



**CDMRP**   
Department of Defense

# Breast Cancer Research Program



*U.S. Army Medical Research and Materiel Command*



## Congressionally Directed Medical Research Programs

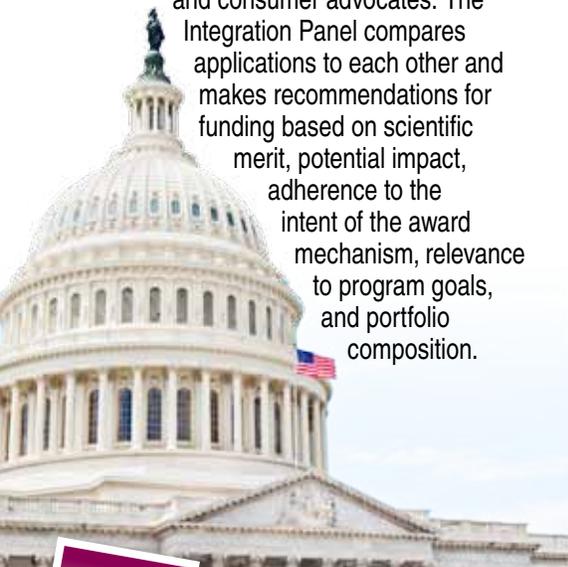
### HISTORY

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 \$8.2 billion (B) in appropriations from its inception through fiscal year 2014 (FY14). 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. 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 Integration Panel, which is composed of leading scientists, clinicians, and consumer advocates. The Integration 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 advocates. Their continued efforts, in concert with the program's accomplishments, have resulted in more than \$3.0B in congressional appropriations through FY14. 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.

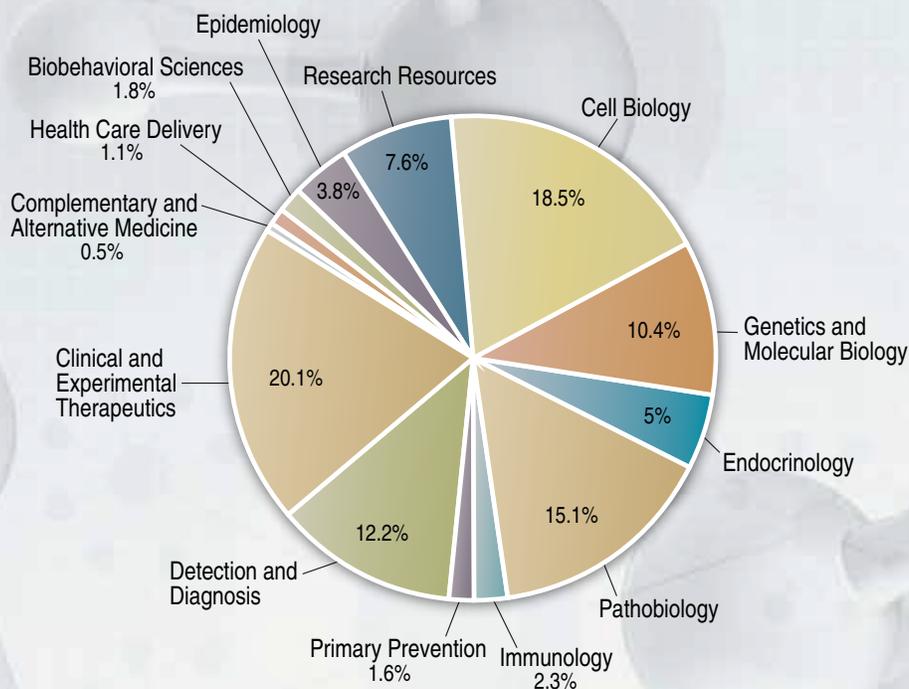


# BCRP Overarching Challenges

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 what makes the breast susceptible to cancer development
- Determine why some, but not all, women get breast cancer
- Distinguish aggressive breast cancer from indolent cancers
- Conquer the problems of overdiagnosis and overtreatment
- Identify what drives breast cancer growth; determine how to stop it
- Identify why some breast cancers become life-threatening metastases
- 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 interventions that have life-threatening toxicities with ones that are safe and effective
- Eliminate the mortality associated with metastatic breast cancer

FY92–FY13 BCRP Portfolio



## The Breast Cancer Landscape

The BCRP has prepared an overview 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% to 30% of women diagnosed with invasive breast cancer will have a recurrence.
- The rate of metastatic breast cancer at initial diagnosis in the United States 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://cdmrp.army.mil/bcrp/pdfs/bc\\_landscape.pdf](http://cdmrp.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 consumer advocates. 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.



“Ending breast cancer is our vision. Serving on the Integration Panel of the DoD BCRP is an extraordinary opportunity to collaborate with visionary scientists and consumer advocates who are focused in finding and rewarding the truly extraordinary scientific applications which will make a difference for the many who are affected by breast cancer every day.”

**Joy Simha**  
**FY15 IP Chair**



“The BCRP provides a great opportunity to truly advocate for breast cancer eradication and to further educate oneself and one's organization on the breast cancer landscape. The consumer advocate's perspective is truly valued.”

**Debra Austin**  
**Sisters Network Dallas**

# Scientists and consumer advocates working together to end breast cancer

## Radiation-Induced Vaccination to Breast Cancer

**Silvia Formenti, New York University School of Medicine**

**Sandra Demaria, New York University School of Medicine**

**William McBride, University of California, Los Angeles**

**Consumer Advocates: Amy Bonoff, Ginny Mason, Jane Permutter, and Michelle Rakoff**

Almost one-third of all patients diagnosed with invasive breast cancer will eventually succumb to metastatic disease. Drs. Formenti, Demaria, and McBride have brought their research teams together in an FY10 Multi-Team Award to test the novel hypothesis that a tumor-specific protective immunity can be induced in breast cancer patients by irradiating a local metastatic site in the presence of transforming growth factor beta (TGF $\beta$ ) blockade. The neutralizing antibody Fresolimumab will be used to block TGF $\beta$  and activate the immune system, while the local radiotherapy will generate a burst of immunogenic and tumor-specific antigens. This combinatorial approach is designed to engage the patient's own immune system in providing systemic control of tumor growth beyond just the targeted metastatic site. Preliminary studies combining radiation with carboplatin or paclitaxel, two combinations chosen to mimic regimens already being used in clinical trials of early stage triple negative breast cancer, showed that chemoradiation produced a dose-dependent induction of immunogenic cell death. Obtained preclinical data demonstrate that TGF $\beta$  is a critical regulator of the ability of radiation to generate a tumor vaccine and that TGF $\beta$  blockade elicits immune responses that are effective against the irradiated tumor and non-irradiated metastases. The team expects to complete a clinical trial in December 2015 assessing the safety of coupling Fresolimumab and local radiotherapy and to see if the combination can generate favorable antitumor immunity and tumor regression in women with metastatic breast cancer. If successful, this approach would create a new therapeutic strategy of individualized vaccination and enable long-term management of metastatic disease.

Four breast cancer survivors have been involved in this intriguing multi-team award, some during development of the proposal and all since the start of the work. The advocates have been excited about the possibilities of this new approach to treating metastatic breast cancer and have learned a great deal by following both the preclinical and clinical progress.



“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 Integration Panel.”

**Silvia Formenti**  
**FY16 IP Chair-Elect**

# Making Breakthroughs in Understanding Breast Cancer

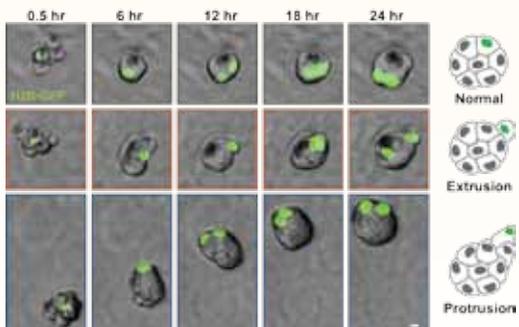


## Deconstructing the Cellular Interactions within the Breast Cancer Microenvironment

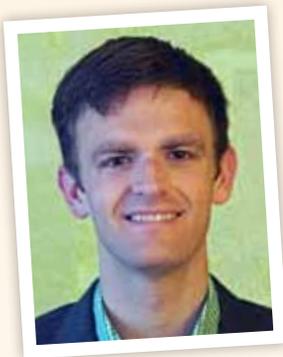
Zev Gartner, University of California, San Francisco

The human body is made up of trillions of cells that organize within the extracellular matrix, a web of molecules filling the space between cells, to form the tissues and organs required to sustain life. However, scientists do not yet understand the molecular and physical rules that determine how individual cells organize within tissues and organs. What is known is that the behavior of epithelial cells is strongly influenced by signals from the tissue microenvironment.

