

Breast Cancer Research Program



Congressionally Directed Medical Research Programs

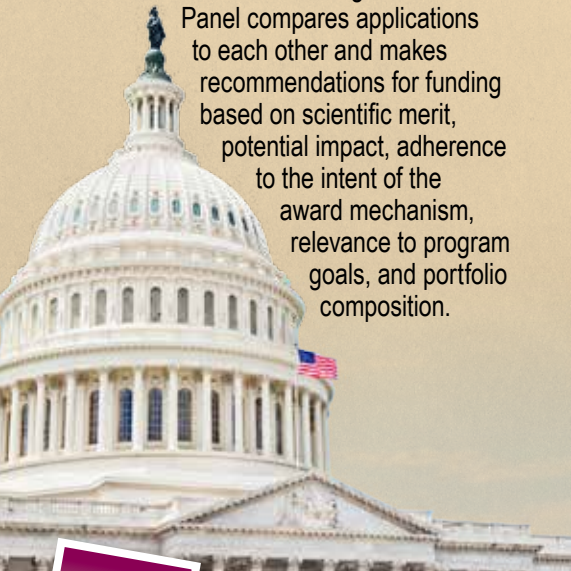
HISTORY

The Office of the Congressionally Directed Medical Research Programs (CDMRP) was created in 1992 from a powerful grassroots effort led by the breast cancer advocacy community that resulted in a congressional appropriation of funds for breast cancer research. This initiated a unique partnership among the public, Congress, and the military. Since then, the CDMRP has grown to encompass multiple targeted programs and has received over \$9.1 billion (B) in appropriations from its inception through fiscal year 2016 (FY16). Funds for the CDMRP are added to the Department of Defense (DoD) budget, in which support for individual programs, such as the Breast Cancer Research Program (BCRP), is allocated via specific guidance from Congress.

APPLICATION REVIEW PROCESS

The CDMRP uses a two-tier review process for application evaluation, with both tiers involving dynamic interaction among scientists and disease survivors (consumers). The first tier of evaluation is a scientific peer review of applications measured against established criteria for determining scientific merit. The second tier is a programmatic review conducted by the Programmatic Panel, which is composed of leading scientists, clinicians, and consumers. The Programmatic

Panel compares applications to each other and makes recommendations for funding based on scientific merit, potential impact, adherence to the intent of the award mechanism, relevance to program goals, and portfolio composition.



Breast Cancer Research Program

VISION

To end breast cancer by funding innovative, high-impact research through a partnership of scientists and consumers

ABOUT THE PROGRAM

The BCRP plays a leading role in the fight against breast cancer through its innovative approaches and its focus on research that will bring an end to this disease. The BCRP was established in 1992 as a result of the dedicated efforts of breast cancer consumers. Their continued efforts, in concert with the program's accomplishments, have resulted in more than \$3.2B in congressional appropriations through FY16. The BCRP enables researchers to propose their best, innovative ideas that address the urgent need to end breast cancer. Scientists are challenged to pursue high-risk, high-reward research, explore new paradigms that could lead to critical discoveries, and make an unprecedented impact on breast cancer. The BCRP also promotes synergistic collaborations across disciplines, and integrates scientists and consumers in unique and meaningful research partnerships.

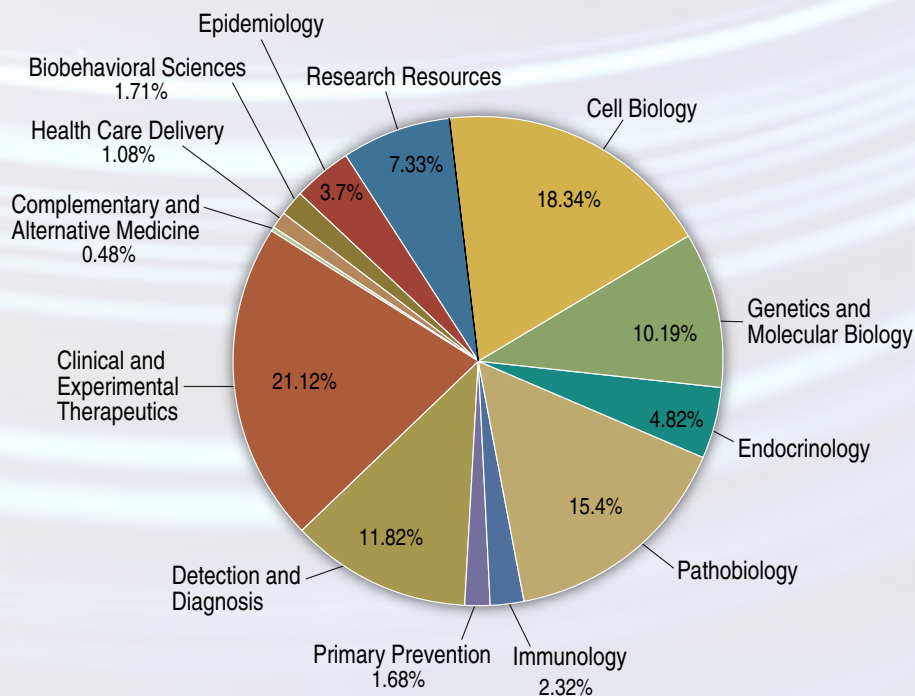


BCRP Overarching Challenges

Considering the current breast cancer landscape, and the BCRP's vision to end breast cancer, the BCRP requires applications to address at least one of the following overarching challenges:

- Prevent breast cancer (primary prevention)
- Identify determinants of breast cancer initiation, risk, or susceptibility
- Distinguish deadly from indolent breast cancer
- Conquer the problems of overdiagnosis and overtreatment
- Identify what drives breast cancer growth; determine how to stop it
- Identify why some breast cancers become metastatic
- Determine why/how breast cancer cells lay dormant for years and then re-emerge (recurrence); determine how to prevent recurrence
- Revolutionize treatment regimens by replacing them with ones that are more effective and less toxic
- Eliminate the mortality associated with metastatic breast cancer

FY92–FY14 BCRP Portfolio



The Breast Cancer Landscape

The BCRP has prepared an overview of the breast cancer landscape, covering the topics most pertinent to the program's vision of ending breast cancer.

Some key points from the Breast Cancer Landscape:

- Worldwide, breast cancer accounts for nearly a quarter of all cancers in women. In 2010, there were 522,000 breast cancer deaths globally.
- Evidence attributes the majority of breast cancers to not one single factor but various physical, environmental, and genetic factors.
- Most risk factors are not modifiable, including age, family history, BRCA status, and breast density. Potentially modifiable risk factors, such as obesity, alcohol consumption, smoking, and exercise, are weakly to moderately associated with breast cancer risk.
- An estimated 30% of all breast cancer cases (both invasive and ductal carcinoma in situ [DCIS]) are considered to be overdiagnosed and overtreated.
- An estimated 20%-30% of women diagnosed with invasive breast cancer will have a recurrence.
- The rate of metastatic breast cancer at initial diagnosis in the U.S. has not changed since 1975.
- Treatments to permanently eradicate metastasis do not exist. There is no cure once metastatic disease has occurred.

Read *The Breast Cancer Landscape* at http://cdmnp.army.mil/bcrp/pdfs/bc_landscape.pdf.

Strategic Partnerships:

The BCRP is widely recognized as a model biomedical research program, and meaningful partnerships have been the foundation of the program's successes from the very beginning. Through this program, the combined efforts of many dedicated individuals foster unique opportunities in breast cancer research. All aspects of the BCRP, including setting program priorities, designing funding opportunities, evaluating and recommending applications for funding, and conducting high-impact research, integrate the expertise of scientists with the perspectives of consumers. Utilizing these innovative approaches is a proven and effective way to support and advance research that has the potential to make a meaningful impact and contribute to the program's vision of ending breast cancer.



"The DoD BCRP has created an unprecedented model of evidence-based, advocacy-informed peer review that rewards relevance, innovation, and impact of breast cancer research. Importantly, it has conveyed to the research community the urgency for a cure and for a successful prevention of this disease. It is an honor and a privilege to serve on the Programmatic Panel."

Silvia Formenti
Weill Cornell Medical College
FY16 Programmatic Panel Chair



"The DoD BCRP is driven by a singular vision: to end breast cancer. While some progress has been made, breast cancer remains the most commonly diagnosed cancer among women, with an estimated 1.7 million women diagnosed each year worldwide. This complex problem requires multi-faceted, innovative solutions, and through a unique partnership between scientists and consumer advocates, the BCRP supports research with clear potential to have high impact. I deeply value and appreciate the opportunity to contribute to the realization of BCRP's vision through serving on the Programmatic Panel."

Chris Li
Fred Hutchinson Cancer Research Center
FY17 Programmatic Panel Chair-Elect



"It has been a distinct honor to serve as a consumer reviewer for the DoD Breast Cancer Research Program, the very agency instrumental in my own training years ago."

Jaime Holloway
Georgetown Lombardi Breast Cancer
Patient Advisory Committee

Scientists and consumer advocates working together to end breast cancer



“While this is an exciting time for cancer immunotherapy, efficacy is not seen in all cancer types, including breast cancer. My group is working hard to develop effective immunotherapy for breast cancer. It is inspiring to work with advocates. They help me focus on what is truly important to patients, and help guide my research toward clinical translation. We have identified important differences in the host immune response to breast cancer versus melanoma, which will guide us in this effort.”

Peter Lee
City of Hope



“Being diagnosed with breast cancer transformed me into an advocate seeking knowledge, one given the chance to “sit at the table” where discussions are being held and decisions are being made, and where I can be a voice for the voiceless.”

Desiree Walker
SHARE



“The most important aspect of being part of the BCRP, for me, has been the interaction with consumer advocates. They have certainly affected the way that I think about breast cancer, but they have impacted the way that I do science more generally. They are a constant reminder that our goal should be to impact people’s lives.”

Greg Hannon
University of Cambridge



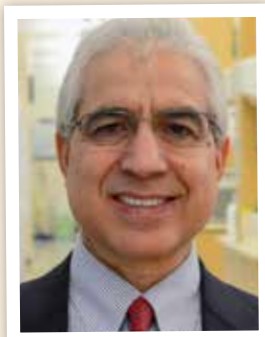
“Besides working in the patient community, I felt like there was no voice for metaplastic breast cancer patients in the medical and research communities. I jumped at the chance to participate with the research community and to give a voice to women with this rare disease. It has become crucial for everyone to join together to closely dissect which applications have the greatest chance of success, but also the greatest chance to impact real lives.”

Maria Fowler
The Metaplastic Foundation

**Maria passed away on December 4, 2015.*

COLLABORATION

Bringing People and Ideas Together



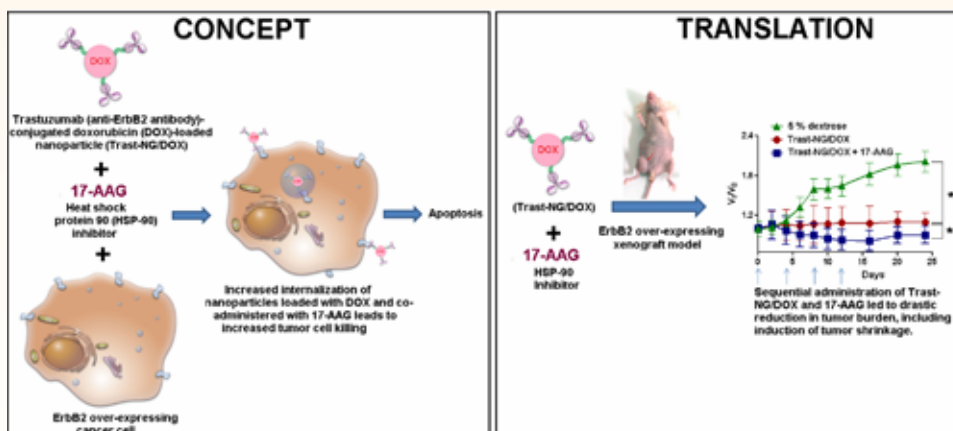
Mechanism-Based Enhanced Delivery of Drug-Loaded Targeted Nanoparticles for Breast Cancer Therapy

Hamid Band, M.D., Ph.D. (pictured left), and Tatiana Bronich, Ph.D., University of Nebraska Medical Center

Up to a third of breast cancers diagnosed in the U.S. overexpress the epidermal growth factor receptor known as ErbB2, or human epidermal growth factor receptor 2 (HER2). The common treatment for ErbB2+ breast cancers is a combination of the anti-ErbB2 antibody Trastuzumab (Herceptin®) and chemotherapy. However, many patients do not respond to the therapy, or they relapse during treatment. When ErbB2 is overexpressed in breast cancer, it is either recycled very quickly back to the cell surface, or there is impairment in endocytosis, the process by which the cell takes in external molecules.

To improve patient outcomes, Drs. Hamid Band and Tatiana Bronich received an FY10 Idea Award to determine a way to make the current treatment of Trastuzumab with chemotherapy more effective. They created nanoparticles with conjugated Trastuzumab. To tackle the problem of endocytosis, the investigators used the heat shock protein 90 inhibitor 17-AAG. They found that adding 17-AAG helped the nanogels penetrate the lysosomes of cells where the nanogels are broken down by the acidic environment, releasing the drugs into the cell.

Drs. Band and Bronich tested nanogels coated with Trastuzumab and filled with Doxorubicin (a common chemotherapeutic agent) in combination with 17-AAG in human administration xenograft tumors, and they found that the tumors regressed with this treatment. They also found that there was lower toxicity with these drugs inside nanogels than there was when the drugs were administered in solution. The combination of Doxorubicin-loaded nanogels and 17-AAG was superior to all other combinations of drugs they tested. This targeted drug-delivery method shows great promise as a future ErbB2+ breast cancer treatment.





Adiposity is Associated with p53 Gene Mutations in Breast Cancer

Peter G. Shields, M.D. (pictured left), The Ohio State University

Jo L. Freudenheim, Ph.D., State University of New York at Buffalo

Mutation of the multifunctional tumor suppressor gene p53 plays a central role in breast carcinogenesis. Mutations in p53 are independent predictive factors of poor prognosis for survival. Although few studies

have investigated the epidemiological origin of p53 mutations, it has been suggested that environmental factors influence the p53 mutational spectra. With support from an FY02 Breast Cancer Center of Excellence Award, Peter Shields (Principal Investigator [PI]) and Jo Freudenheim (multi-site PI) assembled a multi-disciplinary team to examine the epidemiology of breast cancer from a cohort of patients in Western New York.

