

## Hot Topics Scholarship Advocate Reports from the 2007 San Antonio Breast Cancer Symposium

Topic: Preclinical studies

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Anti-estrogens promote an invasive phenotype in intercellular adhesion deficient breast cancer cells.

Dr. Annabel Borley from the United Kingdom shared her research on the effects of anti-estrogen treatment on invasive signaling at the 30<sup>th</sup> San Antonio Breast Cancer Symposium. Anti-estrogens classically exert gene inhibitory effects during responsive phase in estrogen receptor positive (ER+) breast cancer cells. However these drugs are also able to induce the expression of genes which, in the appropriate cell context, may contribute to an adverse cell phenotype. Dr. Borley and her team of researchers have investigated the effects of anti-estrogen treatment on invasive signaling.

Many breast tumors rely on the female sex hormone, estrogen, for their survival.

On this basis, drugs that inhibit the effects of estrogen (such as tamoxifen and fulvestrant) have been extremely useful in the treatment of breast cancer in women whose tumors express one of the major components of the estrogen pathway, known as the estrogen receptor. By removing the ability of cells to respond to estrogen or in fact the production of estrogen itself, these drugs remove one of the major components of tumor survival. Dr. Borley stated that it is widely accepted that tamoxifen prevents deaths from ER + breast cancer. However, ongoing in vitro studies show that in certain contexts tamoxifen may induce an adverse cell phenotype.

Despite the usefulness of drugs that target the effects of estrogen, some of these drugs also stimulate the expression of genes and proteins that actually stimulate the ability of cancer cells to spread around the body, a process known as metastasis. One critical component of this process is the ability of cells to invade tissues away from the breast (invasiveness). To investigate this, Dr. Borley and her team assessed the ability of tamoxifen and fulvestrant to stimulate invasiveness. As an experiment model, cells were cultured in the presence of these drugs and then the ability of the cells to move through a gel that contained other human cells and tissue (a process known as invasiveness) was observed. Researchers finding a way to identify prospectively those who are likely to fare negatively would be a tremendous benefit to patients undergoing therapy.

Research being funded by The Pink is currently underway using a model of MCF7 cells with siRNA suppression of E-cadherin. E-cadherin is a key adhesion molecule. Up to 40% of invasive

ductal carcinomas show reduced or absent E-cadherin expression. This has been associated with a poorer prognosis. Invasion Assay has shown significant increase in invasiveness in cells lacking E-cadherin after tamoxifen treatment. Using Western Blot and Densitometry analysis of 3 separate sample sets, it is observed that tamoxifen induces src kinase activity in E-cadherin deficient MCF7 cells. The inhibition of src kinase prevents tamoxifen-induced invasiveness in E-cadherin deficient MCF7 cells. Ongoing research of fulvestrant has shown fulvestrant promotes invasion and induces src kinase activity in E-cadherin deficient MCF7 cells. Antiestrogens clearly induce expression of genes linked to invasion.

Tamoxifen induces invasiveness in MCF7 cells where E-cadherin mediated intercellular adhesion is disrupted. This appears to be a src dependent process. Fulvestrant is associated with similar effects in E-cadherin disrupted cells. In summary, this analysis demonstrated that neither tamoxifen nor fulvestrant alone could alter invasiveness. However, when the contact between cells in the gel was reduced, both tamoxifen and fulvestrant did increase invasiveness. The ability of tamoxifen or fulvestrant to do this was, in part, controlled by an enzyme known as SRC (pronounced "SARK"). These data demonstrate that anti-estrogens can promote an invasive phenotype in ER+ breast cancer cells under conditions of poor cell-cell contact and suggest a role for Src kinase and associated preinvasive genes in this process.

The invasion-promoting ability of anti-estrogens may have major clinical implications for those patients whose tumors display inherently poor intercellular adhesion. In such cases, combination treatment with anti-Src agents may prove therapeutically valuable.

Submitted by  
Dorothy Phillips, BSN, RN, CHPN  
Alamo Breast Cancer Foundation  
San Antonio, Texas, USA

## Cancer Stem Cells

“Have we been studying the wrong cells?” This is a question that breast cancer researchers and doctors are asking themselves all around the world. The answer could, in the next several years, have profound implications in the treatment of breast cancer.

