



Exploiting DNA repair defects: A new approach to targeted therapy

Targeted therapies have typically been designed to attack tumor cell strengths, such as cell proliferation. Alan Ashworth, PhD, from the Breakthrough Breast Cancer Research Center in London, has proposed an alternative definition for targeted therapy as treatment that exploits the weaknesses of tumor cells, specifically DNA repair defects.

Speaking at the second plenary lecture, on Friday morning, Dr Ashworth described a new treatment approach arising from the observation that *BRCA1/2*-related breast cancers have generally been treated with the same approaches used for sporadic breast cancers, although the biology of the two disease entities may be quite different. Of special interest was the observation that *BRCA1/2* cancers are associated with a florid DNA repair deficit related to DNA double-stranded break repair by homologous recombination. This observation led to the hypothesis that *BRCA1/2* mutant cells might be especially sensitive to DNA-damaging agents, and that this sensitivity could be the focus of new therapeutic strategies. This sensitivity was demonstrated *in vivo* by showing that *BRCA2*-null cells were exquisitely susceptible to platinum-containing agents, which generate DNA crosslinks that require homologous recombination for repair. The hypothesis is now being tested in a clinical trial in which *BRCA1/2* patients experiencing a first metastatic relapse from breast cancer are being randomized to receive either carboplatin or docetaxel.

Dr Ashworth and colleagues are also exploring the idea of inducing genotype synthetic lethality in *BRCA1/2* cells by blocking a second DNA repair pathway, base excision repair. This is accomplished through inhibition of poly (ADP-ribose) polymerase, or PARP. Both *BRCA1*- and *BRCA2*-deficient cells are extremely sensitive to PARP inhibitors *in vitro*, presumably because of the extreme genomic instability caused by PARP inhibitors in these cells.

These researchers have developed a high throughput screen to identify genes other than *BRCA1/2* that may also lead to PARP sensitivity and be useful therapeutic targets. In a phase 1 clinical trial with unselected breast cancer patients, the experimental PARP inhibitor KU-0059436 showed suggestions of activity and very low toxicity when used as a single agent. A phase 2 trial targeting *BRCA1/2* patients is scheduled to begin in 2007.

Treatment options after failure of nonsteroidal aromatase inhibitor therapy

Aromatase inhibitors (AIs) are rapidly becoming the treatment standard for hormonal therapy in breast cancer. Although response rates are high, many patients ultimately reach a point where they no longer respond to first-line AIs and disease progression occurs. At the Friday morning general session, William Gradishar, MD, from Northwestern University Feinberg School of Medicine, presented first results from the EFACT trial. This trial is comparing the efficacy of fulvestrant versus exemestane as second-line hormonal therapy following prior therapy with nonsteroidal aromatase inhibitors in postmenopausal women with advanced breast cancer.

Fulvestrant is a selective estrogen receptor downregulator, while exemestane is a steroidal AI. To achieve a rapid steady state in the blood, a loading dose of fulvestrant was provided on days 1, 14, and 28, followed by monthly therapy. Exemestane was given on a standard dosing schedule. For the major study endpoint, time to progression, there was no difference between the two study arms. There were also no differences in objective response rate or clinical benefit (defined as objective response + stable disease for at least 24 weeks) and no difference in the occurrence of adverse events.

This is the first phase 3 clinical trial in this population, and confirms the efficacy of fulvestrant when used in patients who have shown disease progression on nonsteroidal AIs.

Treatment-induced amenorrhea: Adverse effect or benefit?

A common side effect of chemotherapy in breast cancer patients is treatment-induced amenorrhea. While viewed by some as an adverse event, this indirect endocrine effect can be beneficial, offering a second mechanism for inhibiting the growth of endocrine-responsive tumors. Michael Gnant, MD, from the Medical University of Vienna, presented 11-year results from the ABCSG-05 trial comparing adjuvant treatment with goserelin and tamoxifen versus cyclophosphamide/