The examination of an association between the presence of p53 mutations and obesity has begun by using modern high-throughput sequencing methods in order to probe large quantities of archived tumor tissue. The Western New York Exposures and Breast Cancer (WEB) Study collected breast tumor tissue specimens from 1996 to 2001 (Dr. Freudenheim, FY95 Other Investigator-Initiated Award) from women diagnosed with primary, histologically confirmed, incident breast cancer with no previous cancer history other than non-melanoma skin cancer. Using Gene Chip technology, Dr. Shields and his team analyzed DNA from archived WEB tumor tissue blocks for p53 mutations and compared the mutational status to three measures of adiposity (body mass index [BMI], waist circumference, and waist-to-hip ratios) in pre- and post-menopausal women. In post-menopausal women alone, all adiposity measures were associated with an increased risk of having tumors harboring a p53 mutation. The p53 mutation-positive tumors correlated with a poor clinical outcome, and tumors were poorly differentiated, high-grade, hormone receptor-negative, and highly proliferative.

This study is the first to identify an association between p53 mutational status and adiposity in breast cancer. Although obesity is not recognized as a risk factor for pre-menopausal breast cancer, this study suggests that adiposity is associated with unfavorable tumor characteristics and, thus, obesity prevention may be important for women who are at risk and also for women who have already developed aggressive breast cancer. More studies must be performed to elucidate the underlying mechanisms obesity contributes to an increased risk of post-menopausal breast cancer development.





Organotropic Metastatic Secretomes and Exomes in Breast Cancer

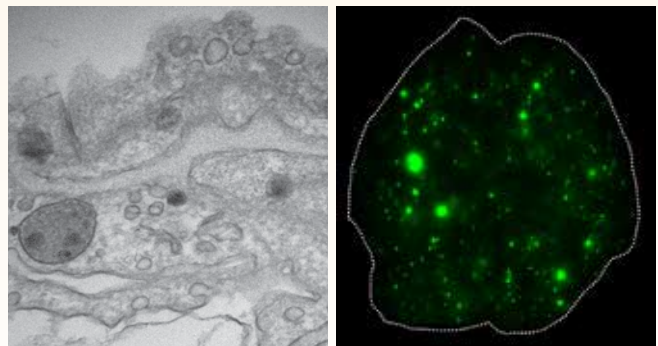
Yibin Kang, Ph.D. (pictured left), Princeton University
Benjamin A. Garcia, Ph.D., University of Pennsylvania
David Lyden, M.D., Ph.D., Weill Cornell Medical College of Cornell University

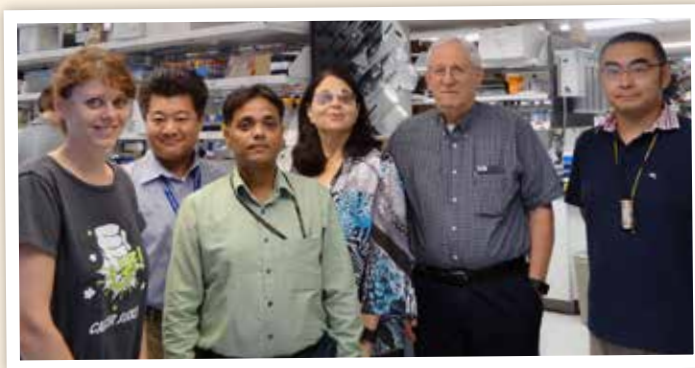
Over 90% of breast cancer deaths result from metastasis. Despite advances made in understanding the complex

components of metastasis, treatments for metastatic lesions remain a crucial, unmet need, and there is no way to predict at initial diagnosis whether a tumor has or will form a metastatic lesion. Exosomes are small-membrane vesicles packaged with biomolecules released by tumor cells and can be found in the circulation of breast cancer patients. Exosomes are emerging as major factors in mediating site-specific metastasis. With the support of a Collaborative Scholars and Innovators Award (FY12), PI Kang, along with co-PIs Garcia and Lyden, set out to comprehensively identify exosomal proteins that mediate bone and lung metastasis to determine the potential of utilizing such factors as biomarkers and therapeutic targets.

A recent publication by A. Hoshino, et al., in *Nature*, supported by this FY12 Award, highlighted the discovery that tumor-derived exosomes have the ability to prime metastatic niches for tumor cell colonization, can be used to predict the site of metastasis, and can redirect tumor cells to different metastatic sites. Using a panel of breast and pancreatic cancer cell lines that preferentially metastasize to the bone, lung, liver, and brain, the investigators were able to demonstrate that isolated exosomes homed to specific organs and could be incorporated by resident cells within the target organ. Target organ homing was dependent on proteins found at the surface of the exosomes, termed “integrins.” Integrins were discovered to mediate the interaction of exosomes with the metastatic niche and resident cells. Furthermore, the collaborative team found that integrin expression profiles correlated with tissue organotropism. Within target organs, exosomes incorporated into resident cells triggered gene changes that promoted inflammation and metastasis. Most notably, the investigators were able to demonstrate that isolation of exosomes from lung-tropic metastatic cells resulted in increased bone-tropic metastatic tumor cell migration to lungs, indicating that exosomes have the ability to redirect metastatic distribution. Finally, their clinical data indicate that integrin expression profiles of circulating plasma exosomes isolated from cancer patients could be used as prognostic factors to predict sites of future metastasis. These findings pave the way for the development of diagnostic tests to predict organ-specific metastasis and therapies to halt metastatic spread.

This study was the first to show that tumor-derived exosomes prepare a favorable microenvironment at future metastatic sites and mediate non-random patterns of metastasis. Overall, the findings suggest that circulating tumor-derived exosomes may be useful not only to predict metastatic propensity, but also to determine organ sites of future metastasis. Future studies must be performed to determine whether combination therapies can be used to block exosomal function to inhibit metastasis, and if these exosomes can be used as biomarkers to predict metastasis in patients.





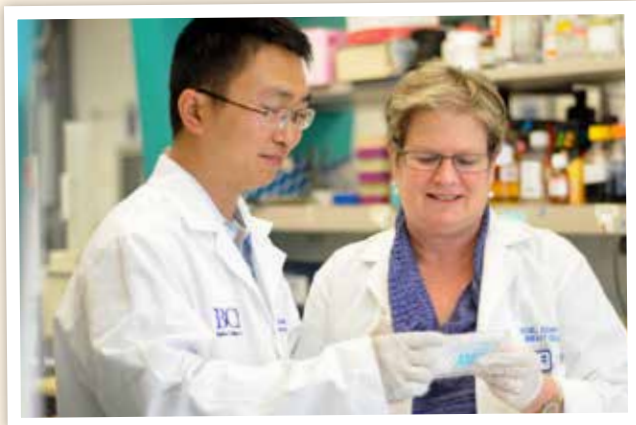
Targeting the Sphingosine-1-Phosphate Axis in Obesity-Promoted Breast Cancer

**Sarah Spiegel, Ph.D., and Dorit Avni, Ph.D.,
Virginia Commonwealth University**

Obesity has been recognized as a risk factor for developing aggressive, therapy-resistant breast cancer. It has been proposed that chronic inflammation as a result of obesity could be an underlying cause for

this increased risk. Sphingosine-1-phosphate (S1P) is generated inside cells by enzymes called sphingosine kinases and is an important signaling molecule involved in regulating many physiological processes related to obesity, inflammation, and cancer. To better understand this link, Drs. Sarah Spiegel and Dorit Avni, with support from an FY13 Breakthrough Award and FY13 Postdoctoral Fellowship Award, respectively, are investigating the involvement of S1P and sphingosine kinases in obesity-related and triple-negative breast cancer (TNBC) development and metastasis, with the goal of developing novel therapies to overcome the problem of hormone-therapy resistance in patients.

Recently, Dr. Spiegel's research team found that FTY720, an FDA-approved drug for the treatment of multiple sclerosis, is phosphorylated by sphingosine kinase 2, serving as a mimetic of S1P and thus interfering with immune cell trafficking. Furthermore, FTY720 has strong anti-cancer effects, but the mechanisms of its action remain unclear. In work published in *Oncogenesis* in June 2015, this group determined that FTY720 treatment can reactivate expression of estrogen receptor (ER) α and enhance the effectiveness of hormone therapy in ER α -negative breast cancer cells. This reversal was found to be due to generation of the active phosphorylated form of FTY720 in the nucleus, where it serves as a potent histone deacetylase (HDAC) inhibitor. HDACs are negative regulators of ER α expression and promising targets for the reversal of tamoxifen resistance in breast cancer. FTY720 treatment enhanced histone acetylation on the promoter of the ER α gene, resulting in increased ER α expression and re-sensitization of ER α -negative cells to tamoxifen in cell culture. This was confirmed using mouse models, where FTY720 treatment suppressed the development and progression of both high-fat, diet-induced spontaneous and ER α -negative breast tumors, by promoting expression of ER α and sensitizing the tumors to tamoxifen treatment. Interestingly, treatment with FTY720 was even more effective than a known HDAC inhibitor, SAHA. These findings show promise for repurposing the FDA-approved drug FTY720 as a novel agent to be used in combination with conventional hormone therapy to treat therapy-resistant or TNBC. Drs. Spiegel and Avni plan to build on these findings by further investigating the mechanism of S1P and sphingosine kinases in inflammation- and obesity-promoted breast cancer progression and prognosis.



Novel Targets for Aggressive Estrogen Receptor Positive Breast Cancer

Xiaosong Wang, M.D., Ph.D. (pictured left), and Rachel Schiff, Ph.D., Baylor College of Medicine

Approximately 70% of all breast cancers are ER+. The most common way to treat ER+ cancers is through endocrine therapy, which includes drugs like tamoxifen that block estrogen from binding to the receptor. However, many ER+ breast tumors treated with endocrine therapy will relapse; therefore, understanding the mechanism of tumor resistance and identifying new therapeutic targets are critical to improving patient outcomes.

Dr. Xiaosong Wang and Dr. Rachel Schiff, with support from BCRP and Breast Cancer Stamp funding, collaborated to study genomic rearrangements in ER+ breast cancer. Genomic rearrangements involving translocation of genes could result in gene fusions and characteristic copy number alterations. By studying genomic data in the Cancer Genome Atlas and creating a tool called Fusion Zoom to process this data (<http://fusionzoom.cagenome.org/>), Drs. Wang and Schiff were able to identify a gene fusion that is more prevalent in the more aggressive luminal B subtype of ER+ tumors. This gene fusion, ESR1-CCDC170, combines the ER gene and CCDC170, whose function is unknown. Further studies showed that the protein product of this gene fusion reduced endocrine sensitivity and increased the movement and metastatic potential of ER+ breast cancer cells. This newly identified gene fusion may lead to a better understanding of the genetic origin of aggressive ER+ breast cancers and may provide a new therapeutic target for effective intervention.

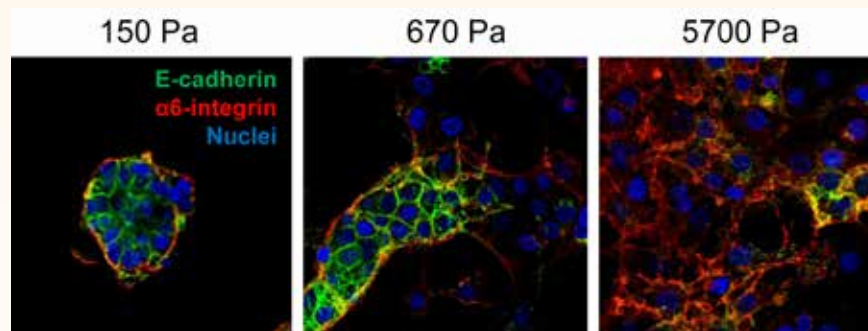
Additionally, the investigators validated and identified the roles of tousel-like kinase 2 (TLK2) and Nemo-Like Kinase (NLK). When TLK2 was overexpressed in cells, correlating genomic instability was observed and DNA repair was disrupted. The cells also become more invasive. The investigators showed that inhibiting TLK2 blocked breast cancer tumor growth in mice, and they determined that TLK2 may be a potential new target for aggressive ER+ breast cancers. In addition, they discovered a crucial role of NLK in a previously unknown survival signaling pathway that endows endocrine resistance of breast cancer cells. Studies showed that when the amount of NLK in endocrine therapy-resistant cancer cells was inhibited, responsiveness to endocrine therapy (tamoxifen) was restored. They also identified a potent NLK inhibitor which has already undergone Phase I and II clinical trials for treating inflammatory disease, and investigated its therapeutic potential in endocrine-resistant ER+ breast cancer cells. The NLK inhibitor was able to sensitize the cancer cells to tamoxifen treatment, thus precipitating cell death. Further, through collaborations they also facilitated the identification of a third kinase target called MAP3K3, which appears to endow resistance of breast cancer cells to cytotoxic chemotherapy. With these studies, Drs. Wang and Schiff identified three novel kinase targets for more aggressive breast cancers and, moreover, have identified a promising drug against one of these targets.



Regulation of Breast Cancer Stem Cells by Tissue Rigidity

Jing Yang, Ph.D. (pictured left), and Adam Engler, Ph.D., University of California, San Diego

The presence of an area of increased stiffness within a breast tumor, or a fibrotic focus, is associated with cancer progression, distant metastases, and overall poor outcome. Also associated with metastases and poor outcome is an enhanced “stemness” of breast cancer cells. Cancer stem cells adopt properties making them better able to migrate, invade, survive in the circulation, and colonize in foreign tissue. While the underlying mechanism for why tissue, or matrix, stiffness promotes cancer progression is not fully understood, Drs. Jing Yang and Adam Engler have hypothesized that tissue rigidity regulates breast cancer stem cell (BCSC) properties and function, thereby promoting tumor development and chemoresistance. With funding from an FY12 BCRP Idea Award and Breast Cancer Stamp funds, Drs. Yang and Engler have found that increasing matrix stiffness promotes epithelial-to-mesenchymal transition (EMT) in human breast cancer cells, and that this regulation depends on the adhesion receptor $\beta 1$ integrin and the transcription factor TWIST1. Their findings, published in a 2015 *Nature Cell Biology* article, suggest a newly identified paradigm in which increasing matrix stiffness induces an integrin-dependent cell signaling cascade and subsequent release of TWIST1 from its cytoplasmic sequestration factor G3BP2. TWIST1 is then able to enter the nucleus and induce the transcription of EMT and invasion-promoting factors. Depletion of G3BP2 promoted breast cancer invasion and metastasis in mice, and reduced expression of G3BP2 in combination with increased matrix stiffness predicts poor survival in breast cancer patients. Cells that have undergone EMT are shown to present many properties of cancer stem cells, thus the $\beta 1$ -G3BP2-TWIST1 mechanotransduction pathway may be a key to the regulation of BCSC properties and chemoresistance. Further investigation into this pathway may lead to novel therapeutic strategies that will be able to halt progression of a tumor before it has the chance to metastasize. Furthermore, this project highlights the effectiveness of the coming together of two labs with complementary sets of expertise, breast cancer biology (the Yang lab), and bioengineering and stem cell biology (the Engler lab). The collaboration has proved to be essential to implement this research program.