The question itself derives from the theory of cancer stem cells. The theory offers a novel interpretation of the way that cancer cells propagate and spread. It remains highly controversial. Some believe that the cure for cancer lies in finding and treating cancer stem cells. Others doubt their very existence. How is this possible?

The issue was addressed in very exciting fashion in San Antonio. There was a mini-symposium that provided an excellent overview of the theory and principles (1,2,3). There were presentations that focused on particular new evidence relating to cancer stem cells (4,5).

To understand the excitement, it is first important to distinguish between embryonic stem cells and cancer stem cells. ‘Normal’ or embryonic stem cells have the ability to regenerate themselves into specific cells in the body in a very controlled manner. Cancer stem cells, on the other hand, have lost control of their regenerating capabilities, have found a way to avoid cell death (apoptosis) and have demonstrated their ability to seed and promote new cancers while remaining resistant to conventional chemotherapy.

The development of cancer, for many years, has been thought to be a ‘clonal evolution’. Normal cells undergo a mutation that generates damaged offspring which go through further mutations and continue to do so until the result is a mass of genetically varied cancer cells. The stem cell concept theorizes that a single adult cancer stem cell found in normal tissue is sufficient to drive a new cancer composed of genetically identical tumor cells. For example, a stem cell can make 2 copies of itself. One is identical; the other, because of some deregulation of the renewal, gives rise to progenitor cells. Treatment may kill the progenitor cells without killing the cancer stem cell.

Using an example from nature, advocates of the theory often describe the stem cell as the ‘root’ while the tumor is the ‘weed’. Without killing the root, the growth will continue to flourish even after apparently successful treatments with chemical agents. Using this analogy, we can see that available treatments that can shrink a tumor may only be acting as a temporary solution.

The implication of the hypothesis of cancer stem cell hypothesis is that these cells are the only ones capable of metastasizing. A major concern is that cancer stem cells, lying in wait after seemingly successful treatment of the original tumor, have the potential to seed the same cancer at a distant site. These cancer stem cells lie dormant awaiting a trigger that will activate them. They can become activated, creating progenitor cells and forming a tumor. This activating mechanism can take place at any point in the course of a woman’s lifetime.

Dr. Max Wicha, of the University of Michigan Comprehensive Cancer Center, one of the speakers at the mini-symposium, has commented that “Within the next year, we will see medical centers targeting stem cells in almost every cancer”(6). Another of the speakers, Dr. Jeremy Rich of Duke University Medical Center, drew on his experience with brain cancer to draw some lessons. He noted the resistance of these stem cells to radiation and chemotherapy and suggested some possible clinical approaches, including targeting the maintenance pathways and finding stem cell specific targets (2).

Two important papers provided some evidence on such approaches.

Dr. Li (5) and the team at the Dan L. Duncan Cancer Center at Baylor College of Medicine reported on a clinical trial involving 30 patients with locally advanced HER2+ breast cancer. Using lapatinib as a single agent for 6 weeks followed by weekly trastuzumab (Herceptin) and docetaxel every three weeks over a 12 week period before primary surgery, Dr. Li had impressive findings. Unlike conventional chemotherapy, the lapatinib arm of the study showed a decrease in the cancer stem cells from 10.6% to 4.7% and a marked decrease in self renewal capabilities. The pathologic complete response rate following the trastuzumab/docetaxel was much higher than expected at 63%. This *in vivo* study showed for the first time that lapatinib decreases cancer stem cells in the tissue of women treated before surgery.

If cancer stem cells are resistant to chemotherapy, the importance of signal inhibitors and regulators on the pathways leading to stem cell self renewal is highly significant. Previous research has been done on various pathways, e.g., the NOTCH pathway, such as those currently being investigated in clinical trials by Dr. Wicha and associates. (1)

G.J. Lindeman (4) and associates at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, have investigated of the GATA-3 transcription factor, endeavoring to demonstrate the hierarchy of stem and progenitor cells in breast development and cancer. In earlier work, they identified a population of mouse mammary markers which expressed 'basal' markers highly enriched with stem cells. The researchers noted that these cells were all negative for ER, PR and ERbB2, similar to a 'triple negative' subset in human breast cancer. The conclusion drawn was that the 'cell of origin' might indeed be the mammary stem cell.

