), a Bayesian hierarchical regression approach was used to estimate adjusted posterior means and credible intervals (CrI) for the adjusted odds ratios (ORs) for PAH exposure sources, considered singly and as groups: active smoking; residential environmental tobacco smoke (ETS); indoor and outdoor air pollution; and grilled/smoked meat intake.

Results. Most women were exposed to PAHs from multiple sources. In a hierarchical model, breast cancer incidence was positively associated with ETS from a spouse (OR=1.20, 95%CrI=1.03, 1.42) and residential synthetic firelog burning (OR=1.30, 95%CrI=1.06, 1.60). Additionally, PAH exposure groups, including ingestion (OR=1.45, 95%CrI=1.16, 1.79), indoor stove/fireplace use (OR=1.30, 95%CrI=1.02, 1.62), and total indoor sources (active smoking, ETS from spouse, grilled/smoked meat intake, stove/fireplace use, OR=1.46, 95%CrI=1.03, 2.05), were associated with increased breast cancer incidence.

Conclusions. Groups of PAH sources, especially those for ingestion and indoor sources, were associated with a 30-50% increase in breast cancer incidence. PAH exposure is ubiquitous and a potentially modifiable breast cancer risk factor.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-09-09

Title: Abstract Withdrawn

Body:
Title: All-cause survival estimates compared to observed survival in older women with breast cancer in CALGB 49907 and 369901 (Alliance A151503)

Kimmick G, Pitcher B, Mandelblatt J, Clapp J, Ballman K, Barginear M, Freedman R, Artz A, Klepin H, Lafky J, Hopkins J, Winer E, Hudis C, Muss H, Cohen H, Jatoi A and Hurria A. Duke University Medical Center, Durham, NC; Georgetown University; North Shore Health System; Dana Farber Cancer Institute; University of Chicago; Wake Forest University School of Medicine; Mayo Clinic; Forsyth Regional Cancer Center; Memorial Sloan Kettering Cancer Center; UNC Lineberger Comprehensive Cancer Center and City of Hope.

Body: Background: Older adults represent 50% or more of all newly diagnosed cancer patients annually; these patients have multiple morbidities, complicating treatment decision-making. Discussions about the risks and benefits of cancer treatments might be improved by having data on estimated all-cause survival. ePrognosis (http://eprognosis.ucsf.edu/carey2.php) is an online tool validated in older adults without cancer. We compared survival estimates using ePrognosis to observed survival in a population of women with early stage breast cancer who volunteered for cooperative group studies.

Methods: Participants in CALGB 49907 (n=194) and 369901 (n=809) who were age 70+ were included (total n=1003). Both studies had comparable eligibility: primary, newly diagnosed, invasive, non-metastatic breast cancer. In 49907, eligibly also included PS 0-2; in 369901 there were no PS restrictions, but women who failed a screening cognitive exam were excluded. The Carey 2-year Index from ePrognosis was used to estimate all-cause 2-year survival, based on age, sex, and daily function. Function (needing help from another person to bath and shop for groceries, difficulty walking several blocks and pushing or pulling a heavy object) was derived from the EORTC QLC-30. The Carey index from ePrognosis generates scores from 1-10, with higher scores indicating higher probability of death. Kaplan-Meier methods were used to obtain point estimates and confidence intervals for the observed 2-yr survival. A two sided z-test was used to test the hypothesis that the observed survival rate is equivalent to the predicted survival rate.

Results: At two years from study entry, 921 women were alive; 56 had died, and 26 were lost to follow-up/withdrawn. The population was, on average, 76 years old (SD 4.8), primarily white (89.3%), and the majority had hormone receptor positive tumors (79.4%). In our population, the Carey 2-years index predicted survival was not significantly different than observed rates in the 0-2 points and underestimated the survival rates for patients who had 3-6 points and 7-10 points.

<table>
<thead>
<tr>
<th>ePrognosis Prediction</th>
<th>49907 &amp; 369901 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Points</td>
<td>Predicted Probability of Survival</td>
</tr>
<tr>
<td>0-2</td>
<td>95%</td>
</tr>
<tr>
<td>3-6</td>
<td>88%</td>
</tr>
<tr>
<td>7-10</td>
<td>64%</td>
</tr>
</tbody>
</table>

Conclusions: In this population of older women with breast cancer, using a few readily available data items, ePrognosis provided accurate survival estimates for women with a low probability of death (0-2 points) and underestimated all-cause survival in women with an increased probability of death (3-10 points). Further studies are needed to assess the validity of this tool in samples of cancer patients with higher risks of 2-year mortality. Extended follow-up to validate the tools in predicting 5- and 10-year all-cause and non-cancer mortality risk will further contribute to decision making in older patients.
Title: Independent prognostic value of age depends on breast cancer subtype


Background: Young women present more often with aggressive breast cancer phenotypes and have worse prognosis. It remains controversial whether age is an independent prognostic factor in early stage breast cancer. Arbitrarily chosen age cut-off values have been proposed in different studies. Furthermore, few studies have examined the impact of breast cancer subtypes on the prognostic value of age. This abstract represents an update of a prior analysis (San Antonio Breast Cancer Symposium, December 4-8 2012, P06-07-29).

Methods: We included all primary operable female breast cancer patients from our prospectively managed database in UZ Leuven, Belgium. We assessed the effect of age on locoregional free interval (LRRFI), distant metastasis interval (DMFI) and breast cancer specific survival (BCSS). In univariate analysis, using Cox regression models, we determined the best categorization of age at diagnosis into two or three age groups by considering all possible combinations of cut-off values. Best categorization was obtained with three age groups. We further determined, using multivariate analysis (correcting for phenotype, tumor size, nodal status, adjuvant chemo -, hormone – and radiotherapy, type of surgery and procedure of axillary staging), whether age at diagnosis remains an independent predictor of outcome (LRRFI, DMFI and BCSS). We further explored whether age at diagnosis is an independent predictor of event risk (LRRFI, DMFI and BCSS) in different breast cancer subtypes. Luminal A-like (grade I or II, ER and/or PR positive, HER 2 positive), Luminal B-like (idem but grade III), Luminal HER 2 like (ER and/or PR positive, HER 2 positive), triple negative (ER/PR negative, HER 2 negative).

Results: We included 4180 patients with a mean/median age of 58/57 year and with a median follow up of 8.9 year. Multivariate analysis confirmed age as an independent prognostic variable for LRRFI, DMFI and BCSS.

Results multivariable analysis with age in 3 groups (HR (95% CI) P-VALUE)

<table>
<thead>
<tr>
<th></th>
<th>LRRFI</th>
<th>DMFI</th>
<th>BCSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Youngest versus middle</td>
<td>1.61 (1.18-2.18) 0.0025</td>
<td>1.54 (1.23-1.93) 0.0002</td>
<td>1.72 (1.26-2.36) 0.0007</td>
</tr>
<tr>
<td>Youngest versus oldest</td>
<td>3.45 (1.85-6.45) 0.0001</td>
<td>1.25 (0.89-1.77) 0.1982</td>
<td>1.31 (0.85-2.02) 0.2220</td>
</tr>
<tr>
<td>Middle versus oldest</td>
<td>2.15 (1.22-3.79) 0.0082</td>
<td>0.81 (0.61-1.09) 0.1706</td>
<td>0.76 (0.55-1.06) 0.1014</td>
</tr>
</tbody>
</table>

We found optimal cut-off values for LRRFI at 44y and 72y, for DMFI at 47y and 71y and for BCSS at 41y and 70y.

In an exploratory analysis, with age as continuous variable, by subtype we found a significant independent association between age and LRRFI (P=0.0169), DMFI (P=0.0344) in luminal A-like, LRRFI (P=0.0022) in luminal B-like and DMFI (P=0.0010) and BCSS (P=0.0053) in triple negative breast cancer. No significant associations were found in luminal HER2 and HER2 like breast cancers.

Conclusion:
This study has shown that young age is an independent prognostic factor for LRRFI, DMFI and BCSS after correction for the most important clinical prognostic factors. The prognostic effect is most important in luminal A and triple negative subtypes. Additional analyses for subtypes with age as a categorical variable will be performed and optimal cut off values will be defined.
Title: Breast cancer characteristics and the levonorgestrel intrauterine device. A monocentric retrospective study


Body: OBJECTIVE: The levonorgestrel-intrauterine device (LNG-IUD) is a widely used contraceptive method. It is not clear if LNG-IUD users are more likely to develop breast cancer. Breast cancer growth through the estrogen and/or the human epidermal growth factor receptor 2 (HER2) pathway could be influenced by a continuous low systemic dose of levonorgestrel. In this study, we compare breast cancer characteristics and the receptor expression of estrogen (ER), progesterone (PR) and HER2 in women with and without a LNG-IUD at the time of diagnosis.

METHODS: In this retrospective, observational study, we included 2599 consecutive breast cancer patients who were younger than 55 years at diagnosis and treated between 2000 and 2014 in the University Hospitals Leuven for a primary invasive, non-metastatic tumor. The non LNG-IUD group was matched by age and parity at diagnosis. ER, PR and HER2 status were reported according to ASCO/CAP guidelines. The Chi-square test was used to compare receptor status between groups. All tests were two-sided, and a 5% significance level was assumed. An additional analysis was performed to detect the occurrence of HER2 expression with or without intake of oral contraception by diagnosis in the control group.

RESULTS: 366 LNG-IUD users and 2233 women without a LNG-IUD were included. Compared to the control group, the LNG-IUD users had a lower Nottingham prognostic index (4.2 vs 4.4; p=0.048), more PR expression (79.2% vs 73.4%; p=0.021) but less HER2 expression (11.6% vs 17.2%; p=0.009). A significant higher rate of ER+PR+HER2- was observed in the LNG-IUD group (63.26 % vs 73.46%; p<0.001). These differences in receptor expression were mainly observed in the age group 45-49 years at diagnosis. Additionally, a trend of more HER2 positivity associated with oral contraceptive use was noticed in the control group.

CONCLUSION: We found in a breast cancer population, matched for age and parity, significant differences in the PR and HER2 expression according to use of LNG-IUD at time of diagnosis. ER positive, PR positive and HER2 negative breast cancers are more frequently seen in LNG-IUD users. There is a trend of less HER2 positivity in LNG-IUD users and it is more common seen in oral contraception users.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-09-13

Title: Multi-institutional evaluation of women at high-risk for developing breast cancer


Body: Background
Well-established risk factors for breast cancer (BC) include family history, BRCA mutations and biopsies with atypical hyperplasia (AH) or lobular carcinoma in situ (LCIS). Several institutions have registries of these high-risk women but outcomes from these registries require large numbers and long follow-up. We sought to compare characteristics between high-risk populations and evaluate early outcomes.

Methods
Women enrolled in IRB-approved high risk registries at NYU Langone Medical Center (NYU) and University of Vermont (UVM) were evaluated for risk category, uptake of prevention and development of breast cancer. Descriptive statistics were used to summarize the data and Pearson's Chi-Square and Fisher's Exact Tests were performed to compare the variables of interest among the two high risk registries.

Results
Between 2003-14, 1035 women enrolled in these high risk registries. There were significant differences in age and risk characteristics but we found a 99% concordance of variables collected between both high risk registries. Among all risk groups there was a low uptake of prevention opportunities, with 8% taking chemoprevention and 7% undergoing risk-reducing surgeries. Women with AH/LCIS accounted for 66% of those choosing chemoprevention while women with BRCA mutations accounted for 76% of those undergoing risk-reducing surgeries. To date, 43 women (4%) have been diagnosed with breast cancer. 86% were diagnosed with stage 0-1 disease and 70% had moderate or poorly differentiated cancers. There was no significant difference in background risk characteristics when comparing those with breast cancer to those who have not yet developed breast cancer.

Table 1. Clinicopathologic Characteristics between UVM and NYU

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>UVM (N=496, 48%)</th>
<th>%</th>
<th>NYU (N=539, 52%)</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIAN AGE (years)</td>
<td>46 (20-75)</td>
<td></td>
<td>50 (20-87)</td>
<td></td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>RISK FACTORS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One or more 1° relative with BC</td>
<td>442</td>
<td>89</td>
<td>286</td>
<td>53</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>BRCA positive</td>
<td>25</td>
<td>5</td>
<td>92</td>
<td>17</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>AH</td>
<td>63</td>
<td>13</td>
<td>245</td>
<td>45</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>LCIS</td>
<td>22</td>
<td>4</td>
<td>112</td>
<td>21</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>UPTAKE OF BC PREVENTION METHODS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemoprevention</td>
<td>27</td>
<td>5</td>
<td>54</td>
<td>10</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Prophylactic bilateral mastectomy</td>
<td>1</td>
<td>0.2</td>
<td>45</td>
<td>8</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Bilateral salpingo-oophorectomy</td>
<td>1</td>
<td>0.2</td>
<td>42</td>
<td>8</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>DEVELOPED BC</td>
<td>31</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>STAGE OF BC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>42</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>I</td>
<td>26</td>
<td>84</td>
<td>6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>IIA, IIB</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>IIIA, IIIB, IIIC</td>
<td>3</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions
Despite the low uptake of chemoprevention and risk reducing surgery, only 4% of patients went on to develop breast cancer in the study period. The majority of cancers involved moderate or high-grade lesions and were early stage, suggesting a benefit to participation in surveillance programs. We have demonstrated a high degree of concordance between high risk registries, suggesting no barriers to multi-institutional collaboration. High risk registries represent an important resource for studies into methods to prevent breast cancer and improve outcomes from this disease.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-09-14

Title: Awareness of breast cancer risk factors among lay persons and physicians

Morère J-F, Viguier J, Blay J-Y, Touboul C, Lhomel C, Eisinger F and Pivot X. Hôpital Paul Brousse, Villejuif, France; Hôpital Bretonneau, Tour, France; Centre Léon Berard, Lyon, France; KantarHealth, Montrouge, France; Roche, Boulogne-Billancourt, France; Institut Paoli Calmette, Marseille, France and CHU Besançon, Besançon, France.

Body: Background
The EDIFICE surveys have been conducted every three years since 2005. The aim of the surveys is to provide a better understanding of the participation of the French population in cancer screening programs. The breast cancer screening program is nowadays widely implemented throughout the female population aged 50-74 years; however, the question of whether it could be adapted according to breast cancer risk factors is currently under debate. This analysis focuses on awareness of the nature of breast cancer risk factors among the lay population and physicians.

Methods
This fourth nationwide observational survey, EDIFICE 4, was conducted by phone interviews using the quota method. A representative sample of 1602 individuals aged between 40 and 75 years old was interviewed between June 12 and July 10, 2014. A mirror survey on a representative sample of 201 general practitioners (adjusted for age and geographical area) and 100 oncologists (adjusted for type of healthcare institution and geographical area) was conducted between July 9 and August 8, 2014. Interviewees were all asked to cite five main risk factors for breast cancer.

Results
For lay persons (737 women with no history of cancer), the breast cancer risk factors reported were: for 54%, heredity and family history; for 29%, unhealthy lifestyle, including smoking, poor diet, stress, alcohol, physical inactivity; for 15%, exposure to exogenous hormone therapy; for 4%, air pollution; for 4%, sunburn on breasts; for 4%, late childbearing or no childbearing, and for 2%, overweight/obesity. Among physicians (70 female general practitioners and 35 female oncologists), the breast cancer risk factors reported were: for 98%, heredity and family history; for 51%, exposure to exogenous hormone therapy; for 39%, late childbearing or no childbearing; for 32%, unhealthy lifestyle, including smoking, poor diet, stress, alcohol, physical inactivity; for 22%, overweight/obesity; for 20%, age; for 22%, no breast feeding, and for 2%, air pollution.

Conclusion
We observed a relatively satisfactory level of understanding regarding the different risk factors for breast cancer despite the lack of indication of any qualitative ranking. Although overweight is a known risk factor for breast cancer, this fact is still not clearly understood among physicians and not widely known by the general public. On the other hand, both physicians and also half of the lay population were well aware of the fact that heredity is a risk factor for breast cancer.
Holmberg C, Bandos H, Fagerlin A, Bevers TB B, Battaglia TA A, Wickerham DL and McCaskill-Stevens W. NSABP; Berlin School of Public Health, Charite; University of Pittsburgh; Center for Bioethics and Social Sciences in Medicine, University of Michigan and VA Ann Arbor Center for Clinical Management Research; The University of Texas M.D. Anderson Cancer Center; Boston Medical Center and Boston University School of Medicine; Allegheny Cancer Center at Allegheny General Hospital and Community Oncology and Prevention Trials Research Group, Breast Cancer Prevention, Division of Cancer Prevention, National Cancer Institute.

Body: Background:
Tamoxifen and raloxifene are two selective receptor modulators (SERMs) that have been shown to reduce the risk of developing breast cancer in women at increased risk of the disease. Both drugs are infrequently used in the general U.S. population. Increased knowledge about the risks and benefits of SERM use for breast cancer risk reduction does not lead to increased uptake of chemoprevention. We know little about what influences decision-making regarding breast cancer risk reduction with SERMs.

Methods:
To better understand what influences SERM decision-making for breast cancer risk reduction we conducted a survey study assessing social, environmental, and psychological factors that may influence a woman's decision. Women who talked to a health care provider (HCP) about SERM use (N=1,023) received a questionnaire immediately after the HCP visit that asked about the counseling session, sociodemographics, experiences with breast cancer, breast cancer risk, and risk perception. After its completion a second survey was administered that inquired about issues surrounding medication intake such as attitudes about taking medicines in general, trust in pharmaceutical companies, and in their HCP. A statistical comparison of survey responses was performed between those who decided to take a SERM and those who decided not to take a SERM. Logistic regression was used to determine a key set of independent factors associated with the decision.

Results:
Of the 1,023 women, 716 made a decision about SERM intake (70%) and were included in the study. Of those, 324 (45%) decided to take a SERM and 392 (55%) decided not to take a SERM. Of SERM users 89.8% received a recommendation to take a SERM by the HCP compared to 44.4% of non-users. Only 15.7% of SERM users reported never having had a breast biopsy compared to 26.3% of non-users. Overall, SERM users had a higher breast cancer risk, risk perception, and worry about getting breast cancer. In multivariate analysis 11 factors were identified as having independent association with SERM use, including: recommendation from HCP, attitudes and perceptions regarding medication intake, influenced by someone's breast cancer diagnosis, breast cancer worry, trust in HCP, a diagnosis of atypical hyperplasia, and others' experiences with SERM intake. Women who had one or more first degree relatives with breast cancer were less likely to take a SERM. Neither breast cancer risk nor risk perception influenced SERM decision-making.

Discussion:
Factors that influence SERM decision-making are related to women's personal experiences with breast cancer, their HCP, and attitudes towards medications.

Conclusions:
Social, environmental, and psychological factors proved to be more important for SERM decision-making than breast cancer risk or risk perception. In addition to presenting risks and benefits in counseling, the importance of personal experiences and attitudes for decision-making need to be considered to understand and support women's decision-making on SERM use for breast cancer risk reduction.

SUPPORT: U10CA37377, -69974; -180868, -180822; -189867.
Title: Patient rather than physician-related barriers to tamoxifen uptake for breast cancer prevention in high-risk women

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Body: Background: Tamoxifen use for 5 years confers 35% risk reduction in breast cancer at 15 years, although the evidence to support its use for BRCA1 or BRCA2 mutation carriers is less clear. Since 2014, it has been our policy to discuss and recommend endocrine prevention to every eligible individual who attends the risk management clinic (for gene carriers or women with a strong family history) at our institution.

Aim: To assess the uptake of endocrine prevention in a high risk population when uniformly recommended and reasons for declining prevention.

Methods: All patients attending risk management clinic between February 2014 until May, 2015 received both verbal and written evidence-based information on endocrine prevention and were registered prospectively. Endocrine prevention use and cessation was captured. Reasons for declining or ineligibility for tamoxifen use were recorded.

Results: During the study period, 314 consultations were carried out on 248 women. They comprised 38 BRCA1 and 42 BRCA2 mutation carriers, 4 Peutz-Jegher syndrome patients, 155 women with strong family history, 9 with moderate family history. Their median age was 39.7 (range: 21-70 years). Despite routine policy, Tamoxifen prevention was routinely discussed, except where a patient required biopsy or was given biopsy results n=14 (7%). One patient had a new cancer diagnosis during this period. In 52 women, tamoxifen was not recommended for the following reasons: patients re-assessed as moderate risk (9), on other prevention trials (2), very young/old age (28), pregnant or breast-feeding (10) and previous mastectomy/cancer/DCIS (3). Of the remaining 182 eligible patients, 14 (8%) were on tamoxifen (median duration 12 months, range= 1-20 months), 8 (4%) had ceased tamoxifen due to side effects. Main reasons for declining were 25 (14%) were trying to conceive, 34 (19%) prefer prophylactic mastectomy, 15 (8%) were concerned regarding the side effects, 5 (3%) were currently suffering from menopause and 4 (2%) wished to await salpingo-oophorectomy. 66 (36%) indicated that they were 'not interested'.

Conclusions: Physician-reluctance is not the reason for poor uptake of endocrine prevention in a specialized risk management clinic. Contraindications are common, many women elect for alternative risk reducing strategies and despite supportive evidence, many women prefer to choose surveillance alone over medical prevention.
**Title:** Does participation in clinical trials influence on survival in patients with metastatic breast cancer?

Kim T-Y, Sohn JH, Kim S-B, Yoon JH, Kim GM, Lee KH, Koh S-J, Park YH, Lee SE, Chae Y, Lee KS, Lee KE, Won HS, Kim JH, Jeong J, Park KH, Cho EK, Im Y-H, Im S-A and Jung KH. Seoul National University Hospital, Seoul, Republic of Korea; Asan Medical Center, Seoul, Republic of Korea; Samsung Medical Center, Seoul, Republic of Korea; Division of Breast-Endocrine Surgery, Chonnam National University Hwasun Hospital, Kwangju, Republic of Korea; Yonsei University College of Medicine, Seoul, Republic of Korea; Chungbuk National University Hospital, Cheongju, Republic of Korea; Ulsan University Hospital, Ulsan, Republic of Korea; Dong-A University Hospital, Pusan, Republic of Korea; Kyungpook National University Hospital, Daegu; Center for Breast Cancer, National Cancer Center, Goyang, Republic of Korea; Ewha Womans University Hospital, Seoul, Republic of Korea; Uijeongbu St. Mary's Hospital, Uijeongbu, Republic of Korea; Seoul National University Bundang Hospital, Sungnam, Republic of Korea; Gangnam Severance Hospital, Seoul, Republic of Korea; Korea University Anam Hospital, Seoul, Republic of Korea and Gachon University Gil Medical Center, Incheon, Republic of Korea.

**Body:**

**Background**
Recently, many clinical trials (TRIAL) especially incorporated with molecular-targeted agents are being conducted in treatment for breast cancer worldwide. However, the relation of participating clinical trials with survival has not been actively studied. This study was designed to evaluate whether participation in clinical trials could improve overall survival (OS) or not in patients with metastatic breast cancer (MBC), compared with conventional treatment.

**Method**
Korean Cancer Study Group (KCSG) has successfully established Nationwide Cohort in KOREA to conduct diachronic analysis (KCSG BR 14-07). Clinical data for patients with MBC were collected from this Cohort. OS was defined as the time duration from first diagnosis of metastasis to any cause of death. This work is supported by National Strategic Coordinating Center for Clinical Research (H110C2020).

**Results**
A total of 575 patients with metastatic breast from 26 institutes in KOREA cancer MBC were consequently enrolled between September 2014 and May 2015. 156 (27.1%) of patients were enrolled to at least one or more clinical trials and 419 patients received only conventional treatment (CONV). Age, hormone status, HER2 status, initial pathologic stage, metastasis versus recurrence, adjuvant treatment, ECOG performance status (PS) (0, 1 vs 2 or more) were similar between TRIAL and CONV. 30% of trials were associated with HER2-targeted agents. As initial treatment, chemotherapy was more frequently used in TRIAL (85.9%) than in CONV (79.0%) (P=0.038). Number of regimens of chemotherapy was greater in TRIAL (2.9+/1.8) than CONV (2.1+/1.6) (P<0.001). Number of regimens of endocrine therapy (E) was similar between TRIAL (1.4+/0.6) and CONV (1.5+/0.7) (P=0.474). Overall survival of all patients was 16.2 months (95% CI, 14.1-18.1). TRIAL showed significant prolongation of survival, compared with CONV [21.1 (95% CI, 17.7-24.6) vs 15.1 months (95% CI, 13.1-17.2); P=0.005]. The differences in OS was constantly observed in HER2-positive [23.8 (16.7-30.9) vs 17.2 months (95% CI, 12.4-21.9); P=0.018] and Triple-negative [15.4 (10.5-20.3) vs 12.0 months (95% CI, 10.2-13.8); P=0.025]. In multivariate analysis, initial metastasis, hormone status, ECOG PS did not influence on OS between TRIAL and CONV (P=0.849)

**Conclusion**
Participating in clinical trials could be associated with prolongation of survival. This results constantly maintained in HER2-positive and triple-negative MBC. These findings suggested that clinical trials are useful for the patients with MBC, even if the patients do not complete the standard treatment.
Title: Factors determining underutilization of core biopsy prior to breast surgery

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Body: BACKGROUND:
The American College of Surgeons National Accreditation Program for Breast Centers, Standard 2.9, requires a palpation-guided or image-guided needle biopsy as the initial diagnostic approach for breast cancer rather than an open biopsy. In replacing excisional biopsies, this minimally invasive technique demonstrates accuracy and precision in determining tumor characteristics and allows for more optimal breast cancer care. Recent studies have suggested that needle biopsy is underused in the United States. In a recently published analysis of U.S. Medicare data from 2003-2007, needle biopsy was used in 68.4% of all patients with breast cancer surgery. In this single-institution study, we analyzed the utilization of image-guided preoperative breast biopsies and which patient and system related factors contributed to the underutilization of image-guided breast biopsies and clinical outcomes.

MATERIALS AND METHODS:
In this retrospective study, we analyzed all breast cancer cases diagnosed over a four year period at Mount Sinai Medical Center (MSMC) from January 1, 2009 to December 31, 2013 (n=485). We performed a detailed chart review of the surgical cases that did not meet breast biopsy standards to identify contributing patient and surgeon level factors. Descriptive statistics and univariate analysis were used to characterize breast biopsy patterns and outcomes as well as delineate the associations between patient and surgical covariates with needle biopsy receipt.

RESULTS:
Needle biopsy was used in 86% (n=419) of all breast cancer surgeries. The median age for the cohort of patients without needle biopsy was 68.0 years (range 35-94). There was no significant variation in utilization of needle biopsy by race or surgeon. The proportion of patients without needle biopsy decreased significantly over time from 2009-2013. The most common reason for the lack of preoperative breast biopsy was the surgeon's preference to proceed with surgery because of very suspicious imaging studies (including mammograms and MRI) (n=9). There were an additional nine cases where the biopsy was attempted but was non-diagnostic. The most common patient-related factors for lack of needle biopsy were advanced age, use of anticoagulation and noncompliance due to a psychiatric diagnosis and patient discomfort. Other factors identified include lack of surgeon consultation before biopsy, biopsy of axillary lymph nodes as a means of diagnosis and patient's inpatient status.

CONCLUSIONS:
In this single institution, the rate of needle biopsy for breast cancer diagnosis was above national benchmarks. The most common reason for lack of a preoperative breast biopsy was the surgeon's preference to proceed with surgery because of very suspicious imaging studies. Patients with advanced age, psychiatric history, and inpatient workup were also more likely to lack a preoperative breast biopsy.
Insulin resistance is associated with luminal B high proliferative subtype in postmenopausal but not premenopausal breast cancer patients

Nam S, Kim S, Park S and Park HS. Yonsei University College of Medicine, Seoul, Korea.

Background: It has been hypothesized that insulin resistance increases breast cancer risk and associated with poor outcomes in breast cancer patients. Women with insulin resistance seems to develop more proliferative cancer, however there is limited information on clinical characteristics in breast cancer patient with insulin resistance, particularly about prognostic factors. The purpose of this study is to investigate the association between insulin resistance defined using HOMA-IR and clinicopathological factors in newly diagnosed breast cancer patients without diabetes.

Patients and Methods: We evaluated 760 breast cancer patients to analyze the relationship between insulin resistance and clinicopathological parameters. Diabetics were excluded at baseline in order to evaluate the characteristics of the patients with insulin resistance without diabetes. We compared clinical factors of patients between patients with and without insulin resistance.

Results: Insulin resistance was found in 26.4% of patients. Age, menopausal status, BMI, tumor size, histologic grade, Ki-67 expression and subtype were significantly different by insulin resistance. In multivariate logistic regression analysis, postmenopausal status and obesity were associated with insulin resistance. In subgroup analysis by menopausal status, age, BMI, tumor size, TNM stage, HER-2, Ki-67 expression and subtype was significantly different by insulin resistance in postmenopausal patients. Multivariate analysis in postmenopausal patients showed that insulin resistance was significantly correlated with obesity, larger tumor size and luminal B/HER-2 negative subtype.

Conclusion: Insulin resistance was significantly associated with highly proliferative luminal B subtype breast cancer in postmenopausal women. These findings suggest that the prevalence of insulin resistance may be a risk factor for prognosis of postmenopausal breast cancer patients.
Title: The impact of body mass index on age at breast cancer diagnosis and breast cancer phenotype


Body: Background
Evidence suggests that premenopausal obesity decreases and postmenopausal obesity increases breast cancer risk. While it has been hypothesized that carcinogenesis may be accelerated by a disrupted metabolic homeostasis in obese women, it is unclear why this dual relationship is observed. We here study whether body mass index (BMI) affects (a) age at breast cancer diagnosis and (b) the probability of being diagnosed with a specific breast cancer phenotype, taking menopausal status into account.

Patients and methods
All patients with non-metastatic operable breast cancer from UZ Leuven diagnosed between January 1, 2000 and December 31, 2013 were included (n=7020). Luminal A like (= grade 1 or 2, ER and/or PR positive, HER2 negative), Luminal B like (= grade 3 ER and/or PR positive, HER2 negative), Luminal HER2 like (ER and/or PR positive, HER2 positive), HER2 like (ER and PR negative, HER2 positive) and triple negative breast cancer (TNBC = ER and PR and HER2 negative). For statistical analysis, linear models and logistic regression were used to study respectively the association between BMI and age at diagnosis and BMI and breast cancer phenotype by menopausal status.

Results
There was a quadratic relationship between BMI and age at breast cancer diagnosis studying the overall population (p<0.0001). A 5kg/m² increase in BMI was associated with the following increases in age at diagnosis: +1.8y (95% CI 1.4-2.3y) at BMI=18, +1.2y (95% CI 0.95-1.5y) at BMI=23 and +0.6y (95% CI 0.4-0.9y) at BMI=28 (corrected for menopause). This relationship was independent of the menopausal status, ER or HER2 status, histology and breast cancer phenotype.

We observed a linear relationship between BMI and the probability of being diagnosed with Luminal B like, Luminal HER2 like and HER2 like breast cancer (table 1). This linear relationship interacts with menopausal status for Luminal B like and HER2 like breast cancers (table 1).

Table 1: Probability of being diagnosed with a certain breast cancer phenotype by BMI (linear model) and the impact of menopausal status.