Mouse mammary epithelial were three-dimensional (3D)-cultured on polyacrylamide gel substrates with increasing rigidities from 150Pa (mimicking normal breast tissue) to 5700Pa (average rigidity of breast tumors), and stained for E-cadherin (green), integrin (red), and nuclei (blue).

INNOVATION

Pursuing Novel Ideas and Technologies



Real-Time Imaging of the Tumor Microenvironment Provides Mechanistic Insight into Tumor Cell Intravasation

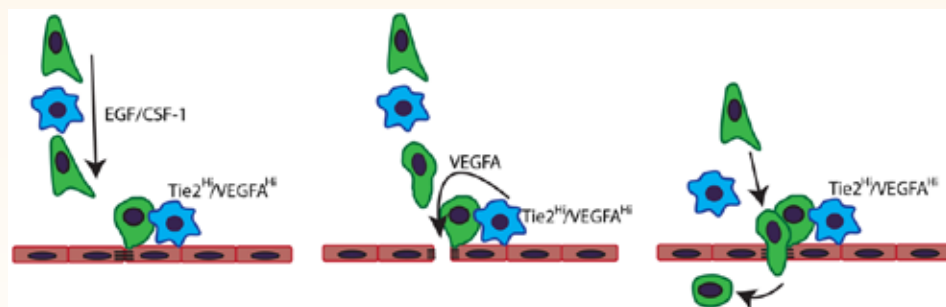
Allison S. Harney, Ph.D., Albert Einstein College of Medicine of Yeshiva University

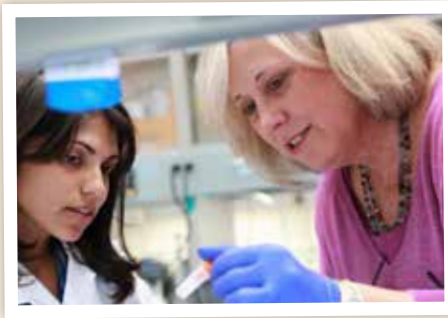
For women diagnosed with breast cancer, the primary cause of morbidity and mortality is the development of metastatic disease. Entry of cancer cells into the bloodstream is an essential first step of metastasis. This process requires complex interactions between multiple cell types in the primary tumor, including immune cells such as macrophages and vascular endothelial cells. With funding from an FY12 BCRP Postdoctoral Fellowship Award, Dr. Allison Harney and her mentor, Dr. John Condeelis, are investigating the mechanism by which macrophages interact with blood vessels to facilitate tumor cell entry into the blood stream (intravasation).

Specific milieus termed “Tumor MicroEnvironment of Metastasis” (TMEM) exist within tumors where tumor-associated macrophages, tumor cells, and endothelial cells come into direct contact. TMEMs are associated with tumor cell intravasation and metastatic recurrence in breast cancer patients. Furthermore, high levels of blood vessel permeability are neither uniform nor constant within tumors. To establish the role of TMEMs in transient vascular permeability and tumor cell intravasation, Dr. Harney and colleagues utilized high-resolution in vivo imaging techniques to observe these interactions in multiple breast cancer mouse models. In findings recently published in *Cancer Discovery*, they revealed that vascular permeability is dynamic and occurs simultaneously with tumor cell intravasation at TMEMs, providing the first direct evidence linking these phenomena. Furthermore, they demonstrated that transient vascular permeability is mediated by vascular endothelial growth factor-A signaling in Tunica interna endothelial cell kinase-2-expressing macrophages within the TMEM, resulting in the disassembly of endothelial cell junctions and transmigration of tumor cells into the blood stream.

These findings provide insight into microenvironments present within breast tumors that facilitate tumor intravasation and distant metastases. Based on these findings, Dr. Harney and colleagues are investigating therapeutics targeting

TMEM formation and function. The group believes that treatment with TMEM-targeting drugs at the time of primary tumor may reduce or eliminate the risk of developing metastases, ultimately improving breast cancer survival rates.





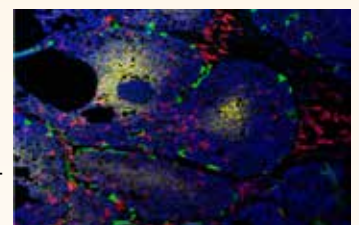
Modulating Immune Response to Improve Therapy for Breast Cancer

Lisa Coussens, Ph.D. (pictured right), Oregon Health & Science University

While standard treatment methods have improved the overall outlook and quality of life for women with breast cancer, too many women still succumb to the disease. Furthermore, 20%-30% of women diagnosed with a favorable prognosis still develop metastatic disease within 10 years. More thorough and accurate prognostic assays that accurately predict risk of disease metastasis or recurrence are needed, as are treatments that suppress tumor growth. Dr. Lisa Coussens, recipient of an FY06 BCRP Era of Hope Scholar Award, and Scholar Expansion Award, has discovered that the research area of tumor-specific inflammation may yield answers to both of these needs. Although breast cancer has not been linked definitively to underlying inflammation or infection, it does display tumor-associated inflammation marked by infiltration of developing tumors by immune cells, the most abundant myeloid cell being macrophages, known to provide growth and survival factors that aid in metastasis. This information warrants investigation into novel treatments that target the tumor-specific immune response, which may be particularly useful for late-stage breast cancer patients.

Previously, Dr. Coussens was able to show that the pulmonary metastasis of mouse mammary tumors is functionally regulated by two types of immune cells: CD4+ T cells and macrophages. Her study determined that blocking macrophage infiltration into the tumors enhanced efficacy of standard-of-care chemotherapy (CTX). This finding correlated highly with data from human breast cancer patients showing that recurrence-free survival could be stratified based upon macrophage and T cell infiltration. Moreover, when macrophage infiltration into mammary tumors was blocked, chemosensitivity to paclitaxel was increased, resulting in reduced primary tumor growth, 85% reduction in metastases, and increased survival. In translating these basic science discoveries to the clinic, Dr. Coussens partnered with clinical colleagues Drs. Hope Rugo (University of California, San Francisco) and E. Shelley Hwang (Duke University) to propose therapeutic targeting of macrophages in women with TNBC. As such, the three collaborators were awarded a Komen Promise grant that is supporting an investigator-initiated clinical trial evaluating efficacy of this approach (NCT01596751).

An FY10 Era of Hope Scholar Expansion Award allowed Dr. Coussens to initiate an integrated, multidisciplinary project to evaluate the clinical benefit of macrophage modulation in preclinical mouse models of breast cancer, with the goals of facilitating biomarker identification and informing clinical trials of CTX in combination with known macrophage-antagonists. The hypothesis is that components of the macrophage response in breast cancer can be identified to serve as biomarkers for risk stratification, and that these components can be effectively targeted for therapeutic intervention. The prediction is that the intervention will result in decreased late-stage breast cancer development and metastasis when combined with CTX. In a recent article in *Cancer Immunology Research*, Dr. Coussens wrote that clinical responses to radiotherapy can also be improved by neutralizing the TH2-based programs that drive tumorigenic and immune-suppressive pathways in mammary tumors. Furthermore, Dr. Coussens expects that these novel strategies will be able to better harness the power of dendritic cell-based vaccines that may induce durable tumor repression. This highly translational project will allow for an entirely new treatment strategy for breast cancer where a novel immune-based approach will be leveraged to potentiate the effect of cytotoxic chemotherapy in patients with breast cancer.



Histology of macrophages (red) in mammary carcinomas (blue) with blood vessels (green), and areas of hypoxia (yellow).



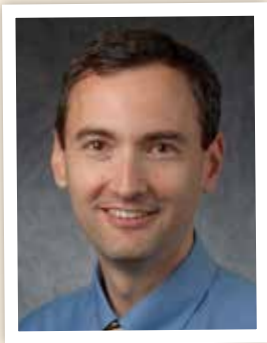
Pasireotide, a Promising Alternative for Breast Cancer Chemoprevention Treatment

David Kleinberg, M.D., New York University School of Medicine

Numerous clinical trials have demonstrated the preventative effects of selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene, in women at increased risk for ER+ breast cancer. However, SERMs have a number of side effects linked to their anti-estrogenic properties, including hot flashes in premenopausal women, increased risk of blood clots, and endometrial hyperplasia and carcinoma, which contribute to reduced compliance among women treated with anti-estrogen therapy. Therefore, new anti-hormone therapies without negative side effects are a significant need in breast cancer clinical care.

To address this need, Dr. David Kleinberg of the New York University School of Medicine received an FY06 BCRP Synergistic Idea Award to examine the efficacy of a somatostatin analog (pasireotide) that had been shown to inhibit the effects of insulin-like growth factor I (IGF-I) and estrogen in the mammary glands of rats, despite the presence of estrogen. Together with Drs. Julia Smith, Deborah Axelrod, and Baljit Singh, Dr. Kleinberg enrolled 15 women volunteers with atypical hyperplasia of the breast to conduct the study in order to assess the effects of pasireotide on IGF-I and breast tissue in humans. The women were treated with the compound for 10 days in the study. By comparing tissue samples before and after the treatment phase, it was found that pasireotide inhibited cell proliferation and stimulated cell death in hyperplastic breast lesions by inhibiting IGF-I action and also reducing growth hormone secretion from the pituitary. Furthermore, the side effects of pasireotide were mild or moderate. The most notable was an increase in blood glucose during treatment.

Based on these promising results, Dr. Kleinberg proposed to further assess the potential clinical utility of pasireotide in a larger cohort of women with DCIS through an FY09 BCRP Idea Expansion Award. Additionally, the treatment phase was extended to 20 days to both study the direct effects on DCIS over a longer time period and examine the persistence of altered blood sugar and growth hormone levels. The study is ongoing at this time. Preliminary imaging results have revealed that two patients with low nuclear grade DCIS experienced complete disappearance of DCIS at excision, two patients with high nuclear grade disease had apparent tumor shrinkage, and three with high nuclear grade disease had no apparent tumor shrinkage. Also, as with the pilot study, it appears that serum glucose levels return to normal following the treatment phase. The results from these two studies represent a major step toward the identification of a treatment that can prevent the development of breast cancer or reverse premalignant lesions of the breast, while maintaining intact circulating estrogen levels.



Multiple Cooperating Oncogenes Drive Recurrent Breast Cancer-Associated Chromosomal Amplifications: Creation of Isogenic Human Cell Line Models

Josh Lauring, M.D., Ph.D., Johns Hopkins University

Dr. Josh Lauring's research project concerns a type of genetic change, called chromosomal amplification, which is common in many types of cancer, including breast cancer. Amplifications are regions of chromosomes that accumulate as extra copies of genetic material in cancer cells. Amplifications cause tumors to make abnormally high levels of proteins encoded by genes within the amplified region. Some of these genes, like Her-2 in breast cancer, are "drivers" of tumor growth, which can be targeted for therapy. However, some of these amplifications are very complex. They contain many genes, and it has been difficult to identify a single targetable driver gene. Dr. Lauring hypothesized that what is driving breast tumor growth in this case is the cooperation among multiple genes within the amplified region. Traditional laboratory techniques that test one or two genes at a time are unsuited to testing this novel hypothesis of genetic cooperation within amplifications. With support from an FY10 Idea Award, Dr. Lauring's laboratory developed a unique genetic engineering technology to model this kind of amplification in the laboratory. The technique involves using gene editing technology to target a selectable marker gene to the chromosomal region to be tested. Drug selection then applies pressure on the cells to amplify the targeted region in order to survive. Using this technique in MCF-7 cells, a widely used human cell line model of ER+ breast cancer, Dr. Lauring was able to engineer MCF-7 cells with amplifications of a chromosomal region that is amplified in 15% of breast cancers, carrying a poor prognosis. These engineered cells showed many of the same features as human breast tumors, with different patterns of gene gain and loss, and simultaneous overexpression of multiple proteins encoded by the amplified genes. The goal of these experiments is to produce engineered cell lines that bear common genetic modifications highly correlated with aggressive breast cancer and then to use the cellular models for drug identification and development. Because investigators will know which mutations were necessary to produce the engineered cell lines, they will be able to conduct experiments of a much more intricate nature – beyond the current state of the art, which tends to rely on knocking out single genes, and struggles to manage complex networks. The initial results of this work have recently been reported in an article in *Breast Cancer Research Treatment*. If successful, these cellular models have great potential to speed the identification and introduction of novel drugs to help in the fight against breast cancer.

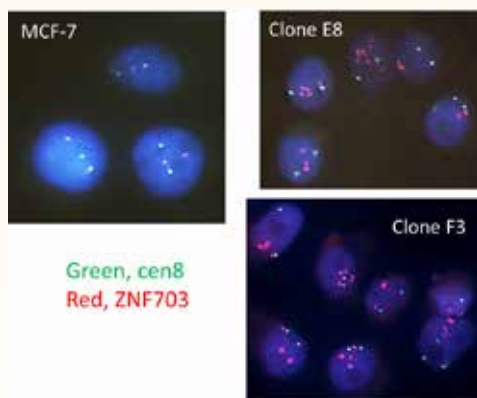


Figure showing fluorescence in situ hybridization analysis of MCF-7 breast cancer cells and two cell lines engineered by Dr. Lauring's laboratory to develop chromosomal amplifications on chromosome 8. The red probe is for a gene called ZNF703, within the region targeted for amplification. The green probe is for a control gene outside of the region. Normal cells should have two signals for each probe, as shown for MCF-7. Cells "E8" and "F3" have many more signals for ZNF703, indicating that amplification of this gene has occurred.