The report by Dr. Lindeman and associates at the Symposium followed up their earlier work. Concentrating on the 'luminal' cells, and using the GATA-3 transcription factor, the group studied its role in normal mammary gland development. A deficiency in GATA-3 led to an increased number of luminal progenitors and poor differentiation of the epithelial cells, while the introduction of GATA-3 promoted differentiation of the cells and cell maturation. The stem cell population showed very low levels of GATA-3, while the differentiated luminal cells showed a propensity of GATA-3, highlighting the importance of this transcription factor.

So, are the cancer stem cells really the important target? Are they resistant to current therapies? Research continues. Scientists are seeking a better characterization of cancer stem cells and, indeed, even working to achieve an agreed working definition of what they are. They are also looking at targeted therapies as a way of overcoming drug resistance and attacking the cancer stem cells. This includes the studies noted above that target the stem cell maintenance pathways, and also work on targeting the cancer stem cells themselves. The implications of this research could have serious consequences in the funding and approval of new drugs. Funding drugs which increase overall survival would take precedence over those which simply increase time to progression.

Certainly, much more research is required on the hypothesis itself. It is important to remember that at the present time, it is just a hypothesis, and we advocates have seen many theories fail to deliver on their early promise. Still, the subject is very exciting and may sometime in the next several years even offer a pathway from treatment to actual cure.

Submitted by:  
Lilla M. Romeo  
SHARE  
MBCN  
New York, NY

Reference:

- (1) Max Wicha, MD University of Michigan Comprehensive Cancer Center Ann Arbor MI. Breast cancer stem cells: Targets for prevention & therapy.
- (2) Jeremy Rich, MD Duke University Medical Center, Durham, NC. Cancer stem cells in therapeutic resistance and as cellular targets.

- (3) Peter Laird, PhD USC/Norris Comprehensive Cancer Center, Los Angeles, CA.  
Epigenetic stem cell signatures in breast cancer.
- (4) Lindeman GI, Asselin Labat M-L, Sutherland KD, Barker H, Thomas R, Shackleton M, Hartley L, Robb L, Grosveld FG, van der Wees J, Visvander JE, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; The Royal Melbourne Hospital, Melbourne, Australia, Erasmus University, Rotterdam, Netherlands. Elucidating the stem and progenitor cell hierarchy in breast development and cancer – an essential role for GATA-3. Abstract #81.
- (5) LiX, Creighton C, Wong H, Hilsenbeck SG, Osborne CK, Rosen JM, Lewis MT, Chang JC, Dan L. Duncan Cancer Center at Baylor College of Medicine, Houston, TX. Decrease in tumorigenic breast cancer stem cells in primary breast cancers with neoadjuvant lapatinib. Abstract #82.
- (6) Dr. Max S. Wicha as quoted in the NYTimes December 21,2007.

## **Combination of nab-paclitaxel and bevacizumab in the eradication of well-established tumors as well as lymphatic and pulmonary metastases**

### Background

Drugs used in chemotherapy work in different ways to stop tumor cells from dividing so they stop growing or die. They block cancer growth in different ways. Some block the ability of cancer cells to grow and spread. Others find cancer cells and help kill them or deliver cancer-killing substances to them.

*Nab*-paclitaxel (Abraxane<sup>®</sup>, ABX) is a albumin-bound paclitaxel that has shown greater anti-tumor efficacy and a better safety profile than solvent-based paclitaxel (Taxol<sup>®</sup>) and docetaxel (Taxotere<sup>®</sup>) in xenograft models (cells, tissues grafted from one species to another) and clinical trials. Bevacizumab (Avastin) is a humanized monoclonal antibody (antibodies that are identical because they are produced by one type of immune cell that are all clones of a single parent cell) against vascular endothelial growth factor (VEGF). It is not yet known whether paclitaxel works better with or without bevacizumab in treating breast cancer.

The stress of chemotherapy can act to protect tumor cells causing reactionary angiogenesis (i.e. the development of blood vessels mediated by VEGFA). Avastin has been shown to decrease the protection of the tumor cells.