With the support of an FY09 BCRP Era of Hope Scholar Award, Dr. Zev Gartner and his team are using a bottom-up approach to directly synthesize three-dimensional (3D) living tissues composed of multiple cell types to identify the contextual cues that bring about the early stages of breast cancer. They are particularly interested in the role of cell-to-cell variability, or heterogeneity, as a driver of malignant behavior in breast cancer. The team focused on the role of Ras, a small GTPase that affects cell behaviors such as cell motility, survival, and proliferation. They used MCF10A cells, a noninvasive type of breast cancer epithelial cell, but expressing a Ras mutant that only moderately increases Ras activity compared to normal Ras. When single members of these MCF10ARas cells were placed within a group of wild-type MCF10A cells to create 3D mosaic aggregates, the MCF10ARas cells consistently displayed basal cell extrusions and motile multicellular protrusions, morphological hallmarks of invasive cancers. They also observed that a single MCF10ARas cell was capable of driving the same motility-promoting changes in the wild-type cells that surrounded it. This was not a surprise, as previous studies suggested that multicellular protrusions led by single cells may play a leading role during tumor invasion and progression in cancer. What was surprising, however, was that these invasive changes were due to only modest differences between MCF10ARas cells and their wild-type neighbors. Rather than absolute levels of Ras activation, multicellular protrusions were led by subtle variability in Ras activation between neighboring cells. More protrusions resulted from this heterogeneous aggregate of cells than from aggregates in which all cells expressed the mutant Ras.



These findings show that the cell-to-cell variability in Ras activation, not simply the absolute level, can affect the global behavior of a tissue. In other experiments, Dr. Gartner and colleagues showed that the morphological protrusions of MCF10ARas cells appeared to depend on both MAPK and PI3K pathway signaling. Future studies aimed at uncovering the mechanisms that maintain or suppress cell-to-cell variability in Ras, and perhaps other signaling pathways in different biological contexts, will lead to greater insight into how tissue heterogeneity impacts the invasive potential of whole cell populations.



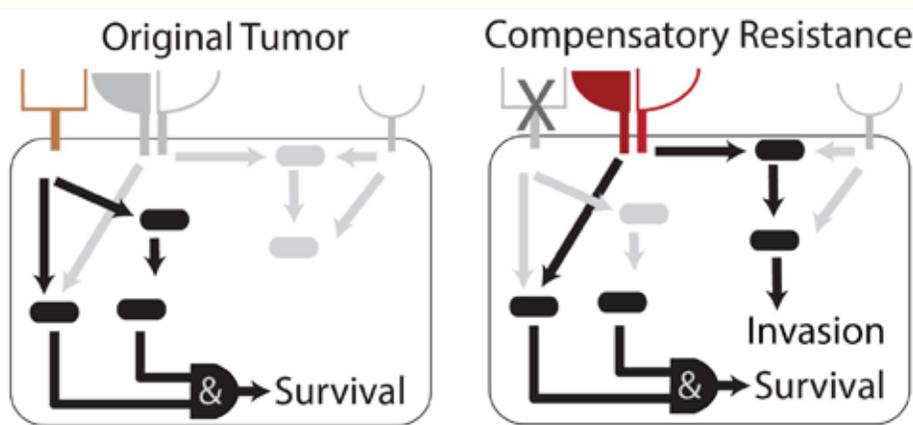
## Determining the Mechanisms Underlying Transformation of Breast Cancer Cells to Migratory and Invasive Behavior

Aaron Meyer, Massachusetts Institute of Technology

Receptor tyrosine kinases (RTKs) are known to be abnormally abundant and dysregulated in cancer cells and have thus been the focus of many targeted therapies. The benefits of RTK inhibitors, however, have been limited due to the development of resistance that renders treatment ineffective. While many studies have recognized that cells resistant to therapy often have the greatest capacity to form metastases, the underlying mechanisms of resistance and subsequent spread are not well understood. Supported by an FY10 BCRP Predoctoral Fellowship Award and mentored by Dr. Douglas Lauffenburger, Dr. Aaron Meyer attempted to identify the link between tumor resistance to drugs targeting RTKs and metastasis.

Using an innovative data analysis strategy designed to minimize false associations, Dr. Meyer and colleagues interrogated a large data set in order to identify genes that may be involved in resistance to RTK-targeted therapeutics. They discovered that expression of another RTK, AXL, is an exceptionally strong predictor of resistance to ErbB receptor family-targeted inhibitors. It had already been shown in previous studies that AXL is widely expressed in cancers and is associated with poor patient outcome.

Following up the computer-based finding, the research team sought to elucidate AXL's role in drug resistance. Using triple-negative breast cancer (TNBC) cell lines, Dr. Meyer and colleagues observed that an AXL receptor inhibitor caused more cell death than the ErbB inhibitor, erlotinib, a drug already being used to treat other cancers. This sensitivity to AXL corresponded to an interaction between the two receptors whereby activation of the ErbB receptor caused transactivation of AXL receptors. Transactivation of AXL by the associated ErbB receptor resulted in the activation of downstream cell signaling pathways in a way not typically activated by ErbB itself that contributed to the migration of TNBC cells. The researchers were able to verify that AXL and ErbB molecules associated in clusters on the tumor cell surface. If future experiments show that this clustering is required for resistance, then drugs that target and disrupt this clustering may bolster RTK-targeted therapies. Furthermore, this synergistic interaction between the RTKs limits the use of ligand-blocking therapies and suggests that ErbB inhibitor treatment should not be withdrawn after the onset of resistance because transactivation-mediated signaling could drive tumors to spread.



Many cancers are driven by the dysregulation of RTKs, which provide signals necessary for cancer cell survival (left). While targeted therapies have shown efficacy in certain breast cancers, ultimate survival benefits are limited by the onset of resistance, which often occurs by dysregulation of another RTK (right). An important finding of this work is that this process can adjust the set of activated signals in cancer cells, promoting migration and invasion.



## Dissecting the Mechanisms of Collective Breast Cancer Cell Invasion

Kevin Cheung, Johns Hopkins University

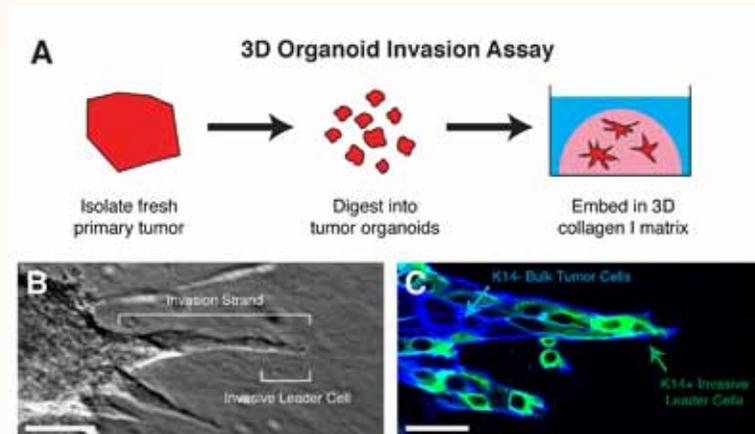
Invasion of tumor cells from the primary tumor to surrounding tissue is a fundamental step in metastasis. A clearer understanding of the cellular and molecular factors that underlie invasion could lead to breakthroughs in preventing invasion and, consequently, metastasis. The difficulty of monitoring tumor progression in vivo, however, poses a major challenge to elucidating the changes cancer cells undergo to execute invasion.

Supported by an FY11 BCRP Postdoctoral Fellowship Award and co-mentored by Drs. Andrew Ewald and Sara Sukumar, Principal Investigator Dr. Kevin Cheung developed an assay to identify the most invasive cells within a primary tumor and the unique molecular markers that could be exploited to target these cells and curb their invasive and metastatic potential.

Dr. Cheung and colleagues developed a 3D organoid assay that models the architecture of breast tissue in greater detail than the more common 2D cultures. The assay utilizes “tumor organoids” – collections of cohesive tumor cells that preserve much of the cellular heterogeneity found in primary tumors – cultured within 3D collagen I gels, which models the tissue microenvironment surrounding invasive breast cancers. Using organoids generated from a breast cancer mouse model, the team observed that tumor organoids extended multicellular strands of cancer cells into the collagen I. Because the cells leading these invasive strands were highly protrusive and migratory, they were termed “invasive leader cells.”

Molecular profiling of the leader cells showed enhanced expression of several basal epithelial markers including the transcription factor p63 and the intermediate filament protein cytokeratin-14 (K14). Tumor organoids triggered p63 and K14 expression before initiation of collective, multicellular invasion, indicating that these genes are components of a dynamically regulated gene program. Remarkably, while only a minority of cells within tumors expressed these genes, knockdown of basal epithelial gene expression was sufficient to block collective invasion in 3D cultures and in vivo.

Dr. Cheung and colleagues observed K14+ leader cells across major breast cancer subtypes, leading them to suggest that a shared molecular program for collective invasion may underlie the most common breast cancers. These data show that the heterogeneous interactions between tumor cell subpopulations are critical to collective invasion and that targeting the key drivers of invasion can significantly disrupt metastatic progression.



A. Tumor organoids are isolated from fresh primary breast tumors through a combination of mechanical disruption and enzymatic digestion. When tumor organoids are embedded in 3D collagen I matrix, they become invasive. B. Tumor organoids extend multiple invasive strands into the collagen matrix led by protrusive “invasive leader cells.” C. Invasive leader cells are molecularly distinct from the bulk tumor cells and express markers of basal differentiation, such as keratin-14 (K14). Scale bar is 50 microns.