Identification of the HER2 Functional Site: Concurrent Blockage of Receptor Homo- and Heterodimerization

Ruth Lupu, Ph.D., Mayo Clinic, Rochester

Overexpression of the HER2 is correlated with highly aggressive breast cancer disease and, as such, has been an excellent target for therapeutic interventions with both monoclonal antibodies (trastuzumab, pertuzumab, and cetuximab) and small molecule kinase inhibitors (lapatinib). HER2 is a receptor that lacks its own ligand but can be activated by overexpression, causing spontaneous homodimerization and activation, or by heterodimerization with another ligand-stimulated HER-family receptor. Unfortunately, these two independent mechanisms of activation negatively impact the response to current HER2-targeted agents and highlight the need for a therapeutic that can inhibit HER2 dimerization via a combination strategy. A recipient of an FY05 Concept Award, Dr. Ruth Lupu found that the specific disruption of a 16-amino acid essential activating sequence on the extracellular subdomain III of HER2 can simultaneously disable HER2 homo- and heterodimerization, ultimately blocking all possible activation of HER2 and its oncogenic downstream signaling pathways. In a 2015 paper published in the *Journal of the National Cancer Institute*, Dr. Lupu and colleagues revealed that the deletion of that sequence blocked several oncogenic properties of HER2-driven signaling in breast cancer cells, including anchorage independent growth, enhanced cell cycle progression, and chemo-resistance. Consequently, the mutant HER2 was able to restore the sensitivity of breast cancer cells to paclitaxel. Therefore, disruption of this critical activation sequence on HER2 may represent a novel targeted approach for the management of HER2-driven breast carcinomas. The Lupu group is currently expanding their approach to design soluble, high-affinity peptide mimetic compounds that specifically interact with this essential activating region of HER2.





Improving the Assessment of Surgical Margins in Breast Conserving Therapy

Anita Mahadevan-Jansen, Ph.D., Vanderbilt University

For certain women diagnosed with early stage breast cancer, breast conserving therapy (BCT), or lumpectomy, is the preferred and most common form of treatment. When BCT is combined with an adjuvant therapy, such as radiation, it has been shown to be as effective as a full mastectomy. In order for BCT to be successful, all cancerous cells must be removed, and the remaining normal tissue must have clear margins that are free of residual tumor cells. Unfortunately, in at least 25% of cases, an incorrect assessment of tumor margins is made during surgery. When a tissue sample is determined to have positive margins after surgery, an additional surgery, called re-excision, is required. A full mastectomy may also be required at this time if the surgeon cannot remove enough tissue to obtain clear margins. In hopes of improving upon the current limitation of the techniques used to examine margins, Dr. Anita Mahadevan-Jansen and her team at Vanderbilt University, with support from an FY08 Idea Award, sought to develop and test a device called “spatially offset Raman spectroscopy (SORS)” that can quickly, accurately, and noninvasively evaluate margins in real time in the operating room.

To design and develop SORS for breast tumor surgical margin evaluation, Dr. Mahadevan-Jansen verified that the SORS probe can detect a ~0.1 mm-thick tumor under 0.5 mm of normal tissue, and be sensitive to breast tumors located up to 2 mm beneath normal breast tissue. After successfully characterizing SORS in soft tissue samples, the team evaluated the performance of the SORS probe in normal and malignant breast tissue in vitro. The analysis showed that SORS had the ability to discriminate positive and negative margin status in breast tissue in vitro with 100% positive predictive value and 94% negative predictive value. The Vanderbilt team then tested the clinical feasibility of SORS to evaluate margins in breast tissue. Evaluating 59 lumpectomy specimens, they demonstrated proof of concept and observed 92% preliminary sensitivity. Further development of the SORS device has moved it closer to being clinically applicable and capable of scanning the entire surface of the excised breast specimen in under 10 minutes. Dr. Mahadevan-Jansen received an FY13 Idea Expansion Award to continue the development and validation of the SORS device, and to use the instrument to continue to unravel the mysteries of metastatic potential and tissue biochemistry associated with the various aspects of breast cancer. This impactful research could potentially reach thousands of patients per year who undergo BCT. The SORS device has the potential to help eliminate the need for re-excision surgery for those battling breast cancer.

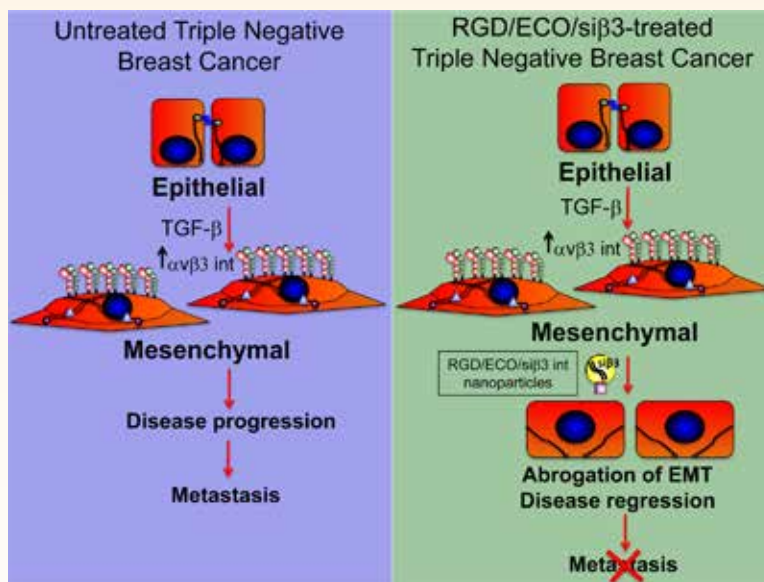


Nanoparticle Delivery of Integrin-Specific siRNAs as Targeted Therapy to Alleviate Triple-Negative Breast Cancer (TNBC) Metastasis

Jenny Parvani, Ph.D. (pictured middle), Case Western Reserve University

TNBC is a highly aggressive subtype of breast cancer that currently lacks FDA-approved targeted therapies. These patients typically respond to chemotherapy initially, but ultimately succumb to disease recurrence and metastasis.

The process of metastasis is complex, and involves a number of events, including tumor cells undergoing EMT, local invasion, intravasation into blood or lymph vessels, survival in the circulation, extravasation out of the vessels, and colonization in the metastatic niche and lesion site. EMT is an important initiator of metastasis and is driven by various factors, including integrin engagement of the tumor microenvironment. Previous studies have demonstrated that integrins are essential for EMT and metastasis, making them promising targets for specific therapies tailored to treat metastatic TNBC. A recipient of an FY13 Postdoctoral Fellowship Award, Dr. Jenny Parvani has demonstrated that silencing the expression of $\beta 3$ integrin can prevent EMT and inhibit TNBC metastasis in vivo. Under the mentorship of Dr. Zheng-Rong Lu and Dr. William Schiemann, Dr. Parvani recently published in *Cancer Research* on utilizing an siRNA delivery system that she has shown to be versatile, safe, and effective in the delivery of $\beta 3$ integrin siRNA to breast cancer cells responsible for metastasis. In order to enhance uptake of the siRNA specifically by post-EMT breast cancer cells, the nanoparticles were modified with a cyclic RGD (Arginine-Glycine-Aspartic Acid) peptide. The effectiveness of the silencing system in a mouse model of breast cancer was evidenced by a decreased primary tumor burden and recurrence post resection. The silencing of $\beta 3$ integrin by this method also prevented pulmonary metastasis after treatment. Overall, this study demonstrates that $\beta 3$ integrin is a powerful target for diminishing the metastatic potential of TNBC cells, and that this highly effective delivery system may be a way to treat TNBC patients for whom targeted therapy is currently lacking.





Co-Treatment with HIF-1 Inhibitors Can Reduce Breast Cancer Stem Cell Enrichment in Triple Negative Breast Cancer

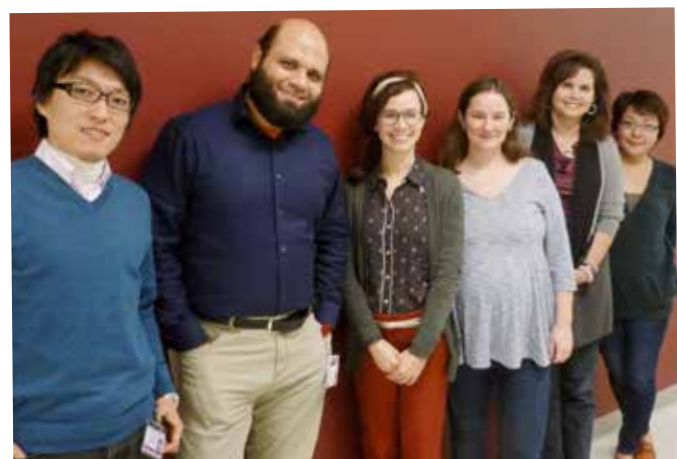
Gregg L. Semenza, M.D., Ph.D. (pictured right), Johns Hopkins University

Targeted therapies against hormone receptors (i.e., estrogen, progesterone) and HER2 have significantly improved therapeutic options for individuals with breast tumors expressing these biomarkers. However, 10% to 15% of all breast cancers lack expression of these three targeted receptors, limiting therapeutic strategies for the subgroup known as triple-negative breast cancer, to radiation or chemotherapy. Identifying the underlying molecular mechanisms that contribute to chemotherapy resistance in TNBC is vital for the development of new therapies and decreasing patient mortality. Overexpression of the hypoxia-inducible factor-1 (HIF-1)

transcription factor has been linked to decreased survival and metastasis in TNBC and has been implicated in chemotherapy resistance. Through support from an FY11 BCRP Impact Award, Dr. Gregg Semenza of Johns Hopkins University is further examining the mechanisms of HIF-1 in TNBC chemotherapy resistance with the goal of developing new treatment strategies to improve patient survival.

Dr. Semenza observed an increase in HIF-1 expression in multiple TNBC cell lines following chemotherapy treatment. HIF-1 activity resulted in a signaling cascade that enriched for breast cancer stem cells (BCSCs), which are a subpopulation of cancer cells that are resistant to chemotherapy treatment and are responsible for recurrent tumors or metastases. Chemotherapy-induced HIF-1 activity led to the preferential survival of BCSCs through the activation of interleukin-6 and -8 signaling, and increased expression of the multidrug resistance 1 protein. Additionally, Dr. Semenza found that chemotherapy treatment of TNBC cells promoted biosynthesis of the natural antioxidant glutathione in a HIF-1-dependent manner. Inhibiting glutathione synthesis prevented the enrichment of BCSCs following chemotherapy treatment. Surprisingly, induction of the BCSC phenotype in TNBC cells by glutathione is independent of its function as an antioxidant. In this case, glutathione increased the expression of pluripotency factors that drive cancer cells toward a stem cell phenotype.

Together, these results demonstrate multiple pathways through which chemotherapy-induced HIF-1 activation contributes to cancer progression in TNBC cells. Given these results, Dr. Semenza hypothesized that HIF-1 inhibitors could be used in combination with cytotoxic chemotherapy to prevent the enrichment of chemotherapy-resistant BCSCs. When breast cancer cells were co-treated with the HIF-1 inhibitor digoxin and chemotherapy drugs that are commonly used to treat TNBC, enrichment of BCSCs was blocked. These results suggest that inhibiting HIF-1 or other HIF-1-regulated pathways, in combination with chemotherapy, could be a promising new treatment strategy to improve TNBC patient outcomes.



Antagonists for E-selectin for the Prevention of Breast Tumor Metastasis

**Takemi Tanaka, Ph.D. (pictured left),
Oklahoma University Health Science Center**

During metastasis, cancer cells invade the bloodstream and adhere to the blood vessel walls of distant organs to establish metastatic foci. Adhesion of circulating cancer cells to endothelial cells is a critical step for colonization and is followed by transmigration through the endothelial lining into adjacent tissue. E-selectin, an adhesion molecule

on vascular endothelial cells, plays a pivotal role in the shear-resistant cell adhesion and transendothelial migration through its interactions with its ligand, CD44, on cancer cells. The expression of E-selectin is induced at distant organs at the premetastatic niche in response to factors released from the primary tumor. Dr. Takemi Tanaka, a recipient of an FY09 Concept Award, aimed to develop an antagonist of E-selectin from an emerging class of small molecule ligands called thioated aptamers. In a recent article published in *Molecular Therapy*, Dr. Tanaka demonstrated that blocking E-selectin with an E-selectin-targeted thioaptamer (ESTA) created a profound reduction in the generation of metastatic foci in mouse xenograft models of ER- breast cancer. ESTA has a very high affinity for E-selectin, and had prolonged activity from a single injection. These results highlight the potential for E-selectin antagonists for the prevention of metastasis in ER- breast cancer. As a successful outcome of the proposed studies, ESTA was U.S. patented (serial number 13/209,866, published as US20120039810).

IMPACT

Making Breakthroughs in Understanding Breast Cancer



Role of Ecd, a Novel Cell Cycle Regulator, in Breast Cancer

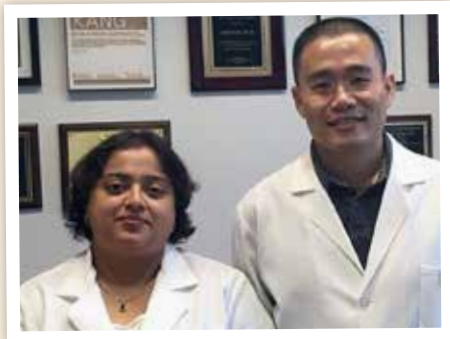
Vimla Band, Ph.D., University of Nebraska

Uncontrolled proliferation is a hallmark of breast cancer and often the consequence of mutations in cell cycle-related genes. Therefore, understanding the mechanisms of cell cycle regulation and the manner in which cancer cells hijack these systems is important for designing cancer treatments and predicting mortality.

With support from an FY10 Idea Award, Dr. Vimla Band examined the role of ecdysonless (Ecd) in cell cycle regulation and breast cancer oncogenesis. Ecd is an embryonically lethal protein that plays a regulatory role in the G1-S transition of the cell cycle in humans. It is believed to do so by binding directly to the Retinoblastoma protein, releasing it from the transcription factor E2F and allowing for expression of proteins required for an efficient transition from G1 to S-phase. Dr. Band compared Ecd expression levels in normal and cancerous breast tissues, the results of which were published in *Breast Cancer Research and Treatment* in 2012. Through development of an antibody specific for human Ecd, it was shown that overexpression of Ecd correlates with breast cancer progression and known markers of poor prognosis, including overexpression of the HER2/neu protein. Furthermore, Ecd overexpression was correlated with decreased survival in HER2/neu overexpressing breast cancer patients. Therefore, Ecd overexpression in HER2+ patients has the potential to serve as a novel biomarker of poor prognosis and could help guide treatment regimens.