The lecture presentation #74 and poster # 1107 were studies done to determine the effects of *nab*-paclitaxel and bevacizumab (Avastin<sup>®</sup>), as a single or combined therapy, on the rate and metastasis of breast cancer tumors. Does this combination improve overall survival? This combination was looked at in a mice model and in a E2100 phase III trial (an intergroup trial for HER2 negative and first line metastatic breast cancer).

### Models

The mice model, breast cancer tumor cells were implanted into the mammary fat pad of female SCID mice (Severe Combined Immunodeficiency mice i.e. mice who are immunodeficient, in which tumors from other species can be transplanted and studied in a whole animal system without being rejected) and allowed to reach 460 mm<sup>3</sup> in size before treatment. A group of 6-8

mice were treated with two cycles of Abraxane (30 mg/kg, qdx5, MTD) followed by injection of Avastin (4 mg/kg) twice a week for 10 weeks. Additional groups received Abraxane or Avastin alone or saline. Mice were monitored for tumor growth and signs of toxicity. Enzyme activity was measured in extracts from proximal and contra lateral lymph nodes and both lobes of lungs.

#### Results:

Abraxane-treated mice did not gain weight for the duration of the treatment (3 weeks) and no additional signs of toxicity were observed in that or other experimental groups. Avastin-treated mice had nearly identical tumor growth rate as the controls indicating lack of the effect of this drug given alone. However, all tumors regrew and reached the same volume as controls 45 days after reaching their minimal size.

In contrast, 100% mice treated with Abraxane plus Avastin therapy had significant tumor inhibition and complete regression of tumors. Tumors did not regrow for the combination treatment. Neither Abraxane nor Avastin alone inhibited metastasis as compared with control mice with 100% mice (7/7 for Abraxane and 6/6 for Avastin alone) being positive for lymph node and pulmonary metastasis. In contrast, 100% mice (6/6) treated with the combination were metastasis-free in either proximal or contra lateral lymph nodes as well as in lungs

The E2100 Phase III is an intergroup trial for HER2 negative, first line chemotherapy for metastatic breast cancer. The parent trial is a multicenter study. Patients were randomized and stratified according to disease-free interval (no more than 24 months vs. more than 24 months), number of metastatic sites (less than 3 vs. 3 or more), treatment with prior adjuvant chemotherapy (yes vs. no), and estrogen receptor status (positive vs. negative vs. unknown).

Patients were randomized to one of two treatment arms.

- Arm I: Patients receive paclitaxel IV over 1 hour on days 1, 8, and 15 followed by bevacizumab IV over 30-90 minutes on days 1 and 15.
- Arm II: Patients receive paclitaxel as in arm I. In both arms, courses repeat every 4 weeks in the absence of disease progression or unacceptable toxicity.

Quality of life was assessed at baseline and on day 1 of weeks 17 and 33.

Patients were followed every 3 months for 2 years, every 6 months for 3 years, and then annually thereafter. The combination of the two drugs showed a doubling of progression free survival and a doubling of responds rates, but did not show a dramatic difference in over all survival.

Poster presentation #1107 looked at the “Association of genetic polymorphisms (changes in form) of VEGF & VEGFR-2 with outcomes in E2100”.i.e. using genetic variability to predict differences in incidence of malignancy, prognosis and altered metabolism of different drugs. VEGF has been associated with increased risk of breast cancer. In this study tumor blocks were extracted from E2100 patients. 363 cases were available for study to look for association between genotype and outcome. This was a correlation study looking at combination arms broken down by genotype correlating for VEGF. This enabled testing for correlation between 5 common polymorphisms of VEGF with efficacy.

## Results

The outcomes from E2100 combination arm in association with genetic polymorphisms of VEGF&VEGFR-2 They found two genotypes that showed a difference in over all survival. They showed 1-2 fold increase in prolonged survival (38-47 months compared to 25months). They found two genotypes that predicted clinical significance for Grade3/4 hypertension (patients required intervention). VEGF-2 did not predict outcome in E2100 patients.

## Discussion

Abraxane plus Avastin combination is highly effective in treatment of primary tumors as well as distant metastases in an aggressive breast cancer model. Neither drug alone was able to reduce the incidence or total metastatic burden in lymph nodes or lungs. In contrast, Abraxane plus Avastin successfully eradicated the primary tumors as well as lymph node and pulmonary metastasis in 100% of the treated mice for the duration of experiment.