<table>
<thead>
<tr>
<th>Effect of BMI on the probability of being diagnosed</th>
<th>p-value</th>
<th>effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A like</td>
<td>0.4430</td>
<td>n/a</td>
</tr>
<tr>
<td>Luminal B like</td>
<td>0.0276</td>
<td>BMI +5kg/m² OR 1.07 (95% CI 1.01-1.14)</td>
</tr>
<tr>
<td>Luminal HER2 like</td>
<td>0.0367</td>
<td>BMI +5kg/m² OR 0.91 (95% CI 0.83-1.00)</td>
</tr>
<tr>
<td>HER2 like</td>
<td>0.0219</td>
<td>BMI +5kg/m² OR 0.88 (95% CI 0.78-0.98)</td>
</tr>
<tr>
<td>TNBC</td>
<td>0.5454</td>
<td>n/a</td>
</tr>
<tr>
<td>Interaction with menopausal status</td>
<td>p-value</td>
<td>effect</td>
</tr>
<tr>
<td>Luminal A</td>
<td>0.2204</td>
<td>n/a</td>
</tr>
<tr>
<td>Luminal B</td>
<td>0.0487</td>
<td>Premenopausal OR 0,996 (CI 0,974-1,019), p=0,7449; Postmenopausal OR 1,023 (CI 1,008-1,038), p=0,0023</td>
</tr>
<tr>
<td>Luminal HER2</td>
<td>0.2571</td>
<td>n/a</td>
</tr>
<tr>
<td>HER2 like</td>
<td>0.0031</td>
<td>Premenopausal OR 1,020 (CI 0,983-1,059), p=0,2923; Postmenopausal OR 0,947 (CI 0,919-0,976), p=0,0004</td>
</tr>
</tbody>
</table>
Conclusion
We could not confirm the hypothesis that increasing BMI decreases (increases) age at diagnosis in postmenopausal (premenopausal) women. Obesity does affect the probability of being diagnosed with certain breast cancer phenotypes, but for certain breast cancer phenotypes an interaction with menopause was observed. We presume a potential biological link through BMI between Luminal B and HER2 like breast cancer that needs further exploration.
Title: Family history and risk of pregnancy-associated breast cancer (PABC)

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Body: Background: The risk of breast cancer is at least doubled in young women with a family history of breast cancer. Pregnancy has a dual effect on breast cancer risk; a short-term increase followed by a long-term protection. It has been hypothesized that women with a genetic predisposition for breast cancer are particularly susceptible to physiological changes during childbearing that may influence short term risk.

Materials & methods: We followed a cohort of women aged 15 to 44 years between 1963 and 2009 identified in Swedish registers. Family history was defined as having a mother or sister with breast cancer. In a case-cohort analysis, we estimated incidence rate ratios of breast cancer during pregnancy and time intervals up to 10 years post-delivery.

Results: In 3,452,506 women, there were 15,548 cases of breast cancer (1,208 were pregnancy-associated (PABC), defined as breast cancer during or within 2 years of pregnancy). Compared to nulliparous women, the risk of breast cancer was decreased during pregnancy, similar during 1st year and increased during 2nd year post-delivery. The pattern was similar in women with or without family history of breast cancer. A peak in risk was observed 5-6 years following the first birth regardless of family history. After a second birth, this peak was only present in women with a family history.

Conclusions: Our results indicate that women with a family history of breast cancer do not have a different breast cancer risk during and within 10 years following pregnancy compared to women without a family history.
Title: Pregnancy associated breast cancer (PABC); no evidence of patients’ or doctors' delays


Body:

A small, but not negligible, proportion of breast cancers in young women are detected in association with childbearing. While pregnancy usually is a period of intense medical observation, signs and symptoms of a malignancy may be overlooked or misinterpreted as pregnancy-related, resulting in diagnostic and treatment delays. Also, a delayed diagnosis in pregnant women has been suggested as a reason for the more advanced disease and poorer outcomes in women with pregnancy-associated breast cancer.

Material and Methods:

For the purpose of the present study, pregnancy-associated breast cancer (PABC) was defined as an invasive breast tumor diagnosed during pregnancy and up to two years post-delivery (non-PABC cases were diagnosed outside this time window, or nulliparous). Based on a systematic review of medical records for women aged 15-44 years at diagnosis with PABC and non-PABC identified in Swedish health care registers, chart information was retrieved by trained nurses for a total of 570 women (285 PABC women and 285 age and hospital matched non-PABC women) treated at 11 hospitals across Sweden between 1992 and 2009. Median waiting times from initial signs or symptoms in days to start of treatment, and time periods within, were computed using the Kaplan-Meier method and compared using the logrank test for the Kaplan-Meier curves. Dates on first symptoms were available for 122 matched PABC/non-PABC pairs, in total 244 patients. Full dates to assess and compare times between first health care contact – diagnosis – start of treatment, were available for 246 PABC/non-PABC pairs, in total 492 women.

Objective:

To examine and compare lengths of several defined waiting times within the time period from initial symptoms to start of treatment in women diagnosed with PABC and non-PABC.

Results:

Patient delay-time between first symptom and first point of contact with health care provider.
Median time between first symptoms and first contact with health care was 36 days and 45 days for women with PABC and non-PABC, respectively (logrank test p-value 0.48).

Time between first health care contact and diagnosis
Median time between first contact and diagnosis was 7 days for both PABC and non-PABC women (logrank test p-value 0.16).

Time between diagnosis and start of treatment
The median waiting time from date of diagnosis to initiation of treatment was shorter in women with PABC (22 days) compared to non-PABC women (26 days) (logrank test p-value 0.14).

Time between first contact and start of treatment
The median delay of start of treatment from first contact with a health care provider was 34 days in PABC women and 37 days in non-PABC women. (logrank test p-value 0.14).

Conclusions:

Patients’ delay and the time between first contact with health care and start of treatment was shorter in women with PABC compared to non-PABC. Taken together, the present results do not support the notion that diagnostic and treatment delays are more common in women diagnosed with breast cancer during or shortly after pregnancy.
Title: Effect of chemoprevention uptake on mammographic density over time in women at high risk for breast cancer

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Background: Mammographic density (MD) is an independent risk factor for breast cancer, but whether chemoprevention can modify this risk biomarker is unknown. MD naturally declines with age, particularly after menopause. Prior studies have shown a significant decrease in MD with tamoxifen correlated with decreased breast cancer incidence, however, other anti-estrogens, such as aromatase inhibitors (AIs) and raloxifene, have not shown significant effect. No studies have examined the long-term effects (>24 months) of chemoprevention on MD.

Methods: We conducted a retrospective cohort study of high-risk women seen at an academic breast center. Patients were considered to be at high risk for breast cancer and eligible for chemoprevention if they had a 5-year predicted breast cancer risk according to the Gail model of ≥1.67%, atypical ductal or lobular hyperplasia, history of ductal or lobular carcinoma in situ, and/or BRCA mutation. We collected demographic, breast cancer risk factor, and clinical data, including prior or current anti-estrogen use, type of anti-estrogen, and duration of use, from a self-administered questionnaire and medical chart review. We dichotomized anti-estrogen use as ever/never. One reader measured MD from digital images using a semiautomated computer-assisted technique with Cumulus software. MD was determined for a baseline mammogram (within 6 months of starting an anti-estrogen), a short-term follow-up (12-24 months) and/or a long-term follow-up (48-60 months) mammogram. We conducted multivariable logistic regression models to assess the association between change in MD over time with and without anti-estrogen use for chemoprevention.

Results: Of 190 evaluable women, 86 (45%) women took an anti-estrogen (53 tamoxifen, 25 raloxifene, 4 AI, 4 multiple anti-estrogens) and 104 (55%) did not. Compared to women who did not take an anti-estrogen, those who took chemoprevention were more likely to be older (age 56 vs. 53 years), postmenopausal (65% vs. 51%), have a higher body mass index [BMI] (28.2 vs. 26.8 kg/m2), and had a lower mean baseline MD (12.7% vs. 15.9%). Comparing high-risk women who initiated anti-estrogens to those who did not, mean absolute short-term change in MD (SD) was -0.249% (SD 0.057) and mean long-term change in MD was -3.25% (8.25) vs. -4.19% (11.98), respectively. There was no significant short-term change in breast density with chemoprevention; however, women who took chemoprevention were 4 times more likely to have at least a 5% decrease in breast density compared to those who did not take chemoprevention after adjustment for age, menopausal status, and baseline MD (OR=4.14, 95% CI=1.03-16.73).

Discussion: Among high-risk women who initiated anti-estrogens, we did not observe a significant short-term change in MD, but chemoprevention uptake was associated with a significant decrease in MD with long-term follow-up. Our data suggests that short-term changes in MD with chemoprevention may be too small to be detected by current methods, therefore other risk biomarkers may be needed to assess short-term response to anti-estrogens.
Title: Association between mammographic density and breast cancer subtypes among Chinese women

Yang XR R, Li J, Li E-N, Guida JL L, Li M, Sung H, Lu N, Hu N and Gierach GL L. National Cancer Institute, Bethesda, MD; Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China and Chinese University of Hong Kong, Hong Kong, China.

Body: Epidemiological studies have shown that associations between breast cancer risk and risk factors vary by tumor pathology such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status. Mammographic density (MD) is a strong risk factor for breast cancer, but data on the association between MD and breast cancer subtypes have been inconsistent and most studies have been conducted among Western women. The goal of this study was to evaluate the association between MD and breast cancer subtypes in an Asian population where the proportion of dense breast tissue is higher but the overall breast cancer incidence rate is much lower compared with Western countries. Breast cancer cases from a cancer hospital in Beijing, China with MD and ER, PR, and HER2 immunohistochemical (IHC) data were included in this analysis. To reduce subtype misclassification, we excluded cases that were HER2 2+ for IHC but had no FISH data. Tumor subtypes were defined as Luminal A (ER+ or PR+ and HER2-, N=376), Luminal B (ER+ or PR+ and HER2+, N=97), HER2-overexpressing (ER- and PR- and HER2+, N=71), and triple negative (TN, ER- and PR- and HER2-, N=66). MD was assessed on digital mammograms and categorized into four levels using the Breast Imaging Reporting and Data System (BI-RADS) scoring system (a=almost entirely fat, b=scattered fibroglandular densities, c=heterogeneously dense, and d=extremely dense). Because there were few cases with almost entirely fat breasts, we combined MD levels "a" and "b" as our reference group. Polychotomous logistic regression was used to assess the association between MD and breast cancer subtypes with the adjustment of age, menopausal status, parity, age at menarche, and body mass index (BMI) since all these variables showed significant inverse associations with MD (P=0.002 for age at menarche and P<0.0001 for all others). Compared with luminal A cases, cases with HER2-overexpressing tumors were significantly more likely to have extremely dense breasts (Odds ratio [OR], 2.6; 95% confidence interval [CI], 1.2-5.7). Cases with luminal B (OR, 2.1, 95% CI, 1.2-3.9) and TN (OR, 2.9, 95% CI, 1.5-5.8) tumors had significantly higher proportions of heterogeneously dense but not extremely dense tissue compared with luminal A cases. Our data suggest that higher MD is associated with more aggressive tumor subtypes, particularly the HER2-overexpressing subtype among Chinese breast cancer cases. If confirmed in larger studies, these results may provide insight into the higher incidence rates of HER2-overexpressing breast cancer seen among young Asian American and Asian women.
Title: State based dense breast legislation and its effect on patient awareness

Lindner D and Vasic J. Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: Recent efforts by advocacy groups to enact state government mandated legislation requiring that patients with dense breast tissue be informed of their breast density has been met with mixed responses by the medical community and cancer advocacy groups. Breast density has been well characterized as a risk factor for breast cancer, and is known to make mammogram interpretation more difficult. There are no formal recommendations for additional screening and management of patients who have dense breasts. There is no standard measurement in place for what defines dense breasts on mammography. Many women's health practitioners seeing radiology reports that comment on breast density are not adequately prepared to counsel patients in terms of best practice, as guidelines do not exist. Despite uncertainty of how knowledge of breast density impacts ongoing imaging or whether it impacts cancer early detection, 21 states have now enacted laws mandating breast density reporting. It is unclear whether in states with mandatory reporting more patients are actually being informed about their breast density. "Are You Dense", the advocacy group driving this legislation, states that 40% of women have dense breast tissue and that 95% of these women are unaware of their breast density. The aim of this patient survey study was to determine whether state legislation for mandatory breast density reporting has altered the number of women informed about their breast density compared to states without mandatory reporting.

A 19 question survey was introduced using the social media site Facebook targeting women who are health conscious. Patients were asked to answer questions about their age, personal and family history of cancer, personal and family history of genetic testing, health behaviors including tobacco and alcohol intake and exercise habits and BMI, and personal history of breast screening results and whether they had been told they had dense breasts by a health care provider. Results were recorded and data analyzed using chi square and student t test.

17,936 women visited the survey site, and 15,392 women completed the survey. Of these, 3,536 women reported to be over 40 and eligible for mammography screening. 1,997 (56%) reported that they had been informed by a health provider that they had “dense breast tissue”. Among patients residing in the 21 states with mandatory reporting, 59% (1231 out of 2098 responding) reported being told they had dense breasts vs 53% (766 out of 1438) of patients residing in states without mandatory reporting. While groups advocating for mandatory reporting of breast density want to ensure that women with dense breast tissue are informed of their risk, and have access to an early breast cancer diagnosis, it is yet unclear whether this legislation impacts early cancer diagnosis or whether patients are actually being informed with increased frequency based on the legislation. Our study did not show a significant difference in the number of patients being informed of increased breast density in states with mandatory reporting. Additional studies are required to determine whether this legislation has any impact on patient awareness, uptake of additional screening, and earlier detection of breast cancer.
Title: Volumetric breast density better predicts tumour characteristics associated with poor prognosis compared to visual BI-RADS


Body: Purpose:
Early detection of breast cancer through mammography screening reduces breast cancer mortality. To improve outcomes from screening, more than half of invasive cancers and a third of high grade cancers should be small at detection (<15mm), and more than 70% of all cancers should be node negative at diagnosis. Although breast density is associated with reduced mammographic sensitivity, it is unclear whether certain tumor characteristics associated with poorer prognosis are more prevalent in women with denser breasts. The study investigated associations between visually- or volumetrically-assessed breast density and tumor characteristics related to poor prognosis.

Methods:
Our IRB-approved study included 755 DCIS, invasive ductal or invasive lobular breast cancers diagnosed in women (aged over 40) between January 2009 and December 2012. Information on the patients' tumor characteristics including stage, size, receptor, grade and lymph node status was collected retrospectively. Women were excluded if they had a previous history of breast cancer or breast surgery, or if they were missing tumour size data or raw digital mammograms taken within 24 months of the cancer diagnosis. For women with multiple cancers, only the first diagnosed cancer was included. Breast density was assessed using visual BI-RADS density categories and Volpara Density Grades (VDG; an automated equivalent to 4th Edition BI-RADS, assigned using preset cut-offs of volumetric breast density: 4.5, 7.5, 15.5 and >15.5%). VDG was calculated from both breasts if the prior negative mammogram was available, and the contralateral breast for positive mammograms.

Results:
Overall, 55% of invasive tumors and 33% of grade 3 tumors were smaller than 15 mm and 83% were node negative. Mean tumor size increased significantly with increasing VDG (VDG 1 = 12.8, 2 = 14.7, 3 = 16.1, 4 = 20.4 mm, p<0.001) and increasing BI-RADS (1 = 12.2, 2 = 13.9, 3 = 16.8, 4 = 18.2 mm, p=0.01). The proportion of node positive tumors also increased significantly with increasing VDG (5.1%, 12.8%, 19.3%, 26.1%, p<0.001) and BI-RADS (5%, 10.6%, 19.5%, 26%, p=0.01). There was a significant increase in grade 3 tumors (11%, 16.1%, 21.7%, 21.7%, p=0.02) and HER-2 positive tumors (5.6%, 10.5%, 13.3%, 14.3%, p=0.02) with increasing VDG that was not seen with visual BI-RADS assessment (p=0.4). Increasing VDG was also significantly associated with increased proportions of larger (i.e. >15mm) node positive cancers (1.7%, 6.3%, 10.5%, 16%, p=0.004) that were not seen with BI-RADS (p=0.2).

Conclusion:
We found that tumour size and node status differed significantly with breast density as measured by VDG and visual BI-RADS. However, grade 3, HER-2 positive and large/node-positive cancers were all significantly associated with increasing VDG, but not BI-RADS density grade. Further research is needed to investigate whether automated volumetric breast density can be used to predict which women are more likely to be diagnosed with tumours that have poorer prognostic features.
Title: Risk of contralateral breast cancer in women with early breast cancer: A systematic review and meta-analysis

Rana P, Parpia S and Levine M. McMaster University, Hamilton, ON, Canada.

Body: BACKGROUND:
There is uncertainty about the lifetime risk of contralateral breast cancer (CBC) in a woman who is diagnosed with early stage breast cancer. Studies report a wide range of rates of CBC between 2% and 35%. An accurate estimate of the risk of CBC is important for informed decision making about contralateral prophylactic mastectomy which appears to be on the rise.

OBJECTIVES:
(i) To determine the risk of CBC in women with early stage breast cancer, and (ii) to compare the risk of CBC between women who undergo adjuvant systemic treatment compared to those who do not, and between women who undergo adjuvant radiation treatment and those who do not.

SEARCH STRATEGY:
We searched PubMed, Ovid MEDLINE, EMBASE, Healthstar, Cochrane Central Register for Controlled Trials. Studies in English were included.

SELECTION CRITERIA:
Studies were included if participants had unilateral invasive breast carcinoma, had a minimum of 5 years of median follow-up, and had a minimum of 100 participants. Studies which included only high-risk participants were excluded (e.g. genetic mutation positive). Only randomized controlled trials were included for the meta-analysis.

DATA COLLECTION AND ANALYSIS:
At least two authors independently abstracted and analyzed the data. A DerSimonian and Laird random-effects meta-analysis was used to estimate the pooled rate of CBC. A sensitivity analysis was conducted based on study quality. All rates presented are mean weighted rates from individual arms of the trials.

MAIN RESULTS:
4571 articles were extracted and reviewed for eligibility. Twenty-three randomized controlled trials were included in the final meta-analysis with a total of 40,700 participants. The median follow-ups were between 5.0 and 20.0 years. The rates of CBC are visualized in a forest plot. The overall pooled rate of CBC is 0.36% per year, (95% confidence interval (CI): 0.33% to 0.42%). The rate of CBC from trials considered good or excellent quality is 0.36% per year (95% CI: 0.32% to 0.40%). The rate of CBC in studies without adjuvant systemic treatment is higher than the rate without such treatment, 0.56% per year (95% CI: 0.40% to 0.77%) versus 0.35% per year (95% CI: 0.31% to 0.40%). The rate of CBC in studies with adjuvant radiation treatment is 0.26% per year (95% CI: 0.18% to 0.39%) which is similar to the rate in studies with radiation (0.30% per year, 95% CI: 0.20% to 0.43%).

CONCLUSIONS:
The rate of CBC in women with early stage breast cancer is relatively low. These results are important for women with early stage breast cancer who are considering contralateral prophylactic mastectomy and their physicians.
Title: CYP2E1 rs6413432 polymorphism is associated with breast cancer: Result from a meta-analysis

Xu Y, Tang C, Zhou N and Yang H. Daping Hospital, The Third Military Medical University, Chongqing, China; Daping Hospital, The Third Military Medical University, Chongqing, China and Toxicology Institute, School of Preventive Medicine, Third Military Medical University, Chongqing, China.

Body: A dose-response manner has been established for alcohol induced breast cancer, a 10-13% of increased risk is found for each additional 10g/day intake of alcohol. Evidence suggests that acetaldehyde (AA) is responsible for the carcinogenic effect of alcohol, which is mainly metabolized by ADH1B, CYP2E1, and ALDH2 in human breast. In individual with inactive ADH1B due to genetic variations, or in chronic alcohol drinkers, or after an excessive drinking, the CYP2E1 pathway becomes a primary pathway for alcohol metabolism, and CYP2E1-dependent alcohol metabolism leads to increased oxidative stress due to the generation of reactive oxygen species (ROS), in which the risk is believed to be higher. Polymorphisms in the rest enzymes alter the concentration of AA, accordingly, these variations are considered to modify individual susceptibility to breast cancer. However, current data remains conflicts. Based on the checklist of the Meta-analysis of Observational Studies in Epidemiology, we conducted this meta-analysis to evaluate possible associations between ADH1B, CYP2E1, and ALDH2 gene polymorphisms and susceptibility to breast cancer. By retrieving relative publications in English language from PubMed and EMBASE using free words, MESH words or Emtree words, 12 case-control publications, between January 1996 and March 2015, which focused on the associations between ADH1B, CYP2E1 or ALDH2 and breast cancer with properly ORs reported were included in the systematic review, data showing the association between breast cancer and ADH1B rs1229984, CYP2E1 rs2031920, rs3813867, and rs6413432 and ALDH2 rs671, were extracted. These conflicting results were pooled using a fixed- or random-effect meta-analytical technique by groups polymorphisms; subgroups were stratified according to menopausal status. Sensitivity analysis was performed by excluding studies, publication bias was estimated with Begg's funnel plot. As a result, 6,109 cases of breast cancer and 6,850 controls were involved in this meta-analysis, the pooled results demonstrated the A allele from CYP2E1 rs6413432 SNP was associated with breast cancer risk at 1.27-fold (95% CI, 1.04-1.57), while the rest did not show significant effect on this risk. This SNP might be a susceptibility biomarker for breast cancer, especially in drinkers. However, more data is still needed for establishing casual relationship.
Lifelong vegetarianism and breast cancer risk in India: A multicentre case control study of 2101 women

Gathani T and Barnes I. University of Oxford, Oxford, Oxfordshire, United Kingdom.

Body: Background
Breast cancer is now the most commonly diagnosed malignancy in women in India with over 150,000 incident cases per year. Interest in the role of diet in the aetiology of breast cancer is stimulated by the observation of the lower incidence of breast cancer in Asian populations where the intake of animal products is lower than that of Western populations. Studies investigating this relationship to date in India have been of small size and provided conflicting results and therefore a large scale case-control study in India addressing this relationship is of interest.

Methods
Between 2011 and 2014 we conducted a multicentre hospital based case-control study in eight cancer centres in India. Eligible cases included women aged 30-70 years with a new diagnosis of primary invasive breast cancer (ICD10 C50). Eligible hospital based controls included the accompanying attendants of the women with breast cancer and patients in the general hospital without cancer. Information about diet, lifestyle, reproductive and socio-demographic factors were collected using interviewer administered structured questionnaires. Cases and controls were frequency matched on age geography. Multivariate logistic regression models were used to estimate the odds ratio and 95% confidence intervals for the risk of breast cancer in relation to lifelong vegetarianism, following adjustment for other known risk factors for the disease.

Results
The study included 2101 cases and 2252 controls. The mean age at recruitment was similar for both cases (49.7 years) and controls (49.6 years). The mean number of children was similar for both cases (2.6) and controls (2.5) and 98% of the study population had ever breastfed. However significant differences were observed in the proportion of cases whose duration of breastfeeding was greater than six years compared to controls (22% versus 29%). Cases were significantly more likely to live in a town (23% versus 16%), have running water (84% versus 78%) and live in a permanent dwelling (88% versus 78%). The proportion of lifelong vegetarians among the cases was 29% compared to 25% amongst the controls. However, on multivariate analysis, with adjustment for known risk factors for the disease, the risk of breast cancer was the same amongst cases and controls (OR 1.01 (95% CI 0.87-1.19)).

Conclusions
The prevalence of the known reproductive and sociodemographic risk factors for breast cancer is similar in India as in other populations. To our knowledge, this is the largest study conducted in India investigating the relationship between lifelong vegetarianism and risk of breast cancer, taking into account known risk factors for the disease. These results show that lifelong exposure to a vegetarian diet does not decrease the subsequent risk of breast cancer.
The effect of C-peptide on the risk of breast cancer by serum concentration levels of IGFBP-3 in premenopausal women

Torres-Mejía G, Lazcano-Ponce E, Ortega-Olvera C and Ángeles-Llerenas A. Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico.

Background. IGFBP-3 has been known for regulating the bioavailability of the IGFs from the circulation to the tissue and therefore having a growth inhibitory effect. However, more recently other mechanisms have been investigated, probably a bit late due to that there are no diseases-causing mutations of the IGFBP3. Therefore we investigated the interaction between C peptide and IGFBP3 on the risk of breast cancer.

Methods. We used samples from a population based case-control study from three big states in Mexico (Monterrey, Mexico City and Veracruz) including 197 premenopausal breast cancer cases and 198 age-matched healthy controls. We measured serum leptin, adiponectin, IGF-I, IGFBP-3, C-peptide and insulin. Anthropometric measures and health information were obtained through an interview. We evaluate the interaction using a Multiple logistic regression models.

Results. We found that the odds of having breast cancer increased 8.8 times with each unit increase of C-Peptide (ng/mL) (OR = 8.6; 95% CI 2.3 to 31.7), this association decreased with increasing tertiles of IGFBP3 serum levels (OR=2.0; 95% CI 1.2, 3.4; OR=1.4; 95% CI 1.0, 1.9, for Tertiles 2 and 3, respectively) (p for interaction = 0.01).

Interpretation. These results suggest that the effect of C-Peptide on the risk of breast cancer might be regulated by IGFBP-3. This contributes to the knowledge about IGFBP-3 regulated pathways. Further studies are required to understand the possible mechanism that explain this interaction.
Rates of prophylactic surgeries among BRCA 1 or 2 mutation carriers: A single institution experience


Background
Women with identified BRCA1/2 mutation have a substantially increased risk of developing several types of cancer, mainly breast and ovarian, during their lifetime. Management options included close surveillance, chemoprevention and prophylactic surgeries. The aim of this study is to assess the rate of prophylactic surgeries among BRCA1/2 carriers counseled and screened at a single institution in the last 2 decades.

Methods
We retrospectively captured all women with a BRCA1/2 mutation that were identified in our genetic clinic between 2000 and 2015. The incidence of breast and ovarian cancer among all BRCA carriers was reported. The rates of prophylactic surgeries were calculated and analyzed in all identified carriers.

Results
Six hundred and eighty four women were identified to carry a deleterious BRCA mutation, among them 364 BRCA1 (53%) and 320 BRCA2 (47%). Three hundred and twenty seven (48%) were diagnosed with breast cancer and 80 (12%) had either ovarian or fallopian tumor. Forty percent (N=271) of the women assessed were healthy carriers. Prophylactic bilateral salpingo-oophorectomies (BSO) were performed in 342 women (50%) and prophylactic mastectomies (PM) (bilateral or unilateral) in 190 (28%). Furthermore, 154 women (23%) had both BSO and PM. Of note, 79 women (12%) were less than 35 years old and 122 were less than 40 years old (18%), the majority of those were waiting to have BSO later on. If we remove the young women from the analysis, 57 and 61% of the women would have had BSO (less than 35 and 40 years excluded respectively). If we only analyze the women who had a recent follow up in our clinic (2014-2015), 422 women would be eligible. Among those, 58 and 84 were less than 35 and 40 years old (y) respectively. For this cohort, the rates of BSO would be 61% (257/422)(whole cohort), 71% (excluding women less than 35y) and 76% (excluding women less than 40y).

Conclusion
A promising rate of BSO was reported in our cohort of BRCA carriers as compared to the literature. This rate was even higher (from 50 to over 70%) when we only analyzed patients with recent follow up in clinic and when we excluded young women waiting to have BSO. More efforts are needed to determine why the rates of PM are lower, for example limited breast reconstruction resources, in order to reduce the incidence of subsequent invasive breast cancer in this high-risk population.
Title: Phyllodes tumor of the breast, clinicopathological features and prognostics factors in a retrospective cohort with 7-year follow-up


Body: BACKGROUND: The phyllodes tumor of the breast is a rare neoplasm that represent less than 1% of all breast tumors and between 2.5% of fibroepithelial tumors. Often develop local recurrence and more infrequently metastatic capability.

OBJECTIVE: The aim of this study was to describe clinical pathological characteristics and identify prognostics factors in terms of local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS) and overall survival (OS) in patients with phyllodes tumors of the breast.

METHODS: We retrospectively analyzed 157 patients diagnosed and treated at the "Instituto Nacional de Enfermedades Neoplasicas" between January 2005 to December 2010. In order to evaluate the relationship between clinical features and histology of the tumors, the Pearson $\chi^2$ test was used. Kaplan-Meier method with Log-rank test or Breslow test (when it was applicable) was used to identify prognostic factors in terms of LRFS, DMFS and OS. RESULTS: The median age was 42 years (range: 13-81 years), 98 (62.4%) cases $\leq$ 45 years and 59 (37.6%) cases $> 45$ years; 88 (56.1%) had tumors in the right breast, 67 (42.7%) in the left breast and 2 (1.3%) had bilateral tumor. The mean of tumor size was 6.3 cm (range: 0.7-30 cm). Sixty three (40%) tumors were $\leq$ 5 cm and 94 (59.9%) were $>5$cm. Regarding the histological classification, 100 (63.7%) cases were benign, 35 (22.3%) were borderline and 22 (14%) were malignant. Age ($p=0.047$), tumor size ($p=0.001$), atypical stromal ($p=0.066$), stromal cellularity ($p<0.001$), pleomorphism ($p<0.001$) and tumor necrosis ($p=0.003$) were associated to the histological type.

Local recurrence occurred in 9 patients, 2 developed distant recurrence and 1 patient presented synchronously local and metastatic recurrence. In the univariate analysis, histological subtype ($p=0.006$) and pleomorphism ($p<0.001$) were identified as prognostic factors of LRFS, while the surgical approach ($p=0.008$), histological type ($p<0.001$) and some histological features such as stromal cellularity ($P<0.001$), pleomorphism ($p<0.001$), heterologous elements ($p<0.001$) and tumor necrosis ($p=0.004$) were identified as prognostic factors for DMFS. In regard to the OS, we found as prognosis factors, histology ($p=0.044$), pleomorphism ($p<0.001$) and tumor necrosis ($p=0.001$).

CONCLUSIONS: In our institution we have a low incidence (2.3%) of Phyllodes tumors of the breast and the majority of our patients were benign tumors and had good prognosis. We found that histological type and degree of pleomorphism are prognostic factors at LRFS and OS. Surgical approach, histological type and some histological characteristics were predictors for DMFS.

Keywords: Phyllodes tumor, local recurrence, distant recurrence, overall survival.
Title: Clinicopathologic features of breast cancers that develop in women with previous benign breast disease


Body: Background

Women with benign breast disease (BBD) have an increased risk for breast cancer (BC). Almost 30% of all BCs develop in women with prior BBD. Information about the features of the expected BCs after BBD would enable individualized surveillance and prevention strategies for these women. We sought to characterize BCs developing in a large cohort of women with BBD.

Methods

Our cohort includes 13,485 women who underwent breast biopsy for mammographic or palpable concern between 1967-2001. Biopsy slides were reviewed and classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH). BCs were identified by follow-up questionnaires, medical records, and Tumor Registry data. BC tissues were obtained and reviewed.