## Prevalence and Characterization of BRCA2 in Male Breast Cancer Cases

**Susan Neuhausen, City of Hope Beckman Research Institute**

Male breast cancer (MBC) is an understudied type of breast cancer, and its causes are poorly understood. BRCA2 mutations, known to increase the risk of breast cancer in women, occur in approximately 10% of MBC cases. Dr. Susan Neuhausen and colleagues sought to characterize the role of BRCA2 mutations in MBC. With funding from the BCRP, they collected 115 MBC cases from around the United States and screened the men for mutations in the BRCA2 gene, and then considered these findings in the context of family and clinical history.

The team identified BRCA2 mutations in 18 of the 115 (16%) MBC cases and found that men with BRCA2 mutations were more likely to have higher grade tumors than those who did not. They also determined that out of the 18 patients with BRCA2 mutations, 4 men had a family history of breast cancer compared to 14 men without a family history of breast cancer, suggesting that family history is not as strong a predictor of BRCA2 mutations in males as it is in females. In addition, the majority (79%) of the 115 MBC cases was diagnosed as invasive ductal carcinoma and nearly 50% of these cases included a family history of breast cancer in at least one first- or second-degree relative. Since BRCA2 is a key component involved in DNA repair in cells, the researchers speculated that the increased frequency of high grade tumors in patients with BRCA2 mutations may be related to the defective BRCA2 protein and the resulting genomic instability in tumor cells. In a follow-up study, they also identified one known pathogenic and one missense mutation predicted to be pathogenic in PALB2. In addition, Dr. Neuhausen and colleagues conducted a genome-wide association study of MBC and found that a genetic variant in RAD51B, another key cellular component involved in DNA repair, was associated with a 50% increased risk for breast cancer. Dr. Neuhausen's findings suggest that genetic testing for BRCA2, and possibly PALB2, should be recommended for any diagnosed MBC case regardless of family history of breast cancer, particularly because DNA repair targeted therapies, such as poly (ADP-ribose) polymerase inhibitors, are in clinical development for patients with mutations in DNA repair pathways.





## Dissecting Breast Cancer Metastasis

**Joan Massagué, Memorial Sloan Kettering Cancer Center**

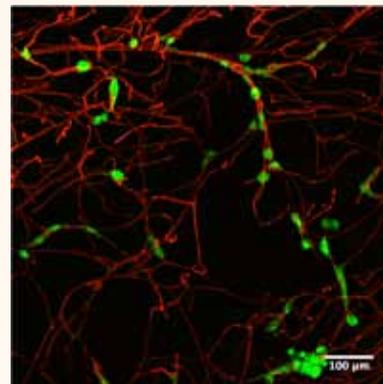
Latent metastasis of breast cancer (LMBC) is represented by a residual population of metastatic breast cancer cells that have infiltrated other organs within a patient but have adopted a low-proliferation state that may protect these cells from death. As these cells in their quiescent state retain the potential to form overt metastasis for years, they represent a latent and potentially devastating risk to patients. Unfortunately, little is known about the cancer cell functions, host tissue signals, and physical niches that promote the survival and fitness of cancer cells during LMBC. Targeting

LMBC with new drugs offers an untapped opportunity to prevent metastasis outgrowth. However, advances in understanding and treating this process have been hindered by the lack of a model system for LMBC.

Dr. Joan Massagué sought to address this unmet need by developing an LMBC model system. With funding from an FY11 BCRP Innovator Award, Dr. Massagué's team labeled breast cancer cells with GFP-luciferase to track these and another gene that enabled the researchers to dissect and isolate them from mouse tissue. The labeled breast cancer cells were injected into mice and followed for two months. In mice that did not develop detectable metastases, tissue was removed to isolate cells that were able to survive as micro-metastases without forming overt metastases. These latent metastatic cells were then grown in cell culture to demonstrate that they have the capacity to actively proliferate.

Dr. Massagué's colleagues then re-injected these latent metastatic cells into mice to verify that they retained the capacity to preserve a latent, low cell-cycling state for months after infiltrating organs. With this approach, he successfully isolated latent metastatic cells from breast and lung cancers.

In parallel with the isolation of these cells, Dr. Massagué also developed a sensitive assay to detect low abundance disseminated cancer cells in mouse tissues using qRT-PCR. Going forward, he plans to develop a similar model using immunocompetent mice so that the role of immunity in LMBC can be studied. The development and use of this experimental model developed by Dr. Massagué, in combination with the ability to detect and isolate LMBC, provides a foundation for developing new targeted therapies for LMBC to prevent breast cancer metastasis.



*Image credit: Danilo Macalinao*



“For more than 10 years, I and thousands of other breast cancer advocates have pushed beyond the belief that raising awareness leads to eradicating the disease. What will make a difference is participating in the establishment of research priorities, providing meaningful feedback during research proposal reviews, and working side-by-side with breast cancer investigators. The BCRP is an excellent venue for the advocacy community to take a leadership role in ending breast cancer as a life-threatening disease.”

**Sandi Spivey**  
**Living Beyond Breast Cancer**



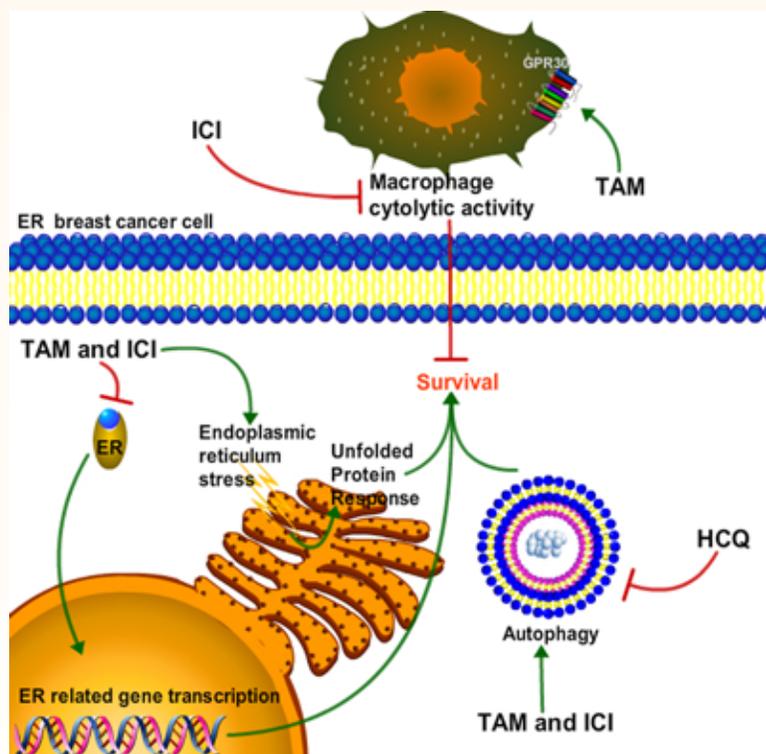
## Understanding the Molecular Mechanisms of Anti-Estrogen Resistance

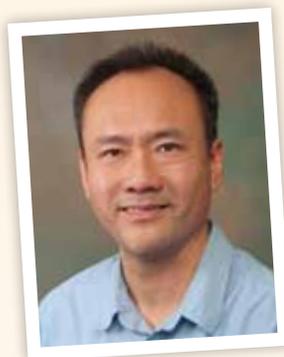
Katherine Cook, Georgetown University

About 70% of breast cancer cases are estrogen receptor positive (ER+), and the standard of care for these patients is endocrine therapy, which includes tamoxifen or fulvestrant. Up to 50% of breast cancer patients will develop resistance to these endocrine treatments due to either de novo or acquired resistance. Endocrine resistance is not well understood but must be addressed in the hopes of developing new therapeutic options for patients.

Dr. Katherine Cook, under the mentorship of Dr. Robert Clarke and with funding from an FY11 BCRP Postdoctoral Award, is studying why ER+ breast cancer becomes resistant to ER-targeted therapies such as tamoxifen or fulvestrant. She is investigating the possibility that autophagy – a biological mechanism that allows the cell to degrade unnecessary or damaged cellular components – may help the cancer cells survive the treatments. This process has been found to be increased in endocrine-resistant breast cancer cell lines. Studies in ER+ breast cancer cells have also shown that both tamoxifen and fulvestrant treatments can induce autophagy.

Dr. Cook tested the use of an autophagy inhibitor, hydroxychloroquine (HCQ), to convert endocrine-resistant cells to endocrine-sensitive cells. HCQ has been used for many years to treat malaria as well as other conditions. Dr. Cook found that treating tamoxifen-resistant breast cancer cell lines with HCQ resensitized the cells to tamoxifen. She also tested the use of HCQ with tamoxifen or fulvestrant against established endocrine-resistant tumors in an orthotopic mouse model. Interestingly, the combination of ICI 182, 780 (fulvestrant), and HCQ was less effective than HCQ alone at inhibiting tumor growth. However, the combination of tamoxifen and HCQ was able to restore anti-estrogen sensitivity and reduce tumor growth. Dr. Cook's research suggests the potential for clinical benefit for patients with ER+ breast cancer by combining HCQ with anti-estrogen therapy. An ongoing BCRP-funded clinical trial ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); NCT01023477) examining the effect of tamoxifen and HCQ in ER+ breast cancer, may provide further clinical evidence to support this new treatment modality.