Paclitaxel with Avastin demonstrated significant improvement in Progression Free Survival (PFS) but not overall survival in the E2100 Phase III. VEGF genotype significantly correlated with overall survival (OS) and Grade 3-4 hypertension. This may help us understand who will benefit and give us some insight into what causes high blood pressure.

Attempts to identify biomarkers to predict which patients will benefit the most were unsuccessful in this study.

For the future this information may help predict a genotype that will predict over all survival and benefit, and therefore this needs to be replicated in a large overall study. Currently there are other on going trials (Ribbon1 and 2) that are similar in design and may help further define which patients should receive the combination of taxane with a VEGF inhibitor.

Submitted by:  
Carole Price  
Breast Cancer Alliance of Cincinnati  
Cincinnati, Ohio

Lecture#74 and Poster#1107

[ClinicalTrials.gov](http://ClinicalTrials.gov)

[www.wikipedia.com](http://www.wikipedia.com)

[www.CommunityOncology.net](http://www.CommunityOncology.net), Abraxane (nanoparticle albumin-bound paclitaxel) in metastatic breast cancer, May/June2005

The VEGF-R inhibitor PTK787/ZK222584 (PTK/ZK) also inhibits aromatase: preclinical studies of PTK/ZK in combination with endocrine therapy.

Data presented by Dr. Susana Banerjee at the 30<sup>th</sup> San Antonio Breast Cancer Symposium on angiogenesis and vascular endothelial growth factor (VEGF) show that they are key components of breast cancer growth, invasion and metastasis. Dr. Banerjee has been investigating whether an oral drug called Vatalanib (PTK787/ZK222584), which is currently being tested as a treatment for bowel cancer patients, could also be an effective treatment for hormone-sensitive breast cancer. Targeting both ER and VEGF signaling may improve clinical outcome in ER+ breast cancer. It is also believed that Estrogen Receptor (ER) and VEGF signaling pathways may be linked and increased VEGF levels have been associated with endocrine-resistance. Many breast tumors rely on estrogen for their survival.

Dr. Banerjee is investigating angiogenesis, i.e. the growth of new blood vessels. Tumors can't grow without their own blood supply, so they produce molecules that cause new blood vessels to grow thereby ensuring the tumor's survival. Scientists believe that blocking angiogenesis could prevent or slow breast cancer from growing. In the laboratory Dr. Banerjee grew different types of breast cancer cells to study the genes and proteins inside them that are responsible for triggering blood vessel growth. Aromatase inhibitors have been developed to inhibit the production of estrogen. Similarly, a number of breast tumors also rely on the effects of a protein known as Vascular Endothelial Growth Factor (VEGF) and again, drugs are available to target the effects of this protein. One of these drugs is vatalanib (PTK/ZK).

Dr. Banerjee and scientists at the Breakthrough Centre in London report a surprising, and totally unexpected, finding; vatalanib, as well as minimizing the effects of VEGF, also inhibits the production of estrogen by inhibiting one of the enzymes involved in estrogen production, known as aromatase. The effects of vatalanib and endocrine therapy were investigated in human breast cancer cell lines engineered to express aromatase: MCF7-AROM1 (ER+ HER2-; endocrine sensitive) and BT474-AROM3 (ER+, HER2+; endocrine –resistant). The effect of vatalanib, tamoxifen or letrozole were

assessed in proliferation and ER alpha-mediated transcription assays. The effect of vatalanib on aromatase activity was measured in the titrated water assay. Research showed that this effect was seen in both tumor cells grown outside the body and on tumor cells that had been introduced into mice.

These findings suggest that drugs such as vatalanib may be used in situations where aromatase inhibitors would normally be used, expanding the potential treatment options for women with breast cancer. Studies are ongoing to assess the degree to which the anti-aromatase activity of vatalanib may contribute to its anti-tumor activity.

Researchers are also looking at other chemicals that have a similar structure to vatalanib, to see if these also have effects on aromatase. This research is still in the early phase of development and clinical trials are still needed to test the drug in breast cancer patients before it can be prescribed by doctors, but the preclinical findings showing blocking of ER and VEGF offer promise for the future.

Submitted by  
Dorothy Phillips, BSN,RN,CHPN  
Alamo Breast cancer Foundation  
San Antonio, Texas