Results

With a median follow-up of 15.8 years, 1273 women developed BC. Most BCs were invasive (81%), among which 61% were ductal, 13% mixed ductal/lobular, and 14% lobular. Two thirds were intermediate or high-grade, and 29% were node positive. Cancer characteristics were similar across the three histologic categories of BBD, with similar frequency of DCIS and invasive disease, tumor size, time to invasive BC, histologic type of BC, nodal positivity and HER2 positivity. Women with AH had a higher frequency of ER+ BC (91%) vs women with PD (80%) or NP (85%), p=0.02.

Conclusion

A substantial proportion of all BCs develop in women with prior BBD. We show that the majority of BCs after BBD are invasive tumors of ductal type, with a substantial proportion node positive. 84% of cancers were ER-positive. Chemoprevention should be strongly encouraged in higher-risk women with BBD.
Title: Cost-effectiveness of pertuzumab in HER2+ metastatic breast cancer

Qian Y, Durkee BY Y, Pollom EL L, King M, Dudley SA A, Shaffer JB B, Chang DT T, Gibbs IC C, Goldhaber-Fiebert JD D and Horst KC C. Stanford University School of Medicine, Stanford, CA; Stanford University School of Medicine, Stanford, CA and Stanford Health Policy, Centers for Health Policy and Primary Care and Outcomes Research, Stanford, CA.

Body: Purpose

The Clinical Evaluation of Pertuzumab and Trastuzumab (CLEOPATRA) study showed a 15.7-month survival benefit with the addition of pertuzumab (P) to docetaxel and trastuzumab (TH) as first-line treatment for patients with HER2 overexpressing metastatic breast cancer. We performed a cost-effectiveness analysis to assess the value of the addition of pertuzumab to docetaxel and trastuzumab.

Patient and Methods

We developed a decision-analytic Markov model to evaluate the cost-effectiveness of TH with or without P in U.S. patients with metastatic breast cancer. The model followed patients weekly over their remaining lifetimes. Health states included: stable disease, progressing disease, hospice, and death. Transition probabilities were based on the CLEOPATRA study. Costs reflected the 2014 Medicare rates. Health state utilities were the same as those used in other recent cost-effectiveness studies of trastuzumab and pertuzumab. Outcomes included health benefits expressed as discounted quality-adjusted life-years (QALYs), costs in U.S. dollars, and cost-effectiveness expressed as an incremental cost-effectiveness ratio. One-way and multi-way deterministic and probabilistic sensitivity analyses explored the effects of specific assumptions.

Results

Modeled median survival was 39.4 months (TH) and 56.9 months (THP). The addition of pertuzumab resulted in an additional 1.81 life years gained (0.62 QALYs) at a cost of $472,668 per QALY gained. Deterministic sensitivity analysis showed that THP is unlikely to be cost-effective even under the most favorable assumptions, and probabilistic sensitivity analysis predicted 0% chance of cost-effectiveness at a willingness-to-pay of $100,000 per QALY gained.

Conclusion

The addition of pertuzumab to docetaxel and trastuzumab in patients with metastatic HER2+ breast cancer is unlikely to be cost-effective in the United States.
Title: Hospitalizations and costs during Implementation of a lay navigation program for older patients with breast cancer in the deep south


Body: Background: Patient-centered strategies are needed to enhance the value of cancer care particularly at the end of life. Lay navigators (LN) can be trained to provide an extra layer of support for cancer patients from diagnosis through survivorship or end of life. We hypothesized that integrating LNs into the care team would reduce healthcare utilization and cost for patients with cancer, including those with breast cancer.

Methods: A prospective, observational study of Medicare claims data was conducted of beneficiaries ≥ 65 years old diagnosed with cancer after 2008 who received care within the UAB Health System Cancer Community Network (12 cancer centers of varying size located in AL, MS, TN, GA, and FL). The first breast cancer (BC) patient was enrolled in navigation in April 2013, and ~18% of BC patients were navigated by the end of 2014. For this analysis, we report on the subset of patients with BC. The outcomes of interest were calculated per quarter from 2012-2014: (1) the proportion of patients with at least 1 hospitalization, (2) the proportion of the 492 deceased BC patients with a hospitalization in the last 30 and 14 days of life and (3) the Total costs for Medicare, excluding prescription drug costs. We used general linear models to evaluate changes in both health care utilization and cost over time, adjusting for age, sex, cancer stage, phase of care, and navigation group. Differential effects for navigated and non-navigated groups were tested with a group*time interaction. Healthcare utilization estimates are presented as Incidence Rate Ratios (IRR), and costs for Medicare as parameter estimates (β) in terms of dollar amounts.

Results: 4835 BC patients received care from 2012-2014: 622 received navigation services. 14.2 % of navigated BC patients were stage III/IV, compared to 9.33% of non-navigated patients. The proportion of hospitalizations trended downward from 7.9% in quarter 1 (Q1) 2012 to 5.7% in Q4 of 2014 (IRR 0.965, p =0.14), with similar decreases for navigated and non-navigated patients (IRR= 1.00, p > 0.05). Hospitalization in the last 30 days and last 14 days of life were 49.7% and 29.3%, respectively, with no between groups difference. Costs per beneficiary per quarter decreased overall from $4,161 in Q1 2012 to $3,010 in Q4 2014 (p <0.0001). In adjusted analysis, the navigated patients had an average $577 greater decline per quarter than the non-navigated patients (βNavigated=-$636; βnon-Navigated=-$59; p<0.0001).

Conclusions: Medicare costs declined during implementation of a lay navigation program, with greater reductions for navigated patients than non-navigated BC patients. Overall hospitalizations also declined, yet rates remain high for breast cancer patients at the end of life. Integration of LNs should be considered by health systems aiming to transition to value-based healthcare delivery.

The project described was supported by Grant Number 1C1CMS331023 from the Department of Health and Human Services, Centers for Medicare & Medicaid Services. The contents of this abstract are solely the responsibility of the authors and do not necessarily represent the official views of the U.S. Department of Health and Human Services or any of its agencies.
Title: A cost effectiveness analysis of baseline left ventricular function assessment for breast cancer patients undergoing anthracycline chemotherapy

Safonov A, Hatzis C, Stratton J, Gross CP P, Russell R, Pusztai L and Abu-Khalaf MM M. Yale School of Medicine, New Haven, CT; Section of Medical Oncology, Yale School of Medicine, New Haven, CT; Cancer Outcomes, Public Policy and Effectiveness Research Center, Yale School of Medicine, New Haven, CT and Section of Cardiovascular Medicine, Yale School of Medicine, New Haven, CT, New Haven, CT.

Body: Background: It is unclear if all breast cancer (BC) patients require baseline left ventricular function (LVEF) assessment prior to anthracycline based chemotherapy (ABC), and the approach is variable in clinical practice. Our objective is to determine the cost effectiveness of obtaining a baseline LVEF assessment prior to (neo) adjuvant ABC in clinical practice.

Methods: We performed a retrospective analysis of the Yale Equilibrium Radionuclide Angiography (ERNA) database for 701 breast cancer patients who had a baseline ERNA scan prior to systemic therapy for an initial diagnosis of stages I-IV BC between July 2003 and May 2013. We found that 14 of 701 (2%) patients had a baseline LVEF < 50%. Age, pre-existing cardiac risk factors and coronary artery disease did not predict an abnormal baseline LVEF <50 %. To evaluate the benefit of obtaining a baseline echocardiogram or ERNA before ABC, we considered the screening scenario in which BC patients with a baseline LVEF < 50% on screening echocardiogram are treated with a second (2nd) generation non-ABC and those with baseline LVEF ≥ 50% receive a third (3rd) generation ABC, and compared this with a non-screening scenario with uniform 3rd generation ABC treatment for all patients who do not have a baseline echocardiogram. We used Adjuvant Online to obtain estimates of the disease free (DFS) and overall survival (OS) for a 3rd generation ABC regimen vs a 2nd generation non-ABC regimen for 50 year old patients with a T2N1 hormone receptor positive BC. We implemented these oncologic clinical outcomes (in addition to cardiotoxicity-related clinical outcomes, costs of screening echocardiogram and treatment of congestive heart failure (CHF), quality of life metrics, as reported in the literature) into a simplified decision-analytic cost-effectiveness analysis that accounts for the different disease states and their associated costs and quality of life outcomes.

Results: Assuming that 20% of the unscreened patients with a LVEF < 50% will develop CHF if treated with ABC regimen without management of baseline cardiac dysfunction, the base case incremental cost effectiveness ratio (ICER) was determined to be 18,520 $USD/QALY. Sensitivity analysis suggested that the cost-effectiveness of baseline LVEF assessment is primarily driven by the prevalence of patients with LVEF < 50%, the incidence of CHF in this high-risk patient group if treated with ABC regimen, and time to CHF development. While our analysis did not reveal risk factors predictive of low baseline LVEF, our model's dependence on prevalence of LVEF < 50% demonstrates the importance of risk factor stratification. A hypothetical predictive marker which enriches the prevalence of an abnormal baseline LVEF 5-fold to 10% would result in a cost-effectiveness of 10,990 $USD/QALY. The model is less sensitive to the cost of baseline echocardiogram testing.

Conclusion: Baseline LVEF assessment was found to be cost-effective under a willingness-to-pay threshold of $50,000/QALY. Our sensitivity analysis suggests that risk factor-guided LVEF baseline LVEF screening may increase the number of high-risk patients in the treatment population, thus further increasing the cost-effectiveness of baseline LVEF assessment.
Title: Use of Mammaprint© (MMP) genetic signature in early breast cancer patients. Economic analysis of a 129 patient cohort treated in three Spanish hospitals


Body: Introduction: Benefit derived from adjuvant chemotherapy (CT) is doubtful in a high percentage of patients with early breast cancer. The 70-genes platform MMP improves prognostic classification and has been proven useful when it comes to individualizing treatment options. At our institution we use this test to try to avoid overtreatment in those patients in which CT benefit is unclear. We defined some criteria in order to discriminate better which patients would benefit most from the MMP assessment as well as to identify a group of subjects where the test would be more cost-effective. These criteria were: age between 35-70 years, tumor size from 1 to 3 cm, histological grade 2, absence of macrometastatic disease in axillary nodes, hormone receptors positive, HER2 negative and Ki67 between 11-25%.

Objective: To analyze the impact of using MMP to help selecting adjuvant treatment, both in clinical and economical aspects.

Material and Methods: Since August 2012 to January 2015, MMP genetic signature was performed in 129 early tumors samples. Most cases met the criteria explained above. Some that did not were also included by decision of the institution multidisciplinary committee when the individual characteristics of the cases where taken into account. We compiled the adjuvant treatment initially planned according to our institution protocol and usual clinical practice and compared it with the actual treatment given after the results of the test were known. We calculated the direct economic costs of chemotherapy and of the diagnostic test.

Results: The clinical characteristics of the patients and the adjuvant therapy they received are shown in Table 1.

**Patients Characteristics**

<table>
<thead>
<tr>
<th>n=129</th>
</tr>
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<tbody>
<tr>
<td>Age, median (range)</td>
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</tbody>
</table>

Histology

- Infiltrating ductal carcinoma: 115 (89%)
- Infiltrating lobular carcinoma: 14 (11%)

pT (mm)

- Median (range): 15 (8-60)
- Mean (range): 18.55 (8-60)

pN

- pN0: 87 (67.4%)
- pN1mic: 30 (23.2%)
- pN1: 7 (5.4%)
- pN2: 1 (0.8%)

Histological grade

- I: 24 (18.8%)
| II | 100 (78.1%) |
| III | 4 (3.1%) |

**Hormone receptors**

| ER 50-100% | 129 (100%) |
| PR <20% | 21 (16.3%) |
| PR >20% | 108 (83.7%) |

**Ki 67**

| <=20% | 74 (57.8%) |
| > 20% | 54 (42.2%) |

| Median (range) | 20 (1-35) |
| Mean (range) | 19.95 (1-35) |
| Her 2 negative | 129 (100%) |

**Adjuvant treatment**

| FEC-paclitaxel + Hormonotherapy | 18 |
| TAC + Hormonotherapy | 27 |
| Hormonotherapy | 84 |

119 Patients (92.2%) would have received adjuvant CT without MMP risk determination. After the results only 45 patients (34.9%) received it. The cost of the genetic study was 306.725€. Direct costs savings estimated from the reduction in CT treatment were 494.771,48 €.

**Conclusion:** The use of the MMP test in a selected group of patients reduced the administration of adjuvant CT in a 57.3%. This represented a saving of 188.046,48€.
Chest imaging in patients with breast cancer treated with curative intent

Dawar R, Palacio S, Monge J, Torres A, Salzberg M, Malpica Castillo LE E, Saravia D, Amarapurkar P and Hurley J. Sylvester Comprehensive Cancer Center & University of Miami Miller School of Medicine, Miami, FL and University of Miami, Miller School of Medicine, Miami, FL.

Body: Background:
With progressive advances in contemporary medicine, care of cancer patients has become increasingly complex and costly. Nationwide demands to regulate health care expenditure have escalated, urging healthcare providers (HCP) to reassess their investigative strategies in patient care for optimal resource utilization. An abnormal chest x-ray (CXR), done routinely as preoperative work up for breast cancer patients, commonly prompts further imaging if found to be abnormal. Data regarding whether this additional imaging is useful is scant.

Method:
This IRB approved retrospective analysis identified all patients diagnosed with breast cancer from 2004 through 2014. Data collected included age at diagnosis, ethnicity, smoking history, insurance status, respiratory symptoms, tumor histology and stage, hormonal receptor status, HER-2 receptor status and radiographic imaging.

Results:
Data from 2400 patients were analyzed. 194 patients were excluded: 117 stage IV disease and 77 incomplete data. 2206 had clinical stages I, II and III. 14% stage 0; 27% stage I; 35% stage II; 23% stage III. Demographics: 99.5% female and 0.5% males. Mean age 54.4 (range 18-92); 4% White, 23% African descent, 1% Asian, 72% Hispanic, 39% uninsured. Smoking history: 79% never smoked, 10% former smokers, 11% current smokers. 2017 (91%) had a preoperative CXR; 83% were normal and 17% abnormal. Abnormalities: nodules (41%), granulomas (18%), atelectasis (11%), infiltrates (4%), masses (4%), other findings (21%). 70% of patients with abnormal CXR had a chest computed tomography (CT) scan; abnormal/normal 88%/12%.

<table>
<thead>
<tr>
<th>Clinical Stage</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients (n = 2206)</td>
<td>14%</td>
<td>27%</td>
<td>35%</td>
<td>23%</td>
</tr>
<tr>
<td>CXR (Abnormal / Total, n = 2017)</td>
<td>11%</td>
<td>17%</td>
<td>17%</td>
<td>20%</td>
</tr>
<tr>
<td>Chest CT (Abnormal / Total, n = 241)</td>
<td>92%</td>
<td>92%</td>
<td>86%</td>
<td>86%</td>
</tr>
<tr>
<td>Finding on abnormal chest CT (n = 211)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nodule</td>
<td>55%</td>
<td>70%</td>
<td>67%</td>
<td>62%</td>
</tr>
<tr>
<td>Granuloma</td>
<td>27%</td>
<td>15%</td>
<td>15%</td>
<td>8%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>0%</td>
<td>0%</td>
<td>9%</td>
<td>15%</td>
</tr>
<tr>
<td>Other</td>
<td>18%</td>
<td>15%</td>
<td>10%</td>
<td>14%</td>
</tr>
<tr>
<td>Patients with follow-up chest CT (n = 111)</td>
<td>55%</td>
<td>43%</td>
<td>47%</td>
<td>63%</td>
</tr>
<tr>
<td>Mean number of follow-up chest CTs per patient</td>
<td>2</td>
<td>1.81</td>
<td>1.85</td>
<td>1.96</td>
</tr>
</tbody>
</table>

Table 1

Abnormalities: pulmonary nodules (65%), granulomas (13%) and metastases (9%), other findings (13%). Pulmonary nodules measured <4 mm (40%), 4-6 mm (35%), 6-8 mm (13%) and >8 mm (13%). Of the 214 patients with abnormal chest CT, 52% had follow-up CT done, with mean follow-up of 1.89 per patient. There were more abnormal CXR in smokers than never smokers (23 vs 16%, p=0.001) but not chest CT scans (91 vs 86%, p = 0.35). There was no difference in rate of abnormal CXR based on age, race, ethnicity or insurance status. Evaluation of abnormal CXR’s with chest CT scans found metastasis: stage 0(0%), stage
1(0%), stage 2(9%), stage 3(15%), p = 0.014.

Conclusions:
Later clinical stage predicts for finding metastasis on chest CT scans, done for evaluation of abnormal CXR. The use of chest CT scans for patients with non-specific pulmonary findings on CXR is not useful for women with clinical stage 0 and stage 1 disease.
Title: Time and value of chemotherapy administration burden in a Swedish hospital


Body: Background
Administration of treatment for cancer patients can require substantial health care resources and differences in administration burden between treatments are therefore important to take into account when comparing them. Besides the direct personnel and bed time, the administration can also have broader consequences on the organisation and process of care delivery, as well as on the patients. How to value differences in these parameters is however not straightforward.

Nab-paclitaxel and paclitaxel are examples of two treatments with important differences in administration burden, and the objective of this study was to perform a time-motion study to compare time required for treatment administration.

Material and methods
Breast cancer patients vising the oncology day care unit at the department of oncology at Akademiska Hospital in Uppsala, Sweden, as part of their regular treatment-course with nab-paclitaxel (n=13) or paclitaxel (n=9) were included in the study. Time data collection was based on a questionnaire answered by hospital staff. Time for infusions and the total patient time in hospital were measured for each patient.

Results
Mean infusion times per administration of nab-paclitaxel and paclitaxel were 42.1 (Standard deviation (SD) 20.7) minutes and 104.3 (SD 43.3) minutes, respectively. Total patient times in clinic per infusion were 82.2 (SD 40.9) minutes and 183.9 (SD 34.8) minutes, respectively.

A 12-week treatment course of nab-paclitaxel (every three weeks), nab-paclitaxel (weekly) and paclitaxel (weekly) would require in total 2.8, 8.4 and 20.9 hours of infusion time, respectively. The corresponding patient time in hospital would be 5.5, 16.4 and 36.8 hours, respectively.

Conclusion
There can be substantial differences in nurse and facility resources required for administration of chemotherapy, our data showed approximately a 7-fold difference in patient, personnel and facility time requirements between the compared treatments. In a time with shortage of both hospital personnel and facilities, the valuation of these resources is not easy and may have broader impact on the organisation and delivery of care. Monetary valuation using rent cost or salaries may in a time of resource shortage underestimate the true opportunity cost, and more research and discussions on how to prioritise use of these resources versus other costs are therefore needed.
Title: Attitudes and practice of breast surgeons towards referring young women with breast cancer (YWBC) for fertility preservation (FP)

Warner E, Yee S, Glass K, Kennedy E, Foong S and Seminsky M. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; University of Toronto; Create Fertility Centre, Toronto; Mt. Sinai Hospital, Toronto; Regional Fertility Program, Calgary, AB, Canada and University of Calgary.

Body: Background: Despite ASCO guidelines (2006, 2013) recommending that young cancer patients be offered referral for FP as soon as possible after diagnosis, the literature consistently shows suboptimal referral rates for YWBC. Surgeons are in a unique position to initiate early FP referral. Surgeon & Patient Oncofertility Knowledge Enhancement (SPOKE) is one of 5 components of the pan-Canadian RUBY research program for YWBC. SPOKE aims to improve breast surgeon FP knowledge and referral rates. The lead surgeon from each of the RUBY sites was previously interviewed about FP. The goal of the current survey was to assess the baseline oncofertility attitudes and practice of the non-lead breast surgeons at those sites.

Methods: An online survey taking approximately 10 minutes to complete was developed specifically for this study. In February 2015, an email invitation with a hyperlink to the anonymous survey was sent to all 86 surgeons identified by the 23 lead surgeons. Repeated reminders were sent by the research assistant over 3 months and a final request was sent by the PI. Participants received a $25 gift certificate.

Results: A total of 55/86 (64%) surgeons with an average of 15 years' surgical practice completed the survey. 53% were male, 56% were under age 50, and 93% worked at a cancer centre or university-affiliated hospital. Thirty respondents (55%) indicated that more than half of their practice was breast cancer. Twenty (36%) never or rarely initiated a fertility discussion, and 23 (42%) never or rarely discussed FP options with their YWBC. Twenty-two respondents (40%) stated it was the duty of the medical oncology rather than the surgical team to initiate fertility discussions. Only a minority were quite or very familiar with egg freezing (n=10, 19%) and embryo freezing (n=11, 20%), while only 7 (13%) felt comfortable discussing egg or embryo freezing with their patients. Twenty-four (44%) did not know a FP centre in their area to which they could refer. Compared to surgeons who assumed responsibility for fertility discussion, surgeons who did not think FP referral was their clinical responsibility were less familiar with egg freezing (21% vs. 63%, p<.001) and embryo freezing (32% vs. 73%, p<.01), and were less likely to know where to send FP referrals (31% vs. 85%, p<.05). The most common patient factors that surgeons stated would deter them from FP referral were: poor prognosis, need to start chemotherapy urgently, and already having children. A quarter of surgeons said they would be less likely to refer a highly anxious YWBC for FP.

Conclusions: Many Canadian breast surgeons are unaware of the importance of early FP referral and nearly half surveyed did not consider FP referral to be their mandate. A majority of these surgeons lack sufficient oncofertility knowledge to feel comfortable mentioning FP options to their patients, and have not created a protocol for FP referral by the surgical team. In the next phases of the SPOKE study, a knowledge translation intervention will be developed and its effectiveness tested.

Support: Canadian Breast Cancer Foundation & Canadian Institute of Health Research (OBW139590).
Title: Association between a dedicated program for young breast cancer patients and discussion about fertility preservation

Srikanthan A, Amir E and Warner E. Princess Margaret Cancer Centre, Toronto, ON, Canada and Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Purpose: To assess whether a dedicated program, including a nurse navigator, improves the frequency of: a) documentation of fertility discussion and b) referrals for fertility preservation (FP).

Methods: A retrospective chart review and prospective survey were undertaken of a cohort of young breast cancer patients diagnosed between 2011-2013 at two academic centres in Toronto, Ontario. The Odette Cancer Center (OCC) has a dedicated program for young breast cancer patients while Princess Margaret Cancer Centre (PM) does not. Documentation of fertility discussion prior to receipt of systemic therapy was extracted from patient records. Prospective surveys were administered to the same cohort to corroborate data collected. Descriptive statistics were used to characterize baseline patient variables. Chi-squared was used to compare categorical variables and t-tests for continuous variables between the two cancer centres. Statistical significance was defined as $p<0.05$.

Results: At OCC and PM respectively, 91 and 81 patient charts were reviewed while 54 and 49 women returned surveys for response rates of 59% and 60%. Chart reviews demonstrated no difference in the frequency of documentation of fertility discussion (80% versus 75% for OCC and PM, $p=0.44$); however, surveys demonstrated higher recall of fertility discussion rates at OCC (96% versus 83%, $p=0.046$). A greater proportion of women were offered FP referrals at OCC, as observed in both chart reviews (53% versus 41%, $p=0.18$) and surveys (70% versus 46%, $p=0.02$). Time to initiation of chemotherapy did not significantly differ between women who underwent FP and those who did not.

Conclusion: A dedicated program for young women with breast cancer including a nurse navigator is associated with a higher frequency of FP referrals without delaying systemic therapy.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-12-03

Title: Delays in diagnosis and treatment of breast cancer patients: A safety-net population profile

Jaiswal KR R, Furniss A, Doyle R, Gayou N and Bayliss EA A. University of Colorado School of Medicine, Denver, CO; Denver Health Medical Center, Denver, CO; Kaiser Permanente Colorado, Denver, CO; University of Colorado School of Medicine, Denver, CO; University of Colorado School of Medicine, ACCORDS, Denver, CO and Denver Health Medical Center, Health Services Research, Denver, CO.

Body: Background: Timely detection and treatment of breast cancer patients is important in survival and recurrence. Given disparities in breast cancer outcomes based on socioeconomic status, we examined the time to diagnosis and treatment in a safety net hospital.

Methods: We conducted a retrospective review of all breast cancer patients identified by cancer registry records from 7/1/2010 to 6/30/2012 (n=124). We excluded patients with primary stage IV (n=8) and those with recurrent breast cancer within 5 years of primary diagnosis (n=4). We determined intervals between presentation to diagnosis, diagnosis to first treatment, last surgery to chemotherapy start, and last surgery to radiation start. We used logistic regression to calculate unadjusted odds of receiving timely treatment (< median time) versus more delayed treatment (≥ median time) as a function of age, language, ethnicity, insurance, Charlson co-morbidity index, cancer stage, method of first presentation (screening mammography vs. care provider), symptoms at presentation, and type of surgical treatment.

Results: Of 112 patients, the median age was 59. 42.9% were Hispanic, 29.5% were White, and 24.1% were African American. Clinical stage distribution was 20.0% stage 0, 31.8% stage I, 40.9% stage II, and 8% stage III. 83.9% of patients had surgery, of which 51.1% had breast conservation. The median time from presentation to diagnosis, time from diagnosis to first treatment, and time from surgery to chemotherapy start, fell within recommended intervals (Table 1). The time from last surgery to radiation start was greater than recommended intervals. Variables with significantly increased odds of taking longer than the median time include: stage, method of presentation, language, Charlson index, surgical treatment, ethnicity, symptoms at presentation (Table 2).

Conclusion: Acceptable diagnosis and treatment intervals were obtained for disadvantaged patients, except for time to radiation therapy. Room for improvement exists: focused interventions to facilitate access to radiation therapy, aid providers in accessing imaging more quickly, aid non-English speaking and Hispanic patients could lead to improved breast cancer care.

Table 1. Intervals of Care

<table>
<thead>
<tr>
<th>Measure: Time From...</th>
<th>Median (days)</th>
<th>25th, 75th % (days)</th>
<th>Recommended Intervals (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation to Diagnosis</td>
<td>19</td>
<td>7, 43</td>
<td>60</td>
</tr>
<tr>
<td>Diagnosis to 1st Treatment</td>
<td>37</td>
<td>30, 48</td>
<td>21-60</td>
</tr>
<tr>
<td>Presentation to 1st Treatment</td>
<td>62</td>
<td>47, 83</td>
<td>n/a</td>
</tr>
<tr>
<td>Last Surgery to Chemo Start</td>
<td>48</td>
<td>31, 59</td>
<td>28-90</td>
</tr>
<tr>
<td>Last Surgery to Radiation Start*</td>
<td>68</td>
<td>53,79</td>
<td>42-56</td>
</tr>
</tbody>
</table>

* for patients needing surgery and radiation only

Table 2. Un-adjusted Odds Ratio of Taking Longer than Median Time

<table>
<thead>
<tr>
<th>Interval</th>
<th>Variable</th>
<th>Reference</th>
<th>O.R.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation to Diagnosis</td>
<td>Stage II or III</td>
<td>Stage 0 or I</td>
<td>2.88</td>
<td>0.008</td>
</tr>
<tr>
<td>Diagnosis to First Treatment</td>
<td>Provider</td>
<td>Method of Presentation: Screening Mammogram</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------</td>
<td>---------------------------------------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>Age &lt; 55</td>
<td>Age ≥ 55</td>
<td>2.18</td>
<td>0.070</td>
<td></td>
</tr>
<tr>
<td>English-speaking</td>
<td>Non-English speaking</td>
<td>0.22</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Charlson Index ≥ 3</td>
<td>Charlson Index &lt;3</td>
<td>0.42</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>Symptomatic Presentation</td>
<td>Asymptomatic Presentation</td>
<td>2.83</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>Breast Conservation</td>
<td>Mastectomy</td>
<td>2.84</td>
<td>0.020</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presentation to First Treatment</th>
<th>Provider</th>
<th>Method of Presentation: Screening Mammogram</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic</td>
<td>Non-Hispanic</td>
<td>2.65</td>
<td>0.022</td>
</tr>
<tr>
<td>Charlson Index ≥ 3</td>
<td>Charlson Index &lt;3</td>
<td>0.52</td>
<td>0.120</td>
</tr>
<tr>
<td>Method of Presentation: Care Provider</td>
<td>Method of Presentation: Screening Mammogram</td>
<td>3.16</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Title: Delayed hospital visits in patients with breast cancer after the great East Japan earthquake and the subsequent Fukushima Daiichi nuclear power plant accident: A retrospective comparative analysis

Ozaki A, Tsubokura M, Nomura S, Morita T, Ochi S, Kato S, Saji S, Yokota T, Leppold C, Tanimoto T, Kami M, Tsukada M and Ohira H. Minamisoma Municipal General Hospital, Minamisoma, Fukushima, Japan; Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo, Japan; School of Public Health, Imperial College London, London, United Kingdom; Soma Central Hospital, Soma, Fukushima, Japan; Fukushima Medical University, Fukushima, Japan; Jyoban Hospital of Tokiwakai Group, Iwaki, Fukushima, Japan and School of Social & Political Science, University of Edinburgh, Edinburgh, United Kingdom.

Body: Introduction

In breast cancer, delay in first presentation and self-interruption of continuous treatment are associated with lower survival. It has been suggested that risk factors for such behavioral patterns include poor social support.

Minamisoma City, located within a 30 kilometers radius from Fukushima Daiichi nuclear power plant, has experienced rapid change in social structures following the Great East Japan Earthquake and the subsequent power plant accident. There has been a mass evacuation among young and middle-aged generations for fear of potential irradiation, and this has resulted in the separation of families and friends. These changes may have resulted in a deterioration of social support for residents, which could consequently lead to changed patterns of behavior in diseases such as breast cancer.

Objectives

This study compared the behavioral patterns before and after the disasters in patients with breast cancer in Minamisoma City.

Methods

We retrospectively analyzed data from patients with breast cancer who were diagnosed from January 2008 through March 2015 in the two main cancer centers in Minamisoma City. Demographic and clinical information was extracted from medical records, including age, stage, pathological findings, treatment, and the reason for the first hospital visit. The main outcome was a change of interval from the appearance of initial symptoms to the first hospital visit before and after the disasters. We also assessed whether continuous follow-up was maintained after the disasters. We used an unpaired t-test for numerical variables and a chi-squared test for categorical variables.

Results

A total of 102 and 97 patients were diagnosed with breast cancer before and after the disasters, respectively. There were no statistically significant differences between the 2 groups concerning average age (61 years old vs. 61 years old, p=1), stage 3 or 4 cancer (18% vs. 17%, p=0.81), invasive cancer (92% vs. 93%, p=0.87) and symptomatic patients (75% vs. 74%, p=0.74), respectively. However, after the disasters, there were significant increases in the ratio of patients with more than a one-year delay from the appearance of the initial symptom to the first hospital visit (5.4% vs. 15%, p<0.05). The patients with more than a one-year delay had a significantly higher ratio of advanced stage cancer compared with patients who visited a hospital earlier. Continuous follow-up was maintained in all patients diagnosed after the disasters.