## Elucidating the Mechanisms of Immune Dysfunction in Breast Cancer

**Peter P. Lee, City of Hope Beckman Research Institute**

Dysregulation of the immune system is an important consequence of cancer development and a major factor in the progression to metastatic disease. However, the specific mechanisms causing immune dysfunction in all cancers, including breast cancer, are poorly understood. Current immunotherapies for the treatment of breast cancer have had limited clinical success; thus, further understanding of the underlying mechanisms causing immune dysfunction could lead to the development of more effective treatment strategies. With support from an FY05 BCRP Era of Hope Scholar Award, Dr. Peter P. Lee has focused his research efforts toward understanding immune system dysregulation in breast cancer development and progression.

Dr. Lee utilized immune cell and tumor samples taken from three sites in breast cancer patients: tumor, peripheral blood, and tumor-draining lymph nodes. He analyzed these samples for immune cell-cancer cell interactions, including dysfunction in a specific immune cell molecular pathway called the interferon (IFN) signaling pathway, which is critical for immune cell activation. He found that IFN-induced signaling was reduced in the immune cells of breast cancer patients, at all clinical stages, in contrast with the immune cells of healthy individuals. Furthermore, when Dr. Lee investigated other molecular gene pathways known to interact with IFN signaling, he found defects, previously undescribed, in the immune proteins, or interleukins, of breast cancer patients. He then studied the dynamics between breast cancer and immune responses by comparing gene expression patterns from tumor-draining lymph nodes within the tumor site versus peripheral blood. Through this analysis, he identified different gene expression patterns between the two and observed an upregulation of gene signatures associated with tumor-promoting immune cells within the tumor site.

Taken together, these results show for the first time that immune cell regulation by IFN-induced gene signaling is deregulated in breast cancer patients. Dr. Lee received further funding from an FY11 BCRP Era of Hope Scholar Expansion Award and continues his work to elucidate the mechanisms of immune dysfunction in breast cancer. Additional understanding of these mechanisms may lead to novel strategies that can correct dysfunction and potentially enhance the success of current immunotherapeutic strategies for breast cancer.



# Pursuing Novel Approaches in Diagnosis and Treatment



## Nanotechnology-Enabled Optical Imaging of Breast Cancer

**Rebekah Drezek, Rice University**

Breast cancer patients who elect to have breast-conserving surgery face the possibility of an incomplete resection that leaves behind residual cancer cells and an increased risk of recurrence. Today, only specialized centers have the ability to evaluate the resected tissue for these residual cells while the patient remains in the operating room. In most cases, patients must wait for pathologic analysis of the tissue and then, if necessary, return for additional surgeries. A fast and accurate way to delineate tissue tumor margins, in real-time during surgery, would reduce risk for recurrence as well as improve patient care.

Supported by an FY06 BCRP Era of Hope Scholar Award, Dr. Rebekah Drezek developed an imaging system designed to rapidly and accurately delineate tumor margins without the need for tissue sectioning and immunohistochemical processing commonly employed following tumor resection. To identify tumor cells at the margins, silica-gold nanoshells were conjugated to antibodies targeting human epidermal growth factor receptor 2 (HER2), a protein overexpressed in 20% to 25% of all breast cancers. These HER2-nanoshells function as contrast agents that, when bound to HER2 receptors found on the surface of breast cancer cells, are bright enough to be visualized with a standard stereomicroscope. Dr. Drezek and colleagues found that treating human breast tissue with HER2-nanoshells for just five minutes was sufficient to detect the same demarcation of HER2 receptors that resulted from conventional immunohistochemical labeling.

In devising their system, Dr. Drezek and colleagues built upon recent advances in optical imaging that utilize the endogenous fluorescence of cancerous tissue for screening. The system includes a Canon digital single lens reflex camera and a lens, a set of excitation light-emitting diodes (LEDs) that excite at two different wavelengths, and several filters that allow the camera to detect light of different wavelengths. Only one excitation LED is needed to visualize the HER2-nanoshells. The second excitation LED was built into the system with the intention of adding an additional molecular marker of breast cancer. Using off-the-shelf components which can, together, fit inside a standard backpack, the device is both low cost and portable, fostering its use in the clinic. The accessibility of this innovative imaging system, combined with the fast action and specificity of HER2-nanoshells, could provide a valuable supplement to current diagnostic methods to reduce time and risk that result from inadequate tissue removal.



### **Novel Methylated Biomarkers and a Robust Assay to Detect Circulating Tumor DNA in Metastatic Breast Cancer**

**Saraswati Sukumar, Johns Hopkins University**

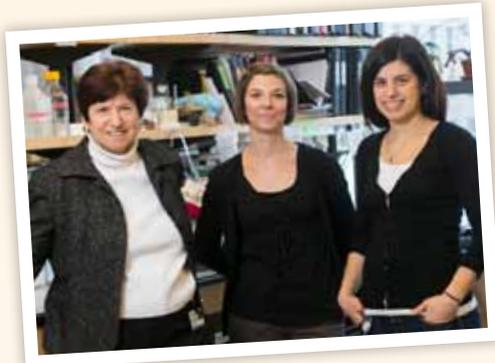
Mutations to the DNA sequence have long been studied for their role in turning a normal cell into a tumor cell. However, it has become clear that other alterations to the genome, independent of DNA sequence, play at least an equally important role in cancer development. Dr. Saraswati Sukumar and her colleagues have exploited one of these sequence-independent alterations to create a test that can detect metastatic breast cancer with an efficiency better than any test currently used. The test, she predicts, will also monitor a patient's response to treatment far more quickly than conventional tests, and it may allow doctors to adjust treatments—minimizing patient exposure to ineffective treatments. Amazingly, the test, developed by Dr. Sukumar with support from an FY03 BCRP Center of Excellence Award, detects breast cancer by sampling the patient's blood.

The key alteration is DNA methylation. Not all genes are active all the time, and one way a gene can be silenced is through the attachment of methyl molecules to the gene's promoter region – the part of a gene that controls where and to what extent a gene is activated. As researchers are now finding out, there are many ways in which this otherwise normal method of gene repression can tip the balance from a gene network that functions normally to one that is cancerous.

In work spearheaded by co-investigator Dr. Mary Jo Fackler, Dr. Sukumar and colleagues developed “cMethDNA,” a multiplexed PCR assay that measures the level of methylation in 10 genes known to be hypermethylated in breast cancer. In pilot studies, cMethDNA detected metastatic breast cancer from the blood serum of patients, with higher than 90% sensitivity and specificity.

A number of technologies are already in development to detect and track breast cancer during treatment, and Dr. Sukumar poses the question, “So why use our test?” Her answer: “Our pilot studies show that the cMethDNA test can detect changes very rapidly, say within 3 weeks of treatment initiation. Tumor shrinkage cannot be that rapidly detected by imaging. In the study, when patients responded to treatment, the levels of methylation dropped within 3 weeks of treatment. When they did not respond to treatment, the methylation levels in serum stayed the same or increased. A less expensive test that can be conducted at weekly intervals throughout the treatment period may provide distinct advantages.”

“The next step is to validate the assay in an independent sample set collected in a trial designed for this purpose (a prospective trial), a set we have recently tested by cMethDNA. If the data obtained through such analysis is promising, this bodes well for the assay and brings it closer to the clinic.”



## Investigating ADAM8 as a Potential Therapeutic Target for TNBC

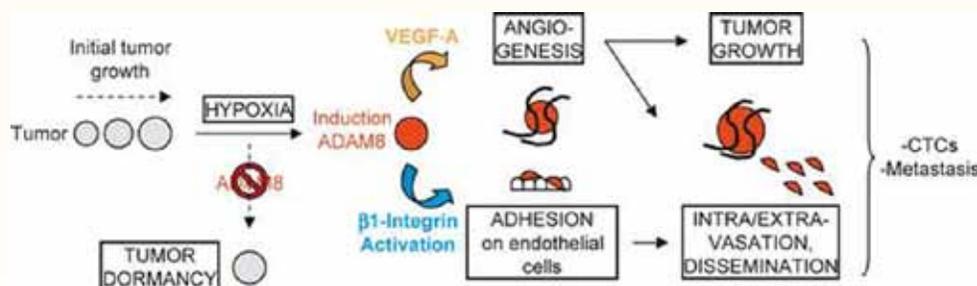
**Mathilde Romagnoli, Tufts University School of Medicine**

TNBC accounts for approximately 10% to 20% of all breast cancers. It is an aggressive form of the disease in which recurrence and metastases are common. While chemotherapy can be used to treat TNBC, targeted and hormone therapies are ineffective because TNBC cells do not express estrogen receptors, progesterone receptors, or HER-

2. With funding from an FY09 BCRP Postdoctoral Fellowship Award and under the mentorship of Dr. Gail Sonenshein, Dr. Mathilde Romagnoli and colleagues identified the metalloprotease-disintegrin protein ADAM8 as playing a pivotal role in breast tumor formation and invasion, thus exposing a promising candidate for TNBC-targeted treatment.