To further examine the role of Ecd in breast cancer progression and prognosis, Dr. Band reported in *Cell Cycle* (2015) her finding that, in the context of oncogenesis, Ecd was found to be a cooperating oncogene when overexpressed with mutant H-Ras in human mammary epithelial cells (hMECs). Overexpression of both Ecd and Ras in hMECs resulted in continued cell cycle progression despite the absence of essential growth factors. Interestingly, co-expression of both Ecd and Ras is required for promoting significant increases in oncogenic phenotypes. This phenomenon was recapitulated in vivo, where only mice implanted with hMECs overexpressing both Ecd and Ras developed tumors in their mammary fat pads. Together, these findings demonstrate that Ecd, in conjunction with mutant Ras, is a driver of breast cancer oncogenesis.

IMPACT: Making Breakthroughs in Understanding Breast Cancer



The Role of p63 in Mammary Stem Cell Regulation and Breast Cancer Progression

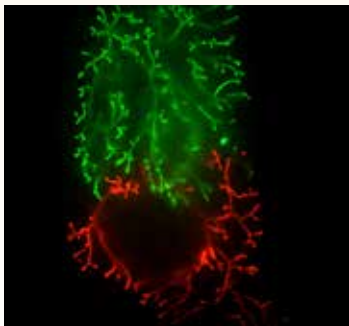
Rumela Chakrabarti, Ph.D. (pictured left),
Princeton University

Increasing evidence points to the existence of tumor-initiating cells (TICs), also known as cancer stem cells, which share many characteristics with normal stem cells but are not regulated properly and, therefore, can contribute to cancer development, metastasis, recurrence, and therapy resistance. Despite this

known correlation, there is little knowledge regarding the molecular mechanisms that link normal stem cells to TICs. Uncovering these shared regulators in the context of breast cancer was the goal of Dr. Rumela Chakrabarti, supported by an FY10 Postdoctoral Fellowship Award, in the laboratory of Dr. Yibin Kang at Princeton University.

Transformation-related protein 63 (p63), a member of the p53 family of transcription factors, is highly expressed in stratified epithelial tissue, like the breast, and whose expression has been linked to cancer. The direct role of p63 in cancer development was previously unknown due to the presence of multiple protein isoforms, the functions of which have not been clearly defined. In a study published in 2014 in *Nature Cell Biology*, Dr. Chakrabarti and colleagues found that the p63 isoform lacking the transactivating domain, known as Δ Np63, is a key stem cell regulator in both normal and malignant mammary tissues, promoting basal-like breast cancer using a regulatory mechanism common to that used in mammary gland development.

Dr. Chakrabarti demonstrated that overexpression of Δ Np63 in primary mammary stem cells promotes their tumor-initiating activity, and the loss of one Δ Np63 allele in a mouse model reduces mammary stem cell function. Microarray analysis revealed downregulation of the Wnt signaling pathway in the mammary epithelial cells and mammary stem cell-enriched basal cells of mice only expressing one functional copy of Δ Np63. The positive regulation of Δ Np63 on the canonical Wnt signaling pathway was confirmed to occur by direct transcriptional regulation of FZD7, a protein that is highly expressed in basal/TNBC and has been shown to promote proliferation. Finally, Dr. Chakrabarti linked Δ Np63, FZD7, and Wnt signaling to initiation of the basal subtype of breast cancer. A strong positive correlation between Δ Np63 and FZD7 in basal breast cancer was observed in both large-scale isoform-specific RNA sequencing data from patients as well as in immunostaining of human breast cancer tissue samples. The initiation and maintenance of breast cancer by the Δ Np63-Fzd7-Wnt signaling axis was confirmed using a variety of in vitro assays and multiple breast cancer mouse models. Together, these findings highlight a novel mechanism of stem cell regulation in mammary cells that also serves as a driver of basal-like breast cancer, presenting the Δ Np63-Fzd7-Wnt signaling pathway as a potential therapeutic target.



Whole-mount fluorescence images of the mammary fat pads after competitive reconstitution assay. Mammary stem cell enriched populations of mammary epithelial cells from Δ Np63 overexpressing-GFP and control-dsRED transgenic mice were mixed equally and then transplanted into cleared mammary fat pads to regenerate mammary ductal structures. Δ Np63 promotes more mammary stem cell activity as shown by increased GFP-colored ductal branching compared to dsRED color.



Cellular and Molecular Characterization of ER+ Breast Cancer

Michael F. Clarke, M.D., Stanford University

Many early stage ER+ breast cancers are sensitive to endocrine therapies, but late-stage ER+ cancers are often resistant. As a recipient of an FY10 Innovator Award, using technology that allowed for single-cell gene expression analysis, Dr. Michael Clarke was able to identify a hierarchy of proliferative potency in early progenitor/mammary epithelial stem cells in ER+ breast cancer. This analysis demonstrated that cancers of different patients display significant heterogeneity due to clonal evolution of certain pools of cell types. Dr. Clarke is currently investigating various factors that may contribute to the proliferative capacity or self-renewal of mammary epithelial stem cells.

It is known that women with Down syndrome, a disease characterized by trisomy 21, do not develop breast cancer, suggesting that a gene on chromosome 21 is protective. Having recently published the role of the USP16 gene in Down syndrome (*Nature* 2013), Dr. Clarke's group had noted that this gene seemed to control cellular growth and self-renewal. Normally, USP16 removes ubiquitin from a histone protein, a critical step in the maintenance of somatic tissues. They were able to establish that the knockout of this gene led to an increased rate of breast cancer in their mouse model. They also found that transduction of human breast cancer cells with USP16 led to markedly reduced rates of tumor formation. As USP16 is deleted in 18% of breast cancers, Dr. Clarke is now examining the effect of USP16 in normal breast stem cells. Subsequent research in the laboratory has identified a second gene regulator, BCL11b, whose mutation or deletion leads to an inability of breast tissue to maintain the population of stem cells that it uses to replenish itself over time. Pilot data suggests that tumors with high levels of BCL11b expression are more likely to relapse, and lead to significantly reduced survival for patients. Translation of these discoveries may represent new therapeutic targets for ER+ breast cancer.



Epigenetic Regulation of microRNA Expression: Targeting the Triple-Negative Breast Cancer Phenotype

Bridgette M. Collins-Burow, M.D., Ph.D., Tulane University

Triple-negative breast cancer, a highly aggressive mesenchymal type of breast cancer, currently lacks effective targeted therapies. One of the hallmarks of the highly metastatic triple-negative phenotype is the presence of the EMT, characterized by the early loss of epithelial markers (e.g., epithelial cadherin CDH1) and emergence of pro-metastatic mesenchymal markers (e.g., vimentin, CDH1 repressor ZEB1). As EMT has been shown to be an early event in TNBC progression, identifying agents that can inhibit the EMT process would be beneficial in the development of anti-metastatic therapies. HDAC inhibitors (HDACi) are a promising class of new multifunctional anticancer agents. Dr. Bridgette Collins-Burow, recipient of an FY08 Concept Award, and her team at Tulane University have shown an increased expression of epithelial markers in TNBC cells following treatment with an HDACi, which together with an inhibition of migration is suggestive of the reversal of the EMT phenotype. As published in a 2014 report in *Breast Cancer Research and Treatment*, exposure to pan-HDACi panobinostat (LBH589) in vitro and in vivo decreased the metastatic potential of TNBC cells via the potent inhibition of the EMT process. These findings indicate a potential use for panobinostat as an EMT-targeted therapy for TNBC patients.

In addition, Dr. Collins-Burow identified EMT-related, HDACi-induced, microRNA (miRNA) changes in TNBC cell lines. The miR-200 family of miRNAs is known to be lost in mesenchymal breast cancer subtypes, such as TNBC. In a recent 2015 *Oncotarget* paper, the Collins-Burow group at Tulane demonstrated through next-generation sequencing analysis that the ectopic overexpression of miR-200b-3p regulates EMT in the TNBC subtype through the inhibition of the pro-metastatic RHO signaling pathways. Furthermore, the group has characterized a novel synergistic mechanism by which miR-200b-3p and miR-200b-5p miRNAs repress non-canonical EMT pathways in triple-negative tumors. These findings demonstrate novel mechanisms for the intervention of TNBC.



Targeting the Immune System's Natural Response to Cell Death to Improve Therapeutic Response in Breast Cancer

Rebecca Cook, Ph.D., Vanderbilt University Medical Center

The first five years postpartum, at any age, carry with them an increased risk of breast cancer. These particular breast cancers are related to wound-healing events that occur during postpartum involution, the process by which milk-producing cells die and are cleared by the immune system, yet the exact mechanism of malignancy remains unclear. One factor that may contribute to the tumorigenic process may be efferocytosis, the natural process by which dead cells are engulfed by phagocytes. Macrophages are guided through the microenvironment using enhanced cytokine and extracellular matrix remodeling, both of which are conducive to tumor progression and metastasis. Dr. Rebecca Cook, recipient of an FY12 Idea Award, is studying how changes in a receptor tyrosine kinase, MerTK, can induce efferocytosis and influence breast cancer development. Her group has shown in a 2014 paper published in the *Journal of Clinical Investigation* that mice lacking MerTK exhibited impaired efferocytosis in postpartum tumors, reduced M2-like macrophage polarization, and decreased transforming growth factor-beta levels. Together, these factors contributed to a reduction in postpartum tumor metastasis. Dr. Cook's work has thus identified a naturally occurring process in breast tissue maintenance, specifically after postpartum, that potentially drives breast oncogenesis. Overall, this study broadens our understanding of the breast microenvironment after pregnancy and the mechanisms that increase a woman's risk for breast cancer. This area of research also identifies the efferocytosis pathway as a novel target for inhibition.

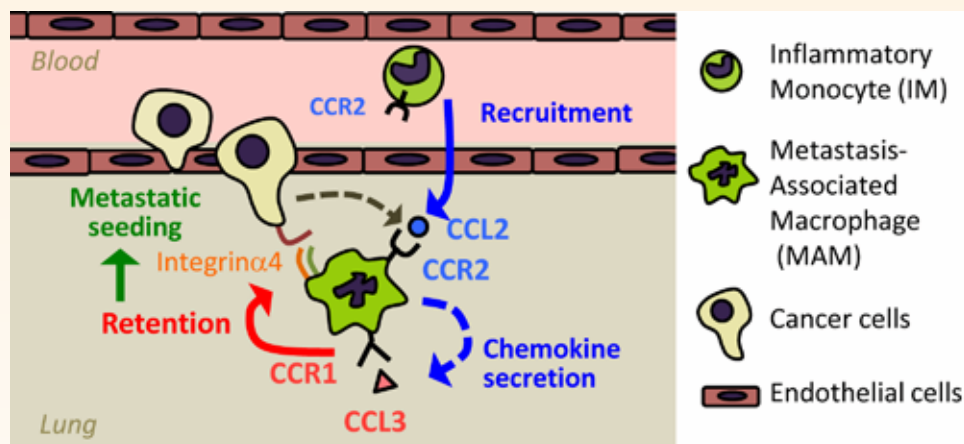
IMPACT: Making Breakthroughs in Understanding Breast Cancer



Molecular Mechanisms by Which Macrophages Promote Breast Cancer Metastasis

Takanori Kitamura, Ph.D., Albert Einstein College of Medicine

Macrophages have been demonstrated to play pivotal roles in breast cancer metastasis. For example, macrophages recruited to the tumor-challenged lung promote extravasation of metastatic cancer cells from blood vessels, and suppress the death of the disseminated cancer cells. Therefore, targeting macrophages is an attractive therapeutic strategy, and thus better understanding is required regarding the mechanisms that regulate accumulation and function of macrophages in the metastatic sites. As a recipient of an FY11 Postdoctoral Fellowship Award under the mentorship of Dr. Jeffrey Pollard, Dr. Takanori Kitamura has investigated the role that macrophages play in the metastasis of breast cancer. In a 2015 article in the *Journal of Experimental Medicine*, Dr. Kitamura and his colleagues demonstrated both in vitro and in vivo that the metastasis of breast cancer to the lung is, in part, attributable to local modification of the breast tumor micro-environment by macrophages. Monocytes (progenitors of macrophages) that encounter CC-chemokine ligand 2 (CCL2) released by stromal cells and tumor cells, become metastasis-associated macrophages and secrete another chemokine ligand, CCL3. CCL3 is predominantly released by macrophages, and stimulates other nearby macrophages by interacting with its receptor, CCR1, which prolongs retention of macrophages in the metastatic sites, in part through enhancing macrophage-cancer cell interaction. Knocking out either CCL3 or CCR1 significantly reduces the number of lung metastatic foci developed by breast cancer cells. This research suggests that the therapeutic competitive inhibition of CCR1 might help to prevent cancer extravasation and metastasis while allowing for a local response to the tumor. This study indicates that inhibition of chemokine signaling can reduce macrophage accumulation and following tumor dissemination, and suggest that chemokine antagonists against CCR1 can be therapeutic agents for metastatic breast cancer.

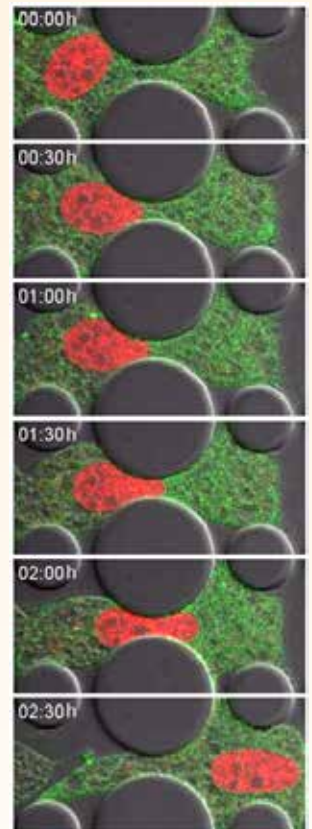




Systematic Analysis of the Functional Relevance of Nuclear Structure and Mechanics in Breast Cancer Progression

Jan Lammerding, Ph.D. (pictured back left), Cornell University

Breast cancer cells and tissues are known to have abnormal nuclear morphology, but little information on how the expression of nuclear envelope proteins impact the metastatic process is known. Nuclear lamins, specifically lamins A/C, are important proteins that provide nuclear shape and stiffness. Dr. Jan Lammerding, a recipient of an FY10 Idea Award, hypothesized that changes in the expression of lamins or lamin B receptors (LBR) may contribute to the irregular cancer cell nuclear morphology and directly modulate cellular functions relevant to cancer progression. The Lammerding group, in 2014, reported in *Cellular and Molecular Bioengineering* their discovery that reduction in lamin A/C led to nuclear deformability as seen by fluorescent microscopy. To complement this finding, they developed and tested multiple cell lines with altered lamin or LBR expression levels using a novel microfluidics device. The device allows for visualization of cells migrating through precisely defined constrictions at high spatial and temporal resolution. They reported that reduced levels of lamin A/C allowed for faster transit through narrow constrictions. To verify this event, the group took highly metastatic breast cancer cells and observed that the cells can adjust their levels of lamin A/C and migrate through 3D environments and narrow constrictions. This means that cancer cells with reduced amounts of lamin A/C have an increased ability to travel through tissues and small capillaries to reach the circulatory system, thus attaining metastatic ability. They also identified this cell population as having a high rate of nuclear rupture. Nuclear rupture will cause severe DNA damage and chromosomal rearrangements that have the capacity to promote further cancer progression. Overall, this work has identified a novel mechanism by which lamin A/C dynamics promote cancer progression and metastasis. These conclusions could have important clinical implications for patient diagnosis and prognosis of breast cancers, where expression levels of nuclear proteins and softer nuclei could indicate a higher risk for the patient. Used as a prognostic, this could be especially impactful when applied to the analysis of circulating tumor cells to determine aggressive subpopulations of cells. Moreover, new nuclear envelope and structural protein targets revealed from Dr. Lammerding's studies may promote the development of new therapeutics aimed at preventing breast cancer progression.