Discussion

The characteristics of patients were not significantly different before and after the disasters, while the ratio of patients with more than a one-year delay of the first hospital visit significantly increased after the disasters. Although information on social capital and other sociodemographic factors was not available, we speculate that poor social support due to changed social structures after the disasters might contribute to delay in first presentation in symptomatic breast cancer patients. Further study is warranted to clarify the factors associated with delayed hospital visits, in order to establish effective health interventions in the aftermath of mass disasters.
Title: Impact of the Middle East unrest on management of breast cancer – A single institution experience


Body: King Fahad University Hospital of Dammam (KFHU) is a major referral teaching Hospital in the Eastern province in Saudi Arabia. With permission, The Breast Division at KFHU accepts expatriate patients for treatment of breast cancer free of charge. Between Jan 2010 and June 2015 the unit has received 435 new and previously treated breast cancer patients, 35% of them were expatriates. We have noticed that most of these patients had an out of their hand delay in initiating either their investigations for a breast lump or start of active treatment; this has resulted in less than optimum prognosis in many cases. We reported that 75% of these patients had an average of 6-19 months delay before they presented for investigations of a breast mass, 25% had received previous irregular treatment and did not complete their management due to war unrest and travelling. More than 70% of patients who needed radiotherapy did not receive it due to unavailability of treatment facility. Conclusion: In spite of the dramatic improvement in diagnosing early breast cancer, after the wide spread of screening programs in the last decade, we are faced again with recent increase in advanced presentations and poor outcome due to war unrest. With the expected increase in the number of similar cases we think that this category of patients should be addressed as a special category.
**Title:** Preoperative chemotherapy regimens and breast cancer subtype in an underinsured Hispanic population


**Body:**

**Background:** Although the Hispanic population is among the fastest growing in the United States, however, less is known about them than other populations. Breast cancer in different racial/ethnic populations display different behaviors. The current study was performed to examine response to preoperative chemotherapy regimen and by breast cancer subtype in a Hispanic safety net population.

**Methods:** A retrospective review of Hispanic breast cancer patients who underwent preoperative chemotherapy from July 2001 to February May 2015 at a safety net hospital. Sociodemographic, clinical, and treatment variables were evaluated. Response to chemotherapy regimen was recorded. Breast cancer subtypes were divided based on IHC and FISH testing. Luminal B subtype was classified based on Ki67 (>15%) and PR (<20%).

**Results:** The average age of the 133 patients was 45 years. 93% of the patients were insured with Medicaid or uninsured (70%). The average size of the cancers at presentation was 5cm. Overall 86% of patients had a clinical response to preoperative chemotherapy and 35% had pathologic complete response (pCR). AC and TC regimens had the lowest rate of pCR at 16%. AC/T (every 3 week and weekly) had similar rates of pCR 23% and 29%, while dose dense regimens showed pCR 40%. Herceptin containing regimens had pCR 57%. 7 patients received TAC and 71% had pCR.

By subtype, Luminal A and B patients had low rates of pCR 9% and 14% respectively. Luminal B patients did benefit from preoperative chemotherapy as 86% of patients who were not candidates for breast conservation at presentation were able to undergo lumpectomy after preoperative chemotherapy. Her2 subtype patients who got Herceptin had pCR 57%. Triple negative patients had pCR 54%.

**Conclusions:** In this underinsured, Hispanic population who presented at advanced stages, differences in response to preoperative chemotherapy were seen based on breast cancer subtype. Differences were also seen based on chemotherapy regimen. TAC maybe a particularly effective regimen in triple negative Hispanic women.
Title: The benefit of preoperative chemotherapy in an underinsured Hispanic population with poor use of screening mammography

Komenaka IK, Djenic B, Walters J, Hsu C-H, Nodora JN, Martinez ME, Bouton M and Mehta D. Maricopa Medical Center, Phoenix, AZ; University of Arizona, Tucson, AZ and University of California, San Diego, San Diego, CA.

Body: Background: Despite prospective clinical trials demonstrating the safety and effectiveness of preoperative chemotherapy for nearly 2 decades, it may still be underutilized in underserved, uninsured populations most likely to present with advanced cancers. The current study was performed to evaluate the effect of preoperative chemotherapy (PC) in a Hispanic safety net population.

Methods: A retrospective review of Hispanic breast cancer patients who presented at clinical stage 2 or higher and were treated from July 2001 to February May 2015 at a safety net hospital. Sociodemographic, clinical, and treatment variables were evaluated. Surgical outcomes were evaluated. Margin status was determined for those who underwent breast conservation. Results: The average age of the 266 patients was 45 years. 93% of the patients were insured with Medicaid or uninsured (70%). Only 24% of patients underwent screening mammography. 133 underwent PC and 133 had primary operations. Patients who underwent PC presented with larger cancers (5cm vs 3.5cm, p < 0.001). 96% of patients in PC group were not candidates for lumpectomy at presentation. However, lumpectomy was performed more often in those who underwent PC (75% vs. 57%, p = 0.01). Re-excision for margins were necessary less often in those who underwent PC (10.3% vs 27%, p = 0.01). Patients who underwent PC were also less likely to require an ALND (33% vs 47%, p = 0.04). Despite presentation at higher average clinical stage in patients who underwent PC, at average follow up of 52 months, risk of IBTR and risk of regional recurrence were similar in the PC and no-PC groups (IBTR: 4.4% vs 3%, p = 0.99 and Regional: 1.5% vs. 1.5%, p = 0.99). Breast cancer specific survival was 86.5% in the PC group compared to 84% in the no-PC group (p = 0.68). Patients in the no-PC group were less likely to comply with recommended chemotherapy.

Conclusions: In this underinsured, Hispanic population who did not use screening mammography, preoperative chemotherapy allowed many women to undergo breast conservation, undergo fewer operations, and were less likely to require ALND. Risk of local and regional recurrence is low in these patients and comparable to those who underwent primary operation.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-12-08

Title: Healthcare barrier profiles in patients navigated for cancer screening and treatment and the impact of the affordable care act


Body: Background: The underserved community experiences barriers to cancer screening that result in overall greater mortality rates across all cancers. Insurance coverage brought forth by the Affordable Care Act has the potential to significantly impact these barriers through reducing the burden of health care cost on the patient. In this study the authors observe the impact of the Affordable Care Act on the barrier profiles presented by the patients navigated for cancer screening and treatment.

Methods: Patient navigation encounters were recorded for a total of 1146 patients navigated for cancer screening and treatment at the Cleveland Clinic Foundation from the years 2012 through 2015. A total of 3259 encounters were classified into barrier types. Health care billed encounters were retrieved from EPIC for this group of patients from the time they entered patient navigation and classified in terms of insurance coverage. Patients were categorized according to their barrier profile. Appropriate generalized linear regression models were used to test for association of these profiles to number and types of navigation and health care encounters and cost, and to test for change in types of encounters and patient barrier profiles through time.

Results: The insurance barrier is present in 23% of all navigation encounters. Patients presenting with an insurance barrier had a greater mean number of navigation (p<0.001) and health care encounters (p<0.006), had a greater proportion of self-paid health care encounters (p<0.001) and a lower total cost billed for health care encounters after controlling for number of encounters (p<0.001). The access barrier is present in 53% of navigation encounters while patients that present with only the access barrier account for 42% of the entire sample. Patients that present with only the access barrier have doubled every year (OR 2.2 per year, 95% CI [1.8 2.6]) from 2012 to 2015, while the proportion of self-paid health care encounters (OR 0.26 per year, 95% CI [0.25 0.28]) and the presence of the insurance barrier (OR 0.55 per year, 95% CI [0.49 0.62]) have more than halved during this time period.

Conclusion: Although the Affordable Care Act has clearly had an impact by lowering the number of insurance barrier navigation encounters through time, it has uncovered access as the predominant remaining barrier. Understanding and targeting the access barrier will be the most effective way to potentiate the effects of the ACA on patients being navigated for cancer screening and treatment.
Title: Uptake of genetic testing for BRCA mutations in a medically underserved population

Thekkekara RJ J, Gaber RS S, Gil DN N, Holden CM M, Aluen Metzner I, Marcus E and Ganschow PS S.  John H Stroger Jr., Hospital of Cook County, Chicago, IL.

Body: Introduction:
Genetic testing for hereditary cancer syndromes is recommended in specific populations to identify individuals at increased risk for developing malignancies. The uptake of genetic testing for BRCA mutations in studies has ranged from 44%-81% but has not been well studied in medically underserved populations. In this study we identified factors that predicted uptake of genetic testing among a medically underserved population undergoing genetic counseling in the Cancer Risk Program at a safety net hospital in Chicago.

Methods:
A retrospective cohort design was used. Data were abstracted from the medical records of 150 consecutive individuals who underwent genetic counseling for BRCA mutation testing in the Cancer Risk clinic at John H Stroger Jr., Hospital of Cook County between October 2013 and July 2014. The primary outcome measure was the uptake of genetic testing among individuals in whom the test was recommended after genetic counseling. Final testing status was assessed as of April 30, 2015. Individuals referred for testing of genetic syndromes other than hereditary breast and ovarian cancers were excluded from this analysis. Data regarding personal and family history of cancer and socio-demographic determinants- age, sex, ethnicity and insurance status were collected.

Results:
Among the 150 individuals who underwent genetic counseling, the mean age was 49 years (range 19-74 years) and only 5 were men. Forty-three percent were African American, 34% were Hispanic and 11% were Caucasian. Sixty seven percent were uninsured and 30% had public insurance. Forty-one percent had a personal history of cancer (36% with breast cancer, 3% with pelvic cancer). Eighty five percent of the individuals had 2 or more relatives with cancer in the family. Genetic testing was recommended in 112 individuals (75%) after assessment and counseling by the genetic counselor. Of those recommended, 89 individuals (80%) underwent genetic testing. Among the men, 4 (80%) were recommended for testing and 3 (75%) underwent testing. Genetic testing was not recommended for 20 individuals (13%) and of the remaining 18 individuals (12%), testing of a living affected relative was recommended first. Only one of the 18 individuals returned with genetic test results from an affected family member during the study period. The only positive predictor of testing was age less than 50 years (OR 3.63; 95% CI 1.31-10.08). Having one or more siblings with cancer was a negative predictor of uptake of testing (OR 0.35; 95% CI 0.13-0.90).

Conclusions:
Uptake of genetic testing was high among our cohort of medically underserved individuals. While prior studies have shown a variable association between age and uptake of testing, younger individuals in our cohort were more likely to undergo testing. Interestingly, while most studies have shown an increased uptake of testing among individuals who have a family history of cancer, having cancer in a sibling was a barrier to testing in our cohort and warrants further study. The very low number of men counseled and tested during the study period also suggests that strategies are needed to extend genetic counseling and testing to at-risk men in medically underserved communities.
Title: Does the quality of systemic therapy for patients with early breast cancer (EBC) differ between adjuvant and neoadjuvant patients?

Enright K, Yun L, Powis M, Gonzalez A, Taback N, Booth C, Trudeau M and Krzyzanowska M. Trillium Health Partners - Credit Valley Hospital, Mississauga, ON, Canada; Institute for Clinical Evaluative Science, Toronto, ON, Canada; Princess Margaret Cancer Centre, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Kingston Regional Cancer Centre, Kingston, ON, Canada and Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Background:
The measurement of the quality of cancer care delivery at a both a system and practice level drives performance improvement within the cancer system by highlighting potential gaps in care. The purpose of our study was to examine whether the performance of the system differs for women with early breast cancer (EBC) who received neoadjuvant chemotherapy (NA) compared with those who received post-operative adjuvant (A) treatment for their EBC.

Methods:
The Ontario Cancer Registry was used to identify all EBC cases diagnosed 2006 – 2010 in Ontario, Canada. The cases were linked deterministically to multiple health care databases to provide comprehensive medical follow-up. We measured a panel of quality indicators (QI) that had been previously developed to reflect the quality of systemic therapy delivery across multiple quality domains (access, delivery, toxicity, safety). We operationalized the QI for use with administrative health care databases to reflect systemic therapy delivery on a population/systems level. The panel of QI was applied to all the patients in the cohort who met the inclusion criteria for the individual indicators. Patients were considered to be NA if they received chemotherapy prior to their breast cancer surgery, all other EBC patients were evaluated in the A cohort.

Results:
We identified 30,006 EBC patients, including 28,427 A and 1579 NA patients. Compared with the A cohort that NA cohort was younger (60.9y vs. 51.9y) and more likely be to be stage 2 or 3 (53.5 % vs 96.7%), thus more likely to reflect locally advanced breast cancer. Both the A and NA cohorts had similar performance in the safety and effectiveness domains (Table 1) , however the NA cohort had higher performance in access and timeliness domains.

Conclusion:
Variation in performance on QI was identified between NA and A cohorts. While some of this variation appears appropriate for the higher risk clinical setting of NA patients, further work is planned to determine the clinical and system-level factors driving this variation.

Table 1. Quality of Systemic Therapy for Adjuvant and Neoadjuvant EBC patients

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Adjuvant</th>
<th>Neoadjuvant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consult with a medical oncologist</td>
<td>91.8%</td>
<td>N/A</td>
</tr>
<tr>
<td>Receipt of chemotherapy</td>
<td>43.5%</td>
<td>N/A</td>
</tr>
<tr>
<td>Receipt of hormonal therapy</td>
<td>77.5%</td>
<td>91.9%</td>
</tr>
<tr>
<td>Timely receipt of chemotherapy*</td>
<td>58%</td>
<td>87%</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adherence to hormonal therapy (HT)</td>
<td>86.8%</td>
<td>85.4%</td>
</tr>
<tr>
<td>Surveillance imaging (lower is better)</td>
<td>58.2%</td>
<td>66.2%</td>
</tr>
<tr>
<td>Toxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency Room visit or hospitalization</td>
<td>47.3%</td>
<td>48.7%</td>
</tr>
<tr>
<td>Febrile Neutropenia</td>
<td>14%</td>
<td>18.2%</td>
</tr>
<tr>
<td></td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Death &lt;60 d from chemotherapy</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate anti-emetics</td>
<td>60.1%</td>
<td>60.2%</td>
</tr>
<tr>
<td>Febrile Neutropenia secondary prophylaxis</td>
<td>20.9%</td>
<td>14.9%</td>
</tr>
<tr>
<td>Inappropriate receipt of HT</td>
<td>&lt;1%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Inappropriate receipt of trastuzumab</td>
<td>4.5%</td>
<td>4.8%</td>
</tr>
<tr>
<td>Appropriate molecular testing</td>
<td>92.3%</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

*within 60 days of diagnosis (NA), within 60 days of surgery A
Title: Functional assessment in early breast cancer in older patients: The FABIO study

Phillips I, Sinha R, Fatz D and Ring A. Royal Marsden NHS Foundation Trust, Sutton, Surrey, United Kingdom and Brighton and Sussex University Hospitals NHS Trust, Brighton, East Sussex, United Kingdom.

Body: Introduction: More than a third of breast cancer diagnoses are made in women aged 70 years or older at the time of diagnosis. Epidemiological studies and practice surveys from the US and Europe have demonstrated lower rates of use of adjuvant chemotherapy and radiotherapy in older women with early breast cancer. It is not clear whether this is due to objective health problems mitigating the benefit of such therapies and increasing the risk of side-effects, or whether decisions are being made on the basis of age bias alone. A prospective cohort study was set-up to describe older patients' fitness according to a Comprehensive Geriatric Assessment (CGA) to investigate if fitness could explain apparent variations from standard care.

Methods: Women aged 70 or over diagnosed with early breast cancer were enrolled. Demographic and tumour details were recorded and patients underwent a CGA which evaluated: cognition (6-Cognitive Impairment Test:6-CIT), functional status (Activities of Daily Living [ADL], Instrumental Activities of Daily Living [IADL], Vulnerable Elders Score-13 [VES-13]), co-morbidities (Charlson co-morbidity index), anaesthetic fitness (ASA grade) and performance status (WHO). All patients also completed a G8 screening score. A predetermined threshold for defining patients as "unfit" (patients who failed one or more elements of the CGA) or "fit" was set for the purposes of this analysis.

Results: Two hundred and ninety-two patients were recruited, of whom 36% were aged 70-74, 54% 75-84 and 10% were aged 85 or over. Twenty-six percent of patients had node positive breast cancer, 29% had high grade tumours, 91% oestrogen receptor positive disease and 12% HER2 positive breast cancer. A full CGA was available for 212 patients. WHO performance status was 0 (42%), 1 (43%) and 2 or more (16%). VES-13 score was 3 or more (associated with functional decline) in 49%. G8 score was < 14 (predictive of failing a CGA) in 56%. Eighty-seven percent and 75% were independent in ADLs and IADLs respectively. Seventy-nine percent of patients has a 6-CIT score of 0-7 (normal). Charlson co-morbidity index was 0 (70%), 1 (18%) and 2 or more (12%). According to the predetermined threshold: 170 (80%) were defined as unfit and 42 (20%) defined as fit.

One hundred and fifty-three patients underwent breast conserving surgery, 91% of those who had breast conserving treatment and were fit received adjuvant radiotherapy. Eighty-two patients had disease at high risk of recurrence meriting consideration of adjuvant chemotherapy (oestrogen receptor negative, HER2 positive, node positive, grade 3). Eighty-three percent of those with high risk disease who were fit received adjuvant chemotherapy.

Discussion: Many patients aged 70 years or older have deficits in a CGA which may indicate a higher risk of death from competing causes, functional decline and increased risks of toxicity, despite an apparently good performance status. Overall the rates of adjuvant chemotherapy use were low (consistent with previous literature). However the majority of patients with high risk disease who were fit received adjuvant chemotherapy, contrasting with the perception that older patients may be being denied access to treatment on the basis of age alone.
Title: How can we improve vulnerability score in breast cancer survivors? A pilot experience in an underprivileged community

Jaouen A, Festa A, Boubaya M, Levy V and Zelek L.  Ac'Sante93, Bobigny, France and Avicenne Hospital, Bobigny, France.

Body: BACKGROUND: We decided to evaluate the evolution of vulnerability in breast cancer survivors receiving an individualized survivorship care plan and living in an area (Seine-Saint-Denis, SSD), which is among the poorest in France with a median household income is 68% lower than in Paris. In SSD, cancer is the leading cause of premature mortality. Whereas it is widely admitted in France that 25% of patients are faced with financial difficulties after breast cancer, this proportion reaches 40% in SSD.

PATIENTS AND METHODS: Ac'Santé93 is a non-profit organization whose aim is to provide supportive care, health education and individualized assistance to patients and families, and to facilitate timely access to quality medical and psychosocial care. Vulnerability was evaluated using a 11-item standardized score (EPICES) previously investigated by French Health Examination Centers. Strictly speaking this score was aimed at measuring precarity, a concept referring to a social condition assumed to face worsening. This score is more strongly related to health status than the administrative classification of poverty (Sass, Sante Publique 2006). Vulnerability was defined by a score ≥30 and considered as severe when ≥40. In SSD two thirds of the population are affected by vulnerability. Patients included in the study were scored after cancer diagnosis (E1) and 6 mos. after the first evaluation (E2). Patients were divided into tertiles according to E1: 30-40, 40-67 and 67-100. Psychosocial comorbidities, demographic data, and supportive care received were also recorded. Actions undertaken were divided in three categories: social/advocacy (e.g. help with filling out administrative forms), individual (e.g. dietician consultation) and group (e.g. group sessions led by a sport instructor).

RESULTS: Over the year 2014, 120 breast cancer survivors were included and had E1 and E2 scores. Median E1 and E2 were 52.1 and 47.3 and the mean difference was 7.2 (p<0.0001). The score improved for 72% of pts but worsened in 16% and remained stable in 12%. Whereas a significant improvement of E2 was observed whatever E1 in patients included in a support group, it was not the case in the other patients (social/advocacy and/or individual). Surprisingly, the effect of support groups on vulnerability score was significantly greater in the highest tertile of pts (E1 from 67 to 100). Being in the highest tertile at inclusion was also the strongest predictor for improvement in all patients (RR=7.7, p=0.007). Younger patients were at significantly higher risk of worsening: median age was 49.2 in case of worsening v 54.3 in case of improvement (p=0.047).

CONCLUSION: Survivorship care plans can improve vulnerability in most pts. Paradoxically, it seems easier to improve vulnerability in pts with highest initial scores. Furthermore these patients are those who benefit the most from support groups. We hypothesize that desocialization is frequently underestimated in this population, and that support groups, besides their primary goal, act through developing social links. Finally, the finding that younger patients are at higher risk for worsening vulnerability underscores the burden of unmet needs in youngest breast cancer survivors.
Title: Awareness and acceptability of skin application of preventive medications for breast cancer

Karavites LC C, Allu S, Khan SA A and Kaiser K. University of Illinois College of Medicine at Mt. Sinai Hospital, Chicago, IL; Northwestern University Feinberg School of Medicine, Chicago, IL and Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: OBJECTIVE: To better understand women's knowledge and perceptions of breast cancer prevention medications and determine if a topical administration of these medications would increase acceptability.

METHODS: Focus groups were conducted with healthy women identified through the breast center of a single institution. An experienced moderator led participants through a discussion of breast cancer risk perceptions, knowledge of and concerns about risk reduction medications, and their views of a skin application of risk reducing medication. Participants provided sociodemographic information, breast cancer risk factors, and prior physician recommendations to take risk-reducing medicines. Trained research personnel examined all qualitative results systematically. Participants' breast cancer risk was estimated using the Gail Model.

RESULTS: Four focus groups (N=32) were conducted. Most participants had at least a college degree (78.2%) and were of either European (50%) or African ancestry (31%). The majority (72%) were at elevated risk for breast cancer, just over half of these women perceived themselves to be at higher than average risk. Only 19% of participants had prior knowledge of preventive medications. Women who perceived themselves to be at high risk were more likely to know about preventive medications than those who did not, 38% vs 0% respectively. Over 90% of the focus group participants stated that they would prefer a topical application of a preventive medication to a pill if their physician advised them to take preventive medication.

CONCLUSION: Awareness of preventive medications was low even in a highly educated sample of high-risk women. If given a choice in the route of administration of chemoprevention, nine out of ten women preferred a gel to a pill. Future work should focus on demonstrating a reduction in side effects of topical over oral medications and raising awareness of chemopreventive options for breast cancer to increase acceptance of preventive medications.
**Title:** Comparative analysis of breast cancer phenotypes in African American, White American, and African patients - Correlation between African ancestry and triple negative breast cancer


**Body:** Introduction: Population-based incidence rates of triple negative breast cancer (TNBC) are higher for African American (AA) compared to White American (WA) women, but it is unclear whether TNBC risk is genetically associated with African ancestry because AA women represent an ancestrally admixed population. Higher frequencies of TNBC have also been observed in sub-Saharan African breast cancer (BC) patients, but comparative analyses of biomarker expression among datasets that include AA, WA, and African women are sparse. We report findings from an international registry that features specimens from a diverse patient population in Detroit, Michigan as well as a hospital in Kumasi, Ghana.

**Methods:** The study dataset included formalin-fixed, paraffin-embedded invasive BC tumors diagnosed between 1998 and 2014 at the Komfo Anokye Teaching Hospital in Ghana and the prospectively-maintained/annotated Henry Ford Health System cohort in Michigan. All Ghanaian tumors underwent pathology confirmation and immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR) and HER2/neu expression at the University of Michigan. Women were classified into five BC phenotypes and dichotomized into two age groups, <50 and ≥50 years. Polychotomous multivariate GLM models were developed to estimate the risk for each BC phenotype. Statistical analyses were performed in SAS v. 9.0 (Carey, NC). This research was approved by the Institutional Review Boards of the participating institutions.

**Results:** A total of 234 Ghanaian cases with mean age 49 years (range 24-92); 271 AA with mean age 60 (range 27-87); and 321 WA with mean age 62 (range 31-91) (P=0.001) contributed to this study. Prevalence of histologic grade 3 was lowest in WA (n=107, 33.7%) which was statistically significant from the observed prevalence in AA (n=135, 50.4%) and Ghanaians (n=84, 53.8%) (P=0.0001). ER-negative and TNBC were more common among Ghanaian and AA compared to WA cases (frequency ER-negativity 67.5%, 37.1%, and 19.8%, respectively, p<0.0001; frequency TNBC 53.2%, 29.8%, and 15.5%, respectively, p<0.001). In the age group <50 years, 82 women (42.5%) were diagnosed with ER+/PR+/HER2-, 65 (33.7%) with TNBC, 27 (14.0%) with ER+/PR+/HER2+, 14 (7.2%) with ER-/PR-/HER2+ and 5(2.6%) with ER-/PR+/HER2- phenotypes. In this young age group, prevalence of TNBC remained highest among Ghanaian women (50.8%), followed by AA (34.3%) and WA (15.9%); (P=.0006). In contrast, highest prevalence of ER+/PR+/HER2+ and ER+/PR+/HER2- phenotypes was observed in WA, followed by AA and Ghanaians. On multivariate analysis histologic grade 3 and racial heritage remained statistically significantly associated with the TNBC phenotype (OR for AA vs. WA with TNBC 1.87, 95% CI 1.15-3.04; OR for Ghanaian vs. WA with TNBC 10.63, 95% CI 5.32-21.25; OR for Grade 3 vs Grade 1 histology with TNBC 33.3, 95% CI 13.45-82.4).

**Conclusions:** This study confirms an association between the TNBC phenotype and African ancestry; furthermore, extent of African ancestry appears to be associated with an increased likelihood of having a TNBC tumor, since frequency of TNBC among AA patients was intermediate between WA and Ghanaian patients.
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-13-01

**Title:** Triplet therapy with ribociclib, everolimus, and exemestane in women with HR+/HER2– advanced breast cancer

Bardia A, Modi S, Oliveira M, Campone M, Ma B, Dirix L, Weise A, Nardi L, Zhang V, Bhansali SG G., Hewes B and Chavez-MacGregor M. Massachusetts General Hospital Cancer Center, Boston, MA; Memorial Sloan Kettering Cancer Center, NY, NY; Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; Institut de Cancérologie de l'Ouest – René Gauducheau Centre de Recherche en Cancérologie, Nantes, France; The Chinese University of Hong Kong, Shatin, Hong Kong; Sint-Augustinus Hospital, Antwerp, Belgium; Barbara Ann Karmanos Cancer Institute, Detroit, MI; Novartis Institutes for BioMedical Research, East Hanover, NJ; Novartis Institutes for BioMedical Research, Cambridge, MA and MD Anderson Cancer Center, University of Texas, Houston, TX.

**Body:**

**Background:** Everolimus (EVE; 10 mg) and exemestane (EXE; 25 mg) doublet therapy improved progression-free survival in women with hormone receptor-positive (HR+)/HER2– advanced breast cancer (aBC; BOLERO-2), but was associated with significant mucositis as well as eventual disease progression. Cyclin-dependent kinase (CDK) 4/6 inhibitors have shown clinical activity in combination with endocrine therapy and could potentially overcome phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitor resistance by acting downstream of the PI3K pathway.

**Methods:** In this ongoing Phase Ib/II study (NCT01857193), postmenopausal women with anastrozole- or letrozole-resistant HR+/HER2– aBC received escalating doses of the CDK4/6 inhibitor ribociclib (LEE011 [LEE]; once daily [QD], days 1–21 of 28-day cycles) with EVE (1–5 mg QD) and EXE (25 mg QD). Dose escalation was guided by a Bayesian logic regression model with overdose control principle and real-time pharmacokinetics. Pre-treatment tumor samples were sequenced by the Foundation Medicine platform. Here we report on the safety and efficacy of triplet therapy, and explore potential predictive biomarkers of response.

**Results:** At the cut-off date March 2, 2015, 84 patients (pts) had been treated; here we present results from the 70 pts in the triplet arm treated with LEE (200–350 mg), EVE (most at 2.5 mg), and EXE. The median number of prior regimens was 5, and 18 (25.7%) pts had received prior PI3K/AKT/mTOR or CDK4/6 inhibitors for metastatic disease. Grade 3/4 treatment-related adverse events (AEs; ≥5% pts) were neutropenia (45.7%), leukopenia (8.6%), and thrombocytopenia (5.7%). Two (2.9%) pts discontinued due to AEs. Grade 3 dose-limiting toxicities were reported in 6 pts treated with LEE (300 mg) and EVE (2.5 mg): increased alanine aminotransferase/aspartate aminotransferase (2 pts), febrile neutropenia and hypophosphatemia (1 pt), oral mucositis (1 pt), rash and thrombocytopenia (1 pt), and thrombocytopenia with bleeding (1 pt). The recommended Phase II dose will be reported at the meeting. Among 55 pts evaluable for best overall response, there was 1 (1.8%) complete response (CR), 2 (3.6%) confirmed and 3 (5.5%) unconfirmed partial responses (PR), 7 (12.7%) non-CR, non-progressive disease (NCRNPND), and 26 (47.3%) stable disease (SD). Disease control rate (CR+PR+SD+NCRNPND) was 70.9%. One pt received treatment for ≥14 months, and 23 (32.9%) pts for ≥4 months. There was a trend towards longer duration of treatment in the CCND1 amplified group (n=10; median 166 days) than in the non-amplified group (n=22; median 60 days). A retrospective analysis of BOLERO-2 showed no differential effect of CCND1 amplification on PFS.

**Conclusions:** Triplet combination of endocrine therapy with mTOR and CDK4/6 inhibition is feasible, permits lower dosing of EVE (resulting in better tolerability), and shows encouraging signs of clinical activity, including in some pts with prior exposure to PI3K/AKT/mTOR or CDK4/6 inhibitors. Duration of treatment was longer in pts with cyclin D amplification, possibly due to inclusion of a CDK4/6 inhibitor. These results suggest that triplet therapy might be beneficial for pts who progress on doublets or who have cyclin D amplification.
A phase 1 dose escalation study of RAD1901, an oral selective estrogen receptor degrader, in healthy postmenopausal women

Hattersley G, David F, Harris A, Clarkin M, Banks K, Glaudemans AWJM, Doorduin J, Koole M, de Vries EFJ and Williams G. Radius Health Inc; Pharmagellan LLC and University Medical Center Groningen.

Estrogen and the estrogen receptor (ER) are known to be prominent drivers of breast tumourigenesis and breast cancer progression. Hormonal agents that target the ER such as selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) are routinely used for the management for ER+ breast cancer. Fulvestrant is currently the only SERD approved for the treatment of ER-positive metastatic breast cancer, however, despite its clinical efficacy, the utility of fulvestrant has been limited by the amount of drug that can be administered in a single injection, the reduced bioavailability and the development of resistance in some patients. To overcome some of the challenges associated with current endocrine therapies there is a need for more durable and more effective ER-targeted therapies.