Dr. Romagnoli demonstrated that ADAM8, a mediator of cell adhesion and migration, was highly expressed in breast cancer samples, as compared with normal breast tissue, and noted that basal-like breast carcinoma (which are typically highly aggressive and mostly TNBC) had the highest levels of ADAM8 gene expression when compared with other breast cancer subtypes. Furthermore, reviewing a publicly available microarray dataset, Dr. Romagnoli found high levels of ADAM8 mRNA were correlated with poor disease-free and overall survival among breast cancer patients. In vitro experiments also revealed that high ADAM8 expression levels were associated with the aggressive TNBC cell phenotype. Dr. Romagnoli then demonstrated that ADAM8 stimulated angiogenesis through release of VEGF-A and transendothelial cell migration via  $\beta$ 1-integrin activation, which suggested mechanisms by which ADAM8 may promote metastatic disease. Importantly, knockdown of ADAM8 expression in an orthotopic mouse model of TNBC led to decreased mammary tumor size, and fewer circulating tumor cells and brain metastases in comparison with mice bearing TNBC expressing baseline levels of ADAM8.

Dr. Romagnoli employed a commercially available antibody targeting ADAM8 to treat mammary tumors in the same orthotopic mouse model. She observed a significant reduction in primary tumor burden and in the size and extent of brain metastases. In a tumor resection model, Dr. Romagnoli found that mice treated with the anti-ADAM8 antibody showed decreased metastases to the brain and lungs. Together, these findings have established ADAM8 as a promising therapeutic target for TNBC and possibly other aggressive forms of breast cancer. Currently, studies are under way to develop a humanized antibody against the ADAM8 protein to test its efficacy in mouse models and ultimately in clinical trials.



*ADAM8 expression in invasive breast cancer promotes tumor dissemination and metastasis.*



## New Method to Determine Best Treatment Regimens

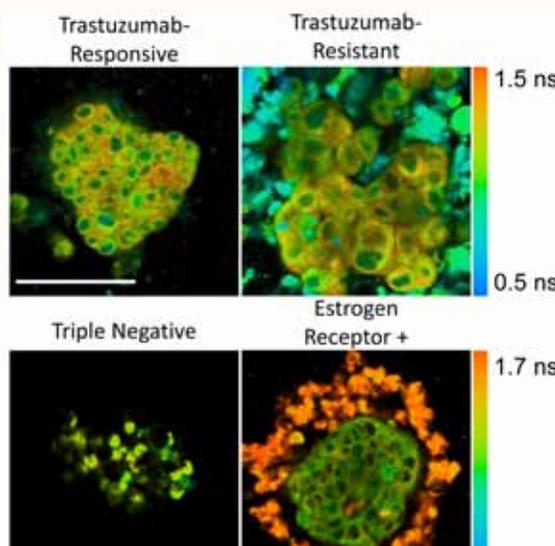
**Melissa C. Skala, Vanderbilt University**

A significant fear for breast cancer patients is completing multiple rounds of therapy only to find out that their tumors are, or have become, resistant to that therapy. With the support of an FY12 BCRP Idea Award, Dr. Melissa Skala and colleagues developed a metabolic assay that measures breast tumor response to multiple treatment schemes enabling the most effective therapies to be chosen for individual patients.

Currently, patients must first complete a therapy regimen before the effectiveness of that therapy can be assessed through the monitoring of tumor size via magnetic resonance imaging and mammography, which can take weeks or months to evaluate. However, changes to individual tumor cells that take place in response to treatment precede the responses observed on a larger scale, such as tumor growth or regression. One of these early changes is tumor cell metabolism.

Dr. Skala and colleagues developed an imaging platform, Optical Metabolic Imaging (OMI), capable of measuring tumor cell metabolism based on the intrinsic fluorescence of metabolic co-enzymes. In animal models, tumor tissue was mechanically disrupted into smaller “organoids” that allowed for simultaneous testing of multiple drugs and their combinations on tissue derived from a single tumor. These organoids were treated with various drugs and OMI was used to calculate a score, called the OMI index, to characterize the metabolic state of the cells following treatment. The researchers found that, not only did the OMI index correlate with responsiveness to the different therapies, but it could predict responsiveness by 72 hours after treatment, as compared with the weeks required to monitor tumor growth. Furthermore, OMI better predicted tumors that began to grow again following a brief regression. Within the organoids, the researchers also observed subsets of cells that displayed different OMI indices. The different indices suggest that the rapid identification of resistant sub-populations of cells that exist within a largely responsive tumor is possible with OMI.

OMI was also able to differentiate between breast cancer subtypes and predict drug responsiveness in several human breast cancer cell lines as well as organoids produced from human breast tumors. Using live tumor specimens from patients with ER+ breast cancer, the group showed, through OMI, that the patients’ tumors were responding differently to treatment, consistent with what is often seen in the clinic. They also tested OMI on tissue from HER2+ and triple-negative breast cancer biopsies and found that OMI predicted a HER2+ sample response to Trastuzumab, but not a triple-negative breast cancer response, as would be expected. Altogether, OMI has the potential to shift the breast cancer treatment paradigm.



*Optical metabolic imaging of primary tumor organoids that are responsive (upper left) and resistant (upper right) to the breast cancer drug trastuzumab. Patient-derived organoids from a triple-negative (lower left) and ER+ (lower right) tumor show that optical metabolic imaging of patient tumors can rapidly inform on the optimal drug regimen for individual patients. Scale bars are 100 micrometers, “ns” – nanoseconds.*



## **STAT5 as a Target for Breast Cancer Prevention Therapy**

**Yi Li, Baylor College of Medicine**

Epidemiological studies have shown that a pregnancy before age 22 lowers a woman's risk of breast cancer, while pregnancies that begin after age 35 increase breast cancer risk. It is unclear what pregnancy-associated changes can increase cancer risk. Supported by BCRP funding, Dr. Yi Li identified a key protein that, during pregnancy, allows pre-cancerous cells to evade the body's natural defenses that normally keep cell proliferation in check. With BCRP funding early in his career, Dr. Li developed a novel mouse model that closely mimics breast cancer initiation in humans. Using this model, he tested the hypothesis that first pregnancies impart different effects on breast cancer risk due to divergent amounts of mutations in the breast cells in younger versus older women. Dr. Li found that the transcription factor Signal Transducer and Activator of Transcription 5 (STAT5) contributed to tumorigenesis in pregnant mice through the suppression of apoptosis in early breast lesion cells, thereby increasing their tendency to become cancerous. Dr. Li's most recent findings have shown that active STAT5 is detectable in human breast lesions, especially in women who have had a pregnancy. Interestingly, the remodeling of breast tissue during pregnancy – specifically, changes in expression levels of lactation hormones – stimulates STAT5 and consequently weakens the cellular mechanisms that kill off precancerous cells by apoptosis. Based on these findings, STAT5 could represent a potential target to prevent and/or reduce the risk of breast cancer in pregnant women over the age of 35. Dr. Li is partnering with industry to conduct a clinical trial to test a STAT5 inhibitor in pre- and post-menopausal women. Dr. Li commented, "I am extremely grateful to the DoD BCRP for its generous support of my research program at Baylor as well as my postdoctoral research training. This support has allowed me to explore some very risky research ideas. I am gratified that some of these ideas have generated potential clinical significance, and we are excited to now be moving toward a clinical trial."



"The DoD BCRP at its inception laid out a new model for the partnership between consumers and scientists. I have been welcomed to the table as a partner with the country's leading scientists, where my unique perspective is honored and my priorities are heard. We need more than awareness. We need more than hope. We need to save lives. We need the science funded by the DoD to end this disease."

**Lori Marx-Rubiner**  
**METAvisor**



### Targeting Master Regulators of the Breast Cancer Metastasis Transcriptome

**Timothy Chan, Memorial Sloan Kettering Cancer Center**

The most common cause of breast cancer deaths is metastatic progression, the spread of cancer to other parts of the body. Effective therapies to halt metastatic progression remain elusive, however, as the cellular mechanisms that drive metastatic progression are poorly understood. One approach to uncovering these drivers is assessing the metastatic transcriptome, the collection of mRNA transcripts – the molecular intermediaries of gene expression – produced by metastatic cancer cells. Supported by an FY12 BCRP Era of Hope Scholar Award, Dr. Timothy Chan is comparing the transcriptomes of primary tumors and metastatic tissue to uncover the genetic “master regulators” that appear to be crucial in reprogramming primary tumor cells to promote metastatic dissemination. His findings thus far have already revealed a new candidate molecule. Reversion-inducing cysteine-rich protein with kazal motifs (RECK) is a protein playing several key roles in normal development including the stabilization of tissue architecture and regulating vascular growth. Comparing primary human breast tumors and the distant metastatic cells derived from them, Dr. Chan and colleagues showed that RECK expression is suppressed in metastatic lesions, compared with primary tumors, suggesting that RECK may also act as a metastasis-suppressor gene. To confirm this, the researchers overexpressed RECK in a mouse model and showed that high levels of RECK did indeed suppress tumor metastasis. Additional work exploring how RECK controls breast cancer metastasis showed that it activates the transcription factor signal transducer and activator of transcription factor 3 (STAT3), which, in turn, induces a number of pro-metastatic genes. Based on these findings, treatments that increase RECK expression or decrease STAT3 activation could impede metastatic progression and significantly improve the prognosis of breast cancer patients.