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Genetic Characterization of Breast Cancer Cells from Primary to Advanced Metastatic Disease

Devon A. Lawson, Ph.D., University of California, San Francisco

The progression of cancer from a primary tumor to metastasis represents the most frequent cause of cancer-related deaths. Determining the mechanisms surrounding the change from primary tumor to metastatic distal tumor growth is crucial to developing targeted therapeutic options to stop the spread and invasion of cancer cells. Supported by an FY10 Postdoctoral Fellowship Award, Dr. Devon Lawson in the laboratory of

Dr. Zena Werb sought to analyze the gene expression of tumor cells at different stages of the metastatic process. These studies were designed to determine the molecular differences of metastatic TICs for novel therapeutic targeting.

Dr. Lawson used a series of human Patient-Derived Xenograft (PDX) models to investigate the role of the microenvironment in human breast cancer metastasis. PDX models are mice, injected with a patient's breast cancer cells, that retain the essential features of the original patient's tumor, including histopathology, clinical markers, and hormone responsiveness. Importantly, the tumors that develop in the mice metastasize to the same organs as those originally observed in the patient. Using this model, Dr. Lawson achieved unprecedented resolution of gene expression in metastatic and primary tumor cells at different stages of the metastatic process. Their data supported a hierarchical model for metastasis, with early metastatic cells having a stem-like phenotype with significant tumor-initiating capacity. These cells then differentiated to a spectrum of cell types more similar to that of the primary tumor as they colonized to develop advanced metastatic disease. Further, Dr. Lawson and colleagues sought to determine if the same tumor-initiating stem-like cells were present in the primary tumor. Interestingly, they identified a correlation between the number of stem-like cells in a primary breast cancer tumor and its metastatic potential, such that the most metastatic PDX model had the most stem-like cells, while, in contrast, the least metastatic PDX model had the least stem-like cells.

Based on these studies, Dr. Lawson and colleagues plan to determine whether the frequency of stem-like cells in primary tumors could be used as a predictive biomarker for metastasis. Moreover, they will begin to use these fine-tuned genetic variation data of the different stages of metastatic cell progression to potentially discover novel therapeutic targets to eradicate stem-like, metastasis-initiating cells.





Drs. Ornella Selmin and Donato Romagnolo, University of Arizona Cancer Center

Gestational Exposure as an Epigenetic Modifier of Breast Cancer Risk

Donato F. Romagnolo, Ph.D., M.Sc. (pictured right), University of Arizona

While genetic mutations in the BRCA1 gene are well-characterized in breast cancer, it is less understood why a significant proportion of individuals with breast cancer have reduced BRCA1 expression despite the presence of intact BRCA1 alleles. CpG hypermethylation, an epigenetic modification that leads to gene silencing, of the BRCA1 promoter has been linked to decreased BRCA1 protein levels and the development of high-grade breast carcinomas. Critical stages of mammary gland development occur in utero. Thus, prenatal exposure to environmental factors that alter the BRCA1 epigenetic landscape could result in increased breast cancer susceptibility. This idea was explored by Dr. Donato Romagnolo in work funded by two BRCP grants, an FY09 Idea Award and an FY13 Idea Expansion Award.

Ligands that bind to and activate the aromatic hydrocarbon receptor (AhR), such as 2,3,7,8-Tetrachlorodibenzodioxin (TCDD), are present in the environment and in certain foods. Dr. Romagnolo demonstrated that exposure to TCDD enhances AhR occupancy and CpG island hypermethylation at the BRCA1 promoter in breast cancer cells, resulting in decreased BRCA1 protein expression. TCDD exposure in utero resulted in reduced mammary gland differentiation and BRCA1 expression in female mouse offspring. These findings indicate that prenatal exposure to compounds that activate the AhR and alter the epigenetic state of the BRCA1 promoter could be a potential breast cancer risk factor in female offspring, suggesting that pregnant women should avoid exposure to such agents. The potential implications of these studies, however, extend beyond exposure to agents such as TCDD since other factors that are ubiquitous in the environment can modulate the AhR. These include photoproducts of ultraviolet light and certain metabolites of dietary compounds.

Resveratrol, a natural compound found in a variety of foods, is known to block AhR activation and, therefore, could protect against the silencing of BRCA1 by TCDD or other AhR-stimulating compounds. In an animal model, pre-exposure to resveratrol stimulated expression of the AhR repressor and its recruitment to the BRCA1 gene, preventing the harmful effects of TCDD in the mammary tissue of female offspring. These results provide evidence to suggest that pregnant mothers could decrease their female offspring's susceptibility for sporadic breast cancer through diets containing agonists of the AhR, like resveratrol.

To further build on these exciting findings, Dr. Romagnolo has recently been awarded an FY14 Breakthrough Level 2 Award providing an opportunity to investigate the impact of in utero exposure to genistein, a dietary soy isoflavone and known DNMT inhibitor, on BRCA1 promoter hypermethylation, gene expression, and the development of TNBC in female offspring. If successful, these results could identify an additional maternal dietary factor that may have preventative effects on breast cancer in offspring. Dr. Romagnolo shares a laboratory at the University of Arizona Cancer Center with Dr. Ornella Selmin, who was a co-author on the cell culture and animal studies that investigated the epigenetic changes on the BRCA-1 gene associated with activation of the AhR. Dr. Selmin is also a co-investigator on the FY14 Breakthrough Level 2 Award.

IMPACT: Making Breakthroughs in Understanding Breast Cancer



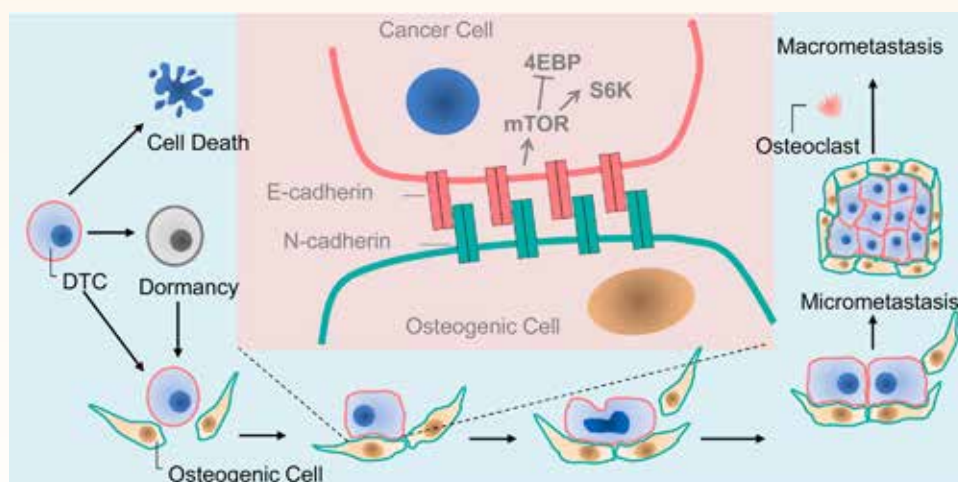
Mechanisms Promoting the Colonization of Breast Cancer Metastases in Bone

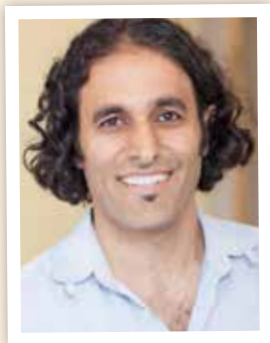
Hai Wang, Ph.D., Baylor College of Medicine

Breast cancer mortality is highly associated with cancer metastases, which frequently appear in bone. Vicious interactions between cancer cells and the cells that build and break down bone are the driving force behind the progression of bone metastases during late stages. Current therapies targeting these cellular interactions limit tumor progression, but ultimately fail to improve patient survival. Little is known about the early stages of cancer metastasis, when microscopic metastases can remain quiescent for long periods of time before growing into detectable tumors. The eradication of micrometastases represents an important therapeutic opportunity for the prevention of cancerous bone lesions.

Dr. Hai Wang, with funding from an FY12 BCRP Postdoctoral Award and under the mentorship of Dr. Xiang Zhang, developed a mouse model to examine how breast cancer cells survive and colonize in bone during early stages of metastasis. Bone samples obtained from these mice revealed that micrometastases reside in a specific niche and preferentially associate with osteogenic (bone-forming) cells. Dr. Wang found that cancer and resident niche cells made direct contact through the E-cadherin proteins on the cancer cell and the N-cadherin proteins on the niche cell, leading to the investigation of a role for cell-to-cell connections called heterotypic adherens junctions (hAJs) in the support of tumor progression within this niche. Dr. Wang observed that the spread of breast cancer metastases can be reduced by disruption of hAJs, suggesting that hAJ formation may be a critical step in the early colonization of cancer cells. Using a 3D cell culture model, Dr. Wang discovered that the presence of bone-forming osteoblasts promoted the survival and proliferation of breast cancer cells, potentially through the activation of the protein kinase mTOR. Dr. Wang made the important discovery that activation of the mTOR signaling pathway is associated with the progression from single cells to osteolytic metastases.

Dr. Wang's research provides a foundation for the clinical development of novel therapies that might specially target the mTOR signaling pathway or hAJs. This work could illuminate new therapeutic avenues for the prevention of breast cancer metastases, as well as potential strategies to protect cancer patients who appear tumor-free yet are still at high risk for metastatic recurrences. Additional information regarding Dr. Wang's research can be found in the February 2015 issue of *Cancer Cell*.





Understanding Tumor Dormancy as a Means of Secondary Prevention

Sohail Tavazoie, M.D., Ph.D., The Rockefeller University

Tumor progression is often marked by cycles of tumor dormancy, prolonged periods of minimal or no cell growth. Dormancy of cancer cells is often due to a lack of signaling cues in the local microenvironment that promote cell proliferation. Eventually, through mechanisms not well understood, a subset of cancer cells can overcome these limitations and proliferate again. Greater understanding of the mechanisms of tumor dormancy and how cancer cells eventually overcome these roadblocks in the context of ER- breast cancer is the goal of research being performed by Dr. Sohail Tavazoie, supported by an FY12 Collaborative Scholars and Innovators Award in collaboration with Dr. Gregory Hannon.

To identify drivers of primary and metastatic tumor re-initiation, Dr. Tavazoie developed an in vivo selection system for isolating subpopulations of ER- human breast cancer cells that have an enhanced tumor-forming ability. This method consists of injecting breast cancer cells at low cell numbers into the mammary fat pads of immunodeficient mice, followed by the generation of single-cell suspensions from the resulting tumors and re-injection over multiple rounds of serial dilution. Therefore, this protocol selects for cells that have an increased ability to initiate tumor formation rather than simply proliferating at the highest rate. Microarray analysis of the tumorigenic-enriched and highly metastatic sublines revealed that three genes (LAMA4, FOXQ1, and NAP1L3) were consistently overexpressed when compared with the parental cell populations. Jason Ross, a student in Dr. Tavazoie's lab, further characterized the most abundantly overexpressed gene, LAMA4, and found that it promotes the re-initiation of tumors in the mammary gland and in other organ microenvironments. One important feature that drives cancer cell malignancy is the ability to proliferate in the absence of attachment to an underlying matrix, though the mechanisms for this are unknown. Expression of LAMA4 was found to contribute to this phenotype both in vitro and in vivo, and requires $\beta 1$ -integrin for cell adhesion. Finally, in the context of breast cancer patients, LAMA4 expression was more highly expressed in malignant cancer cells relative to the adjacent pre-malignant cells, and positively correlated with a decrease in relapse-free survival. Together, these results (published in May 2015 in *Nature Cell Biology*) identify LAMA4 as a novel regulator of primary and metastatic ER- breast cancer re-initiation, which could serve as a potential therapeutic target in these patients.



In the Clinical Pipeline

VACCINES

E75 Her2-derived Peptide Vaccine (NeuVax™)

Constantin Ioannides
Elizabeth Mittendorf

The BCRP supported a study that sought to identify cytotoxic lymphocyte-recognized epitopes on HER2-overexpressing human breast tumors, during which Dr. Ioannides together with Dr. Bryan Fisk discovered E75, an immunodominant HER2 peptide. The E75 peptide combined with GM-CSF has since been developed into an immunogenic peptide-based vaccine under the commercial name of NeuVax (Galena Biopharma). The vaccine reportedly targets 50%-60% of HER2+ patients. NeuVax is now in Phase III clinical trials to evaluate the effectiveness of the vaccine to prevent or delay breast cancer recurrence after standard of care therapy. Also supported by BCRP is a newly opened Phase II clinical trial, run by Dr. Elizabeth Mittendorf, testing NeuVax and trastuzumab in high-risk HER2+ breast cancer patients. In the Phase II trial, NeuVax will be given in the adjuvant setting to prevent recurrences in patients with HER2+ breast cancer who were administered neoadjuvant chemotherapy plus trastuzumab and failed to achieve a pathologic complete response.