methotrexate/5-fluorouracil (CMF) treatment in 1034 premenopausal women. For this study, amenorrhea was defined as a ≥ 4 -month cessation of menstruation after 3 months of treatment. Treatment-induced amenorrhea occurred in the majority of CMF patients and all goserelin/tamoxifen patients. The main analysis of this study, presented in 2001, showed a 3.5% benefit in 5-year relapse-free survival for the combination treatment arm of the study. The current analysis, undertaken at 11 years, demonstrated that the 3.5% benefit for goserelin/tamoxifen persisted for 10 years after treatment, but then declined to a statistically insignificant difference. The presence of amenorrhea in either treatment group was associated with a significant increase in relapse-free survival in both univariate and multivariate analysis. This effect was most significant in patients under the age of 40 and in those who were HER2-negative. The authors of this study have suggested that additional ovarian suppression might be beneficial in patients receiving chemotherapy who do not experience treatment-induced amenorrhea, but this was not demonstrated in the current trial.

In discussion, it was pointed out that the study effects are necessarily confounded in these later results by the occurrence of natural menopause, which may have greatly contributed to the loss of significant difference in outcome between the two study arms over the last few years.

The William L. McGuire Memorial Lecture: The future of aromatase inhibitors

The William L. McGuire Memorial Lecture was presented Friday morning by Richard J. Santen, MD, from the University of Virginia School of Medicine. Dr Santen, who is a trained endocrinologist, has played a pivotal role in establishing AIs as standard hormonal treatment for endocrine-responsive breast cancers. As both a clinician and a researcher, he was a pioneer in translational research. In this lecture, Dr Santen provided a broad overview of the history of AI development, a look at current research, and an idea of possible directions for research over the next 10 years.

Much of Dr Santen's research has been directed at discovering the mechanisms by which cells can become immune to AIs. He was able to demonstrate both in vitro and in vivo that

estrogen-responsive breast cancer cells deprived of estrogen for a prolonged period can develop estrogen hypersensitivity related to upregulation of growth factor pathways. Such cells can then respond to extremely low levels of estrogen. This suggests that inhibitors of growth factor pathways might serve to prevent or delay resistance to aromatase inhibitors or tamoxifen. Research into HER-family inhibitors supports this, as they have been shown to significantly delay development of tamoxifen resistance, especially when used in combination. This concept was also demonstrated in the study presented Thursday by John Mackey, MD, from the University of Alberta Cross Cancer Institute, showing that anastrozole + trastuzumab had a significant clinical benefit compared with anastrozole alone in HER2-positive/ER-positive patients with metastatic breast cancer.

While low doses of estrogen can stimulate cell growth in estrogen-deprived cells, very high doses can induce estrogen-associated apoptosis. Trials are now underway or being developed using an estrogen apoptotic purge: Cells that initially responded to an AI and then developed resistance to the AI are treated with large doses of estrogen to induce apoptosis in the resistant cells, after which the cycle begins again. It is hypothesized that this cycle can be repeated multiple times.

Another therapeutic approach supported by preclinical evidence involves a secondary function of estrogen in the cell. Although most emphasis has been put on the role of estrogen in inducing cell growth, estrogen is also broken down in the cell into toxic metabolites that may themselves be carcinogenic. While tamoxifen blocks only the cell growth pathways, AIs potentially block both pathways, indicating that they may prove to be much more potent than tamoxifen for the prevention of breast cancer.

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Better predictors of response to aromatase inhibitors

Serdar Bulun, MD, from Northwestern University, presented the results of a study that looked at mRNA levels of estrogen-related genes to predict responsiveness to aromatase inhibitors. Aromatase inhibitors have shown a $\geq 50\%$ response rate in endocrine-responsive postmenopausal patients.

Although this response rate is greater than that seen with tamoxifen, it means that many patients still receive treatment that will be ineffective for them, and clinical or molecular predictors of response are not yet known.

This study involved 116 breast tumor samples taken from patients who relapsed after primary therapy and received AI treatment for metastatic disease. Molecular analysis was conducted in the primary tumors for estrogen receptor- α (ER α) and progesterone receptor (PR) proteins and for mRNA levels from 6 estrogen-related genes. For all patients, ER α and PR proteins were very sensitive for the detection of AI response in the primary tumor, but the specificity was low, ranging from 22% for ER α to 31% for PR. On the other hand, for PR-negative patients, mRNAs provided significantly increased specificity, ranging from 65% for ER α mRNA to 100% for a combination of ER α mRNA and *BRCA1* mRNA. In comparison with current practice, a combined analysis of PR protein and ER α and *BRCA1* mRNA in PR protein-negative tumors significantly improves specificity for response, preventing 15% of nonresponders from being unnecessarily treated with an AI.

