



Team Progress Updates

SU2C Breast Cancer Dream Team:

“An Integrated Approach to Targeting Breast Cancer Molecular Subtypes and Their Resistance Phenotypes”



During the past several years, researchers have come to understand that breast cancer is not a single disease but rather a spectrum of conditions that vary in their biology and response to treatment. This team focuses on three major subtypes of breast cancer—ER positive, HER2 positive, and triple negative (that is, simultaneously ER negative, PR negative, and HER2 negative)—with the hope of developing novel therapies for women with this disease.

Over a period, cancer cells can develop ways of “outsmarting” the drugs and agents designed to kill them, a phenomenon known as drug resistance. This Dream Team studies the driving mechanisms that lead to drug resistance and the role that cancer stem cells play in resistance.

The team has gathered the vast amount of information that exists about breast cancer into an integrated database to form a “discovery platform”—a basis for identifying and validating new drug combinations and targets that can be pursued in clinical trials.

The team has reported the following progress:

December 2014

- In continued mouse studies of lapatinib resistance, combination of lapatinib with an inhibitor of the PI3K pathway can eliminate cells that are resistant to lapatinib; combination of lapatinib with an inhibitor of the enzyme MEK does not.
- Because the promising combination of lapatinib + ABT-737 (a dual BCL2/BCL-XL inhibitor) is limited by toxicity, the Dream Team has turned their attention to other HER2-targeted therapies in combination with ABT-737.
- ABT-737 combined with trastuzumab-DM1 (an approved toxin linked conjugate of trastuzumab) was dramatically effective in two patient-derived xenograft mouse models. This combination has significant near-term potential; discussions between Genentech and the Slamon Lab are ongoing to plan the most effective preclinical evaluation of this combination and to seek funds to support this project.

June 2014

- The Dream Team reports that their analysis of the TRIO-B07 clinical trial samples provides a rationale for exploring the combination of BCL2 inhibition with HER2-targeted therapy in the treatment of HER2+ER+ disease.
- Studies of lapatinib resistance in mouse models indicate that the drug ABT-737, a dual inhibitor of the protein BCL2 plus a related protein, BCL-XL, can eliminate cells that are resistant to lapatinib.





Team Progress Updates

- The Dream Team plans to similar tests of lapatinib plus a newly developed selective BCL-XL

December 2013

- The Brugge and Slamon laboratories have now completed their analyses of patient samples from the TRIO-B07 clinical trial. The evaluation of the results is ongoing, but seems to confirm that elevation of BCL2 in intraductal tumor cells correlates with poor response to treatment. The Dream Team is still optimizing the dosage regimen for combinatorial therapies to alleviate toxic side effects observed in their mouse models.
- The Ashworth and Slamon laboratories have continued their collaboration to determine if the increased potency exhibited by the PARP inhibitor, BMN673, would translate into an improved therapeutic window. They have now obtained strong evidence, in various mouse models, that BMN673 is highly potent in inhibiting tumor cells with mutation in genes like PTEN, BRCA1 or BRCA2.
- Using patient-derived tumor xenograft models, they established a treatment regimen that has subsequently been validated in human in a Phase 1 clinical trial.

June 2013

- Following the end of their 3-year grant term, the Slamon, Brugge, and Ashworth laboratories have continued to work in two areas.
- The first project is a collaboration between the Brugge and Slamon laboratories that expands upon previous work that demonstrated the role of Bcl-2 in breast cancer cell survival and suggested a possible use of Bcl-2 inhibitors (e.g., ABT-737) in breast cancer treatment. Their initial in vivo analyses suggest that ABT-737 does indeed sensitize a subset of the protected cells. However, the drug combination, ABT-737 plus an inhibitor of HER2 (lapatinib), also appeared to induce unknown toxic side effects on the mice.
- The Team is currently optimizing the treatment regimen to alleviate these issues. In further studies of xenografted tumor cell lines, the Team discovered that BCL2 expression is not upregulated in invasive lesions lacking contact with the basement membrane, which suggests a role for the microenvironment in the upregulation of BCL2.
- The group will continue these analyses, as well as the analysis of clinical samples from the Slamon-Hurvitz B07 clinical trial of HER2-targeted therapies +/- chemotherapy (carboplatin + docetaxel). The latter seem to confirm that there is elevation of BCL2 in intraductal tumor cells in patients' samples from this trial.
- The second project focuses on triple negative breast cancer, and is a collaboration between the Ashworth and Slamon laboratories. These studies also build on preclinical work performed during the Dream Team's three-year grant term.



Team Progress Updates

- The group has continued their collaboration with Biomarin, the company that developed BMN-673, a new and extremely potent PARP inhibitor the drug, to determine if the increased potency exhibited by BMN673 would translate into an improved therapeutic window. The results are highly encouraging but still very preliminary.
- During this period, the Team has also continued their studies of the protein ARID1A, whose reduced expression is strongly associated with advanced clinical stage and poor prognosis. They have obtained evidence that dasatinib, and inhibitor of certain tyrosine kinases (e.g., BCR/Abl and Src), could be used to treat patients with tumors harboring a mutation in the ARID1A gene.