RAD1901 is a novel, non-steroidal, orally bioavailable SERD. Preclinical studies demonstrated a favorable tissue selectivity profile, dose dependent ER degradation and potent inhibition of in vitro breast cancer cell proliferation. Significant tumor regression was consistently observed with RAD1901 treatment, compared to Tamoxifen or Fulvestrant in MCF7 and patient derived xenograft models, including those harboring ESR1 mutations. Here we describe a Phase 1 clinical study that was conducted in 52 healthy postmenopausal female volunteers. Cohorts of 10 subjects (2 placebo, 8 RAD1901 treated) were dosed with 200 mg, 500 mg, 750 mg or 1000 mg for 7 days. Additional subjects were enrolled to an 18F-estradiol positron emission tomography (FES-PET) cohort to evaluate the 200 mg (n=7 subjects) and 500 mg (n=6 subjects) dose levels. All dose levels were generally well tolerated with a total of 43/52 subjects completing the study. Pharmacokinetic analysis demonstrated good plasma exposure with dose proportional increases. FES-PET was performed at baseline and after 7 days of treatment with RAD1901, to assess estrogen receptor engagement. Standardized uptake values (SUV) pre- and post-treatment demonstrated a complete attenuation of FES-PET signal in ER-rich tissues such as the uterus at the 500 mg/day dose level, whereas almost 80% reduction in the signal was observed at the 200 mg/day dose level. Based on these preclinical and clinical results, RAD1901 is currently being investigated in a Phase 1 study for the treatment of hormone driven and hormone resistant metastatic breast cancers.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-13-04

Title: A phase I trial to evaluate the safety of the addition of alisertib to fulvestrant in hormone receptor positive (HR+), advanced breast cancer

Haddad TC C, D'Assoro AB B, Suman VJ J, Opyrchal M, Goetz MP P and Ingle JN N. Mayo Clinic, Rochester, MN and Roswell Park Cancer Institute, Buffalo, NY.

Body: Background: During tumor progression, activation of Aurora A kinase (AURKA) is associated with epithelial to mesenchymal transition (EMT) reprogramming and expansion of a subpopulation of tumor initiating cells harboring a CD44+/CD24low/− phenotype [D’Assoro, Oncogene 2014]. These cells are characterized by their capacity to self-renew, resist drug therapies, and promote distant metastases. In ER+ breast cancer (BC) models, activation of AURKA is associated with down-regulation of ERα expression and resistance to endocrine therapy. Alisertib, a selective inhibitor of AURKA, can reverse EMT and restore tumor ERα expression and sensitivity to endocrine therapy [Opyrchal, PLoS One 2014]. As a single agent in HR+ advanced BC, alisertib was associated with a 6-month clinical benefit rate of 54% and median PFS of 7.9 months [Melichar, Lancet Oncol 2015]. The objectives of this phase I trial were to determine the maximum-tolerated dose (MTD) and evaluate the toxicities and clinical activity of alisertib with fulvestrant in patients (pts) with HR+ advanced BC.

Methods: In this standard 3+3 dose-escalation phase I study, pts were assigned to two different oral doses of alisertib (40-50 mg BID on days 1-3, 8-10, 15-17 q 28-day cycle) in combination with standard dose fulvestrant (500 mg IM on day 1 and 15 of cycle 1 and then day 1 q 28-day cycle thereafter). Eligibility included HR+ advanced BC, postmenopausal status, measurable disease or nonmeasurable bone disease by RECIST v1.1, ECOG performance status ≤ 1, unlimited prior endocrine therapies, and ≤ 2 chemotherapy regimens in the metastatic setting.

Results: Ten pts enrolled September 2014 - April 2015, and 9 were evaluable for the primary endpoint (one excluded due to ineligibility). The median pt age was 59 (range 48, 73). Prior endocrine therapies included AI (9, 100%), fulvestrant (6, 67%), and everolimus/exemestane (5, 56%). Eight pts (89%) had prior chemotherapy.

A median of 4 cycles of therapy have been administered (range 1+, 9+). There were no severe (grade 3+) toxicities reported during cycle 1 at either dose level, thus the MTD was not reached. The cycle 1 dose 1/2 adverse events regardless of attribution were fatigue (6, 67%), neutropenia (5, 56%), anemia (5, 56%), leukopenia (4, 44%), diarrhea (3, 33%), nausea (3, 33%), and mucositis (1, 11%). As of June 3, 2015, 2 pts have discontinued treatment due to disease progression, and 7 remain on treatment with stable disease (Table). One pt with bone only disease had a near CR on PET scan.

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Alisertib Dose (BID)</th>
<th>Treatment Cycles</th>
<th>≥ Grade 3 Toxicity, All Cycles</th>
<th>Progression-Free Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=3)</td>
<td>40 mg</td>
<td>4, 7+, 9+</td>
<td></td>
<td>117, 170+, 223+</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>50 mg</td>
<td>1+, 2, 3+, 3+, 4+, 5+</td>
<td>grade 4 neutropenia (1 pt)</td>
<td>28+, 56, 56+, 57+, 112+, 116+</td>
</tr>
</tbody>
</table>

+ indicates patients still receiving treatment

Conclusion: Alisertib in combination with fulvestrant was well-tolerated. The recommended phase II dose is 50 mg twice daily on days 1-3, 8-10, and 15-17 q 28-day cycle with standard dose fulvestrant. Promising antitumor activity was observed. Correlative tissue evaluation of AURKA expression and other EMT biomarkers is underway.

Funding: This work was funded by Takeda Oncology and supported by NIH Grant K12 CA90628 [TCH].
**2015 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-13-05

**Title:** Androgen receptor (AR): A novel target and mechanism for radiosensitization and treatment in triple-negative breast cancers (TNBC)

Speers C, Zhao SG, Liu M, Rae JM, Hayes DF, Feng FY and Pierce LJ. University of Michigan Hospital, Ann Arbor, MI.

**Body:**

**Background:** Increased rates of locoregional recurrence have been observed in TNBC despite chemotherapy and radiation (RT). Thus, approaches that result in radiosensitization in TNBC are critically needed. We characterized the RT response of 21 breast cancer cell (BCC) lines using clonogenic survival assays. We paired this with high-throughput drug screen data to identify AR as a top target for radiosensitization and assess AR inhibition as a radiosensitization strategy for TNBC.

**Methods:** Clonogenic survival assays were used to determine the intrinsic RT sensitivity of 21 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 and protein expression was measured using RPPA arrays in human tumor samples (n=2,061) and BCC lines (n=51). AR function was assessed using siRNA knockdown or functional inhibition with MDV3100 (enzalutamide). We measured in vivo tumor growth with varying control and treatment groups (16-20 tumors/group). Kaplan-Meier analysis was performed to estimate local control and survival. A Cox proportional hazards model was used to identify factors of survival, and MVA was used to determine variables associated with LRF survival.

**Results:** Our radiosensitizer screen nominated bicalutamide as a most effective drug in treating RT-resistant BCC lines (R2=0.46, p-value <0.01). We interrogated the expression of AR in >2000 human breast tumor samples and found significant heterogeneity in AR expression with enrichment of expression at the protein and RNA level in TNBC. This same heterogeneity was also identified in human BCC lines. There was a strong correlation between AR RNA expression and protein expression (R2=0.72, p <0.01). Inhibition of AR using both siRNA and MDV3100 (enzalutamide) induced radiation sensitivity in vitro with an enhancement ratio (ER) of 1.35-1.42 in AR-positive TNBC lines. No such radiosensitization was seen in AR-negative TNBC or ER-positive, AR-negative BCC lines. Radiosensitization was at least partially dependent on impaired dsDNA break repair mediated by DNAPKcs. AR inhibition either with MDV3100 significantly radiosensitized TNBC xenografts in mouse models and markedly delayed tumor tripling time and tumor growth (median tumor tripling time 17.4 days for RT alone vs. not reached after 50 days for MDV3100 + RT, p-value <0.001). Biomarker analysis identifies DNAPKcs as a potential biomarker of response. Clinically, analyses of patients with TNBC showed that patients whose tumors had higher than median expression of AR had markedly higher rates of LR after RT (HR for LR 2.9-3.2, p-value <0.01, 2 independent datasets). There was no difference in LR in TNBC patients not treated with RT when stratified by AR expression. In MVA, AR expression was the variable most significantly associated with worse LRF survival after RT (HR of 3.58;p-value < 0.01).

**Conclusion:** Our results implicate AR as a mediator of radioresistance in breast cancer and support the rationale for developing clinical strategies, including clinical trials, to inhibit AR as a novel radiosensitizing target in TNBC.
Title: Novel combination therapies for triple negative breast cancer identified by high-throughput screening

Wali VB B, Langdon CG G, Held MA A, Platt JT T, Safonov A, Aktas B, Stern DF F, Pusztai L and Hatzis C. Yale Cancer Center, Yale University, New Haven, CT.

Background: Finding effective novel therapies for triple negative breast cancer (TNBC) remains an unmet challenge. Our objective was to assess the sensitivity of TNBC to single drugs and to combinations using a systematic screen of existing FDA approved drugs combined with other approved or experimental agents and to characterize the mechanism of action of the most promising combinations.

Methods: We conducted a comprehensive high-throughput drug combination screen in six TNBC cell lines with different genetic backgrounds. We tested a panel of 128 agents with known targets for their growth inhibitory potential individually and in pairwise combinations with six FDA approved anticancer drugs in MDA-MB-231, MDA-MB-436, MDA-MB-468, BT-20, BT-549, and HCC-38 TNBC cell lines. High-throughput assays were performed in 384-well plates and dose response curves were generated using 5 concentrations of the secondary drug, while keeping the first drug at fixed dose. Cell viability was measured 72h after treatment exposure using CellTiter-Glo®. Drug combinations were assessed for overall inhibitory effect and also for superadditivity, assessed as deviations from non-interaction using Bliss model of synergy. Selected synergistic and effective drug combinations identified from the high-throughput screen were subsequently validated in a 96-well low-throughput format in all TNBC cell lines.

Results: Cell cycle and apoptosis regulators were more inhibitory as single agents across TNBC cell lines relative to other drug classes. Bortezomib, carfilzomib, YM155, Flavopiridol, KP372-1, and dactinomycin were highly toxic to all TNBC lines as single agents. Combinations with either everolimus or erlotinib were particularly effective in BT-20 and MDA-MB-468, and combinations with crizotinib in MDA-MB-231 cells. Bay-11-7082/erlotinib and MK-1775/everolimus were few of the promising combinations that elicited potentiated response in all the cell lines. ABT-263/crizotinib was one of the top synergistic combination most effective in MDA-MB-231 cells that express highest relative mRNA and protein levels of respective drug targets, Bcl-xL, and AXL. The combination treatment with ABT-263 and crizotinib also resulted in apoptosis in MDA-MB-231 cells, indicated by marked PARP cleavage in these cells, while MDA-MB-436 cells with lowest expression of Bcl-xL were resistant to apoptosis or growth inhibition, indicating a potential efficacy of this combination in a subset of TNBCs.

Conclusions: This study reveals novel combinations of cell cycle or apoptosis regulators and crizotinib, everolimus, or erlotinib with enhanced anticancer activity. Combinations of Bay-11-7082 and erlotinib; and MK-1775 and everolimus had mild synergistic activity in all TNBC lines, while ABT-263 and Crizotinib showed large synergistic antiproliferative activity in a subset of TNBCs. These promising drug combinations have great potential to improve cure rates in TNBC patients. Since crizotinib, everolimus, and erlotinib are US FDA-approved while ABT-263 and MK-1775 are in advanced clinical trials, there is a rapid path for clinical translation for these drug-combinations after validation in animal studies.
**Title:** The taccalonolides are novel microtubule stabilizers that covalently bind tubulin and have *in vivo* efficacy in drug resistant tumors

Risinger AL L, Li J, Benavides R, Kuhn JG G and Mooberry SL L. The University of Texas Health Science Center at San Antonio, San Antonio, TX; The Cancer Therapy and Research Center, San Antonio, TX and The University of Texas at Austin, School of Pharmacy, Austin, TX.

**Body:** Some of the most effective drugs used in the treatment of breast cancer are microtubule stabilizers. However, there are limitations to their clinical efficacy, including inherent and acquired drug resistance. All microtubule stabilizers that are currently approved for clinical use bind within the taxane pocket on β-tubulin in a reversible manner. The taccalonolides are a novel class of microtubule stabilizers that have a similar profile of microtubule stabilization as the taxanes, but circumvent drug resistance mediated by expression of drug efflux pumps, mutations in the taxane binding site, or overexpression of the βIII isotype of tubulin. We have shown that one important difference between the taccalonolides and clinically approved microtubule stabilizers is that the taccalonolides form a covalent bond to β-tubulin. This distinct interaction allows for irreversible binding, which explains their ability to avoid drug efflux mechanisms and likely belies their exquisite potency in *in vivo* antitumor models which allows for delivery in aqueous solvents. Serum stability and binding studies, microsomal clearance and pharmacokinetic analysis were performed with both taccalonolides AF and AJ to more fully understand the properties of this class of compounds. We found that both taccalonolides had low microsomal intrinsic clearance rates with no evidence of serum binding and had half-lives similar to paclitaxel *in vivo*. Like other microtubule targeted agents, taccalonolide AF has a narrow therapeutic window with antitumor effects accompanied by body weight loss. Interestingly, direct injection of taccalonolide AF into a xenograft tumor was highly effective with no associated toxicities at low doses, indicating that targeted delivery to the tumor would greatly increase the efficacy and decrease toxicities. To this end, efforts to promote the targeted delivery of taccalonolide AF to the tumor are being evaluated.
Title: Palbociclib and paclitaxel on an alternating schedule for advanced breast cancer: Results of a phase Ib trial

Clark AS S, O'Dwyer P, Troxel A, Lal P, Feldman M, Gallagher M, Driscoll A, Colameco C, Lewis D, Rosen M, Matro J, Bradbury A, Domchek S, Fox K and DeMichele A. Perelman School of Medicine, University of Pennsylvania; Division of Hematology/Oncology, Philadelphia, PA; Abramson Cancer Center, Philadelphia, PA; Center for Clinical Epidemiology and Biostatistics, Philadelphia, PA; Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA and Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Body: Background: Palbociclib (P) is an oral CDK 4/6 inhibitor (CDKi) that was recently FDA approved in combination with endocrine therapy for metastatic breast cancer. We have performed a Phase I trial of P in combination with paclitaxel (T) based on preclinical studies suggesting that P synergizes with T when given on an alternating schedule, enabling cell cycle synchronization in tumor cells. We now present the dose expansion cohort.

Methods: Patients (Pts) enrolled on the trial had Rb-expressing tumors of any estrogen/progesterone/HER2 receptor type, adequate organ function, and ≤3 prior chemotherapy regimens for metastatic breast cancer (mBC). Prior adjuvant or metastatic taxane was allowed. Dose escalation led to expansion at P100mg or 75mg, starting with 3 days of P (run-in) and reduction of P dosing from 5-day to 3-day intervals (days 2-4, 9-11, 16-18 of each 28 day cycle). T at 80mg/m2 was given weekly for 3 cycles; thereafter, T was administered days 1, 8 and 15 of 28 day cycle. Weekly toxicity assessments were performed; RECIST 1.0 response was assessed every 2 cycles as partial response (PR), stable disease (SD) or progressive disease (PD). Pts had the option to discontinue T and continue on P alone (3 on/1 off schedule) if they attained SD after cycle 6.

Results: 27 pts enrolled on study (15- dose escalation, 12- dose expansion). Results are shown in the Table. 21 pts had received prior taxane; pts had received a median of 2 chemotherapy regimens for mBC. DLTs were grade 3 AST/ALT (n=1, at 125 mg) and febrile neutropenia (FN) (n=1, at 100 mg). Uncomplicated grade 3/4 NTP was common and frequently led to dose reduction or dose interruption during the first cycle of therapy. Frequency of NTP did not change with reducing the days of P. Among 24 evaluable patients, 14 (58%), had PR or SD ≥ 6 months across all dose levels. Of 14 pts who responded, 10 (71%) had received prior taxane. 20 pts are off study; 19 for PD, and 2 for toxicity (NTP in cycle 17 and FN in cycle 1); 7 pts remain on study. Prolonged tumor responses were seen.

Conclusions: P and T can be safely combined on an alternating dosing schedule; the optimal combination dose is 75 mg of P and 80mg/m2 of weekly T. The high response rate warrants a randomized trial to determine the incremental benefit over T alone. Additional mechanistic studies are in progress to understand the in vivo effects of the alternating dosing schedule on cell cycle activity and tumor proliferation.

<table>
<thead>
<tr>
<th>Starting Dose Level P (mg)</th>
<th>Number (Total 27)</th>
<th>DLT</th>
<th>Grade 3/4 NTP (n)</th>
<th>Final Dose P mg (n)</th>
<th>Dose Interruption (n)</th>
<th>Best Response (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>50 (1) 50 (1) 50 (1)</td>
<td>No (2) Yes (1)</td>
<td>PR (1) SD (1) PD (1)</td>
</tr>
<tr>
<td>75</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>75 (1) 50 (1) 25 (1)</td>
<td>No (1) Yes (2)</td>
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<tr>
<td>100</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>100 (2) 75 (3) 25 (1)</td>
<td>No (1) Yes (5)</td>
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</tr>
<tr>
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<td>3</td>
<td>1-LFT</td>
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<td>75 (1) 50 (2)</td>
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<tr>
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<td>0</td>
<td>1</td>
<td>75 (5) 50 (1)</td>
<td>No (4) Yes (2)</td>
<td>PR (1) SD (2) PD (1) N/A (2)</td>
</tr>
<tr>
<td>100 (Run-In)</td>
<td>6</td>
<td>1-FN</td>
<td>5</td>
<td>100 (1) 75 (4)</td>
<td>No (1) Yes (5)</td>
<td>PR (4) SD (1) N/A (1)</td>
</tr>
</tbody>
</table>
*2 pts not yet evaluable. ^1 pt went off study due to FN after cycle 1.
Title: Dual PI3K and Wnt pathway inhibition is a synergistic combination against triple negative breast cancer

Solzak JP P, Atale R, Hancock B and Radovich M. Indiana 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 disproportionally higher rate of morbidity and mortality compared to other breast cancer subtypes. Because these tumors lack expression of the estrogen, progesterone, or HER-2 receptors (“triple negative”), TNBC patients do not respond to targeted therapies that have been successfully used against tumors that over-express these proteins. Thus, there exists a critical need to improve the outcomes of TNBC patients through the implementation of novel targeted agents.

Methods: RNA-seq data from 94 TNBCs (from Indiana University and TCGA) and 20 microdissected normal breast tissues (Komen Tissue Bank) were merged and analyzed using Partek Genomics Suite. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify therapeutic targets. For in vitro studies, we tested a panel of TNBC cell lines using Buparlisib (a PI3K pathway inhibitor) and WNT974 (a WNT pathway inhibitor), individually and in combination. Cell viability was assessed via Celltiter-Fluor. Synergy between the two drugs was calculated using the Chou-Talalay method. In vivo studies were performed using the TMD-231 cell line and a patient derived xenograft (PDX) from the Jackson Laboratory. Dosing of the mice was performed using 30 mg/kg and 3 mg/kg of Buparlisib and WNT974 respectively, both in combination and individually.

Results: Using next-generation RNA sequencing data of TNBCs and microdissected normal breast tissues (Komen Tissue Bank) were merged and analyzed using Partek Genomics Suite. Statistically significant genes were imported into Ingenuity Pathway Analysis (IPA) to identify therapeutic targets. For in vitro studies, we tested a panel of TNBC cell lines using Buparlisib (a PI3K pathway inhibitor) and WNT974 (a WNT pathway inhibitor), individually and in combination. Cell viability was assessed via Celltiter-Fluor. Synergy between the two drugs was calculated using the Chou-Talalay method. In vivo studies were performed using the TMD-231 cell line and a patient derived xenograft (PDX) from the Jackson Laboratory. Dosing of the mice was performed using 30 mg/kg and 3 mg/kg of Buparlisib and WNT974 respectively, both in combination and individually.

Conclusion: PI3K/mTOR/AKT and Wnt pathways are strong candidates for the development of novel targeted agents for TNBC. Using two small molecule inhibitors that are currently in clinical trials as single agents (Buparlisib and WNT974) we observe significant in vitro synergy when inhibiting both pathways at low nanomolar doses. Furthermore, confirmatory in vivo xenograft studies display a similar synergy for the combination compared to single agent and vehicle controls.
Title: Therapeutic efficacy of HER3-targeted nanobiologics on resistant tumors

Cedars-Sinai Medical Center, Los Angeles, CA; University of California-Los Angeles, Los Angeles, CA; California Institute of
Technology, Pasadena, CA and Technion-Israel Institute, Haifa, Israel.

Body: Elevated cell surface levels of the human epidermal growth factor receptor subunit 3 (HER3) are associated with
resistance to a number of signal-blocking breast cancer treatments, including inhibitors of EGF-R (lapatinib), HER2 (lapatinib,
trastuzumab, T-DM1), HER2-3 (pertuzumab), and combination therapy. Additionally, HER3 elevation has been identified on
"untarget-able" tumors such as triple-negative breast cancer (TNBC), including TNBC with acquired resistance to EGF-R
inhibition. Patients with such refractory tumors currently have limited treatment options and a poor prognosis. Moreover, as up to
70% of cases resist or acquire resistance to signal-blocking therapies, an alternative approach addressing this important clinical
problem has the potential for significant clinical impact.

We have developed a protein construct, HerPBK10, which self-assembles with a variety of payloads (including nucleic acids,
chemotherapy agents, and imaging agents) and uses HER3 as a portal for targeted entry into cells. In contrast to
receptor-targeted antibodies and tyrosine kinase inhibitors currently used in the clinic, HerPBK10 circumvents the need to
modulate signaling by inducing rapid entry of toxic molecules into tumor cells through receptor-mediated endocytosis and
membrane penetration.

We have previously shown that nanobiological particles formed between HerPBK10 and therapeutic payloads can elicit targeted
toxicity to HER2+ tumors due to the prevalence of HER2-3 heterodimers on the tumor cell surface, while sparing heart and liver
tissue. The particles that form (20-40 nm dia.) exhibit stability in serum and no detectable immunogenicity. Here we show that
such particles resolve breast tumor cells with acquired resistance to HER2 and/or EGFR inhibitors in contrast to trastuzumab,
pertuzumab, and combination treatment. Additionally, therapeutic efficacy is augmented on resistant over parental tumor cells,
due in part to the elevated HER3 expression associated with resistance to these inhibitors. Our studies in preclinical models show
that these nanoparticles ablate the growth of tumors with both acquired and pre-existing resistance to trastuzumab. Moreover, we
have found that signal-inhibitors currently used in the clinic, such as trastuzumab, effectively augment the efficacy of our
nanobiologic on both naïve and inherently-resistant breast tumor cells, in part through induced elevation of HER3. Thus, current
targeted molecules such as trastuzumab or lapatinib may act as adjuvants to enhance tumor cell-sensitivity to
HerPBK10-particles. Such an approach may address the tumor-heterogeneity associated with resistance, and corner tumors for
attack by our particles.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-13-11

Title: Phase I study of alpelisib and T-DM1 in trastuzumab-refractory HER2-positive metastatic breast cancer

Jain S, Nye L, Santa-Maria C, Bontemps L, Williams A, Garrett H, Dammrich E, Giles F and Gradishar W. Northwestern University, Evanston, IL.

Body: BACKGROUND: Constitutive activation of the phosphatidylinositol-3-kinase (PI3K) signaling pathway is a mechanism of trastuzumab resistance in HER2-positive metastatic breast cancer (MBC). Alpelisib (BYL-719) is the first oral PI3K inhibitor that selectively inhibits the PI3K alpha isoform. We aimed to determine the maximum tolerated dose (MTD), safety, and activity of alpelisib in combination with ado-trastuzumab emtansine (T-DM1) in HER2-positive MBC that has progressed on or after trastuzumab and/or taxane-based regimens. METHODS: This phase I study enrolled patients with HER2-positive MBC who received alpelisib daily (cohort 1: 300 mg, cohort (-)1: 250 mg) and T-DM1 3.6 mg/m2 on Day 1 of a 3 week cycle using a 3+3 design with dose expansion at the MTD. Treatment was continued until progression, unacceptable toxicity, or patient preference. Blood was collected for pharmacokinetic (PK) and pharmacodynamic (PD) markers. Dose-limiting toxicity (DLT) was defined as CTCAE Grade 3 or 4 adverse events (AE) during the first cycle. Imaging and echocardiogram were obtained every 3 cycles. RESULTS: Dose escalation is completed and included in this analysis (N=8). Median age was 53 (range 46-79) with median ECOG Performance Status of 1. Median prior lines of therapy in the metastatic setting was 6 (range 0-12) including 5 patients who progressed on prior T-DM1 (after median 6 cycles). Two patients had de novo MBC and 3 with ER and/or PR positive disease. Median number of metastatic sites was 2 (range 1-5) including brain (inactive), liver, and lung. Median number of cycles of alpelisib and T-DM1 per patient was 5.5 (range 1-12). Five patients were enrolled in cohort 1 with 2 DLTs (both grade 3 rash), leading to cohort (-)1, in which there were no DLTs. The most common treatment-related AEs were fatigue (n=7, 86%), nausea (n=6, 75%), aspartate aminotransferase increase (n=4, 50%), and thrombocytopenia (n=4, 50%). Grade 3 AEs were rash (n = 3), hyperglycemia (n=1), hypertension (n=1) and thrombocytopenia (n=1), which occurred in only cohort 1. Grade 3 rash typically occurred during cycle 1, which resolved with temporary interruption and subsequent dose reduction of alpelisib and the use of topical steroids for median 9.3 days (range 6-12). Grade 3 hyperglycemia was reversible with oral anti-diabetic treatment. A total of 4 dose reductions occurred in cohort 1. No Grade 3/4 AEs or dose reductions occurred in cohort -1. In total, only 0.07% of all AEs were Grade 3 and none Grade 4. The MTD for alpelisib was established as 250 mg daily. In 7 evaluable patients, there were 6 objective responses after 3 cycles, a response rate of 86% (1 confirmed complete response, 2 confirmed partial response [PR], and 3 unconfirmed PR). Five (67%) of these patients continue to have durable responses (median 6.5 cycles) at doses 200-250 mg daily. One patient had progression of disease. PK/PD results and PIK3CA mutation testing are pending. CONCLUSION: The combination of alpelisib and T-DM1 appears to be safe, well tolerated, with clinical activity in HER2-positive MBC patients including those who progressed on prior T-DM1 therapy. The study is currently in dose expansion with goal of enrolling 10 additional patients with T-DM1 and alpelisib 250 mg daily.
Title: Comparative similarity of ABP 980 and trastuzumab: Results of functional similarity and human pharmacokinetic assessment


Body: Background: ABP 980 is being developed as a biosimilar to trastuzumab, a monoclonal antibody (mAb) that binds human epidermal growth factor receptor 2 (HER2), resulting in proliferation inhibition (PI) and induction of antibody-dependent cell-mediated cytotoxicity (ADCC). Biosimilar mAbs are likely to have minor differences with the reference product due to variances in expression systems, bioprocess, and purification; demonstration of analytical, pharmacologic, and pharmacokinetic (PK) similarity is the foundation for biosimilarity. Functional (PI, ADCC, and tumor xenograft models) and PK similarity between ABP 980 and EU-authorized trastuzumab reference product has been previously evaluated.

Objective: To demonstrate functional and PK similarity between ABP 980 and FDA-licensed trastuzumab reference product.

Methods: Potency of ABP 980 and FDA-licensed trastuzumab was compared by measuring PI and recombinant HER2 (rHER2) binding affinity. PI activity was evaluated using BT-474 cells. Surface plasmon resonance analysis was used to determine the equilibrium binding affinity (Kd) to rHER2. PK similarity was evaluated in a randomized, single-blind, single-dose, parallel-group study in healthy adult men. Primary endpoints were area under the serum concentration-time curve from time 0 to infinity (AUC_{\text{inf}}) and maximum observed serum concentration (C_{\text{max}}). Secondary endpoints were safety, tolerability, and immunogenicity.

Results: ABP 980 displayed PI and rHER2 binding activities comparable to trastuzumab. The range of relative potency by PI was 82%–114% for ABP 980 and 91%–116% for trastuzumab. The Kd ranged from 75–79 pM for ABP 980 and 67–80 pM for trastuzumab. PK similarity was demonstrated for the comparison of ABP 980 with trastuzumab. The geometric mean (GM) ratios for C_{\text{max}} and AUC_{\text{inf}} were 1.04 (90% CI, 0.995–1.079) and 1.06 (90% CI, 0.997–1.117). Both CIs fell within the standard prespecified bioequivalence criteria of 0.80–1.25. After 6 mg/kg intravenous infusion, the GM of C_{\text{max}} and AUC_{\text{inf}} were 135.90 µg/mL and 34061.43µg·h/mL for ABP 980 and 131.19µg/mL and 32271.67µg·h/mL for trastuzumab. The incidence of treatment-emergent adverse events (TEAEs) was comparable between treatment groups. TEAEs occurred in 84% subjects receiving ABP 980 and 75% of subjects receiving trastuzumab; no anti-drug antibodies were detected.

Conclusions: ABP 980 has been shown to be similar to FDA-licensed trastuzumab in functional tests and binding affinity to antigen, as well as in a phase 1 human PK study.
Title: Developing silastic tubing for local delivery of hormonal therapy: A novel approach to breast cancer prevention


Body: Background: For women at high risk for breast cancer, preventative interventions are limited to bilateral mastectomy with or without oophorectomy or prolonged anti-estrogen therapy. For many, these two options are unacceptable due to drug related toxicity or the irreversible consequences of prophylactic surgery. As such, many women will choose neither preventive measures. This highlights the need for prevention alternatives that are less invasive, less toxic, and less permanent. Ideally, only the tissue at risk should be treated. Therefore, we have developed a novel approach to release an anti-estrogen to the breast tissue only, with the goal to reach high breast tissue concentrations with minimal systemic exposure.

Method: Cultured ER-positive breast cancer cells were used to validate the activity of fulvestrant released from the silastic tubing. CD-1 mice were used to demonstrate accumulation of fulvestrant released from the silastic tubing in the target breast tissue. LC-MS/MS was used to quantify released fulvestrant in both in vitro and in vivo experiments.

Results: After 10 cm of silastic tubing was loaded with dry fulvestrant (0.076 mg/cm), it was placed in tissue culture media. Media was collected and replaced with fresh media every 3.5 days for 30+ weeks. It was then used to treat MCF7 and T47D cancer cells for 3 days. Within 7 days, fulvestrant released from the tubing was sufficient to modulate the ER signaling pathway of both cell lines and inhibit cell growth comparable to cells directly treated with a clinically feasible concentration of fulvestrant (100 nM). LC-MS/MS analysis demonstrated a fulvestrant release rate of rate of 10.7 ng fulvestrant per cm of tubing per day in culture media. To date, sustained release at this rate has been confirmed for 7+ months.

To ascertain differential uptake of fulvestrant in the mammary tissue, we implanted fulvestrant-loaded tubing proximal to the inguinal mammary fat pad of CD-1 female mice and characterized biodistribution of fulvestrant. Various organs (blood, heart, lung, liver, kidney, and mammary fat pad) were harvested over time post-implantation. Using LC-MS/MS, we determined that fulvestrant preferentially accumulates in the mammary fat pad (175 nM), with minimal to no detection in other organs.