Angiogenesis as a therapeutic target in breast cancer

New tumors cannot grow to more than 1 to 2 mm³ without the development of new capillary blood vessels. Because of this requirement, antiangiogenic agents were initially hailed as a “magic bullet” that would surely change the face of cancer treatment. Although early attempts to capitalize on this concept met with only limited success, a vast body of research has accumulated in the past 15 years. In Mini-Symposium 3, presented on Friday afternoon, three researchers reviewed much of the work of the last 15 years, and gave some intriguing ideas about the future of angiogenesis research.

Douglas Hanahan, PhD, from the University of California, San Francisco, discussed the mechanisms of angiogenesis that have been elucidated using mouse models of human cancer. Such studies have provided several important lessons. First, most tumors are likely to progress eventually, even when the VEGF pathway is inhibited. Other proangiogenic factors are activated in these tumors, including fibroblast growth factor, and the number of proangiogenic factors in tumors increases with tumor stage. These alternative factors may themselves form

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therapeutic targets. Second, pericytes support and help protect the mature tumor vasculature from the effects of VEGF inhibition. These pericytes can be targeted with platelet-derived growth factor receptor inhibitors, such as imatinib and dasatinib.

Adrian Harris, MD, from Oxford University, discussed his research on the role of hypoxia in regulating angiogenesis in tumors. Hypoxia is a major physiologic difference between tumor and normal tissue, and hypoxia-inducible factors (HIF) have an important role in breast cancer. Both HIF1 and HIF2 have been shown to significantly increase tumor cell growth in a xenograft model. Gene expression profiles show a large array of genes that cluster with known hypoxia-related genes, such as vascular endothelial growth factor (VEGF) and hexokinase 2 (HK2). Based on occurrence in at least 50% of clusters, 99 genes that are upregulated have been identified, along with 57 that are downregulated. A hypoxia score based on median expression of the upregulated genes has been associated with survival in multiple data sets from different institutions. Interestingly, although many genes are involved in the hypoxic signature, no two individuals appear to have exactly the same profile, indicating the importance of therapeutic strategies based on more than one target.

George Sledge, MD, from Indiana University School of Medicine, a 2006 Brinker Award recipient, presented a clinician's view of current ideas and future plans for antiangiogenic therapy for breast cancer. He considered several questions: How does antiangiogenic therapy work? Who does it work in? Why doesn't it work in some cases? How can we make it better?

There are a variety of mechanisms for how antiangiogenic therapy might work in breast cancer. Inhibition of blood vessel growth is an obvious choice, but these agents may also inhibit survival pathways in the tumor endothelium, alter tumor vascular function, affect VEGF receptors on tumor cells, or have an effect on cells, such as dendritic cells, that are related to immunologic response.

Response to antiangiogenesis agents does not appear to be related to any obvious factors, including ER/PR status, type of previous therapy, age, disease-free interval, or number of metastatic sites. Endothelial cell heterogeneity or inter-patient heterogeneity in selected proangiogenic factors may play a role. In addition, as mentioned by others in this symposium, there is significant growth factor redundancy so that multiple pathways are available if one is blocked. Angiogenesis-independent growth is also possible, using such mechanisms as vessel cooption or vascular mimicry. Finally, it is likely that some cases of "resistance" to antiangiogenesis agents are in fact reflections of pharmacokinetic resistance, the inability to deliver the right dose of a biologically active agent to the right cells for the right period of time. Clinical trials are in preparation to determine appropriate dosing for the anti-VEGF antibody bevacizumab in combination or in sequence with traditional chemotherapy agents. Future trials must still consider optimal duration of treatment, the potential for treatment of micrometastases, and the possibility of adverse effects with long-term antiangiogenesis treatment.