“It is my work as a consumer reviewer for the BCRP, in particular, that gives me hope—hope that we WILL find a way to end the disease. Dedicated to high-risk/high-impact research that is both innovative and paradigm-shifting, the program aims to stop people from dying from the disease and prevent people from developing the disease in the future. Today, I am grateful to be alive, to be a mother, and to have my Lily; as a consumer reviewer, I will continue to advocate for an end to the disease—for my own daughter and all of our collective daughters, too.”

**Teri Fuller**  
**Young Survival Coalition**

# Developing Immunotherapies to Prevent and Treat Breast Cancer



## Development of a Vaccine Targeting Triple-Negative Breast Cancer

**Denise Cecil, University of Washington**

TNBC derives its name from a lack of overexpressed HER2, estrogen, and progesterone receptors, proteins whose overexpression distinguishes the other major forms of breast cancer. Patients who develop metastatic TNBC have poor prognoses, with survival times shorter than those associated with other types of breast cancer. A significant challenge to treating TNBC is the lack of a molecular target against which therapies could be developed. One promising candidate target is the insulin-like growth factor-I receptor (IGF-IR), which has been shown to play an important role in breast cancer growth and metastasis and is expressed in almost 50% of TNBCs. With the support of an FY09 BCRP Postdoctoral Fellowship Award, and under the mentorship of Dr. Mary Disis, Dr. Denise Cecil aims to recruit the body's immune system to seek out and destroy TNBC cells with a vaccine that targets IGF-IR.

In preliminary studies, Dr. Cecil and colleagues immunized TgMMTV-neu mice with two vaccines carrying DNA that codes for both ends, the N-terminus and the C-terminus, of the IGFBP-2 protein. T cell activation, an indicator of immune response, that resulted from the two vaccines was then assessed by infusing the activated T cells into tumor-bearing mice and comparing tumor growth. T cells from mice vaccinated with the N-terminus vaccine induced the production of TH1 cytokines, small molecules that powerfully activate the immune response, and inhibited tumor growth when infused into tumor-bearing mice. In contrast, T cells from mice receiving the C-terminus vaccine did not inhibit tumor growth. Further analysis showed that, in contrast to the TH1 cytokines induced by the N-terminus vaccine, mice immunized with the C-terminus vaccine secreted more TH2 cytokines, known to be less effective in stimulating antitumor immunity. Moreover, TH2 cytokines have been shown to suppress TH1 cells and could thus limit the effectiveness of the N-terminus vaccine. To test whether the C-terminus vaccine was in fact limiting the capacity of the N-terminus vaccine to slow tumor growth, the researchers treated mice with both vaccines together. Indeed, the two vaccines together had a decreased antitumor response compared to the N-terminus vaccine alone. Dr. Cecil and colleagues concluded that the N-terminus of IGFBP-2 is immunostimulatory and causes potent antitumor activity. This work could lead not only to a vaccine to treat TNBC but could also illuminate new ways to maximize the potency of other vaccines.

# Developing Immunotherapies to Prevent and Treat Breast Cancer



## Complement Inhibition in the Immunotherapy of Breast Cancer

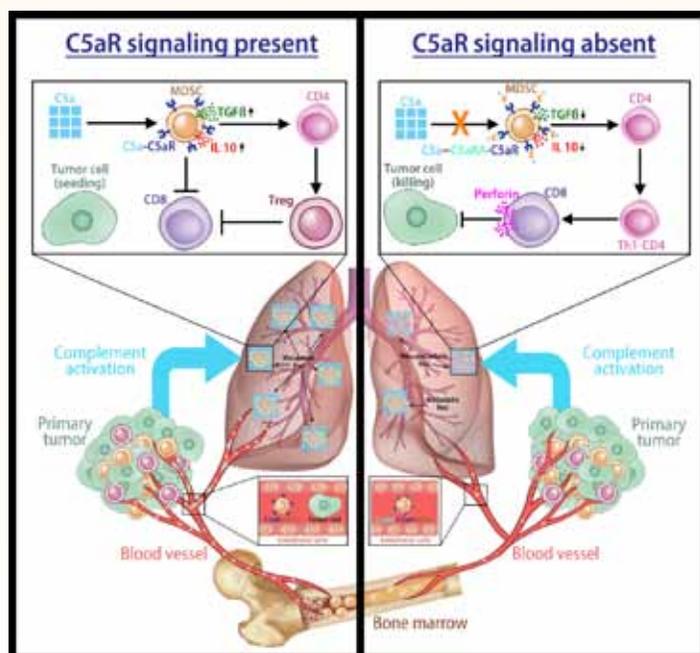
Maciej Markiewski, Texas Tech University Health Sciences Center

The complement anaphylatoxin receptor (C5aR) has been shown to promote tumor growth through the activation of myeloid-derived suppressor cells (MDSCs), one of the cell populations recruited by tumors to help them evade detection by the immune system. It is therefore hypothesized that MDSCs may be responsible for the failure of many anti-cancer vaccine strategies and that inhibition of C5aR may improve therapeutic vaccine efficiency.

Dr. Maciej Markiewski received an FY11 BCRP Idea Award to investigate whether C5aR blockade improves the effectiveness of an anti-cancer vaccine at reducing growth of breast tumors and limiting breast cancer metastases.

Dr. Markiewski demonstrated that inhibition of C5aR reduced tumor growth in two mouse models of breast cancer. The combined treatment of a C5aR inhibitor and a *Listeria monocytogenes*-based HER2 vaccine resulted in greater tumor suppression than either treatment alone. This antitumor therapeutic response was associated with increased recruitment of tumor-specific T cells to the tumors. As expected, Dr. Markiewski found that C5aR inhibition led to a reduction in the number of MDSCs in tumors, suggesting that C5aR regulates MDSC tumor infiltration by recruiting these cells from the circulation. He concluded that the robust response of the combination treatment resulted from attenuation of tumor-mediated immunosuppression by MDSCs. These studies demonstrate that inhibition of C5aR may be an efficient monotherapy for breast cancer and could be used to enhance the effectiveness of some anticancer vaccines.

Dr. Markiewski has also begun exploring the role of C5aR in metastases. Using a mouse model of spontaneously metastasizing breast cancer, he has demonstrated that C5aR promotes metastasis by regulating the pre-metastatic niche – C5aR signaling results in changes that “prime” a metastatic site before the arrival of tumor cells. Based on these findings, Dr. Markiewski believes that C5aR represents a novel therapeutic target to reduce breast cancer metastases.



*C5aR functions in the pre-metastatic niche and its therapeutic implications.*

**Left panel:** C5aR recruits MDSCs to the lung pre-metastatic niche and activates these cells to produce immunosuppressive cytokines. This cytokine milieu favors generation of regulatory T (Treg) cells that inhibit anti-tumor CD8+ T cell responses. In addition, MDSCs activated by C5aR block these T cells directly. Overall, C5aR-mediated immunosuppression facilitates seeding of the lungs by tumor cells.

**Right panel:** C5aR blockade results in the reduced infiltration of the pre-metastatic lungs by MDSC, a decrease in immunosuppressive cytokines and the activation of anti-tumor CD4+ and CD8+ T cell responses. When C5aR is blocked, cytotoxic T cells, armed with perforin, kill metastasizing tumor cells, thereby, preventing lung metastasis.



## Rapid Translation of a Novel and Potent Vaccine in HER2+ Metastatic Breast Cancer Patients

**H. Kim Lyerly, Duke Comprehensive Cancer Center**

HER2 is overexpressed in about 20% of breast cancers. While HER2-targeted therapies – most notably the antibody trastuzumab – provide clinical benefits to many patients, the majority of patients with HER2+ breast cancer will eventually develop resistance to therapy and experience progressive disease; however, trastuzumab-refractory tumors maintain high expression levels of HER2 which can serve as a target for attack by immune cells and antibodies. Seeking to exploit the constitutively high expression of HER2 by trastuzumab-refractory tumors, Dr. H. Kim Lyerly and colleagues received an FY05 BCRP Clinical Translational Research Award to develop vaccines that have the potential to activate an immunological response against HER2 in refractory tumors.

The VRP-HER2 vaccine was developed using an attenuated strain of an alphavirus that has been modified in order to induce a T cell and antibody response against HER2. This antibody response, in addition to helping immune cells recognize tumors, also had the ability to block the signaling activity of wild-type HER2 in human breast cancer cells, thus reducing their tumorigenicity in orthotopic xenograft mouse models. In addition, Dr. Lyerly's team treated tumors in human HER2-transgenic mice resulting in the retardation of tumor growth.