Summary: Our data from in vitro and in vivo breast cancer models suggest that implantable silastic tubing has the capacity for long-term release of the anti-estrogen fulvestrant at high local concentrations that are sufficient to inhibit ER signaling and tumor cell proliferation with minimal systemic exposure. Further work is underway to design the optimal design for delivery. If successful, this option will provide a more acceptable alternative for breast cancer prevention and allow women at high risk to delay or forgo bilateral mastectomies.
Body: INTRODUCTION
Chemotherapeutic synergy enhancement can be obtained when drugs are delivered in a sequential manner. However, clinical translation of synergistic combinations is not feasible due to different pharmacokinetic properties arising from distinct drug formulations that negate proper sequential delivery. Our laboratory has developed a platform for temporospatial delivery of agents in a sequential fashion for purposes of enhancing site-specific synergy. The system comprises a drug-containing poly(lactic-co-glycolic acid) (PLGA) core and an outer shell of drug complexed with β-cyclodextrin (β-CD). In this study, we demonstrate the therapeutic potential of this nanoparticle system by encapsulating two drugs, rapamycin (RAP) and paclitaxel (PTX). This combination has been shown to result in cell-killing synergy enhancement in when administered sequentially, and in a time staggered manner, in triple negative breast cancer (TNBC) in in vitro cell-based models.

EXPERIMENTAL METHODS
The final platform was fabricated by resuspending PLGA-PTX nanoparticles in an aqueous solution of RAP cyclodextrin complexes (RAP-CD) under sonication. Nanoparticles were characterized for size and surface charge. Drug concentration and release kinetics were determined by HPLC. In vitro growth inhibition in SUM159 TNBC cells was determined via MTT assay. Western blotting was used to confirm target knockdown. For in vivo efficacy analysis, SUM159 breast cancer cells (3×106) were inoculated in the mammary fat pads of 4- to 6-week–old female nu/nu mice, and treatments were administered intravenously once a week for a duration of 21 days.

RESULTS AND DISCUSSION
Nanoparticles had a mean diameter of 140 nm and zeta potential of -30 mV. After complexation with RAP-CD, the surface charge became 25 mV. Release kinetics depicted a fast release of RAP at early timepoints, while PTX showed a prolonged release. Nanoparticles showed concentration dependent cell-killing efficacy in SUM159 breast cancer cells in vitro, with knockdown of pS6, characteristic of RAP treatment, observed. Upon administration of NNPs to murine models of TNBC, NNPs showed significant tumor growth inhibition compared to controls, and knockdown of pS6.

CONCLUSION
We have developed a novel nanoparticle for the temporospatial release of drugs. The ability to localize agents site-specifically and deliver them sequentially should enable clinical translation of synergistic regimens observed in in vitro scenarios, in turn yielding more efficacious outcomes following combination chemotherapy.
Title: Lyso-thermosensitive liposomal doxorubicin shows efficacy with minimal adverse events in patients with breast cancer recurrence at the chest wall

Rugo H, Pabbathi H, Shrestha S, Aithal S, Borys N, Musso L and Zoberi I. University of California San Francisco, San Francisco, CA; CTCA - Southeastern Regional Medical Center, Newnan, GA; CTCA - Southwestern Regional Medical Center, Tulsa, Ok; CTCA - Eastern Regional Medical Center, Philadelphia, PA; Celsion Corporation, Lawrenceville, NJ and Washington University School of Medicine, St. Louis, MO.

Body: INTRODUCTION: Local-regional recurrence after definitive treatment of breast cancer is reported in 5 – 40 % of patients depending risk factors and initial treatment. Chest wall recurrence is associated with poor quality of life and limited treatment options. Lyso-thermosensitive liposomal doxorubicin (LTLD, Thermodox®) is an intravenously administered agent designed to selectively release doxorubicin when exposed to temperatures ≥39.5º C at a targeted tumor. Hyperthermia, the elevation of tissue temperature in the range of 40º C to 44º C, causes direct cytotoxicity, enhanced blood flow, and oxygenation. We are reporting the interim findings of an ongoing Phase I/II Study Evaluating the Maximum Tolerated Dose, Bioequivalence/Pharmacokinetics, Safety, and Efficacy of LTLD in Patients with Local-Regional Recurrent Breast Cancer. Final results will be presented in December.

METHODS: Patients with breast carcinoma on the chest wall with progression following radiation were eligible; prior chemotherapy and hormone therapy were allowed. LTLD was administered intravenously followed immediately by hyperthermia in up to two treatment fields for 1 hour per field for a goal of 40–42º C. Response was measured using clinical assessment, CT, and digital photos. All subjects were assessed for safety. Up to six cycles of LTLD/hyperthermia were administered depending on disease progression or tolerance. A total of 11 patients were enrolled on the Phase I portion of the study and 9 were evaluable for efficacy review. To date, 17 patients are enrolled on the Phase II portion of the study and 13 are evaluable for efficacy review. Once 12 patients are found to be evaluable for PK the primary endpoint of bioequivalence can be evaluated.

RESULTS: A dose of 50 mg/m² was recommended by the DSMB at completion of the Phase I study. Seven subjects were dosed at 50mg/m², two patients developed a localized reaction in the treatment area consisting of erythema, woody induration, and pain which resolved with discontinuation of treatment. Twenty patients were dosed at 40mg/m² without recurrence of symptoms. To date, twenty-eight patients were enrolled in the phase I/II study; one subject was excluded due to dose modification following 2 cycles of ThermoDox at a dose of 50mg/m² to 40mg/m² at cycle 3. This subject had a durable partial response. The tables summarize the safety and efficacy data. All efficacy data is investigator reported.

Combined Phase I/II Safety Data

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<tr>
<th></th>
<th>40 mg/m2 (N=20)</th>
<th>50 mg/m2 (N=7)</th>
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<td>Any AE Event (n, %)</td>
<td>17 85.0</td>
<td>7 100.0</td>
</tr>
<tr>
<td>Grade 3+ AE (n, %)</td>
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</tr>
<tr>
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<tr>
<td>Hematological AE (n, %)</td>
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<tr>
<td>Efficacy Data</td>
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</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-----------------</td>
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</tr>
<tr>
<td>Responders (Partial &amp; Complete) (n, %)</td>
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<td>3 42.9</td>
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<td>Complete Response (n, %)</td>
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</tr>
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<td>Partial Response (n, %)</td>
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<td>2 28.6</td>
</tr>
<tr>
<td>Durable Response (lasting ≥ 3 months) (n, %)</td>
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CONCLUSION: These findings suggest that LTLD at a dose of 40 mg/m² combined with hyperthermia offers a promising and well tolerated treatment option for patients with recurrent chest wall disease from breast cancer. Additional data will be presented based on full trial accrual.
Title: Ropidoxuridine (IPdR) potentiates alisertib (MLN8237) activity in triple-negative breast cancer

Rampurwala MM M, Choudhary A and Burkard ME E. University of Wisconsin Carbone Cancer Center, Madison, WI.

Body: Introduction and Background: An estimated 234,190 new cases of invasive breast cancer will be diagnosed in 2015 with 40,730 deaths (American Cancer Society, 2015). Triple-negative breast cancer (TNBC) has an overall poor prognosis due to aggressive and early pattern of metastasis and a relative lack of therapeutic targets. Chemotherapy remains the cornerstone of treatment albeit with a dismal response rate (~25%) to monotherapy. Synergism between two drugs could provide a key advance to enhance effectiveness, decrease resistance and reduce toxicities. Here we focused on discovery of synergism of antiproliferative agents given TNBC is highly proliferative.

Experimental Methods: We assembled a library of antiproliferative agents with unique mechanisms and targets that have subtly different effects on cancer cell proliferation. We performed a novel synergy screen testing 105 unique two-drug combinations in MDA-MB-231 TNBC cells. Our screen was designed to distinguish between synergism and additivity through presence of an internal control and performed in sufficient replicates to identify changes in viability exceeding 5%. We validated hits through Chou Talalay Combination Index (CI), xenograft and mechanistic analyses.

Results: We discovered strong synergy between Ropidoxuridine (IPdR) and Alisertib (MLN8237). Ropidoxuridine is an orally bioavailable pro-drug of IUdR (5-iodo-2'-deoxyuridine), with an improved therapeutic index and with promising activity as a radiosensitizer, although it lacks single agent activity. Alisertib is an inhibitor of Aurora A kinase and acts by impairing assembly of a bipolar mitotic spindle, thereby activating the spindle assembly checkpoint leading to cancer cell death. Alisertib has demonstrated activity in breast cancer with a response rate of 18% in heavily pretreated patients (Melichar B et.al. Lancet Oncol 16:395-405, 2015). First, Ropidoxuridine and Alisertib combination was validated in cell culture using CI analysis with a mean CI of 0.14; range 0.01-0.67 suggesting strong synergism. A separately performed Live Dead Cell Assay also confirmed synergism (mean CI of 0.79; range: 0.53-1.76) suggesting enhanced anti-proliferative activity of Alisertib in presence of Ropidoxuridine. To assess generalizability, this combination was validated in two other TNBC cell lines, MDA-MB-468 and CAL-51. To confirm our hypothesis that in vitro activity of Ropidoxuridine is from its metabolism to IUdR in cancer cells, we demonstrated strong synergy of IUdR and Alisertib (mean CI of 0.12; range 0.01-0.74) at clinically relevant concentrations. To validate synergy in an orthotopic tumor model, Ropidoxuridine and Alisertib single agents and in combination were tested in mice treated with Ropidoxuridine (750 mg/kg/day) and Alisertib (30 mg/kg/day) by gavage. This in vivo model also demonstrated strong synergism. Mechanistically, Ropidoxuridine and IUdR enhance G2/M arrest in response to Alisertib, allowing low dose exposures of Alisertib to be effective.

Conclusions and Future Directions: This study identifies and validates a novel synergy between Ropidoxuridine and Alisertib with a potential for significant clinical implications. We propose evaluating this synergistic drug combination in an early phase clinical trial.
The combination of eribulin and everolimus results in enhanced suppression of tumors in mouse models of triple negative breast cancer

Marcinkowski E, Luu T, Yuan Y, Mortimer J, Leong L, Portnow J, Xing Q, Wen W and Yim J. City of Hope, Duarte, CA.

INTRODUCTION. Triple negative breast cancer (TNBC) is an aggressive form of breast cancer with poor overall and relapse free survival. TNBC does not have targeted or matched therapies. Patients have worse outcomes after chemotherapy than with other subtypes of breast cancer. TNBC accounts for 12-17% of all breast cancers, leaving an unmet need for targeted therapy. Efforts to profile these tumors have revealed several potential targets.

The PI3K/AKT/mTOR pathway is a signal transduction pathway that links growth related hormone receptor interaction to downstream targets such as AKT and mammalian target of rapamycin (mTOR). This pathway targets affect cell proliferation, survival, and apoptosis. Patients with TNBC have high levels of AKT expression and activation of this pathway. Microtubule-targeting agents have been used in TNBC. Eribulin mesylate is a microtubule-targeting agent with benefits in treating taxane and anthracycline refractory breast cancer via a microtubule targeting anti-mitotic mechanism. It has been approved for the treatment of TNBC in heavily pretreated patients.

Despite targeted therapy, breast cancer cells can grow resistant. Targeting multiple cancer growth pathways has been used in patients that progress on therapy or fail to respond. We hypothesized that targeting both mitotic blockade and PI3K/AKT/mTOR pathway may provide enhanced suppression of TNBC growth in both syngeneic and xenogeneic mouse models.

MATERIALS AND METHODS. MDA-MB-468 is a human TNBC cell line. 4T1 is a highly metastatic mouse TNBC cell line derived from a spontaneously arising Balb/c mammary tumor. 4T1 and MDA-MB-468 tumor cells were injected into the mammary fat pad of female Balb/c and NOD/SCID/IL2Rgamma null (NSG) mice (with matrigel) respectively. After tumors were formed Balb/c mice were treated three times per week with vehicle, eribulin (0.75 mg/kg i.v.), RAD001 (5 mg/kg via oral gavage) or a combination of both. NSG mice were treated three times per week with vehicle, eribulin (0.5 mg/kg i.v.), RAD001 (5 mg/kg by oral gavage), or a combination of both. Tumor volumes and body weights were measured. Student t-test was used to compare the means of two groups and determine the p value (p<0.05 is significant). N=3-8 per group.

Table I. 4T1 mouse breast cancer model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Volume (mm3)+/-SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>511.6+/-56.82</td>
</tr>
<tr>
<td>Eribulin</td>
<td>445.6+/-92.17</td>
</tr>
<tr>
<td>Everolimus</td>
<td>324.9+/-24.55</td>
</tr>
<tr>
<td>Combination</td>
<td>171.4+/-16.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination vs. Vehicle</td>
</tr>
<tr>
<td>Combination vs. Eribulin</td>
</tr>
<tr>
<td>Combination vs. Everolimus</td>
</tr>
</tbody>
</table>

Table II. MDA-MB-468 human breast cancer cells in immune deficient mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor Volume (mm3)+/-SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>966.8+/-69.2</td>
</tr>
</tbody>
</table>
RESULTS. In the 4T1 syngeneic breast cancer mouse model, the combination of Eribulin and Everolimus resulted in marked suppression of tumor growth which was statistically significant versus vehicle treatment alone, or Eribulin or Everolimus alone (Table I). In the MDA-MB-468 model, the combination of Eribulin and Everolimus demonstrated marked suppression of tumor growth which was statistically significant compared to either agent alone (Table II).
Title: Triple-negative kinase profile guides in the selection of the multi-kinase inhibitor EC70124 as an active antitumor agent

Perez-Peña J, Serrano-Heras G, Corrales-Sánchez V, Nieto-Jimenez C, Gascón-Escribano MJ, Montero JC, Moris F, Martin M, Pandiella A and Ocana A. Translational Oncology Unit, Albacete University Hospital, Albacete, Spain; IBMCC-CSIC, Universidad de Salamanca, Salamanca, Spain; Entrechem sl, Universidad de Oviedo; Oviedo, Spain, Oviedo, Spain and Medical Oncology Service Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Body: Introduction: Identification of the kinase profile of triple negative tumors can guide in the selection of active multi-kinase inhibitors. We describe the preclinical selection of a novel kinase inhibitor based on the kinase profile identified in human samples.

Methods: phosphokinase profiles of human samples were performed using two kinase arrays: human phospho-RTK array kit (# ARY001, R&D Systems, Abingdon, UK) and the PathScan RTK Signaling Antibody Array Kit (# 7982, Cell Signaling Technology). Based on the kinase profile, inhibitors against activated proteins were evaluated in a panel of triple negative cell lines. Proliferation and growth was measured by MTT uptake. Evaluations of apoptosis and cell cycle were performed by flow cytometry using Annexin V and propidium iodide, respectively. Gene-set enrichment analyses were performed to identify relevant functions mediated by the drug and the identified genes were confirmed by RT-PCR. The in vivo antitumoral effect was evaluated using xenografted animals.

Results: Several kinases were constitutive activated in human tumors, including AKT and pS6, among others. Pharmacological screening identified PI3K/mTOR inhibitors as the most active agents. The novel multi-kinase inhibitor, EC-70124 showed clear antitumor activity. Doses of EC70124 in the nanoMolar range reduced proliferation and cell growth in a panel of triple negative cell lines including HS578T, BT549, MDA-MB231 and HCC3153. Treatment with EC70124 inhibited the PI3K/mTOR pathway and induced DNA damage. Gene set enrichment analyses showed different cellular functions induced by the compound. EC70124 showed activity in xenografted animals. Pharmacodynamic analyses supported the inhibition of the PI3K/mTOR pathway in vivo.

Conclusion: the kinase profile of human triple negative tumors identified major activated pathways including the PI3K/mTOR route. The novel kinase inhibitor EC70124 inhibited the PI3K/mTOR pathway showing clear antitumor activity. Evaluation of the kinase profile followed by functional studies is an attractive approach to rationally select active antitumoral agents.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-13-19

Title: Adding zoledronic acid to neo-adjuvant chemotherapy may improve the efficiency of chemotherapy in locally advanced breast cancer: Results from the prospective randomized study NEOZOL

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Body: Background: There is extensive preclinical evidence that bisphosphonates, especially zoledronic acid (ZOL), exhibit anti-tumor effects (inhibition of tumor cell adhesion, migration, invasion and proliferation, and induction of cell death). ZOL reduces tumor-associated angiogenesis and vascular endothelial growth factor (VEGF) production in different animal models of cancer. In the Adjuvant Treatment with Zoledronic Acid in Stage II/III Breast Cancer Study (AZURE), it was observed a reduction in residual invasive tumor size, ∼ 2-fold improved complete pathologic response rates, and lower mastectomy rates with chemotherapy plus ZOL versus chemotherapy alone. The current hypothesis is that adding ZOL to neoadjuvant chemotherapy will increase the efficacy of this neoadjuvant treatment, thanks to a direct anti-cancer activity of ZOL and/or a synergic anti-cancer effect with chemotherapy. The aim of this study was to evaluate the clinical, pathological and biological impact of adding ZOL to neoadjuvant chemotherapy in locally advanced breast cancer.

Methods: A multicenter prospective phase II trial (NEOZOL) was performed in France to compare the effects of adding ZOL to neoadjuvant chemotherapy with that of neoadjuvant chemotherapy alone. Patients were randomized 1:1 to receive 4-mg ZOL monthly or no ZOL with chemotherapy cycles. Patient’s eligibility criteria were women 18 years of age or older with absence of contraindication to treatment with ZOL and breast cancer (TNM IIa, IIb, IIIa) larger than 2cm in maximal diameter able to benefit from neoadjuvant chemotherapy. The primary end point was circulating concentrations of VEGF. The secondary end points were clinical, radiological, pathological and biological tumor response rates, and the breast conservation rate.

The planned sample size was 38 patients per arm with a power of 95.4%. The protocol has been funded by Novartis and was reviewed by an Ethical Committee (CPP Lyon IV). This study is registered in the NCI database.

Results: due to low accrual, the study was stopped after inclusion of 71 patients between May 2010 and July 2013. 53 patients were randomized and 3 patients were excluded after randomization due to deviation to the protocol. Of the remaining 50 patients, 24 were randomized in arm A (ZOL treatment) and 26 in arm B (control arm). Baseline clinicopathologic characteristics were well balanced. No side effect related to ZOL treatment was reported. Circulating VEGF levels did not statistically significantly differ between both arms. Regarding secondary end-points, there was a trend towards a more conservative surgery in arm A, compared with arm B.

Conclusion: adding ZOL to neoadjuvant chemotherapy in locally advanced breast cancer may increase the efficiency of chemotherapy, improving surgical treatment.
Title: Blockade of breast cancer xenograft growth by combinatorial hyperthermia therapy with docetaxel delivered by novel magnetic nanoparticles

Salcido J, Bandyopadhyay A, Bouamar H, Sun L and Cheng X. Southwest Research Institute, San Antonio, TX; University of Texas, San Antonio, TX and The University of Texas Health Science Center, San Antonio, TX.

Body: Background: Current breast cancer treatment using chemotherapeutics have several drawbacks including severe side effects, drug resistance, and need for multiple doses. Magnetic nanoparticles (MNPs) have been investigated for hyperthermia therapy to circumvent these drawbacks; however, several limitations have yet to be overcome, including optimal formulation and inherent cytotoxicity. Furthermore, the use of MNPs in conjunction with chemotherapeutic agents is not well characterized. Therefore, we developed a novel combination treatment with hyperthermia and chemotherapy utilizing a proprietary MNP formulation [1] and investigated its efficacy in blocking the growth of breast cancer in a murine xenograft model.

Materials and Methods: Magnetic calcium phosphate (MCaP) nanoparticles (NPs) were surface enriched with chitosan as described previously [1]. Advantages of MCaP NPs include: high colloidal stability and low toxicity; ease of surface modification; and the delivery of heat and tumor suppressive agents to selective tissues. MCaP NPs were modified by PEGylation with a heterobifunctional crosslinker to increase colloidal stability and to provide functional reactive sites. PEGylated MCaP NPs were conjugated to an imaging agent-IR-Dye 800 CW (LI-COR) and a chemotherapeutic agent – docetaxel for combined hyperthermia therapy, imaging, and chemotherapy of breast carcinoma. One single dose of five NP formulations ± drug (Table 1) were directly injected into xenograft tumors generated by the inoculation of 3x10⁶ human breast cancer MDA-MB-231 cells in the mammary fat pad of female athymic nude mice. Each treatment group contained five mice. Tumors were measured twice a week for two weeks. Experimental heat therapy groups were treated with an alternating magnetic field every 3 to 4 days with localized internal temperature of 45 °C for 25 minutes.

Results: Tumors in mice receiving combined hyperthermia and chemotherapy (G4) showed no growth during the 14-d experiment while average tumor volume increased by 2.7 fold in the control group (G1). The tumor volume of G4 was significantly smaller than those in all other groups (Table 1). Both G3 and G5 had similar tumor inhibition, though drug efficacy was not seen past day 7.

<table>
<thead>
<tr>
<th>Groups (n=5)</th>
<th>Initial avg tumor vol (mm³) ± SD</th>
<th>Day 14 avg tumor vol (mm³) ± SD</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 NP vehicle</td>
<td>202 ± 45</td>
<td>548 ± 216</td>
<td>-</td>
</tr>
<tr>
<td>G2 NP heat</td>
<td>206 ± 43</td>
<td>320 ± 86</td>
<td>p&lt;0.059 v G1</td>
</tr>
<tr>
<td>G3 NP DTX (20 mg DTX/kg bodyWt)</td>
<td>211 ± 48</td>
<td>341 ± 54</td>
<td>p&lt;0.070 v G1</td>
</tr>
<tr>
<td>G4 NP heat, DTX (20 mg DTX/kg bodyWt)</td>
<td>207 ± 43</td>
<td>190 ± 115</td>
<td>p&lt;0.011 v G1*, p&lt;0.015 v G5*, p&lt;0.029 v G3*, p&lt;0.077 v G2</td>
</tr>
<tr>
<td>G5 Free DTX (20 mg DTX/kg bodyWt)</td>
<td>208 ± 50</td>
<td>408 ± 107</td>
<td>p&lt;0.22 v G1</td>
</tr>
</tbody>
</table>

* denotes statistical significance

Conclusion: Our results suggest that our MCaP NPs are capable of delivering hyperthermia therapy while serving as a drug vehicle to effectively inhibit tumor growth in the breast cancer model. Similar results were obtained in a prostate cancer model. Thus, our MCaP NPs may have novel utility for the treatment of certain cancers.

Acknowledgements: This study was funded by SwRI IR&D program.
Title: Phase 1 study of GR antagonist mifepristone (M) in combination with eribulin (E) in advanced solid tumors, with dose expansion in patients (pts) with GR-positive triple-negative breast cancer (TNBC)

Wilks S, Modiano M, Spira A, Becerra C, Walling J, Nguyen D, Baker G, Conzen SD D and Nanda R. Cancer Care Centers of South Texas, San Antonio, TX; ACRC/Arizona Clinical Research Center and Arizona Oncology, Tucson, AR; Virginia Cancer Specialists Research Institute, Fairfax, VA; Texas Oncology-Baylor Charles A Sammons Cancer Center, Dallas, TX; JW Consulting, Hillsborough, CA; Corcept Therapeutics, Menlo Park, CA and University of Chicago Medicine, Chicago, IL.

Body: Background: High tumor GR expression is associated with poor prognosis in estrogen receptor (ER) negative early-stage breast cancer. Co-treatment with M, a GR antagonist, potentiates effects of chemotherapy in ER- breast cancer xenograft studies. Herein we describe results of a phase 1 dose-escalation study of M plus E, with an ongoing dose expansion cohort in pts with GR+ TNBC.

Objectives: Determine 1) safety and tolerability, 2) recommended phase 2 dose (RP2D) of M + E, and 3) characterize pharmacokinetics (PK) and clinical activity of M in pts with GR+ TNBC.

Methods: Eligibility: 1) relapsed/refractory breast, ovarian, prostate, urothelial, sarcoma, or non-small cell lung cancer; 2) 2-5 prior chemotherapy regimens for advanced disease; 3) ECOG PS 0-1; and 4) adequate end-organ function. Study used a 3 + 3 dose escalation scheme. After a 7 day lead-in of M alone, M was administered by mouth daily in combination with E given IV on days 1 and 8 of a 21 day cycle.

Results: 13 pts in Part 1 Dose escalation with metastatic breast cancer (MBC) were treated with M+E: 5 TNBC, 8 GR+ tumors, 2 GR- tumors, and 3 of unknown GR status. Pts were treated at 3 dose levels (DL) [M mg/d, E mg/m^2]: 3 at DL1 [600, 1.1], 4 at DL-1a [300, 1.4], and 6 at DL-1 [300, 1.1]. Median duration of treatment was 90+ days. Neutropenia leading to delay of E was dose limiting in 4 pts. CTAE Grade 3/4 neutropenia was observed in 10 pts over all DL, but easily managed (9 pts with growth factor support). Other grade 3+ toxicities were neuropathy (2 pts) and onycholysis (1 pt). No other significant toxicity was noted. RP2D was determined as 300mg/d M and 1.1mg/m^2 E. At this DL there were no DLTs. PK of M and E were as predicted from published literature with no evidence of drug-drug interaction (DDI). A total of 6 pts received this dose (3 TNBC; 3 MBC). All 3 TNBC were GR+. 1 had partial response, 1 had stable disease, and 1 had progressive disease.

A phase I/II study of M+E is now in progress. To date, 3 GR+ TNBC pts have been treated for a median of 28+ days.

Conclusion: M + E is a novel combination designed to improve antitumor activity. It is well tolerated with evidence of clinical activity and no evidence of DDI. RP2D is 300mg M + E 1.1mg/m^2. Study is ongoing in expansion phase where recruitment is limited to pts with GR+ TNBC. Additional PK and clinical data will be presented.
Title: Pharmacodynamics and consequences of PI3K inhibition in ER+ breast tumors


Body: PI3K inhibitors have shown promise for the treatment of anti-estrogen-resistant ER+ breast cancers. Current PI3K inhibitor treatment regimens may incompletely and transiently inhibit the pathway in carcinomas, and are accompanied by significant adverse effects in patients. We hypothesized that short-term, complete inhibition of PI3K will have a greater anti-tumor effect and reduce systemic toxicity compared to chronic, partial inhibition.

Pharmacokinetic analysis of the orally available pan-PI3K inhibitor GDC-0941 at low (100 mg/kg) and high (800 mg/kg) doses in mice revealed that plasma levels peaked after 15-30 min, and decreased below the limit of detection within 24 h (low dose) and 72 h (high dose), respectively. Administering 2 doses at 100 mg/kg 12 h apart provided continuous drug exposure. Drug pharmacokinetics in MCF-7 tumors was similar.

Mice bearing s.c. MCF-7 tumors were treated with the anti-estrogen fulvestrant (fulv; 5 mg/wk) three days before GDC-0941 treatment to assess pharmacodynamic effects. Phospho-AKT and -S6 levels (markers of PI3K and mTORC1 activities, respectively) were inversely correlated with tumor drug concentrations. Mice bearing MCF-7, fulv-resistant T47D/FR, or HCC-003 patient-derived xenografts were treated with vehicle, fulv, GDC-0941 (100 mg/kg QD 5 d/wk; 100 mg/kg BID 3 d on/4 d off; 800 mg/kg QW), or combinations. Tumor growth curves indicated that different schedules of PI3K inhibition with fulv similarly induced tumor regression. Molecular analysis of MCF-7 tumors showed that fulv plus GDC-0941 QW induced 30.14% apoptosis (assessed by TUNEL) at 48 h, which dropped to baseline (2.72%) at 72 h. Fulv plus GDC-0941 BID induced 18.27% apoptosis at 24 h, and maintained apoptosis rate near 10% until 96 h (when GDC-0941 washed out), which is a rate similar to that observed with fulv plus GDC-0941 QD. Fulv plus GDC-0941 QW decreased cell proliferation (assessed by geminin and Ki67 staining) from 34.89% (baseline) to 11.46%, which rebounded to 60.54% at the time of GDC-0941 washout (at 96 h). Fulv plus GDC-0941 QD or BID modestly decreased cell proliferation to 28.84% and 24.32%, respectively, after 24 h, which returned to baseline after 36 h and 72 h, respectively, then maintained the level for a week. Temporal analysis of PI3K signaling revealed that fulv plus GDC-0941 QW and BID maximally decreased phospho-AKT levels after 3 h, which returned to baseline within 48 h and 72 h, respectively. With fulv + GDC-0941 QD, phospho-AKT levels decreased after 3 h, but rebounded to baseline within 24 h. These results indicate that 2 approaches to PI3K inhibition provide similar anti-tumor efficacy: 1) complete/intermittent (QW) PI3K inhibition induces a burst of apoptosis with a rebound in cell proliferation after drug clearance; and 2) metronomic/repeated (QD) PI3K inhibition repeatedly induces a smaller amount of apoptosis without significantly affecting cell proliferation. These findings may inform clinical testing of PI3K inhibitors to maximize therapeutic index.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-14-01

Title: Final results of the TANIA randomized phase III trial of bevacizumab (BEV) after progression on 1st-line BEV therapy for HER2-negative locally recurrent/metastatic breast cancer (LR/mBC)

Vrdoljak E, Marschner N, Zielinski C, Gligorov J, Cortes J, Puglisi F, Aapro M, Fallowfield L, Fontana A, Inbar M, Kahan Z, Welt A, Lévy C, Brain E, Pivot X, Putzu C, Gonzalez-Martin A, Ebel K, Easton V and von Minckwitz G. Center of Oncology, Split, Croatia; Outpatient Cancer Center, Freiburg, Germany; Comprehensive Cancer Center, Medical University Vienna and Central European Cooperative Oncology Group (CECOG), Vienna, Austria; Hôpital Tenon, Paris, France; Vall d’Hebron University Hospital, Barcelona, Spain; University Hospital of Udine, Udine, Italy; Institut Multidisciplinaire d’Oncologie, Clinique de Genolier, Genolier, Switzerland; Sussex Health Outcomes Research & Education in Cancer (SHORE-C), Brighton & Sussex Medical School, University of Sussex, Falmer, United Kingdom; University Hospital of Pisa, Istituto Toscana Tumori, Pisa, Italy; Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; University of Szeged, Szeged, Hungary; West German Cancer Center, University Hospital Essen, University Duisburg-Essen, Essen, Germany; Centre François Baclesse, Caen, France; Institut Curie – Hôpital René Huguenin, Saint-Cloud, France; University Hospital Jean Minjoz, Besançon, France; University Hospital of Sassari, Sassari, Italy; MD Anderson Cancer Center, Madrid, Spain; F Hoffmann-La Roche Ltd, Basel, Switzerland; Stamford Consultants AG on behalf of F. Hoffmann-La Roche Ltd, Basel, Switzerland and German Breast Group, Neu-Isenburg, and University Women’s Hospital, Frankfurt, Germany.

Body: BACKGROUND: The open-label randomized phase III TANIA trial (NCT01250379) evaluated 2nd-line BEV-containing therapy in BEV-pretreated LR/mBC. The primary objective was met: 2nd-line PFS was statistically significantly improved in patients (pts) receiving further BEV (hazard ratio [HR] 0.75, 95% CI 0.61–0.93; p=0.0068) [von Minckwitz, Lancet Oncol 2014]. We report final efficacy, safety, and health-related quality of life (HRQoL) results.