HER2 Peptide-Based Vaccine

Mary (Nora) L. Disis

Dr. Disis developed a vaccine that, when concurrently administered with trastuzumab strongly elicits an immune response to the growth factor receptor HER2, generating long-term tumor-specific immunity. The HER2 intercellular domain (ICD) peptide-based vaccine is designed to treat breast cancer by stimulating the immune

destruction of remaining cancer cells after primary cancer therapy. The HER2 ICD peptide vaccine was evaluated in a Phase II clinical trial in stage III and stage IV HER2+ breast cancer patients concurrently receiving trastuzumab. Results of the trial indicated considerable improvements in relapse-free survival, as well as minimal toxicity and prolonged, robust, antigen-specific immune responses. Long-term follow up of patients receiving HER2 vaccination against the ICD shows potential improved survival in patients with advanced stage HER2+ breast cancer when administered early in the course of treatment. Analysis shows a median RFS of 33% at 6 years, and greater than 80% of patients are alive at 8 years from enrollment. The vaccine has been licensed by EpiThany for further investigation.

STEMVAC

Mary (Nora) L. Disis

Dr. Disis has successfully created a multi-antigen vaccine, STEMVAC, comprised of Th1 epitopes derived from five BCSC/EMT immunogenic proteins. The vaccine has been shown to be safe and to inhibit tumor growth in mouse models of breast cancer, and Dr. Disis has recently started enrollment for a Phase I clinical trial in patients with HER2-negative, advanced stage breast cancer. The goals of this trial are to assess the vaccine's safety in patients and to determine the immunogenicity of specific doses of the vaccine. Furthermore, the trial will determine whether immunity can be enhanced or maintained by additional booster vaccinations as well as how the vaccine impacts development of immunologic memory. If the vaccine proves safe, further development will proceed to testing in the prevention setting.

Mammoglobin cDNA Vaccine

William Gillanders

Mammoglobin-A, a member of the secretoglobin superfamily, is a novel breast cancer-associated antigen and an exceptional target for breast cancer vaccine therapy. The renewed interest in cancer vaccines has been sparked by recent advances in cancer immunotherapy. With support from a BCRP Clinical Translational Research Award, Dr. Gillanders has recently completed a Phase I clinical trial of a mammoglobin-A cDNA vaccine. The results of the trial showed that the mammoglobin-A vaccine is safe, able to induce specific IFN- γ -secreting CD8 T cells, and results in longer progression-free survival for patients. Based on the success of that trial, and with support from a BCRP Breakthrough Level 3 Award, Dr. Gillanders has opened a Phase Ib trial of the mammoglobin-A DNA vaccine in patients receiving neoadjuvant endocrine therapy.

IMMUNOTHERAPIES

HER2 Bi-Armed Activated T Cells

Lawrence G. Lum

The BCRP supported preclinical studies on HER2 bi-armed activated T cells, which induces the development of "memory" antigen-specific cytotoxic T lymphocytes directed at Her2. This led to a Phase 1 clinical trial in women with Her2+ metastatic breast cancer, which indicated that the treatment infusions are safe and induced long-term antitumor responses. The Her2 bi-armed activated T cells are currently in Phase 2 clinical trials for treating breast cancer.

Products in Clinical Trials and Development

TRC105 Antibody

Ben Seon

The BCRP supported the development of TRC105, a monoclonal antibody which targets endoglin and inhibits angiogenesis. Preclinical results indicated that systemic administration of TRC105 and other anti-endoglin antibodies could suppress the growth of established tumors as well as new tumor growth. These results led to a current Phase I clinical trial of TRC105 in combination with capecitabine in breast cancer patients, as well as several other early phase clinical trials in other cancer types.

NOVEL TECHNIQUES IN TREATMENT

Prone Radiotherapy

Silvia Formenti

With BCRP support, Dr. Formenti conducted clinical trials to assess the efficacy of an accelerated, hypofractionated whole-breast radiation therapy designed to minimize the length of radiotherapy required after partial mastectomy in patients with DCIS. In this method, patients are treated in the prone position rather than in the supine position on a specially designed table, greatly reducing unnecessary radiation exposure of the heart and lungs. Importantly, prone radiotherapy

offered heart and lung protection regardless of breast size. Prone radiotherapy is poised to become a standard choice in breast radiotherapy.

HER2-Targeted Drug Delivery

John Park
James Marks

Dr. Park proposed to develop a novel breast cancer therapy by combining the targeting properties of a monoclonal antibody and the drug delivery advantages of liposomes to deliver the anti-neoplastic drug doxorubicin to tumor cells. Dr. Park collaborated with another BCRP award recipient, Dr. James Marks, to develop a new anti-HER2/neu monoclonal antibody (now called MM-302) and demonstrate its ability to efficiently target HER2 overexpressing breast cancer cells. Moreover, they showed that doxorubicin delivered via the antibodytargeted liposomes inhibited tumor growth in mouse models of HER2-positive breast cancer while at the same time lowering the toxicity of doxorubicin to normal tissue. MM-302 has been licensed by Merrimack Pharmaceuticals and is currently in a multiinstitutional, Phase 2 clinical trial involving patients with advanced stages of HER2-positive breast cancer.

Targeted HER2 Radiotracer

Gary Ulaner

Growing evidence suggests that HER2 expression may change between the primary breast lesions and metastases, an example of tumor heterogeneity. The BCRP is supporting a proof-of-concept trial that uses a targeted HER2 radiotracer (89Zr-trastuzumab) to determine the proportion of patients with HER2-negative primary breast cancer who develop imagable HER2-positive metastases and, in those patients, determine if HER2-targeted therapy results in a measurable treatment response. A Phase

1 clinical trial with 50 subjects is ongoing. Preliminary data indicates the ability to detect HER2+ metastatic foci in patients with confirmed HER2+ primary tumors.

THERAPEUTICS

IDO Inhibitor

George C. Prendergast

Indoleamine 2,3 dioxxygenase (IDO) is an enzyme that is commonly activated in breast cancer and is implicated in preventing the antitumor immune response by blocking T-cell activation. The BCRP supported preclinical studies that identified and characterized lead inhibitors of IDO that have pharmacological properties suitable for testing in clinical trials. As a result of this work, Dr. George C. Prendergast demonstrated that the D isomer of an IDO inhibitor called 1MT (D-1MT) has potent antitumor properties, and his group discovered IDO2, an IDO-related gene, as one of its molecular targets. D-1MT is now in clinical trials for breast cancer and other solid tumors.

PD0332991 (Palbociclib)

Dennis Slamon

Preclinical research supported by the BCRP led to the identification of cyclin-dependent kinases (CDKs) as a target for ER+ breast cancer and the discovery that ER+ breast cancer cells are sensitive to a CDK inhibitor, PD-0332991. These and other findings provided the basis for Phase I and Phase II clinical trials, supported by Pfizer, in which PD-0332991 in combination with the aromatase inhibitor letrozole demonstrated an increase in median progression-free survival. These results prompted "Breakthrough Therapy" status by the FDA and Pfizer's initiation of a Phase III clinical trial in 2013. In 2015, the FDA granted accelerated approval of PD-with letrozole for

In the Clinical Pipeline

the treatment of ER+ HER2+ breast cancer in postmenopausal women. With BCRP funding, Dr. Slamon will perform molecular and correlative studies on patient samples from the clinical trials, to determine what characteristics best determine sensitivity to this combination therapy. With this knowledge, PD-0332291 can be administered to patients who will benefit most from its use.



Anne Abate, American Cancer Society

"Recently, my wonderful oncologist recommended adding a brand new drug, Ibrance®, to the mix of things we are using to

ward off any progression of my disease. I was thrilled to discover that some of the initial research on this drug had been supported by the DoD BCRP. What an amazing thing to know that, as a consumer reviewer, I am helping to advance research that could one day actually help me and thousands of other women stay alive. I now have even more reasons to be involved in this program."

5-Flouro-2'deoxyctidine (FdCyd)

Edward Newman

DNA methylation inappropriately turns several genes off in cancer cells. Preclinical studies supported by the BCRP demonstrated the effects of FdCyd with tetrahydrouridine on reversal of DNA methylation in several genes expressed by breast cancer cells. This combination treatment not only reversed DNA methylation, but also induced mRNA expression. A Phase I clinical trial funded by the BCRP was completed, and a Phase II clinical trial in breast and other cancer types has been initiated by the National Cancer Institute.

Combining Aromatase and Src Inhibitors

Joyce Slingerland Isabel Chu

The proliferative effects of estrogen in breast cancer are due in part to its ability to induce cells to enter the cell cycle. Through work supported by the BCRP, Dr. Slingerland found that estrogen stimulation of cell cycle progression was dependent on inhibiting p27, a negative regulator of the cell cycle protein, cyclin E-cdk2. She found that both estrogen-stimulated progression and resistance to anti-estrogen drugs, such as Tamoxifen, involved a decrease in p27 and subsequent increase in cyclin E-cdk2 activity, leading to cell cycle entry and proliferation in breast cancer cells. Further work performed chiefly by Dr. Slingerland's graduate student, Isabel Chu, also supported by the BCRP, showed that inhibition of p27-required phosphorylation of another protein, Src, led to p27 degradation in cells, and that inhibiting Src prevented p27 degradation. These studies suggest that a two-pronged approach that includes both anti-estrogens and drugs that preserve p27 may be effective in arresting cell cycle progression in breast cancer. Dr. Slingerland has begun Phase I and II trials to test the tolerability and efficacy of anastrozole, an aromatase inhibitor that stops estrogen production, together with the Src inhibitor, AZD0530, in post-menopausal women with ER+ breast cancer.

Aspirin as Adjuvant Therapy for Node-Positive Breast Cancer

Eric Winer Michelle Holmes

Epidemiological and preclinical data suggest that aspirin may have the potential to reduce breast cancer recurrence and improve survival. With support from the BCRP, Eric

Winer and Michelle Holmes have proposed a Phase III randomized, placebo-controlled trial of aspirin among survivors of breast cancer who are node positive. The trial will use invasive disease-free survival as the primary end point, assess adherence to and toxicity of long-term aspirin use, and create a longitudinal biospecimen and epidemiologic data repository. A cohort of 3,000 subjects with node-positive stage II or III breast cancer will be recruited from Brigham and Women's Hospital, Dana-Farber Cancer Center, and the Alliance for Clinical Trials in Oncology. The trial is expected to open in Spring 2016.

Mechanisms and Treatment of Oligometastases

Andy Minn

Dr. Minn received an FY08 Era of Hope Scholar Award to investigate how previously identified gene programs promote metastasis or treatment resistance, how these programs are regulated, and how oligometastases can be effectively treated. Based on this pre-clinical work, Dr. Minn and colleagues have opened a Phase I clinical trial with assistance from Merck that examines radiation to a metastatic lesion in combination with the immune checkpoint inhibitor pembrolizumab (PD-1 inhibitor) for patients with metastatic cancers for which anti-PD-1 therapy has failed, or for patients who have progressed after at least one regimen of systemic therapy (<https://clinicaltrials.gov/ct2/show/NCT02303990>). A second Phase I trial will soon open to test radiation in combination with dual immune checkpoint blockade using tremelimumab (anti-CTLA-4) and MED14736 (anti-PDL1) for patients with breast, melanoma, or pancreatic cancer.

Products in Clinical Trials and Development

BRAIN METASTASIS

Studies Directed Toward the Eradication of Brain Metastasis of Breast Cancer

Patricia Steeg

Brain metastases of breast cancer are increasing in incidence and are devastating in terms of neurocognitive complications and mortality. With support from a BCRP Center of Excellence award, Dr. Steeg formed the first group to examine this topic in a comprehensive multi-disciplinary manner. The goals are to (1) develop infrastructure to research this disease, including tumor collections and mouse model systems; (2) investigate the contribution of the blood-brain barrier in the uptake of drugs into brain metastases, and to explore new methods to accelerate this process; (3) identify molecular alterations correlated with brain metastasis and determine their functional contributions to the process; (4) test drugs for the prevention and treatment of brain metastases, alone and in combination with radiation; (5) conduct a Phase I presurgical trial to determine drug uptake in human brain metastases; and (6) communicate the findings to the public. A clinical trial based on the group's preclinical work with temozolomide, pazopanib, and possibly lapatinib to identify the drug with the best secondary prevention signal is under initial trial design at the National Cancer Institute.

DIAGNOSTICS

MenaCalc™

John Condeelis Jeanine Pignatelli

Breast cancer cells enter the blood stream at sites called TMEM and spread elsewhere in the body. TMEM sites are correlated with

the expression patterns of Mena protein isoforms, specifically high levels of MenaINV and low levels of Mena11a (MenaCalc). In a prospective clinical trial supported by BCRP, Drs. Condeelis and Pignatelli demonstrated that the MenaCalc score in fine needle biopsies predicted the TMEM score (i.e. a high number of TMEM sites) in resected primary breast tumor tissue. In addition, two retrospective trials showed that the MenaCalc score can be used as a prognostic marker for distant recurrence. This test can also be run on fine needle biopsies from patients at the time of office visit, making it ideal for selecting patients who will respond to TMEM-directed inhibitors to prevent metastatic breast cancer. MenaCalc™ has been licensed to MetaStat, Inc. where it has been clinically validated for use in breast cancer treatment decision making.

Intraductal Techniques

Susan Love

Most breast tumors appear to arise in the cells lining the milk ducts of the breast. With BCRP funding, Dr. Love looked for early evidence of cancer in the ducts by modifying an endoscope to enter and examine milk ducts through their openings at the nipple. Her research increased understanding of duct architecture, most importantly in providing evidence that early-stage breast cancer is confined to a single duct system. She laid the groundwork for the development of increasingly sophisticated and miniaturized endoscopes that allow the retrieval of cell samples for analysis, the precise location of intraductal lesions for excision, and the potential to deliver breast cancer therapy intraductally.

Molecular Breast Imaging

Carrie Hruska

Molecular breast imaging (MBI) is a nuclear medicine technique that uses high-resolution dual-head gamma cameras to detect the

functional uptake of a radiotracer in the breast. Following a Mayo Clinic study that demonstrated MBI to be more sensitive than conventional mammograms for detecting breast cancer in women with dense breast tissue, the BCRP funded work to evaluate the concordance of MBI with magnetic resonance imaging of the breast, to investigate the effects of fluctuating hormonal levels on the appearance of MBI, and to develop important quantitative analysis software for MBI. Later clinical trials show promise of MBI as a tool for monitoring patients' response to neoadjuvant chemotherapy. MBI is an FDA-approved, commercially available technology that is growing in use both in the United States and internationally.

Advancements in the camera technology and patient preparation procedures now allow MBI to be performed at low radiation doses that are acceptable for use in screening. Recent work has demonstrated MBI to offer improved cancer detection and low false-positive rates when used for supplemental screening in women with mammographically dense breasts.