December 2012

- During this last period of the initial grant term, the Dream Team has continued to make significant progress in all studies initiated. They have completed some, and will pursue others through monies leveraged from other sources.
- The Team has continued to develop the work demonstrating the role of Bcl-2 in breast cancer cell survival, including the possible use of Bcl-2 inhibitors (e.g., ABT-737) in breast cancer treatment.
- They have also continued their efforts to develop the new PARP inhibitor (BMN-763) for possible use in breast cancer patients. Supplemental funding of \$1.0 million was approved to allow a subset of the original Dream Team to translate these two promising avenues of research into the clinic.
- In this period, the Team reports the development of a robust system to assess genomic aberrations and drug responses in cells grown on cell spot microarrays (CSMA). Numerous studies are now underway using this system.
- Analyses of the 32 completed genome-wide RNAi screens using the third generation shRNA library (74,304 shRNAs targeting > 19,000 genes) have also continued; data has been analyzed for 16 of the 32 so far. For the sequencing, the Team has transitioned to high capacity platforms and developed a new protocol that will allow completion of sequencing in the first half of 2013.
- The Team has continued to develop and improve computational methods to analyze and visualize the wealth of data generated by this and other SU2C-funded Dream Teams. These include: PARADIGM, PARADIGM-SHIFT, Differential Pathway Signature Correlation (DiPSC), and the SU2C Cancer Genome Browser.
- The Haussler group is requesting further support from SU2C to support the UCSC Cancer Genomics Browser as a common resource to service all SU2C teams. The browser will be a platform for data sharing, and provides a coherent mechanism for teams to release data to the public. The browser will serve as a living data portal to view, access and retrieve past and current SU2C team data.



Team Progress Updates

- As each team concludes their projects, the datasets generated under their awards could be made available at a common site that provides storage and retrieval, dynamic access and online exploration. If funded, The Haussler lab would work with all the teams to process and host their data sets.

June 2012

- By the end of this period, the Dream Team has initiated or joined a significant portfolio of breast cancer clinical trials, clearly contributing to improving breast cancer outcomes.
- Of note are attempts to improve treatment of HER2 positive breast cancer using the new drug T-DM1. A neoadjuvant trial of the conjugate given in alone and in various combinations (including with Pertuzumab and no cytotoxic chemotherapy) should discern the magnitude of the treatment effect.
- Another promising study involves the new PARP inhibitor BMN-673 that appears to be so much more potent than the other existing PARP inhibitors.
- The Dream Team reported a fascinating opportunity for triple negative breast cancers with PTPN12, which is lost in 65-70% of triple negative breast cancer cases, exhibits tumor suppressor actions when reintroduced into breast cancer cells devoid of the enzyme. The Team is now exploring the possibility of a clinical trial with sunitinib and crizotinib, two drugs that are FDA-approved and marketed by Pfizer; contacts with the oncology development group at Pfizer have been initiated.
- Also, several key preclinical concepts generated by the Team are advancing to clinical assessment. For instance, the Team has continued its effort to tease apart mechanisms of adaptive resistance to targeted drugs exhibited by breast cancer cells in the context of 3D cultures.
- They identified the gene Bcl-2 as a critical component of treatment resistance. The Dream Team reported a number of clinical observations that provide a strong rationale for the use of Bcl-2 antagonists in combination with HER2 treatment.
- The various screens using shRNA libraries developed by the Dream Team since the inception of the project appear to have the potential to discriminate adaptive versus selected resistance mechanisms. Several screens have been completed; others are in the pipeline.
- Finally, the combined cell line screening studies and enhanced bioinformatics efforts have begun to produce genomic signatures related to drug sensitivity and resistance as well as prognosis for different breast cancer types and are suggesting ways to link drugs to treatment of cancers with specific genomic signatures. The Team intends to disseminate data and analysis tools broadly to other SU2C-funded Dream Teams and eventually the broader scientific community.



Team Progress Updates

December 2011

- At the two-year mark, the Dream Team had made significant progress at integrating “omics” data from various sources. They launched the SU2C Cancer Genomics Browser that contains both SU2C-generated and other published data from hundreds of cell lines. Most of the data from other platforms, such as those generated by other SU2C Dream Teams, or non SU2C-affiliated laboratories, can be easily ported to the SU2C Cancer Genomics Browser and added to the collection.
- The Team also continued to make progress in defining both prognostic and predictive genomic signatures. Notably, their preclinical work using cell lines and animal models led to the identification of genes potentially involved in the development of resistance to hormone therapy. These included MEK1/2, mTOR, B1-intergrin, FAK, c-Src, and PTEN. From these efforts, a new clinical trial was planned to test the effect of specific inhibitors of MEK1/2, mTOR, B1-intergrin, and FAK.
- The studies on the effect of the microenvironment on drug sensitivity continued moving forward with linkages to the clinical research components of the Team. Specifically, the up-regulation of Bcl-2 in the microenvironment suggested that it might create an opportunity for combination therapy using a Bcl-2 antagonist.
- The Dream Team also reported plans to move forward with a PARP inhibitor that was shown to be 100-fold more potent than other agents currently in the clinic; a phase 1 trial was initiated. The Dream Team plans to take the drug into trials for ER positive and HER2 positive breast cancers.