METHODS: Eligible pts had HER2-negative LR/mBC that had progressed on/after 1st-line BEV plus chemotherapy (CT). Pts were randomized to receive 2nd-line CT (investigator’s choice) either alone or combined with BEV (15 mg/kg q3w or 10 mg/kg q2w) until disease progression (PD), unacceptable toxicity, or consent withdrawal. At 2nd PD, pts in the CT arm received 3rd-line CT without BEV (no crossover); pts initially randomized to BEV–CT received 3rd-line BEV–CT. Secondary endpoints included 3rd-line PFS, 2nd- and 3rd-line PFS (from randomization to 3rd PD/death), overall survival (OS), HRQoL, and safety. HRQoL was assessed using FACT-B at baseline, every 8/9 weeks (depending on treatment schedule) during 2nd-line therapy, and at the time of 2nd PD. Prespecified HRQoL analyses included differences between treatment arms in mean change from baseline for each FACT-B subscale.

RESULTS: At the time of data cut-off for the prespecified final analysis (April 30, 2015, 24 months after the last pt was randomized), median follow-up was 32.1 vs 30.9 months in the CT vs BEV–CT arms, respectively. Of the 494 pts randomized to 2nd-line therapy, 234 began 3rd-line therapy (105 initially randomized to CT; 129 from the BEV–CT arm, of whom 17 received CT without BEV). The most commonly selected 3rd-line CT was vinorelbine (33% of CT pts vs 31% of BEV–CT pts).

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>No. of events/pts (%)</th>
<th>Median, months (95% CI)</th>
<th>Stratified HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>BEV–CT</td>
<td>CT</td>
<td>BEV–CT</td>
</tr>
<tr>
<td>3rd-line PFS</td>
<td>99/105 (94)</td>
<td>124/129 (96)</td>
<td>2.9 (2.2-3.9)</td>
<td>3.8 (2.4-5.1)</td>
</tr>
<tr>
<td>2nd- and 3rd-line PFS</td>
<td>177/247 (72)</td>
<td>206/247 (83)</td>
<td>10.7 (9.2-12.5)</td>
<td>12.8 (10.7-14.5)</td>
</tr>
<tr>
<td>OS</td>
<td>156/247 (63)</td>
<td>163/247 (66)</td>
<td>18.7 (15.4-21.2)</td>
<td>19.7 (17.6-21.0)</td>
</tr>
</tbody>
</table>

Subgroup analyses of 3rd-line PFS and OS according to stratification factors were consistent with the overall ITT result. Before study closure, 68% and 61% of pts in the 3rd-line ITT population CT and BEV–CT arms, respectively, received further CT.
3rd-line safety results showed no new safety signals. At week 8/9, mean change from baseline for all FACT-B subscales was <1.5 points in either direction in both treatment arms, representing no significant difference. Similarly, exploratory HRQoL analyses of the physical and functional wellbeing subscales using mixed-model repeated measures and responder analyses revealed no meaningful significant differences between treatment arms.

CONCLUSIONS: Although BEV given after PD on 1st-line BEV-containing therapy showed improvement in 2nd-line PFS, no OS benefit was demonstrated. No new safety signals were observed. There were no differences in HRQoL between treatment arms, suggesting that the PFS benefit with BEV is achieved with maintained HRQoL.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-14-02

Title: Abstract Withdrawn

Body:
Title: Genome wide association study (GWAS) to identify variants conferring ramucirumab-associated hypertension in the ROSE/TRIO-012 breast cancer trial

Mackey JR R, Lipatov O, Martín M, Webster M, Hegg R, Verma S, Ramos-Vázquez M, Fresco R, Thireau F, Houé V, Press MF F, Kumaran M and Damaraju S. Cross Cancer Institute; University of Alberta, Edmonton, AB, Canada; Bashkortostan Clinical Oncology Center, Ufa, Russian Federation; Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain; Tom Baker Cancer Centre and University of Calgary, Calgary, AB, Canada; Centro de Referência da Saúde da Mulher. Hospital Pérola Byington, Sao Paulo, Brazil; Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Centro Oncológico de Galicia, A Coruña, Spain; Translational Research in Oncology (TRIO), Montevideo, Uruguay; Translational Research in Oncology (TRIO), Paris, France and University of Southern California, Los Angeles, CA.

Body: Background: In a candidate single nucleotide polymorphism (SNP) association study, we previously identified VEGFR-1 and VEGFR-2 SNPs strongly associated with treatment emergent hypertension (HT) in the ramucirumab (RAM) and docetaxel (Doc) arm in the ROSE/TRIO-012 study, a double-blinded multinational phase III trial that randomized 1,144 patients with advanced breast cancer to receive first-line Doc in combination with RAM or placebo (Mackey et al, JCO Jan 10, 2015:141-148; Mackey et al, JCO, Volume 33, Issue 15_suppl, May 20, 2015: 547). However, candidate SNP studies limit the number of genes for interrogation and a more comprehensive genome wide search may identify critical variants associated with the phenotype of HT. Preliminary analysis indicated that patients experiencing HT with RAM showed better overall survival (Mackey JR, et al (2015). Reply to H. Lee, et al. JCO; in press). These observations provide the potential to identify those patients with genetic variants for predisposition to RAM-associated HT to inform therapeutic decisions.

Methods: Genotyping of samples is underway using Affymetrix SNP 6.0 arrays. Genotype data will be filtered for deviations from Hardy Weinberg Equilibrium and minor allele frequency of >0.05. Study subjects (n=792) provided ethics-committee approved prospective consent for this genetic study of whom 478 subjects were allocated RAM + Doc arm. Toxicity grades 0-1 (n= 394 controls; low toxicity) vs. grade >2 (n= 84 cases, high toxicity) is our binary outcome. Dominant genotypic model is assumed. Chi-square test, FDR and/or 10000 permutation tests will be employed (Golden Helix-SVS v8.3) and p<0.05 considered statistically significant. Population stratification will be identified (EIGENSTRAT) and association statistics will be corrected using Eigenvectors along with age as covariates. Fine mapping of loci showing significant associations will be attempted using imputation tools.

Results and conclusions: We expect up to 700,000 SNPs to be retained after filtering based on our previous breast cancer GWAS analyses (Damaraju et al. Cancer Research (suppl); Vol 70 (24), page 258s, 2010 and Sehrawat et al Hum Genet. 2011 Oct;130(4):529-37) and 30,000 SNPs to show significance at a nominal p-value (0.05); these will be analysed for regions of high linkage disequilibrium to narrow down potential loci showing association with HT to serve as candidate markers in further independent validation studies. Cumulative dose to adverse events will be considered in the analysis. Identified loci will be interrogated for potential genes in the flanking regions with biological relevance based on pathway analysis. Identified variants from candidate SNP and GWAS may allow developing predictive tools to enable stratification of patients for therapies. The analysis is expected to be completed by mid-November, 2015.
Triple negative breast cancer is vulnerable to Pan-HER, an antibody mixture simultaneously targeting EGFR, HER2 and HER3

Choi DS, Qian W, Davila-Gonzalez D, Ensor JE E, Lantto J, Kragh M, Horak ID D and Chang JC C. Houston Methodist Cancer Center, Houston, TX and Symphogen A/S, Ballerup, Denmark.

**Body: Background:** Triple negative breast cancer (TNBC) is a highly heterogeneous and aggressive subtype of cancer, lacking expression of estrogen and progesterone receptors as well as human epidermal growth factor receptor (HER) 2 protein. Limited standard therapeutic options, absence of effective targeted therapies, and early metastatic spread have contributed to poor prognosis and outcomes associated with this disease. Although overexpression of EGFR has been reported in nearly 80% TNBC, EGFR-targeted therapy has yielded little clinical benefit, and the outcome is still under debate. In conjunction, we also found mixed effects of EGFR-targeted therapy on TNBC xenograft tumors despite significant target engagement, suggesting that tumor heterogeneity and compensating mechanisms may contribute to the variable drug responses to the EGFR-targeted therapy. Recently, we reported superior anti-cancer effects of Pan-HER, a mixture of antibodies targeting the HER family members EGFR, HER2 and HER3 on various types of cancer by overcoming drug resistance and tumor heterogeneity. To this end, we hypothesized that Pan-HER can effectively inhibit tumor growth in TNBC by inhibiting tumor heterogeneity and drug resistance.

**Objective:** The goal of this study is to test the effect of Pan-HER antibody mixture (Sym013) on tumor growth and recurrence of 14 patient-derived (PDX) TNBC orthotopic xenograft tumor models and to investigate molecular biomarkers which can predict drug response to Pan-HER.

**Methods:** We evaluated in-vivo anti-tumor effects of Pan-HER (50 mg/kg, i.p. three times/week, 10 doses in total for 3 weeks) over vehicle on tumor growth and tumor recurrence on 14 PDX TNBC models with known expression levels of EGFR and HER3 (n=3/group). HER family proteins and related downstream molecules (Akt, Erk, Stat3, FAK) in the tumor tissues were evaluated by Western blot assay and immunohistochemistry analysis. Additionally, using dCHIP and ingenuity pathway analysis, we compared microarray data from the tested cohorts and other TNBC PDX models with known HER family receptor status.

**Results:** We found that Pan-HER alone effectively inhibited tumor growth in all 14 PDX models and showed statistical significance (p=0.0103) when compared to the vehicle groups. Among these, one PDX model, BCM-3186, showed substantial tumor reduction and additional two (MC1 and BCM-4913) showed complete response with no recurrence after the last treatment of Pan-HER. The significant anti-tumor effects of Pan-HER were positively correlated with inhibition of phosphorylation and expression of EGFR, HER3, Akt, Erk, and FAK, but not Stat3, and this was consistent in all PDX models tested. Additionally, the microarray and the pathway enrichment analyses suggest that loss of PTEN expression and up-regulation of FAK and RAS pathways may be the predictive markers for the Pan-HER drug response in TNBC.

**Conclusion:** Our in-vivo data suggest that simultaneous targeting of the three HER family receptors is a potential new approach for treatment of TNBC. Further confirmation of our in-vivo results will warrant a phase I clinical trial and lend support to single agent Pan-HER as a viable treatment strategy for TNBC patients in the clinic.
Title: Synthetic lethality in TNBC mediated by an anti-Trop-2 antibody-drug conjugate, sacituzumab govitecan (IMMU-132), when combined with paclitaxel or the PARP inhibitor, olaparib


Body: Background: In current clinical trials (ClinicalTrials.gov, NCT01631552), triple-negative breast cancer (TNBC) patients treated with IMMU-132, which is composed of the active metabolite of irinotecan, SN-38, conjugated to an anti-Trop-2 antibody, shows manageable toxicity and very encouraging responses in relapsed/refractory cases. Synthetic lethality is a concept in which a cell harboring one out of two possible gene or protein defects is viable, while a cell containing both defects is nonviable. BRCA1/2 mutations are linked to deficiencies in DNA repair and are associated with TNBC. Other repair mechanisms involve poly(adenosine diphosphoribose) polymerase (PARP), which can be used by cancer cells to overcome loss of BRACA1/2. Treatment of TNBC cells with either IMMU-132 or paclitaxel results in cleavage and deactivation of PARP, whereas the small molecule olaparib directly inhibits PARP. Therefore, the rationale of combining IMMU-132 with either paclitaxel or olaparib to effectively knock-out PARP activity was investigated in TNBC xenografts to ascertain if these combinations will result in synthetic lethality.

Methods: Mice bearing human TNBC xenografts (MDA-MB-468 or HCC1806) were treated with 15 mg/kg paclitaxel weekly for 5 weeks. IMMU-132 was administered either at 10 mg/kg or 12.5 mg/kg on days 1, 8, 22, and 29. In vitro, various human TNBC cell lines were incubated with either a constant amount of IMMU-132 in combination with various amounts of olaparib or constant olaparib with varying amounts of IMMU-132. A combination index number was calculated to determine whether the interaction was synergistic, additive, or antagonistic. Mice bearing TNBC tumors were treated with olaparib (50 mg/kg, qdx5d, for 4 wks), or IMMU-132 (10 mg/kg, 2xwkly x 4 wks), or the combination of both.

Results: Mice bearing MDA-MB-468 tumors treated with the combination of IMMU-132 and paclitaxel exhibited superior anti-tumor effects with >11-fold shrinkage of tumors in comparison to 1.4-fold shrinkage in the IMMU-132 group alone (P=0.0003) or 11.4-fold increase in tumor size in those mice treated with paclitaxel alone (P<0.0001). In the more aggressive HCC1806, the combination improved median survival from 17.5 and 17 days for paclitaxel and IMMU-132, respectively, to 38 days for those in the combination group (P<0.0015). IMMU-132 and olaparib demonstrated synergy in all TNBC cell lines tested in vitro. In an ongoing experiment, this same combination is proving to be superior to single agent therapy in mice bearing MDA-MB-468 tumors (P<0.0032). In all studies, the combination of IMMU-132 with either paclitaxel or olaparib was well tolerated, with no observable toxicities. DNA breaks as determined by TUNEL staining of excised xenografts are being assessed.

Conclusions: Targeting the PARP DNA repair pathway in BRCA1/2 mutant TNBC tumors by combining IMMU-132 therapy with either paclitaxel or olaparib achieved synthetic lethality in this disease model with no observable toxicity. These data provide the rationale for the clinical evaluation of IMMU-132 in combination with other chemotherapeutics that likewise target DNA-repair mechanisms in patients with TNBC.
**Title:** Differences in patterns of change of bone turnover markers during treatment with bone-modifying agents of breast cancer patients with bone metastases


**Body:** Background: Bone-modifying agents have demonstrated their efficacy for treatment by suppressing osteoclast function. The activity of bone-modifying agents can be monitored by means of bone resorption markers such as c-terminal crosslinking telopeptide of type I collagen (1CTP) and N-telopeptide of type I collagen (NTX) as well as bone forming marker bone-specific alkaline phosphatase (BAP). In contrast to these markers which indirectly indicate bone turnover, tartrate-resistant acid phosphatase-5b (Tracp-5b) has been established as a direct marker showing osteoclast number and activity. The aim of this study was to identify the relative significance of these bone turnover markers as indicators of treatment efficacy induced by bone-modifying agents for breast cancer patients with bone metastases.

Patients and Methods: For this study, 52 breast cancer patients with bone metastases treated with bone-modifying agents were recruited. Zoledronic acid and denosumab were administered as bone-modifying agents to 36 and 22 patients, respectively (for 6 patients, denosumab was used after zoledronic acid). Serum Tracp-5b, 1CTP, NTX and BAP were measured with, respectively, the EIA (enzyme immunoassay), RIA (two-antibody radioimmunoassay), ELISA (enzyme-linked immunosorbent assay) and CLEIA (chemiluminescent enzyme immunoassay) method. Blood samples were obtained pretreatment and 1, 3 and 6 months after treatment. Changes in these bone turnover markers were statistically analyzed with Friedman's test, and correlation between serum markers and clinicopathological factors was calculated with Mann-Whitney's test.

Results: Serum tracp-5b decreased significantly after treatment (p<0.0001). The baseline median value of Tracp-5b (457.5mU/dl, range: 173-1630mU/dl) had been reduced to 137mU/dl (91-795mU/dl) 1 month after treatment, but no further reduction was observed after that. For 13 out of 15 patients to whom Tracp-5b was administered, abnormally high levels (above 420mU/dl) decreased to normal range with one month treatment. Serum NTX was also significantly reduced after treatment (p=0.0007). The median baseline value (16.5nmolBCE/L, 6.1-52.2nmolBCE/L) was diminished after 1 month (to 10.9nmolBCE/L, 7.0-49.5nmolBCE/L), and further reduction of NTX was observed after 3 months (9.55nmolBCE/L, 6.4-56.0nmolBCE/L). Similarly, baseline BAP (15.1µg/L, 6.4-81.3µg/L) decreased significantly (p=0.0032), a reduction which was obtained after 3 months (10.15µg/L, 6.1-51.7µg/L), but not after 1 month (13.0µg/L, 7.7-137.0µg/L). On the other hand, reduction in 1CTP was not significant (p=0.83).

Conclusion and discussion: Although baseline values of the bone turnover markers Tracp-5b, NTX and BAP decreased significantly after treatment with bone-modifying agents, the pattern of reduction for these three markers varied. Tracp-5b appears to reflect efficacy of bone-modifying agents most quickly and sensitively, possibly due to its direct link to the number and activity of osteoclasts. These findings may prove usefulness of Tracp-5b when considering the efficacy of various bone-modifying agents in clinical practice.
Title: Treatment of skeletal metastatic breast cancer with bone seeking matrix metalloproteinase inhibitors

Tauro M, Laghezza A, Tortorella P and Lynch CC C. H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL and University of Bari "Aldo Moro", via Orabona 4, Bari, Italy.

Body: Background. Breast to bone metastasis is a common event during breast cancer progression. The resultant lesions are painful and currently, despite medical advances, are incurable. 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. Given that previous broad-spectrum MMP inhibitor (MMPI) trials were unsuccessful due to dose limiting systemic side effects, 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 (IC$_{50}$=140 nM). In vitro, we tested the effect of BMMPIs at varying doses (1nM-100 µM) 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 (1x10$^5$) 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.

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).

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:** Central nervous system metastases at diagnosis in patients with HER2+ MBC: Baseline characteristics, HER2-targeted treatments and clinical outcomes from the SystHERs registry

Hurvitz S, O'Shaughnessy J, Mason G, Yardley D, Jahanzeb M, Bruisky A, Rugo H, Swain S, Kaufman P, Tripathy D, Mayer M, Ogale S, Yoo B, Beattie M and Cobleigh M. UCLA Jonsson Comprehensive Cancer Center and Translational Research in Oncology, Los Angeles, CA; Baylor Charles A. Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX; Inflammatory Breast Cancer Research Foundation, West Lafayette, IN; Tennessee Oncology, Nashville, TN; University of Miami Sylvester Comprehensive Cancer Center, Deerfield Campus, Deerfield Beach, FL; University of Pittsburgh Cancer Institute, Pittsburgh, PA; University of California San Francisco Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH; University of Texas MD Anderson Cancer Center, Houston, TX; AdvancedBC.org, NY, NY; Genentech, Inc., South San Francisco, CA and Rush University Medical Center, Chicago, IL.

**Body:**

**Introduction**

HER2+ MBC patients with CNS metastases are often excluded from clinical trials. The Systemic Therapies for HER2+ Metastatic Breast Cancer Registry (SystHERs), a real-world, prospective registry study, is uniquely positioned to evaluate this understudied population over time. Here we describe the clinical characteristics, HER2-targeted treatments, and survival outcomes of patients with and without CNS metastases at MBC diagnosis.

**Methods**

SystHERs is enrolling patients with HER2+ MBC who are >18 years of age and within 6 months of MBC diagnosis. Patients with CNS metastases are identified by their physicians according to local clinical practice. Patient-reported outcomes are obtained at enrollment and throughout the study. Median PFS and OS were estimated using the Kaplan-Meier method and Cox regressions were used to estimate hazard ratios (HRs).

**Results**

As of February 2, 2015, data are available for 579 eligible patients, of whom 53 (9%) had CNS metastases at MBC diagnosis (median time from diagnosis to enrollment was 2.3 months). Clinical characteristics and patient-reported functioning in patients with and without CNS metastases at MBC diagnosis are shown in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th></th>
<th>CNS (n=53)</th>
<th>Non-CNS (n=526)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>57 (36-86)</td>
<td>56 (21-88)</td>
</tr>
<tr>
<td>De novo MBC(^a), n (%)</td>
<td>15 (28)</td>
<td>275 (52)</td>
</tr>
<tr>
<td>Hispanic or Latina, n (%)</td>
<td>7 (13)</td>
<td>52 (10)</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>46 (87)</td>
<td>407 (77)</td>
</tr>
<tr>
<td>BMI (range)</td>
<td>29 (17-43)</td>
<td>28 (9-60)</td>
</tr>
<tr>
<td>ECOG PS (0-1), n (%)</td>
<td>36 (68)</td>
<td>471 (90)</td>
</tr>
<tr>
<td>Hormone receptor status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+,PR+</td>
<td>21 (40)</td>
<td>247 (47)</td>
</tr>
<tr>
<td>ER+,PR-</td>
<td>11 (21)</td>
<td>106 (20)</td>
</tr>
<tr>
<td>ER-,PR+</td>
<td>0</td>
<td>10 (2)</td>
</tr>
<tr>
<td>ER-, PR-</td>
<td>21 (40)</td>
<td>153 (29)</td>
</tr>
<tr>
<td>FACT-B(^b,c), median (IQR)</td>
<td>(n=42)</td>
<td>(n=422)</td>
</tr>
<tr>
<td></td>
<td>93 (78-110)</td>
<td>102 (85-118)</td>
</tr>
<tr>
<td>Rotterdam Symptom Checklist(^b,d), median (IQR)</td>
<td>(n=43)</td>
<td>(n=429)</td>
</tr>
</tbody>
</table>
First-line MBC treatment data are available for 502 patients, of whom 488 (97%) reported first-line HER2-targeted therapy at the time of data cutoff (Table 2).

Table 2.

<table>
<thead>
<tr>
<th>First-line HER2-targeted therapy, n (%)</th>
<th>CNS (n=40)</th>
<th>Non-CNS (n=448)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any trastuzumab + pertuzumab</td>
<td>22 (55)</td>
<td>313 (70)</td>
</tr>
<tr>
<td>Any trastuzumab, no pertuzumab</td>
<td>11 (28)</td>
<td>117 (26)</td>
</tr>
<tr>
<td>Any lapatinib</td>
<td>14 (35)</td>
<td>14 (3)</td>
</tr>
<tr>
<td>Any T-DM1</td>
<td>5 (13)</td>
<td>29 (6)</td>
</tr>
</tbody>
</table>

At a median follow-up time of 12.9 months, median first PFS was 8 months in patients with CNS metastases, and 11.9 months in those without (HR = 0.442; 95% CI 0.304 – 0.641). Median OS was 22 months in patients with CNS metastases, and was not reached in patients without CNS metastases (HR = 0.299; 95% CI 0.174 – 0.514).

Conclusions

Patients with CNS metastases at MBC diagnosis represent 9% of this registry population. Poor baseline function and an increased risk of progression and death characterized these patients compared to those without baseline CNS metastases. Treatments administered to these patients differ from ASCO consensus recommendations (Ramakrishna, 2014). Further studies are needed to determine the associations between treatments and both clinical and patient-reported outcomes. At the time of presentation, detailed treatment data and additional patient-reported data will be available.
**Title:** Ado-trastuzumab emtansine (T-DM1) is effective against established HER2-positive breast cancer brain metastases in mice

Askoxylakis V, Ferraro G, Kodack D, Badeaux M and Jain R. Edwin L. Steele Laboratories, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

**Body:** Background: Brain metastases represent a major problem in the treatment of HER2-positive breast cancer (1). The antibody-drug conjugate ado-trastuzumab emtansine (T-DM1) has shown efficacy in trastuzumab-resistant systemic breast cancer. Here, we tested the hypothesis that T-DM1 could overcome trastuzumab resistance in murine models of brain metastases.

Methods: We used previously established animal models of HER2-positive breast cancer brain metastases and organotypic brain slice cultures that recapitulate clinical scenarios (2). We treated mice bearing HER2-positive breast cancer brain metastases with trastuzumab or T-DM1 at equivalent or equipotent doses. Using intravital imaging, molecular techniques and histological analysis we determined tumor growth, mouse survival, cancer cell apoptosis and proliferation, tumor drug distribution, gene expression, and HER2 downstream signaling.

Results: T-DM1 significantly delayed the growth of HER2-positive breast cancer brain metastases compared to trastuzumab. These findings were consistent between HER2-driven and PI3K-driven breast tumors. The activity of T-DM1 resulted in a striking survival benefit compared to trastuzumab (median survival for BT474 tumors: 28d for trastuzumab vs 112d for T-DM1, HR=6.2, P<0.001). A comparison of T-DM1 with trastuzumab revealed no difference in their tumor distribution, HER2 downstream signaling inhibition or immune cell enrichment. T-DM1, however, led to a significant increase in tumor cell apoptosis. Electron microscopy studies revealed increased numbers of abnormal mitotic figures in brain tumors treated with T-DM1.

Whole-transcriptome microarray analysis of BT474 brain tumors treated with trastuzumab or T-DM1 showed an enrichment of genes that are associated with mitotic catastrophe in the group treated with the antibody-drug conjugate. These mechanistic studies support the hypothesis that the efficacy of ado-trastuzumab emtansine in the brain microenvironment is mediated through the cytotoxic chemotherapeutic effect of the DM1 component.

Conclusions: Our findings suggest that T-DM1 can overcome resistance to HER2-targeted therapies in the CNS, and warrants clinical investigation for the effective treatment of HER2-positive breast cancer brain metastases.

References:
Title: Trastuzumab emtansine (T-DM1) in patients with HER2-positive breast cancer and brain metastases

Jacot W, Pons E, Guiu S, Levy C, Frenel J-S, Bachelot T, D'Hondt V, Firmin N, Romieu G, Thezenas S and Dalenc F. Institut Régional du Cancer de Montpellier, Montpellier, France; Institut Claudius Regaud, IUCT- Oncopole, Toulouse, France; Centre François Baclesse, Caen, France; Institut de Cancérologie de l'Ouest, Nantes, France and Centre Léon Bérard, Lyon, France.

Body: Purpose: Few data are currently available regarding the efficacy and safety of T-DM1 in breast cancer (BC) patients with brain metastases (BM), since clinical trials excluded these patients or included highly selected ones. We report here the experience of our institutions with the T-DM1 use in daily care practice BM BC patients.

Patients and methods: HER2+ BC patients presenting with BM treated by T-DM1 in one of our institutions, using a standard dose of 3.6 mg per kilogram intravenously every 21 days, were considered in this retrospective study. Dose delays, reductions, and discontinuations due to toxic effects were performed according to the product guidelines. Treatment was continued until progression or unacceptable toxicity. We analyzed efficacy data by recording tumor response rates, progression-free survival (PFS) and overall survival (OS), treatment compliance (Relative Dose Intensity [RDI]) and safety by analyzing clinical and biological toxicities using NCI CTCAE v4.03.

Results: 17 patients were treated between 2012 and 2015, with a median age of 52.8 years (range 35.2-68.8 years). 81.3% of the tumors were of the invasive ductal carcinoma subtype. No tumor was recorded to be Scarf, Bloom and Richardson grade I, 47.1% were estrogen receptor negative. 94% of the patients presented with concomitant extra-cerebral disease, mainly bone (71%), liver (47%), lymph node (47%) and lung (12%) metastases. The number of previous chemotherapy and trastuzumab regimens in the metastatic setting were 3 (1-7) and 2 (1-7) respectively. 15 out of the 17 patients previously received the capecitabine – lapatinib association. All patients previously received a locoregional treatment for their BM (whole brain radiation therapy in 88.2% of the cases).

After a median follow-up of 4.3 months (95%IC 3.5 – 13.6), 9 patients presented a disease progression (first site of progression: brain 5; meningeal 2; outside of the CNS 2), 4 patients died due to progressive disease and 13 patients are still alive. The median number of T-DM1 cycles was 6 (range 1-27). There were 5 partial responses (29.4%), with an additional 35.3% disease stabilization, for a total 64.7% of patients with clinical benefit. Median PFS was 5.5 months (95%CI: 2 – Not Reached). Median OS was not reached at the moment of the present statistical analysis. There were no presumed treatment-related deaths. No dose reduction was required, the median RDI was 1. Treatment was well tolerated, without unexpected toxicities, treatment delay or dose reduction. Only one patient discontinued T-DM1 after 27 cycles due to bilirubin increase, while experiencing sustained disease stability. There were only one grade 3 toxicity (fatigue), and no reported grade 4 toxicities.

Conclusion: In this limited population of unselected, heavily pretreated, patients affected by BM from HER2+ BC, T-DM1 appears to be a safe option, with clinical activity, even if it appears inferior to the ones reported in the pivotal trials. These results could be linked to the more advanced status of the population. A larger population, altogether with a longer follow-up appears mandatory to more accurately evaluate this agent in the BC BM. Thus this study will be updated for the meeting in term of number of patients and follow-up.
A phase II, open-label, multi-center study of ANG1005, a novel brain-penetrant taxane derivative, in breast cancer patients with recurrent CNS metastases

Tang S-C, Bates S, Kesari S, Brenner AJ J, Anders CK K, Garcia A, Ibrahim NK K, Tkaczuk KHR HR and Kumthekar P. Georgia Regents University Cancer Center, Augusta, GA; National Cancer Institute, NIH, Bethesda, MD; UC San Diego Moores Cancer Center, La Jolla, CA; Cancer Therapy and Research Center at UTHSCSA, San Antonio, TX; University of North Carolina at Chapel Hill, Chapel Hill, NC; University of Southern California - Norris Comprehensive Cancer Center, Los Angeles, CA; M.D. Anderson Cancer Center, Houston, TX; University of Maryland Greenebaum Cancer Center, Baltimore, MD and Northwestern University, Chicago, IL.

**Body: Background:** Treatment options for brain metastases are limited to local therapies due to the inability of most anti-cancer agents to cross the blood brain barrier (BBB). ANG1005 is a novel taxane derivative, being developed for targeted treatment of brain metastases. It consists of 3 paclitaxel molecules covalently linked to Angiopep-2 designed to cross the BBB and to penetrate malignant cells, regardless of location, via the low density lipoprotein (LDL) receptor related protein-1 (LRP-1) transport system.

**Methods:** Adult patients with measurable recurrent brain metastases from breast cancer with, or without, leptomeningeal disease are currently being enrolled in this multi-center, open-label study (planned n=56). ANG1005 is administered IV at 600 mg/m² every three weeks (one cycle) until disease progression, unacceptable toxicity or consent withdrawal. HER2+ patients are allowed to continue HER2 targeted therapies. The primary endpoint is intracranial objective response rate, as assessed by MRI using CNS RECIST 1.1. Secondary endpoints include duration of intracranial response, median progression-free survival, 3/6/12-month progression-free survival rate, overall survival at 6 months, extracranial objective response rate, safety and tolerability. Extracranial response is also assessed by CT using RECIST 1.1. An imaging sub-study, evaluating the use of 18F-FLT-PET in comparison to MRI, is also ongoing in 10 patients with measurable brain metastases from breast cancer, receiving ANG1005 IV at 550 mg/m².

**Results:** Accrual is ongoing and to date, 48 patients have been treated with a range of 1-18 cycles of ANG1005. Median age is 47 years (range: 26-65). Safety profile is similar to that of paclitaxel with myelosuppression as the predominating toxicity. Based on data from patients evaluated to date for intracranial response, 6/30 (20%) patients had a partial response (PR) and 17/30 (57%) had a stable disease (SD), as best response. A sub-analysis, based on breast cancer sub-type is presented below:

<table>
<thead>
<tr>
<th>Outcome by CNS RECIST</th>
<th>HER2- (n=13)</th>
<th>HER2+ (n=17)</th>
<th>TNBC (n=6)</th>
<th>LMD (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR, n (%)</td>
<td>1 (8%)</td>
<td>5 (29%)</td>
<td>1 (17%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>SD, n (%)</td>
<td>6 (46%)</td>
<td>10 (59%)</td>
<td>2 (33%)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>PD, n (%)</td>
<td>6 (46%)</td>
<td>2 (12%)</td>
<td>3 (50%)</td>
<td>2 (18%)</td>
</tr>
</tbody>
</table>

TNBC, triple-negative breast cancer; a sub-group of HER2-; LMD, leptomeningeal disease, including 3 HER2- and 8 HER2+ patients

The longest duration on treatment is for 18 cycles, seen in a patient with an intracranial PR that sustained for 10 cycles; the treatment is still ongoing. Extracranial tumor evaluations were completed in 14 patients, all showing disease control including in those previously treated with paclitaxel. One (7%) patient had a PR and 13 (93%) patients had an SD.