Skp2 Oncogene

Michele Pagano

Skp2 and p27 are genes involved in the regulation of the cell cycle. The BCRP supported the establishment of Skp2 as an oncogene that is overexpressed in human breast tumors. High Skp2 expression correlating with destabilization of p27 was found to be associated with poor prognosis in breast cancer patients. These findings contributed to the practice of Skp2/p27 immunohistochemical analysis as a prognostic test performed in clinical pathology laboratories. Furthermore, in collaboration with Dr. Peng Lee at the NYU Medical Center, Dr. Pagano is working to develop the expression of Skp2 by immunohistochemistry as an *in vivo* diagnostic test for breast cancer.

Making an Impact

RESEARCH RESOURCES

Expression Arrest™ shRNA Libraries

Gregory Hannon
Stephen Elledge

RNA interference (RNAi) is a cellular system that controls which genes are active or silent. The selective effect of RNAi on specific gene expression makes it a valuable research tool. Small hairpin RNAs (shRNAs) are one of the gene silencing mechanisms of RNAi. The BCRP supported the development of whole genome shRNA libraries that target over 30,000 genes. This commercially-available research tool provides researchers with ready-to-use, rapid RNAi screens for the entire human and mouse genomes to study gene regulation and identify new therapeutic targets in many diseases and conditions, including cancer.

Three-Dimensional Culture Systems

Mina Bissell

The BCRP supported the development of 3D culture systems that have made important contributions in understanding the tissue microenvironment and how interactions between epithelial cells and the extracellular matrix control cancer development. As surrogates for in vivo studies, 3D culture models have enabled the elucidation of oncogenic and other cell-signaling pathways that are controlled by cell-matrix interactions. 3D culture models are currently utilized across academic laboratories and by drug companies as screening assays to test for therapeutic response.

Novel Models for Breast Tumor Growth and Metastasis

Alana Welm

Orthotopic breast tumor models can replicate the diversity of human breast cancer through patient-centric models for tumor growth, metastasis, drug efficacy, and prognosis. These models exceed the current standard of cell line xenograft models. Funding from BCRP has supported the generation of publicly available tumor graft mouse models. Since some of the most promising therapies affect the immune response and need to be tested in pre-clinical models before entering trials, work is now under way to develop immune-competent mouse models representing each subtype of human breast cancer to predict response to therapy. Researchers interested in obtaining the models should refer to <http://www.ncbi.nlm.nih.gov/pubmed/22019887>. Additional unpublished models are also available by contacting Dr. Welm.

THERAPEUTICS

Herceptin®

Dennis Slamon

Herceptin (trastuzumab) is a monoclonal antibody that targets the HER2 receptor. HER2+ breast cancer accounts for approximately 25% of all breast cancers. The BCRP was instrumental in supporting the preliminary in vitro and in vivo studies needed to test the efficacy of Herceptin, which later led to clinical trials and commercialization. Herceptin revolutionized breast cancer treatment and the field of targeted therapeutics. Herceptin is now part of standard-of-care treatment regimens for HER2+ early-stage and metastatic breast cancers.

ATLAS Clinical Trial

Richard Peto

BCRP funds supported initiation of the Phase 3 clinical trial ATLAS (Adjuvant Tamoxifen Longer Against Shorter), the largest breast cancer treatment trial ever undertaken. Adjuvant tamoxifen is the first-line treatment for ER+ breast cancer in premenopausal women.

The ATLAS trial examined whether 10 years of tamoxifen confers greater benefit than 5 years of tamoxifen. Results of the trial indicated that the risk of recurrence or death from breast cancer was reduced in women who took tamoxifen for 10 years versus 5 years.

DIAGNOSTICS

Sentinel Lymph Node Biopsy

Douglas Reintgen
Kathryn Verbanac

The previous standard for detecting breast cancer invasion into the lymph nodes was to remove the majority of axillary lymph nodes and search for cancer cells. In a significant proportion of women, this causes lymphedema of the arm, a condition that compromises functionality and quality of life.



Beth Emery, Alamos Breast Cancer Foundation

"I am alive today because of a research grant funded by the Department of Defense Breast Cancer

Research Program (DoD BCRP) to Dr. Dennis Slamon. That groundbreaking research led to the development of my personal miracle drug: Herceptin."

Products Currently in Use by Patients and Researchers

In sentinel lymph node biopsy, lymph node removal is limited to only the first few nodes that receive lymph drainage from the breast. This diagnostic/prognostic technique enables clinicians to determine tumor staging, and if more extensive lymph node surgery is necessary. The BCRP provided funding for multicenter clinical trials to validate lymph node mapping and sentinel lymph node biopsy as an accurate method to predict the presence of disease in the axillary lymph nodes.

Digital Mammography and Breast Tomosynthesis

Laurie Fajardo
Daniel Kopans

Digital mammography allows for an expanded detection range of X-ray signals than standard film mammography. The BCRP provided support to optimize technology and to conduct a multicenter clinical validation of digital mammography. The study demonstrated that digital mammography is superior to film mammography in detecting breast cancer in women with dense breast tissue, leading to a change in clinical practice. The BCRP also supported the development and clinical evaluation of digital breast tomosynthesis. This 3D digital mammography tool offers an additional 3D view to capture images for improved sensitivity. A tomosynthesis system is now FDA approved and commercialized for clinical use.

MetaSite Breast™

John Condeelis
Allison Harney

TMEM sites are composed of a stable interaction between three specific cells: an endothelial cell, a tumor-associated macrophage, and a MenaCalc-positive tumor cell (expressing high levels of the MenaINV protein isoform and low levels of the Mena11a isoform). In work funded by BCRP, Drs. Condeelis and Harney found that TMEM sites are the only doorway for tumor cell entry into blood vessels. In other studies, TMEM were found in all primary and secondary sites, and in all stages of breast cancer progression, making TMEM the common dissemination marker in all breast tumors and their associated distant sites. In collaboration with MetaStat, Inc., Dr. Condeelis and colleagues clinically validated the MetaSite Breast test, which measures TMEM levels to predict the metastatic potential of the primary tumor. MetaSite Breast has been licensed to MetaStat, Inc. and is CLIA (Clinical Laboratory Improvement Amendments) certified and publically available.

PATIENT RESOURCES AND REGISTRIES

BreastCancerTrials.org

Laura Esserman

Breast cancer patients can benefit from objective information about clinical trials. The process of identifying an appropriate clinical

trial by performing independent research is challenging. BCRP funding contributed to the development of an online resource (BreastCancerTrials.org) that educates patients about breast cancer clinical trials and matches them with appropriate trials.

Carolina Mammography Registry

Bonnie Yankaskas

The Carolina Mammography Registry was first funded by a BCRP award to create the infrastructure for a population-based mammography registry in North Carolina, focusing on a largely rural population. The registry became a member site of the National Cancer Institute Breast Cancer Surveillance Consortium, a national registry that provides a resource for researchers to study mammography screening on a national level.

Margaret Dyson Family Risk Assessment Program

Mary Daly

The BCRP supported the establishment of a high-risk breast cancer registry to gather genetic and environmental risk information about women with familial breast cancer. This registry evolved into the Margaret Dyson Family Risk Assessment Program for individuals at risk for breast or ovarian cancers. This program, which serves Philadelphia and its surrounding communities,

Making an Impact

provides a range of risk assessment, screening, and preventive services.

BrainMetsBC.org

Patricia Steeg

Breast cancer advocates on this team-based award led the efforts to develop an online resource (BrainMetsBC.org) that provides the latest information about brain metastases. The website, which is available in English and Spanish, includes updates on current research, treatments, and clinical trials, as well as personal experiences written by patients.

RISK ASSESSMENT

BRCA2 617delT Mutation

David Goldgar Susan Neuhausen

Breast cancer and ovarian cancer risk is greater in individuals with mutations in the BRCA1 and BRCA2 tumor suppressor genes. The likelihood of BRCA1 or BRCA2 mutations is higher in certain populations, including individuals of Ashkenazi Jewish descent. BCRP funding contributed to the discovery of the BRCA2 617delT mutation, one of the three founder BRCA1/2 mutations that occur in Ashkenazi Jews. The BRCA2 617delT mutation is now part of a commercialized test for BRCA1/BRCA2 gene mutations in this risk group.

OncoVue®

Eldon Jupe

Risk association studies funded by the BCRP formed the foundation for a breast cancer risk assessment test. Single nucleotide polymorphisms (SNPs) are DNA

sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. OncoVue is the first genetic-based breast cancer risk test that incorporates a woman's SNPs with personal history to estimate her risk for breast cancer. This test can identify high-risk patients and enable clinicians to individualize breast cancer screening and monitoring. OncoVue is commercially available and is currently offered at more than 30 breast care centers in the United States.

PTEN

Michael Wigler

BCRP funding contributed to the original discovery of the PTEN (phosphatase and tensin homolog) gene. In normal cells, PTEN functions as a tumor suppressor protein that helps regulate cell division and growth. PTEN is one of the most frequently mutated genes in several cancers, and PTEN mutations have been shown to be predictive of aggressive cancer. PTEN mutations also occur in a group of genetic syndromes that are characterized by malignant and benign tumors. A PTEN test is commercially available to confirm PTEN mutations for clinical and prenatal diagnoses and identification of at-risk family members.

PALB2 Mutations

Bing Xia

BCRP funding contributed to the discovery of PALB2, a BRCA2 binding protein. PALB2 and BRCA2 work together to mend broken strands of DNA, which helps to maintain the rate of cell growth. While BRCA1 and BRCA2 gene mutations are high-risk factors for breast cancer, these mutations do not account for all familial breast cancers. Identification of mutations in the PALB2 gene indicates an approximate twofold increase in breast cancer susceptibility due to its inability

to interact with BRCA2. A commercialized PALB2 genetic test is available for those with familial breast cancer.

BROCA Cancer Risk Panel

Tomas Walsh Mary-Claire King

An estimated 70% of families with multiple cases of breast cancers have no known gene mutations that increase their risk to the disease. Dr. Walsh in collaboration with Dr. King identified and validated rare mutations termed copy-number variants, which led to development of a comprehensive test named "BROCA" that enables assessment of all known breast cancer genes and all mutation types in a single assay. The BROCA test is currently available through the University of Washington by physician request.

PROGNOSTICS

Breast Cancer IndexSM

Dennis Sgroi

Women with ER+ breast cancer have an increased risk of relapse many years after their initial diagnosis. To identify women with an increased risk of disease recurrence, Dr. Sgroi validated biomarkers that correlated with relapse-free survival and tumor grade, leading to a risk assessment test termed the Breast Cancer Index (BCI). BCI, which is now commercially available through bioTheranostics, provides a quantitative assessment of the likelihood of early and late recurrence, as well as benefit from extended endocrine therapy.

Research on the Horizon

There were 102 awards funded in FY14. For additional information on FY14 awards, go to <http://cdmrp.army.mil/search.aspx>.

Yumei Huang, CellMosaic, Inc.

FY14 Breakthrough Award Level 1

Precision AqT-Warhead to treat breast cancer

Mark Labarge, Lawrence Berkeley National Laboratory

FY14 Era of Hope Scholar Award

Metastable tissue states that result from aging after susceptibility to breast cancer

Julie Sterling and Craig Duvall, Vanderbilt University

FY14 Breakthrough Award Level 2

Targeted drug nanocarriers for inhibiting bone metastatic cancer

Lali Medina-Kauwe, Cedars-Sinai Medical Center

FY14 Breakthrough Award Level 2

Nanobiologic targeting of metastatic breast tumors

Cyrus Ghajar, Fred Hutchinson Cancer Research Center

FY14 Era of Hope Scholar Award

Targeting tumor dormancy to prevent metastatic relapse

Chun-Ju Chang, Purdue University

FY14 Breakthrough Award Level 1

Targeting cell polarity machinery to exhaust breast cancer stem cells

Keith Knutson and Edith Perez, Mayo Clinic and Foundation Jacksonville

FY14 Breakthrough Award Level 4 – Clinical Trial

Folate receptor alpha vaccines for preventing progression of TNBC following first-line conventional therapy

Leif Ellisen, Massachusetts General Hospital

FY14 Breakthrough Award Level 1

Defining high-risk precursor signaling to advance breast cancer risk assessment and prevention

Saraswati Sukumar, Johns Hopkins University

FY14 Breakthrough Award Level 2

Identification of early epigenetic changes in African-American progenitor cells and their role in breast cancer initiation

Kevin Burgess, Texas A&M University Zheng Li, Methodist Hospital Research Institute

FY14 Breakthrough Award Level 2

Theranostics targeting metastatic breast cancer

Todd Giorgio, Vanderbilt University

FY14 Breakthrough Award Level 1

Systematic characterization of olfactory receptor expression and function in breast cancer

Eric Winer, Dana Farber Cancer Institute

Michelle Holmes, Brigham and Women's Hospital

FY14 Breakthrough Award Level 4 – Clinical Trial

Randomized trial of aspirin as adjuvant therapy for node-positive breast cancer

Joyce Slingerland, University of Miami Coral Gables

FY14 Breakthrough Award Level 2

Epigenetic targeting of the cancer stem cell hierarchy in triple-negative breast cancer

Darren Roblyer, Trustees of Boston University

FY14 Era of Hope Scholar Award

Development of less toxic treatment strategies for metastatic and drug-resistant breast cancer using noninvasive optical monitoring

Edgar Engleman, Stanford University

FY14 Breakthrough Award Level 2

Alloantibodies in the treatment of breast cancer

Qin Yan, Yale University

FY14 Era of Hope Scholar Award

Epigenetic mechanisms of breast cancer metastasis

Harikrishna Nakshatri, Indiana University at Indianapolis

FY14 Breakthrough Award Level 2

The impact of ethnicity-dependent differences in breast epithelial hierarchy on tumor incidence and characteristics

Siyuan Zhang, University of Notre Dame

FY14 Breakthrough Award Level 1

Targeting neuronal-like metabolism of metastatic tumor cells as a novel therapy for breast cancer brain metastasis

Sharon Pitteri, Stanford University Ingrid Oakley-Girvan, Cancer Prevention Institute of California

FY14 Breakthrough Award Level 1 – Parterning PI Option

Distinguishing benign from malignant breast lesions: Does breast interstitial fluid hold the answers?

Venkatram Mereddy and Lester Drewes, University of Minnesota Twin Cities

FY14 Breakthrough Award Level 2

Development of novel drugs for high-risk triple-negative breast cancer treatment

William Gillanders, Washington University

FY14 Breakthrough Award Level 3 – Clinical Trial

Phase IB clinical trial of a candidate breast cancer prevention vaccine



For more information, please visit

<http://cdmrp.army.mil>

or contact us at:

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