Currently, Dr. Lyerly and his team are conducting a Phase I clinical trial to evaluate the safety of VRP-HER2 (now called AVX901) in patients with HER2-overexpressing breast cancer. Thus far, they have successfully treated 14 patients with AVX901 alone or in conjunction with other HER2-targeted therapies, and they have reported no dose-limiting toxicity, supporting its safety in humans.

Ultimately, more advanced clinical studies will be conducted in order to establish the safety and efficacy of the HER2 vaccine in the prevention of breast cancer progression and the effective treatment of patients suffering from refractory disease.



“The BCRP has played a significant role in bringing much needed funding and resources to breast cancer scientists and to the women who are counting on them to be accurately diagnosed and effectively treated. It was an honor to serve as a consumer reviewer alongside breast cancer research scientists in determining which projects have the greatest scientific merit and will make the biggest difference in women's lives.”

**Noreen Fraser**  
**Noreen Fraser Foundation**

# Developing Immunotherapies to Prevent and Treat Breast Cancer



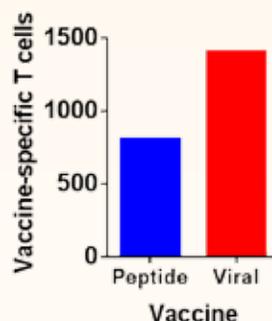
## Educating Normal Breast Mucosa to Prevent Breast Cancer

**Keith Knutson, Vaccine and Gene Therapy Institute of Florida**

The mucosal lining of the breast mammary gland has an integrated immune system that functions much like the lining of the gut to maintain epithelial integrity and protect against microorganisms. Research has shown that defects in this immune regulation may contribute to the onset of breast cancer development; thus, reversing these immunological defects may help prevent breast cancer. Dr. Keith Knutson has shown that, in human samples, lack of breast-specific immune protection can lead to higher rates of atypical hyperplasia, a precancerous condition. With support from an FY11 BCRP Idea Award, he aims to further understand the immune biology of the breast mucosa and develop methods to enhance this local immune system to target and kill tumor cells.

Dr. Knutson and colleagues have found that a majority of the immune cells in the breast mucosa are a specific type of immune cell, called CD8+ T cells, which attack abnormal cells such as cancer cells. The research team is now attempting to elicit a CD8+ T cell response in breast mucosa as a means by which to destroy breast cancer cells; they have developed a vaccine targeting many of the tumor antigens found on most breast cancers, including on breast cancer stem cells. In preliminary mouse studies, they found that this multi-antigen vaccine elicited a prominent T cell response and, importantly, led to significant delays in outgrowth of implanted tumors as compared with animals that did not receive vaccine.

With these encouraging results, Dr. Knutson plans to further develop vaccines that can generate strong, durable immune responses in the breast mucosa to prevent spontaneous development of breast cancer. The researchers are thus creating vaccines utilizing the cardiovirus Theiler's murine encephalomyelitis virus as a delivery and immune stimulatory vehicle. In preliminary investigations, they found that direct vaccination into the mammary gland led to robust immune responses in the mammary gland, better than that achieved with peptide vaccine. Investigations are now under way to determine how long the immunity persists and whether it can prevent spontaneous development of breast cancer. Ultimately, Dr. Knutson hopes that this strategy can be used as a preventive immunotherapy for breast cancer.



*Viral vaccination results in mammary gland immune surveillance. Shown are the levels of tumor-specific T cells in the mammary gland (per million total cells) following immunization with peptide or viral vaccine.*



“The scientific review experience is like no other experience I have had before or since. The mutual respect between the scientists and the consumers is profound. We, the consumers, help remind the scientists why they do the research that they do. The scientists make us aware of how passionately they care for their work—and for our mutual cause—to prevent and treat breast cancer. I am grateful for the opportunity to participate in the BCRP, and I am grateful for the passion of the scientists and my fellow consumers.”

**Mara Ginsberg  
To Life!**



## Overriding Systemic and Local Immunologic Checkpoints to Maximize Breast Cancer Immunotherapy

Leisha Emens, Johns Hopkins University

Approximately 20% of breast tumors overexpress a growth factor receptor called HER2. Treatment for HER2+ breast cancer changed dramatically with the development of trastuzumab (Herceptin). However, not all HER2+ patients respond to trastuzumab, and thus development of additional therapies is needed.

Cancer immunotherapy is a therapeutic approach designed to enhance the antitumor immune response to treat cancer. Dr. Leisha Emens is developing an immunotherapy for HER2+ breast cancer that combines an anti-HER2 antibody with two immunological stimuli: an HER2+ granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting breast tumor vaccine and vaccine-boosting doses of the chemotherapeutic agent cyclophosphamide (CY). With support from an FY06 BCRP Clinical Translational Research Award, Dr. Emens and her research team found that the combination of an anti-HER2 antibody with a GM-CSF-secreting tumor vaccine and immune modulating doses of CY greatly enhanced the immune response to HER2, as well as tumor-free survival in a mouse model of breast cancer. Dr. Emens' team recently conducted a clinical trial to look at the safety and clinical benefit of this combination in 20 research subjects with HER2+ metastatic breast cancer. She found that up to four sequential GM-CSF-secreting and CY-modulated vaccinations in addition to weekly trastuzumab were safe and well-tolerated. Furthermore, treatment with this combination resulted in a 6-month clinical benefit rate of 55%, median progression-free survival of 7 months, and overall survival of 42 months. These clinical results compare favorably to other HER2-targeted trials for metastatic breast cancer.

Dr. Emens further investigated the immune cell profile in the patients who underwent the combination therapy. She found that HER2-specific CD8+ T cells proliferated across the vaccination cycles. Additionally, Tregs and MDSCs, mediators of immune tolerance and suppression, decreased as a result of the immunotherapy. Dr. Emens hypothesizes that the combination of a decrease in Tregs and MDSCs and a greater proportion of HER2-specific T cells may be associated with the longer progression-free survival and overall survival.

Results achieved under her award have aided Dr. Emens in obtaining additional funding to continue clinical trial work on this vaccine-CY-trastuzumab combination therapy in a larger breast cancer study. While improving patient outcomes when integrated with traditional drug therapies, cancer vaccines such as this one may also have the potential to treat tumors that have developed resistance to standard cancer therapies—a crucial, unmet need in breast cancer.



# Developing Immunotherapies to Prevent and Treat Breast Cancer



## Vaccination of High-Risk Breast Cancer Patients with Carbohydrate-Mimicking Peptides

Thomas Kieber-Emmons, University of Arkansas

Tumor-associated carbohydrate antigens (TACAs) include, as their name suggests, carbohydrates that are abundantly expressed on the surface of tumor cells. While the surfaces of all cells express carbohydrates, TACAs arise due to changes in glycosylation that occur when normal tissue becomes cancerous and thus represent a promising target for cancer therapies. Also, as with other carbohydrate molecules, TACAs are expressed on multiple types of cell surface proteins and lipids. This means that targeting TACAs on cancer cells represents a more potent approach than therapies that target single-protein antigens, such as HER2. Additionally, TACAs affect a number of processes that are key to tumor cell communication and survival. Together, the broad spectrum nature of TACA-expressing antigens and their link to cancer cell survival make them potentially powerful targets for immunotherapies designed to treat breast and other types of cancer.

With support from an FY05 BCRP Clinical Translational Research Award, Dr. Thomas Kieber-Emmons developed a therapeutic vaccine for breast cancer that targets TACAs. One challenge to any immunotherapy, however, is the fact that the targets are self-antigens – the immune system recognizes them as non-foreign and thus they do not induce a robust, T cell-activated immune response. Because of this, Dr. Kieber-Emmons and colleagues created novel carbohydrate mimetic peptides (CMPs) that function as T cell-dependent surrogates of TACAs to induce a cross-reactive immune response. These CMPs induced the production of antibodies directed against molecules important in cell proliferation, adhesion, and migration, and were shown in preclinical studies to inhibit tumor growth. To increase potency, the most promising of these CMPs, called P10 short (P10s), was conjugated with a peptide called PADRE that boosts the body's immune response. In 2011, the resultant vaccine, P10s-PADRE, was entered into a Phase I clinical trial to assess its safety and tolerability.



Six participants in the trial were immunized five times over a period of 23 weeks. Although the trial was strictly intended to ensure that P10s-PADRE was safe and tolerable – which it was shown to be – correlative studies revealed an elevation of antibodies against P10s in the blood of all participants following immunization. Serum and plasma isolated from 5 of the 6 participants was subsequently shown in the lab to be cytotoxic to human breast cancer cells, including trastuzumab-resistant cells. One participant displayed significant clinical benefit attributed to a combination of docetaxel and vaccine. Incubation of cancer cells in the lab with a combination of vaccine-induced serum and docetaxel resulted in more cell death than treatment with docetaxel alone.

A Phase II trial is set to begin soon that will test the P10s-PADRE vaccine in combination with chemotherapies in patients with Stage I, II, or III estrogen receptor-positive breast cancer.