**Conclusions:** CNS activity was observed in all subsets of breast cancer, suggesting that ANG1005 is a promising therapy for treatment of brain and leptomeningeal metastases from breast cancer. ANG1005 treatment also resulted in disease control in extracranial lesions, including patients previously treated with paclitaxel. The dose and treatment regimen were well tolerated with
a safety profile similar to paclitaxel. Updated efficacy and safety data will be presented at the meeting.
Title: A corrole nanobiologic crosses the blood-brain-barrier and recognizes triple negative breast cancer: Implications for targeting brain metastases

Patients with breast cancer metastases to the brain on average survive less than one year. These tumors tend to be resistant to current therapies, and the majority of targeted therapeutics are unable to breach the blood brain barrier (BBB) to reach these tumors, thus improved alternatives are urgently needed.

Elevated cell surface levels of the human epidermal growth factor receptor subunit 3 (HER3) is associated with metastatic breast tumors, including those that spread to the brain. Elevated HER3 is also associated with resistance to a number of targeted therapies currently used in the clinic, including inhibitors of EGFR (lapatinib), HER2 (lapatinib, trastuzumab, T-DM1), HER2-3 (pertuzumab), and combination therapy.

Whereas a number of targeted therapies are currently used to combat peripheral breast tumors, the delivery of these molecules to brain metastases is limited by the blood brain barrier (BBB). This is exemplified by HER2+ breast tumors that metastasize to the brain: these tumors, while targetable outside of the central nervous system (CNS) by HER2 antibodies such as trastuzumab, are unreachable by these same antibodies because the HER2 subunit, though present on the brain endothelium, does not mediate antibody transcytosis across the blood vessel wall.

HER3, on the other hand, undergoes rapid transcytosis across the brain endothelium upon ligand binding, which normally occurs to mediate the delivery of neuregulin growth factors for neural growth and maintenance. We have developed a self-assembling nanobiological particle, HerMn, which uses HER3 as a portal for targeted entry of toxic molecules into tumor cells. HerMn is a 10-20 nm diameter serum-stable particle comprised of a HER3-targeted cell penetration protein non-covalently assembled with a sulfonated manganese(III) corrole (S2Mn or Mn-corrole). Tumor-targeted toxicity by HerMn occurs by mitochondria membrane disruption and superoxide-mediated damage to the cytoskeleton. HerMn can also elicit tumor-selective detection by magnetic resonance imaging (MRI) due to the paramagnetic property of the corrole. HerMn distributes to the brain after systemic injection in mice, in addition to showing preferential homing and toxicity to subcutaneous tumors expressing the HER2-3 dimer. Interestingly, the Mn corrole is known to exhibit neuroprotective effects due to its antioxidant activity on normal tissue. Consistent with this, we have found that HerMn supports human cardiac cell survival ex vivo. Our studies interrogating the therapeutic potential of HerMn suggest that this nanobiologic bears the capacity for targeting toxicity to brain-metastatic breast tumors while sparing off-target tissue due to both its targeting capacity and ability to provide beneficial protective effects to normal tissue such as the brain and heart.
Relevance of the hyaluronidase-1 (HYAL1) in brain metastasis formation of breast cancer patients

Oliveira-Ferrer L, Anna M, Wickman H, Matschke J, Schumacher U, Milde-Langosch K, Müller V and Witzel I. University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Institute of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany and University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Body: The incidence of brain metastases in breast cancer (BMBC) patients has increased in the last years and represents the major life-limiting problem for metastatic breast cancer (BC) patients. However, the knowledge about tumor cell invasion in the brain is still very limited and new markers for brain metastasis incidence are urgently needed in order to early detect high risk patients.

In a recent study based on cDNA microarray data of primary breast tumors, we could show that mRNA expression of certain glycosylation enzymes significantly correlates with an organ-specific metastatic spread. Interestingly, hyaluronan synthase 2 (HAS2) and hyaluronidase-1 (HYAL1), both genes involved in hyaluronan (HA) metabolism, showed an independent prognostic value and a significant correlation with brain metastasis formation.

In order to corroborate the role of these enzymes at protein level, we examined the expression of HYAL1 and HAS2 on a tissue microarray including 200 primary BC samples. Here, the prognostic impact of HAS2 could not be validated, whereas for HYAL1 shorter disease free survival (DFS) was observed for patients with high HYAL1-expression levels. This trend could be additionally verified in a second cohort of 107 BC samples, using western blot analysis. Moreover, significantly higher HYAL1 expression was detected among primary tumors with subsequent brain metastases compared with those without brain metastases using immunohistochemistry (IHC).

No impact of HYAL1 expression on disease progression of BCBM patients could be observed after analysis of 87 brain metastasis samples. Here, quantification of tumor-associated HA revealed a significant positive correlation with triple negative tumors and a trend towards shorter progression-free survival.

Taken together, our data suggest a role of the enzyme HYAL1 for progression and especially for the development of brain metastases in breast cancer patients.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-17-07

Title: Changing molecular profile of brain metastases compared with matched breast primaries and impact on clinical outcomes

Thomson AH H, Purvis G, McGrane J, Palmer J and Jenkins R. Royal Cornwall Hospital, Truro, Cornwall, United Kingdom.

Body: Background
Over 10% of patients with metastatic breast cancer develop symptomatic brain metastases. Limited treatment options can result in significant morbidity and a dismal prognosis. Little is known about the molecular profile of brain secondaries, differences compared with the patient's breast primary and whether any changes impact on prognosis.

Methods
Patients with resected or biopsied brain metastases from a breast cancer were identified from an electronic database, with clinical data collected from hospital notes for patients in the south west of the UK. Patients were included if tissue from the primary breast cancer and brain metastasis were available for testing. Immunohistochemical analysis was performed for oestrogen receptor (ER), progesterone receptor (PR), p27kip1, cyclin D1, epidermal growth factor receptor (EGFR), insulin like growth factor 1 (IGF1) and receptor (IGF1-R), vascular endothelial growth factor A (VEGF A), vascular endothelial growth factor receptor (VEGFR-2), transforming growth factor beta (TGFB) and Her 2 receptor on both the brain and breast samples. Borderline Her 2 results were analysed by fluorescent in situ hybridisation.

Results
41 patients were identified. Median age of patients was 49, with a 26 month interval between breast cancer diagnosis and development of brain metastases. Median time from brain metastases to death was 15 months. 13 patients (32%) had a HER2 positive breast primary, 12 receiving HER2 directed therapy prior to development of brain metastases. 15 patients (37%) had an ER positive breast cancer at initial diagnosis. 11 patients had a biopsy of their brain secondary, 30 more extensive surgical resection. 28 patients received whole brain radiotherapy (WBRT), 1 patient stereotactic radiotherapy (SRT) alone, 6 patients both WBRT and SRT, 6 unknown.

Changes in the molecular profile of the breast primary compared with the brain secondary are illustrated in Table 1.

Number of patients with a change in molecular profile from breast primary to brain metastasis

<table>
<thead>
<tr>
<th></th>
<th>Breast positive to brain negative</th>
<th>Breast negative to brain positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PR</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Her2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>EGFR</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>IGF1</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>IGF1-R</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>TGFB</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>p27 kip1</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>cyclin D1</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>VEGF</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VEGFR-2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

There was a change in more than 10% of patients from a positive breast primary to a negative brain secondary for p27kip1 and from a negative breast cancer to a positive brain metastasis for EGFR, IGF1, cyclin D1 and p27kip1. These alterations did not have a significant impact on time from brain metastasis to death. However, there was a significant improvement in survival from brain metastasis diagnosis for secondary lesions that were ER positive (p=0.005) or PR positive (p=0.013). Survival from time of brain metastasis improved with a longer time to brain metastasis from initial diagnosis (p=0.001).
Conclusions
In this cohort there were demonstrable phenotypic differences in the expression levels of EGFR, IGF1, p27Kip1 and cyclin D1 in metastatic brain tumours compared with primary breast tumours of the same patient, although survival was not affected. 8 patients (20%) had a change in ER or Her 2 that could impact on current therapeutic decisions. Hormone receptor positive brain metastases had superior survival compared with negative lesions.
Title: Brain metastases in breast cancer network Germany (BMBC, GBG 79): First analysis of 548 patients from the multicenter registry

Body: Background: The incidence of brain metastases (BM) in breast cancer patients is rising and has become a major clinical challenge. So far, limited therapeutic options and insights into the biology of BM exist since only a few studies analyzed exclusively data of breast cancer patients. In order to improve this situation, our multicenter registry was initiated in 2014: Brain Metastases in Breast Cancer Network Germany (BMBC, GBG79).

Materials and Methods: Patients with BM diagnosed since 2000, a history of breast cancer and no history of other malignant or neurologic disease can be included. Registration is allowed retrospectively as well as prospectively into a web–based database ("MedCodes"). Characteristics of the primary tumor, metastatic disease and BM as well as treatment details are documented. For this first analysis, 548 patients from 39 German centers were included.

Results: Median age at first diagnosis of BM was 55 years (25 – 90 years). 43% of patients (233/548) were HER2 positive, 19% (n=105) were triple–negative and 25% (n=138) had luminal primary tumors indicating a selection of patients with specific tumor biology who develop BM. 54 % of the patients (n=267) had up to three BM whereas 45% (n=223) had more than three BM. 19% of patients (n=106) had BM without evidence of extracranial disease. 27% of the patients (n=146) underwent surgery of the BM. Of these patients, 61% (n=89) were treated with whole brain radiotherapy and 16% (n=23) with stereotactic radiotherapy. In patients without surgery (n=397), 73% (n=289) received whole brain radiotherapy and 7% (n=28) stereotactic radiotherapy. Median time from diagnosis of primary breast cancer to BM was 38.5 month for the entire cohort (CI95% 35.4 – 43.3). The time from first diagnosis to BM was shorter for triple–negative patients (20.9 month, CI95% 15.5 – 25.9) compared with patients with HER2–positive (37.0 month, CI95% 30.5 – 42.0) or luminal tumors (48.3 month, CI95% 38.2 – 54.0) (p<0.001). Median time from first diagnosis of BM to death in the entire cohort was 6.1 months (CI95%: 5.2 – 7.3). One year survival rate from diagnosis of BM was 32.2 % (CI95%: 2.2 – 67.8). Regarding tumor subtypes, HER2–positive patients had the longest median survival with 9.4 months (CI95%: 7.1 – 13.4) compared with 6 months (CI95%: 4.0 – 7.3) for luminal primary tumors and 3.2 months (CI95%: 2.1 – 4.6) for triple–negative patients (p<0.001). HER2 positive patients receiving HER2–directed therapy after the diagnosis of BM lived longer than those without (median 9.6 vs. 5.5 months, p=0.029). Regarding the number of BM, no difference in survival was observed between one, two or three BM (median survival of 7.8 months). However, survival was shorter in those patients with more than three BM (5.2 months; p=0.007).

Conclusion: This is so far the largest analysis of breast cancer patients with BM treated in Germany. In this cohort, triple–negative subtype or more than three BM were associated with shorter survival from the diagnosis of BM. HER2 positive patients with no HER2 directed therapy after the diagnosis of BM showed a shorter survival. The recruitment of the registry is ongoing and we aim to include more than 1000 patients by the end of 2015.
Whole brain radiation therapy (WBRT) with or without concurrent temozolomide for brain metastases in breast cancer patients

El-Sadda WM M, Abdel-Halim II I, El-Ibrashi MM M and Magdi MM M. Mansoura University School of Medicine, Mansoura, Dakahliya, Egypt and Al-Ghad International Colleges for Health Sciences, Najran, Saudi Arabia.

Body: Background:
Breast cancer remains the second leading cause of CNS metastases. HER2 positive and triple negative subtypes are especially and independently at higher risk of developing brain metastases. Although surgical resection and radiosurgery may play a role in the management of selected cases, whole brain radiation therapy remains the standard treatment but with limited efficacy. Temozolomide (TMZ); an oral alkylating agent that cross the blood brain barrier has shown radiosensitizing characteristics as well as synergistic effects of radiation therapy (RT).

The aim of the study was to assess the efficacy and safety of WBRT with concomitant TMZ in treatment of brain metastases in breast cancer patients.

Patients and methods:
A prospective randomized study included 60 patients with newly diagnosed brain metastases (BMs) from breast cancer not candidate for surgical resection or radiosurgery. All patients received WBRT 30 Gy/10 fractions with or without TMZ, administered at a dosage of 75 mg/m2/day during the irradiation period. The primary end point was objective response rate (ORR) 6 weeks after the end of treatment defined as partial response (PR) or complete response (CR) on brain MRI (WHO criteria). Secondary end points were progression-free survival (PFS) and overall survival (OS), neurological symptoms and tolerability.

Results:
Between October 2011 and October 2013, 60 patients were enrolled in the study, 30 patients received WBRT+TMZ (group A) and 30 patients received WBRT (group B). Median age was 54 years (30-65), median follow up was 10.4 months (3-36). ORR was 80% in the WBRT+TMZ arm versus 50% in the WBRT (p = 0.017). The median PFS were 11.8 versus 5.8 respectively (p = 0.005). The median OS was 13.8 for group A and 10.8 for group B (p = 0.59). WBRT+TMZ was well tolerated with mostly grade 2 non-hematologic toxicity; headache, nausea & vomiting were 6%, 15% & 12% in group A versus 12%, 9% & 6% in group B, respectively. While the most evident hematologic toxicity recorded was lymphopenia grade 2 & 3 (15% & 12% in group A) and (12% & 9% in group B). No grade 4 toxicities were noted.

Conclusion:
WBRT plus TMZ improve local control and response rate of BMs from breast cancer, which was reflected by improved PFS.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-18-01

Title: Novel genetic susceptibility loci for inflammatory breast cancer identified by whole exome sequencing

Ye Z, Li B, Wang C, Zhong X, Wei Q, Mu Z, Austin L, Jaslow R, Avery T, Palazzo J, Biederman L, Yang H, Cristofanilli M and IBC Inflammatory Breast Cancer International Consortium. Vanderbilt University, Vanderbilt, TN; Division of Population Science, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; Vanderbilt University, Vanderbilt, TN; Division of Solid Tumor Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA; Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA and Only.

Body: Background: Inflammatory breast cancer (IBC) is an extremely aggressive form of locally advanced breast cancer that affects approximately 5% of breast cancer patients. The prognosis of IBC patients is remarkably poor, with a three-year survival rate of approximately 30% compared to 60% for non-IBC breast cancer patients. The etiology of IBC is largely unknown. A few risk factors have been reported such as body mass index (BMI) and educational level. Prior evidence has also implicated genetic components in IBC etiology. For instance, the reported familial cases and racial incidence disparity of IBC patients, as well as the fact IBC patients typically have a younger age onset than non-IBC patients, all indicated the possible involvement of genetic factors. Nevertheless, as yet no genetic epidemiological study has been reported to evaluate IBC genetic predisposition.

Methods: To test the hypothesis that genetic variants and mutations may affect IBC susceptibility, we performed whole exome sequencing in a pilot case-control study that contained 70 IBC cases and 119 unrelated cancer-free controls. Sequencing data were de-multiplexed, filtered, assessed for various quality control metrics, mapped to reference genome and annotated. Comprehensive single variant-based, gene-centered, and pathway-based analyses were conducted to identify variants, genes, and pathways that may be involved in IBC predisposition.

Results: We obtained > 50x on-target sequencing coverage of the whole exome in > 90% of the patients. In single variant analysis, we identified six variants reaching genome-wide significance. Four variants were encoded by genes that have been implicated in breast cancer development including MALAT1, MAP3K9, POLR3B, and FIP1L1. Two variants were encoded by novel genes that have not been related to breast cancer, including CCDC30 and LINC01565. Two types of analyses based on a gene-centered strategy identified top genes such as SLC39A4, CDHR1, AP5Z1, GNB3, ITGA10, etc. However, possibly due to the limited sample size, none of these genes reached genome-wide significance. Ingenuity Pathway Analysis (IPA), using the complete list of significant genes identified by each of these analyses all reported “cancer” as the highest possible disorder associated with these genes, demonstrating the biological plausibility of our findings. Moreover, canonical pathways such as IL4 signaling, glycogen degradation, epithelial adherence junction signaling, and CCR3 signaling in eosinophils were among the top pathways that were found involved in IBC predisposition.

Conclusion: Overall, we provided novel preliminary evidence that genetic variants are potentially associated with the risk of developing IBC. We are currently conducting validation studies with larger sample sizes are warranted to confirm these findings and identify additional genetic susceptibility loci.
2015 San Antonio Breast Cancer Symposium

Publication Number: P6-18-02

Title: Patterns of breast reconstruction in patients diagnosed with inflammatory breast cancer

Nakhlis F, Regan M, Chun YS, Dominici LS, Jacene HA, Yeh ED, Bellon JR, Warren LE, Hirko K, Hirshfield-Bartek J, Hazra A and Overmoyer BA. Brigham and Women's Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Brigham and Women's Hospital, Boston, MA; Brigham and Women's Hospital and Harvard Medical School, Channing Division of Network Medicine, Boston, MA and Harvard T.H. Chan School of Public Health, Boston, MA.

Body: Background Inflammatory Breast Cancer (IBC) is a rare and aggressive type of breast cancer treated by multimodality therapy. Due to the presence of dermal lymphatic involvement by disease it is unknown whether a skin sparing mastectomy (SSM) would be safe, even after completion of preoperative chemotherapy, therefore immediate breast reconstruction (BR) following modified radical (MRM) is discouraged. We sought to explore the patterns of BR outcomes in IBC patients (pts) and to evaluate their surgical outcomes.

Methods A retrospective analysis was performed using an IRB-approved database of IBC pts evaluated at Dana Farber Cancer Institute (DFCI) from 1997 until 2014. Pts with stage III IBC who received preoperative systemic therapy followed by MRM and post-mastectomy radiation (PMRT) were analyzed. Receipt and timing of BR, post-operative morbidity and subsequent esthetic issues were collected. We also analyzed oncologic events which may have hindered receipt of BR.

Results 318 pts were enrolled in the IBC registry at DFCI between 1997 and 2014. 181 pts with stage III IBC were identified, with a median follow-up of 57 months (mo) from MRM. 33/181 pts (18%) underwent BR; 2 pts were not evaluable due to lack of details concerning BR. 12 pts had immediate BR, 10 of which were performed elsewhere, prior to initial evaluation at DFCI. These included 3 tissue expander (TE) reconstructions, 3 single stage implants, 1 deep inferior epigastric perforator (DIEP) flap, 4 transverse rectus abdominis myocutaneous (TRAM) flaps, 1 latissimus dorsi (LD) flap. 19 had delayed BR. These included 1 TE, 5 DIEP flaps, 2 LD flap, 1 TE+LD flap, 10 TRAM flaps. Delayed BR occurred at a median of 13 mo (range 3-64 mo) following completion of PMRT.

Complications post-BR were rare. Among the immediate BR pts, 1 pt with a TRAM flap BR required a reoperation 15 days (d) following BR for a partial TRAM flap necrosis. Among the delayed BR pts, one had a reoperation for abdominal TRAM flap donor site wound dehiscence 29 d after BR. Another delayed BR pt, who had a TE+LD flap BR, had her reconstruction implant removed due to chronic hematoma 79 mo after her initial BR; this occurred following 3 operative attempts to salvage her reconstruction by evacuating the hematoma. Overall, 12 reoperations were performed including 6 immediate BR (6/19 (31.6%) and 6 delayed BR pts (6/12 (50.0%); 6 of these reoperations (50%) were done for minor esthetic issues, such as reconstruction revisions for fat necrosis and capsular contracture, in addition to the more significant surgeries described above. Among 148 pts who did not undergo BR, 69 (47%) had disease recurrence following MRM (66 distant +/- local-regional recurrence (LRR); 3 LRR only); within 12 mo of MRM disease recurrence developed in 22% of pts (33/148).

Conclusion Only 11% of pts presenting with stage III IBC received delayed BR in this retrospective analysis of 181 pts. The majority of these pts achieved successful BR, except for 1 pt. It is possible that BR was not sought more frequently due to a fairly high rate of distant disease relapse (47% in this cohort). Further studies addressing the outcomes of BR in IBC pts are needed in order to assist in counseling pts regarding their reconstructive expectations.
Title: Tumor profiling of inflammatory breast cancer: Advancing the tools needed for precision medicine


Body: Introduction: Inflammatory breast cancer (IBC) is a rare and highly lethal form of breast cancer, accounting for approximately 10% of breast cancer mortality in the US. The clinical presentation of IBC includes rapid onset of symptoms, erythema > 1/3 of the breast, and edema. The genomic changes underlying the clincopathologic manifestations of IBC are yet unknown. Identification of a unique molecular signature in de novo IBC may provide insight into the biology of this disease, allowing further investigation into the etiology and treatment of this aggressive disease. In previous studies, supervised analysis of gene expression data from surgical tissue specimens identified a molecular-subtype independent 79-gene signature associated with IBC compared to locally-advanced non-IBC. In this study, we propose to identify a gene expression signature associated with IBC using breast specimens collected from patients with non-metastatic IBC prior to initiating preoperative systemic treatment.

Methods: Formalin fixed paraffin embedded (FFPE) core biopsy specimens were collected from patients with inflammatory breast cancer prior to initiating systemic therapy. All specimens underwent centralized pathology review at Brigham and Women's Hospital, and the clinical diagnosis was confirmed through evaluation by the Dana Farber Cancer Institute Inflammatory Breast Cancer Program. Sufficient RNA and DNA were simultaneously extracted from 14 biopsy specimens using the Qiagen AllPrep Kit. The RNA was amplified using the Sensation kit and profiled using the Affymetrix Human Transcriptome Array (HTA) 2.0. DNA was profiled for druggable somatic mutations and genome-wide copy number variations using the Affymetrix OncoScan Array.

Results: Pearson correlation coefficients for overall gene expression for 4 technical replicates included in the HTA ranged from r=0.993 - 0.994 and suggest excellent reproducibility in archival biopsy tissue. In preliminary analyses, 765 mRNA transcripts and 335 non-coding transcripts were differentially expressed based on clinical presentation features. The strongest differential association for rapid onset of disease was observed for alternately spliced variants in the TSPAN1 gene. Somatic mutations in PIK3CA were detected in 3 of the IBC patients. Additional paired assays as well as single-gene and pathway analyses, and integrated analyses of the genome and transcriptome using the R/Bioconductor packages are ongoing.

Conclusion: An understanding of the genomic changes that contribute to the unique presentation and biologic features associated with IBC should lead to a significant impact on identifying etiologic risk factors and in optimizing treatment strategies. Our findings to date suggest a robust and reproducible method for genomic investigation using standard diagnostic breast core biopsies among IBC patients, and may inform profiling of biopsy specimens for other cancer types. The completion of this study will provide biological insights into the molecular mechanisms driving IBC and may identify clinically actionable targets for novel IBC therapies that warrant further exploration.
Body: Background: Male breast cancer (MaBC) is a relatively uncommon disease, representing less than 1% of all breast cancers. Given its rarity, information about prognostic factors is unclear and mainly extrapolated from data from female breast cancer. This represents an important challenge for the risk assessment and treatment decisions in men. The aim of this study was to analyze the characteristics of patients with MaBC and factors associated with prognosis over the past decade.

Methods: We evaluated men with microscopically confirmed invasive breast cancer diagnosed between 2003 and 2012, reported to the Surveillance, Epidemiology and End Results (SEER) 18 registries program. Patients (pts) with other primary malignancy either before or after breast cancer were excluded. Pt characteristics were compared between tumor grades. Univariate and multivariate analyses were performed to determine the effects of each variable on overall survival (OS).

Results: We included 2992 pts. Median age was 65 years (range 23-97). Median follow-up was 36 months (range 0-119). At diagnosis, ductal histology represented 85% of cases, ER positive 95.1% and PR positive 86%. Thirty-one percent were Stage I, 42% stage II, 18% stage III and 9% stage IV. Only 12.8% of pts had breast conservation and 23.7% received adjuvant radiotherapy. Tumor grade distribution was: 12.4% grade 1, 51.5% grade 2 and 36% grade 3/4. Pts with grade 3/4 tumors were more likely mixed ductal and lobular histology (p<0.0001), more often ER and PR negative (p<0.0001), presented with more advanced stage (p<0.0001), were more likely to have mastectomy and radiotherapy (p<0.0001 and p=0.001, respectively) and to die from breast cancer (p<0.0001). Univariate analysis showed that older age, black race, grade 3/4 tumors, stage IV disease, no surgery, no radiotherapy, ER negative tumors, PR negative tumors and unmarried pts had worse prognosis. Most deaths in the ER negative group occurred within the first 5 years (OS rate at 5 years 66.2%). OS rates between ER positive and ER negative groups were similar after 7.5 years (60.9% and 61.9%, respectively). In multivariate analysis, older age, grade 3/4 tumors, stage IV disease, no surgery, no radiotherapy, ER negative tumors and unmarried pts had shorter OS.

<table>
<thead>
<tr>
<th></th>
<th>Univariate p</th>
<th>Multivariate p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race</td>
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<td>NS</td>
</tr>
<tr>
<td>Grade</td>
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<td>0.006</td>
</tr>
<tr>
<td>Stage</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Surgery</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
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<tr>
<td>Histology</td>
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</table>

Conclusions: MaBC is most commonly diagnosed at early stages of disease. Tumors are frequently ductal in histology with high rates of ER positivity, however grade 1 is uncommon. We observed significant differences in pt characteristics according to tumor grade. The main difference in OS by ER status is seen during the first 5 years. Age at diagnosis, tumor grade, stage, surgery, radiotherapy, ER and marital status have clear influence on OS in MaBC over the past decade.
Title: Clinicopathologic features and radiation therapy utilization in patients with male breast cancer: A national cancer database (NCDB) study

Algan O and Herman T. Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Body: Introduction: Male breast cancer (MBC) is an uncommon malignancy, accounting for approximately 1% of all breast cancers (BC). The purpose of this study was to compare the clinicopathologic features of and radiation therapy (RT) utilization by patient age in patients with non-metastatic MBC and female breast cancer (FBC) using a large national database.

Methods: The NCDB is a comprehensive national database that captures approximately 70% of newly diagnosed cancer patients in the US. Data for patients meeting study criteria were extracted including patient demographics, tumor characteristics, treatment modalities used, and overall survival (OS). Comparison of clinicopathologic factors between MBC and FBC were made using the Chi-Square test. Overall survival curves were estimated using Kaplan-Meier method. All results are presented as MBC vs FBC patients.

Results: A total of 23,305 MBC patients and 2,678,061 FBC patients with non-metastatic breast cancer were identified in the NCDB database. The median patient age was 65 vs 60 yrs (MBC vs FBC, p < 0.001). Median tumor size was 19mm vs 15 mm (p < 0.001). In patients who had a LN evaluation, the median number of LN examined was 4 vs 3 and the median number of positive LN's were 1 vs 0. Percentage of patients undergoing breast conserving surgery was 25.6% vs 54.8%. Central tumor location was most common in MBC (30.3% vs 6.3%) and UOQ location was most common in FBC (17.2% VS 33.6%). The pathologic tumor characteristics for patients with MBC and FBC is shown in Table 1. Invasive lobular histology was less common in MBC patients (3.1% vs 7.5%). Higher percentage of female patients had non-invasive and Grade I cancers. MBC patients were more likely to have ER or PR positive tumors and less likely to have triple negative tumors. Lymph vascular invasion (LVI) was more likely to be present in MBC patients. There was a tendency towards earlier stage tumors in female patients. Regardless of gender, postoperative RT use was lower in patients 70 years or older compared to younger patients. Doing a stage-by-stage comparison, median survival was lower for patients with MBC compared to FBC.

Conclusion: MBC is an uncommon malignancy accounting for approximately 1% of all breast cancers. Compared to FBC, patients with MBC presented with more advanced stage BC, and were more likely to have LVI. Median survival rates were lower for patients with MBC compared to FBC.

<table>
<thead>
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<th>Behavior</th>
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<th>FBC</th>
<th>p-value</th>
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<td>86.5%</td>
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<tr>
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<td>13.7%</td>
<td>18.1%</td>
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<td>Grade III/undif</td>
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<td>35.5</td>
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<tr>
<td>ER or PR positive</td>
<td>92.2%</td>
<td>81.6%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Her2/neu positive</td>
<td>11.6%</td>
<td>14.6%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>5.3%</td>
<td>12.9%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LVI Present</td>
<td>27.7%</td>
<td>16.6%</td>
<td>&lt; 0.001</td>
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<tr>
<td>Tumor Stage</td>
<td>Tis</td>
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<td></td>
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<td>11.1%</td>
<td>16.4%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>41.5</td>
<td>47.3</td>
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<td>Stage Grouping</td>
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<tr>
<td>RT after BCS</td>
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<tr>
<td>All patients</td>
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<td>60.4%</td>
<td>73.5%</td>
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<tr>
<td>RT after Mastectomy</td>
<td></td>
<td>44.9%</td>
<td>45.6%</td>
</tr>
<tr>
<td>Older RT after BCS</td>
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<td>51.6%</td>
<td>63.1%</td>
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<tr>
<td>Older RT after Mastectomy</td>
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<td>40.1%</td>
<td>31.9%</td>
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<td>Median OS (Mo)</td>
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<td></td>
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<tr>
<td>Stage 0 / I</td>
<td></td>
<td>180 / 168</td>
<td>NR / 189</td>
</tr>
<tr>
<td>Stage II / III</td>
<td></td>
<td>120 / 78</td>
<td>180 / 98</td>
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Older - 70 years or greater; NR - Not reached
Title: Overexpression of FOXM1 is a potential prognostic marker in male breast cancer


Body: Background
The Forkhead box M1 (FOXM1) transcription factor is known to play an important role in the development and progression of many cancer types including breast cancer. The important role of FOXM1 in cancer affirms its significance for clinical use and therapeutic intervention. Elevated expression of FOXM1 in female breast cancer correlates with undifferentiated tumor phenotype and negative clinical outcome. However, whether FOXM1 has any indication for prognosis in male breast cancer (MBC) patients is still unknown. The purpose of this study was to examine the expression levels of FOXM1 in MBC and to identify the relationship between FOXM1 expression and patient survival.

Methods
Immunohistochemical analysis for FOXM1 was performed in a total of 80 male breast cancer specimens, all with linked clinical outcome data. Kaplan-Meier method and Cox proportional hazards analysis were used to relate FOXM1 expression to clinicopathological variables and overall survival (OS).

Results
We observed high expression of the FOXM1 protein in 39% of MBC samples (31/80). FOXM1 overexpression was significantly associated with higher histological grade (p=0.05), lymph node metastasis (p=0.04), tumor size (p=0.05), and estrogen receptor expression (p=0.04). Patients with FOXM1 expression had a significantly poorer overall survival than those without FOXM1 expression (p=0.02). Multivariate analyses indicated that FOXM1 positivity was an independent prognostic factor for OS (p=0.03).

Conclusion
These results show that FOXM1 may represent a novel MBC marker with prognostic significance that could be included into the limited marker panels for MBC.