June 2011

- At the half-way point, the Dream Team translated some of their preclinical studies into the clinic; multiple clinical trials were started for each of the different types of breast cancers, and more were still in the planning phase.
- The Dream Team also reported extensive preclinical evaluation of T-DM1, a HER2-targeted agent which appears to be effective against HER positive breast cancer cell lines, even when Trastuzumab and/or Lapatinib failed.
- The Dream Team reported on their continued effort to discover new targets and agents for triple negative breast cancers, particularly those targeting DNA repair pathways which tend to be crippled in many triple negative breast cancers.
- Of note, the product of the gene PTPN12 seems to act as a tumor suppressor by interfering with the activity of a number of receptor tyrosine kinases, and appears to be mutated in 5% of



Team Progress Updates

triple negative breast cancers and absent from as many as 60%. This finding suggests that Sunitinib (or Crizotinib) and Lapatinib might have beneficial activity against these tumors.

- The Team also reported follow-up studies based on their observation that cells located in the outer layer of 3D cultures were activating a variety of survival signaling pathways leading to treatment resistance. They confirmed that this phenomenon was also evident for model breast cancer cells propagated *in vivo*, and for breast cancer tissue specimens in women treated with anti-breast cancer agents. These preclinical experiments have nominated combinations of targeted drugs and the BCL-2 antagonist ABT-737 for future clinical trials.

December 2010

- In this period, the Dream Team continued their preclinical studies to determine how resistance arises in each subtype of breast cancer, and the best drugs that can be employed to overcome this resistance.
- Some of the planned targets, such as c-SRC, failed to be confirmed as drug resistance mechanisms in ER positive breast cancers. They began planning clinical trials to test other potential targets, such as VEGFR2, or cdk-4/6. Positive results for HER2 positive breast cancers were reported as the Team identified the loss of PTEN, combined with mutations in the PI3K pathway, as a potential mechanism of resistance.
- The Dream Team also reported the results of clinical trials demonstrating that the drugs Lapatinib and Trastuzumab have a synergistic effect when used in combination.
- The "discovery group" within the Dream Team assembled and classified several hundred breast cancer cell lines, and the bioinformatics Team began working to extract novel information from this large dataset.
- Progress on 3D models of breast cancer cells was also reported. There, the Team has made the interesting observation that those cells in the outer layer in 3D cultures seem to be protected from treatment with PI3K inhibitors.
- Their next step was to explore the molecular mechanism responsible for this phenomenon.

June 2010

- In the first six months, the Dream Team focused on implementing a variety of integrated approaches to identify novel targets and mechanisms that may lead to later stage clinical trials opportunities.
- These so-called "discovery" studies included the development of (1) techniques that can measure drug impacts on single cells; (2) xenografts model from human tissues; and (3) 2D and 3D assays to examine the interaction of tumor cells with their environment in response to drug treatment.



Team Progress Updates

- In addition, they completed the construction of a library of “short hairpin RNAs” (shRNAs) that will be used to systematically inactivate genes in order to identify potential new targets for therapy. The Dream Team also established a state-of-the-art bioinformatics and data analysis platform aimed at maximizing multi-site communication and collaboration.
- On the clinical side, the Dream Team made progress in the validation of new drug treatments to circumvent anti-hormone resistance in ER positive breast cancers. Potential candidates include VEGFR2 inhibitors, buthionine sulfoximine (BSO), c-SRC inhibitors, as well as PI3K inhibitors.
- They also showed that cells expressing the gene ZNF217 were more resistant to chemotherapy, suggesting that this gene could be good target for new therapies. For HER2 positive breast cancers, the Dream Team focused on the development and characterization of tumor cell lines model that demonstrate either acquired or de novo resistance to two different drugs: lapatinib or trastuzumab.
- Analyses of these preclinical data are ongoing, and potential targets will be selected for in vivo validation studies.
- The Dream Team also reported an interesting correlation between a deficiency in the product of the gene RAD51, and strong response to chemotherapy for triple negative breast cancers.
- Finally, the Dream Team developed a method to identify cancer stem cells (CSCs) in breast cancer cell lines. They reported data indicating that Herceptin may target those CSCs when used in combination with a chemotherapeutic agent.