# Research on the Horizon

**Kerrie Bouker, Georgetown University**  
**FY13 Postdoctoral Fellowship Award**

*In utero estrogen exposure increases anti-estrogen resistance by inducing epithelial-to-mesenchymal (EMT) transition*

**Richard Hynes, Massachusetts Institute of Technology**

**FY13 Innovator Award**

*Extracellular matrix biomarkers for diagnosis, prognosis, imaging, and targeting*

**Sara Huvitz, University of California, Los Angeles**

**FY13 Breakthrough Award Level 2**

*Safe and effective targeting of breast cancer progenitor cells with a Notch 3 antibody-drug conjugate*

**David Shapiro, University of Illinois at Urbana – Champaign**

**FY13 Breakthrough Award Level 2**

*An inhibitor that induces rapid tumor regression by targeting a new ER upregulated pathway in metastatic breast cancer*

**Christopher Umbricht, Johns Hopkins University**

**FY13 Breakthrough Award Level 2**

*Total RNA sequencing analysis of DCIS progressing to invasive breast cancer*

**Elizabeth Mittendorf, M.D. Anderson Cancer Center**

**FY13 Breakthrough Award Level 3**

*Combination immunotherapy for the treatment of high-risk HER2-positive breast cancer*

**Eli Gilboa, University of Miami School of Medicine**

**FY13 Breakthrough Award Level 2**

*Enhancing therapeutic index by targeting co-stimulation to the tumor stroma with bispecific oligonucleotide aptamers*

**Mikala Egeblad, Cold Spring Harbor Laboratory**

**FY13 Era of Hope Scholar Award**

*Targeting the tumor microenvironment to terminate drug-resistant breast cancer*

**Donato Romagnolo, University of Arizona, Tucson**

**FY13 Idea Expansion Award**

*Gestational exposure as an epigenetic modifier of breast cancer risk*

**Michel Sadelain and Clifford Hudis, Memorial Sloan Kettering Cancer Center**

**FY13 Breakthrough Award Level 4**

*Tumor antigen-targeted T-cell therapy for metastatic breast cancer*

**Yi Huang and Nancy Davidson, University of Pittsburgh**

**FY13 Breakthrough Award Level 2**

*Targeting histone abnormality in triple-negative breast cancer*

**Eun-Sil Hwang, Duke University**  
**Carlo Maley, University of California, San Francisco**

**FY13 Breakthrough Award Level 4**

*Genomic diversity and the microenvironment as drivers of progression in DCIS*

**Sandra McAllister, Brigham and Women's Hospital**

**FY13 Era of Hope Scholar Award**

*New diagnostic and therapeutic approaches to eradicating recurrent breast cancer*

**Dario Marchetti, Baylor College of Medicine**

**David Sanghyn Hong, M.D. Anderson Cancer Center**

**FY13 Breakthrough Award Level 2**

*Mechanisms of CTC biomarkers in breast cancer brain metastasis*

**Andrew Thorburn, University of Colorado at Denver**

**Daniel Gustafson, Colorado State University**

**FY13 Breakthrough Award Level 2**

*Identifying and targeting autophagy dependence to eliminate metastatic breast cancer*

**Ruth Keri, Case Western Reserve University**

**FY13 Breakthrough Award Level 1**

*An epigenetic regulatory hub in triple-negative breast cancer*

**Sohail Tavazoie, Rockefeller University**

**FY13 Breakthrough Award Level 2**

*A peptide therapeutic for suppressing breast cancer metastasis*

For additional information on awards recommended for funding: <http://cdmrp.army.mil/search.aspx>

# In the Clinical Pipeline

## DIAGNOSTICS

### 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.

## VACCINES

### Therapeutic and Preventative Vaccines

**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. The vaccine has been licensed commercially for further investigation. Another vaccine developed by Dr. Disis takes a different approach to treating breast cancer by targeting proteins associated with breast cancer stem cells and EMT. A Phase I clinical trial to test the stem cell/EMT vaccine is anticipated to begin in 2015.

### E75 HER2-Derived Peptide Vaccine (NeuVax™)

**Constantin Ioannides**

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). NeuVax is now in Phase III clinical trials.

## IMMUNOTHERAPIES

### ErbB2/ErbB3 Bispecific ScFv (ALM) Antibody

**Gregory Adams**

The BCRP supported preclinical studies to develop and test an engineered single-chain Fv antibody capable of simultaneously engaging both HER2 and HER3. This novel agent was designed to enhance therapeutic effects on breast cancers that express both HER2 and HER3, and block the potent pro-growth signaling that occurs when these tumor-associated antigens engage each other upon ligand binding. Resulting technology and parent antibodies were licensed by Merrimack Pharmaceuticals, which developed the agents and concepts into a drug called MM-111, which is currently in early-phase clinical trials for treating patients with HER2+ advanced breast cancer.



## HER2 Bi-Armed Activated T Cells

### Lawrence G. Lum

The BCRP supported the 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 I clinical trial in women with HER2+ metastatic breast cancer, which indicated that the treatment infusions are safe and induced long-term anti-tumor responses. The HER2 bi-armed activated T cells are currently in Phase II clinical trials for treating breast cancer.

## TRC105 Antibody

### Ben K. Seon

The BCRP supported the development of TRC105 (also known as c-SN6j), a monoclonal antibody (mAb) which targets endoglin and inhibits angiogenesis. Preclinical results indicated that systemic administration of anti-ENG mAbs could suppress the growth of established tumors as well as new tumor growth. These results led to several clinical trials including an ongoing Phase Ib clinical trial to test the safety and efficacy of TRC105 in combination with capecitabine in metastatic HER2-negative breast cancer patients.

## 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 and 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 antibody-targeted 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 multi-institutional, Phase 2 clinical trial involving patients with advanced stages of HER2-positive breast cancer.

## TARGETED THERAPIES

### Targeting Autophagy to Eradicate DCIS

#### Lance Liotta and Kirsten Edmiston

Although most DCIS lesions remain dormant and do not invade or spread to the lymph nodes, some lesions progress to eventually become invasive and metastatic. There are no methods to predict which DCIS lesions will become invasive and no therapeutic options to prevent the invasive phenotype. Drs. Liotta, Espina, and Edmiston of George Mason University and Inova Fairfax Hospital tested the hypothesis that some DCIS lesions are preprogrammed with invasive properties, and that the mammary duct microenvironment provides a unique niche for DCIS cell survival. Their findings indicated that autophagy may play a key role in regulating the emergence of DCIS invasive progenitor cells and that chloroquine is a potential new therapeutic for treating DCIS. They are now conducting a neoadjuvant clinical trial using chloroquine as a potential DCIS treatment to arrest or kill pre-invasive lesions and prevent progression to invasive breast cancer.

### 5-Flouro-2'Deoxycytidine (FdCyd)

#### Edward Newman

DNA methylation inappropriately turns off several genes in cancer cells. Preclinical studies supported by the BCRP demonstrated the effects of FdCyd with tetrahyouridine 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.

# In the Clinical Pipeline

## IDO Inhibitor

### George C. Prendergast

Indoleamine 2,3 Dioxygenase (IDO) is an enzyme that is commonly activated in breast cancer and is implicated in preventing the anti-tumor immune response by blocking T cell activation. The BCRP supported the 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. Prendergast demonstrated that the D isomer of an IDO inhibitor called 1MT (D-1MT) has potent anti-tumor 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.

## PD-0332991 (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 recent initiation of a Phase III clinical trial. With BCRP funding, Dr. Slamon will perform molecular and correlative studies on patient samples from these clinical trials, to determine what characteristics best determine sensitivity to this combination therapy. With this knowledge, PD-0332991 can be administered to patients who will benefit most from its use.

## Anti-Androgen Therapy

### Jennifer Richer and Anthony Elias

The BCRP supported several discoveries that revealed a critical role of androgen as a driver of breast cancer growth. Dr. Richer in collaboration with Dr. Elias showed that higher levels of androgen receptor are expressed on ER+ breast cancers that are resistant to anti-estrogen therapy versus those that are responsive. Such cells were shown to proliferate in response to androgen, and this effect was inhibited by an anti-androgen (enzalutamide). These preclinical results led to current Phase I and II clinical trials to test the safety and efficacy of enzalutamide in combination with other breast cancer treatments.

## Combining Aromatase and Src Inhibitors

### Joyce Slingerland and 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.

# Making an Impact

## RESEARCH RESOURCES

### **Expression Arrest™ shRNA Libraries**

**Gregory Hannon and Stephen Elledge**

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 more than 30,000 genes. This commercially available research tool provides researchers with 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.

## THERAPEUTICS

### **Herceptin®**

**Dennis Slamon**

Herceptin (trastuzumab) is a monoclonal antibody that targets HER2. HER2+ breast cancer accounts for approximately 25% of all breast cancers. The BCRP was instrumental in supporting preliminary 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 III 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. 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.

# Making an Impact

## 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.

## PATIENT RESOURCES AND REGISTRIES

### **BreastCancerTrials.org**

**Laura Esserman**

Patients with breast cancer 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](http://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, 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](http://BrainMetsBC.org)) that provides the latest information about brain metastases. The web site, 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 and 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 30 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 and 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 Index<sup>SM</sup>

### 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.

### 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 patients with breast cancer. These findings contributed to the practice of *Skp2/p27* immunohistochemical analysis as a prognostic test performed in clinical pathology laboratories.



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