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 $7.5 billion in appropriations from its inception through fiscal year 2013 (FY13). 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

ABOUT THE PROGRAM

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

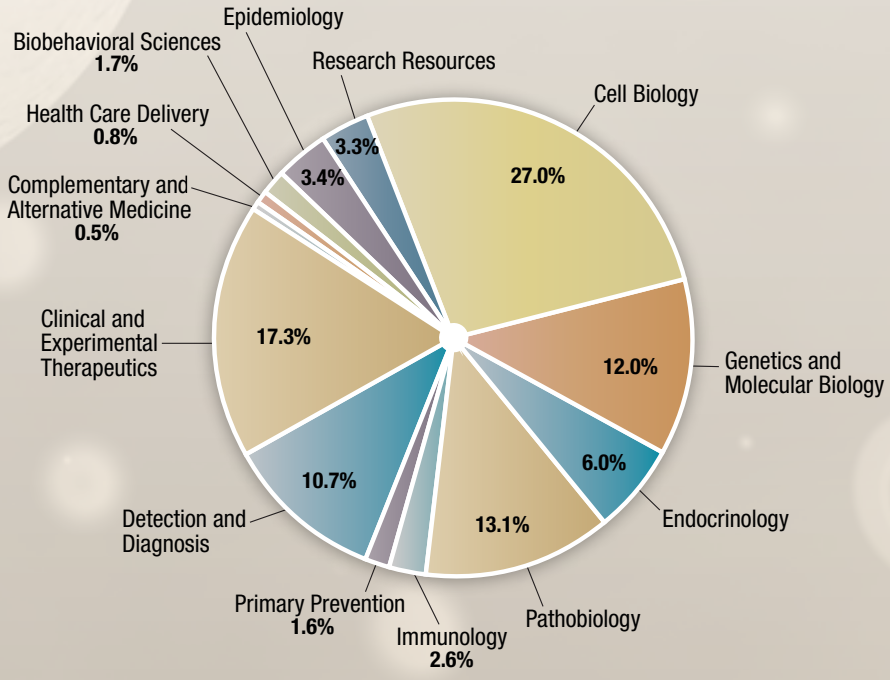
The BCRP plays a leading role in the fight against breast cancer through its innovative approaches and its focus on research that will bring an end to this disease. The BCRP was established in 1992 as a result of the dedicated efforts of breast cancer advocates. Their continued efforts, in concert with the program's accomplishments, have resulted in more than $2.9 billion in congressional appropriations through FY13. 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, set 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. The BCRP's training and early-career awards have provided the foundation for many of today's leading breast cancer researchers, and the program continues to invest in the **"best and brightest"** next generation of breast cancer experts.

BCRP Overarching Challenges

Considering the current breast cancer landscape, and the BCRP's vision to end breast cancer, the BCRP encourages applications that address these overarching challenges:

- Eliminate the mortality associated with metastatic breast cancer
- Prevent breast cancer (primary prevention)
- Distinguish aggressive breast cancer from indolent cancers; overcome the problems of overdiagnosis and overtreatment
- Revolutionize treatment regimens by replacing drugs that have life-threatening toxicities with safe, effective interventions
- Identify what drives breast cancer growth and metastasis; identify why some breast cancers become life-threatening metastases
- Identify what makes the breast susceptible to cancer development
- Determine why some, but not all, women get breast cancer
- Determine why/how breast cancer cells lay dormant for years and then re-emerge (recurrence); determine how to eliminate dormant cells early

FY92–FY12 BCRP Portfolio



The Breast Cancer Landscape

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

Important information from the Breast Cancer Landscape:

- Worldwide, breast cancer accounts for nearly a quarter of all cancers in women. In 2010, there were 438,000 breast cancer deaths globally.
- The rate of metastatic breast cancer at initial diagnosis in the U.S. has not changed since 1975.
- An estimated 20%-30% of women diagnosed with invasive breast cancer will have a recurrence.
- An estimated 30% of all breast cancer cases (both invasive and ductal carcinoma in situ [DCIS]) are considered to be overdiagnosed and overtreated.
- Most risk factors are not modifiable (e.g., age; family history; BRCA status). Potentially modifiable risk factors, such as obesity, alcohol consumption, smoking, and exercise, are weakly to moderately associated with breast cancer risk.

(Read The Breast Cancer Landscape at: http://cdmrp.army.mil/bcrp/pdfs/bc_landscape13.pdf)

Strategic Partnerships:

Did you know...

More than 3,600 scientists and 780 consumers representing 390 advocacy organizations have contributed their expertise to the BCRP two-tier review process?

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 breast cancer survivors (consumers). Utilizing these innovative approaches, which have been adopted by other funding organizations, 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.

"I feel privileged to have the opportunity to be part of a panel of scientists and breast cancer advocates who are truly committed to finding the most innovative scientific solutions towards the eradication of breast cancer. Serving on this panel is a reminder of how we need to seek non-incremental scientific agendas that can truly transform the face of this disease."

Nimmi Ramanujam
FY14 IP Chair

"What an invaluable experience for anyone who truly wishes to contribute to the fight against breast cancer! At the end of the peer review session, my heart was filled with nothing but hope that all things are possible when people work together toward the same mission: To find a cure and eradicate breast cancer."

Ghecemy Lopez
USC/Norris Comprehensive Cancer Center
Cancer Survivorship Advisory Council

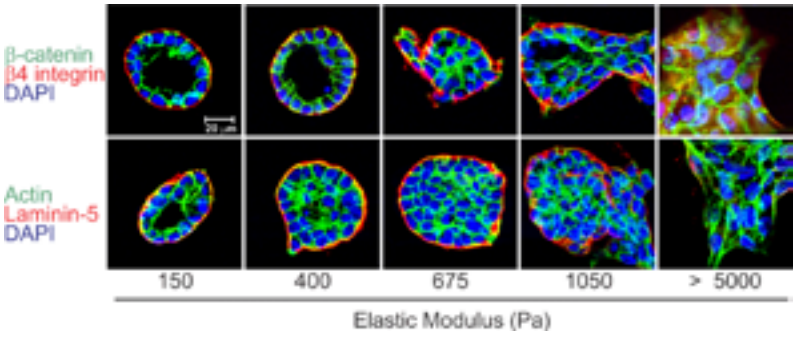
Scientists and consumer advocates working together to end breast cancer

The Interplay among Tension, Breast Cancer, and Treatment

Valerie Weaver, University of California, San Francisco

Tumor progression has traditionally been linked to the sequential accumulation of genetic mutations within cells. However, clinical data have shown that the microenvironment in which a tumor resides can influence its ability to survive and resist treatment. Instead of deciphering which of the puzzling array of factors to target and how best to treat individual patients, Dr. Valerie Weaver believes fundamental properties shared by all breast cancers should be targeted as the drivers of tumor aggression and treatment resistance. Under an FY04 Era of Hope Scholar Award, Dr. Weaver assembled an interdisciplinary team of researchers and consumer advocates to combine the strengths of quantitative sciences such as engineering, bioinformatics, and physics together with molecular and cellular biology, as well as the patient's perspective. Collaboratively, they explored the interplay between a noncellular component of the microenvironment, the extracellular matrix (ECM), and breast cancer pathogenesis. In both preclinical models and clinical samples, they found that ECM tension increases prior to and during malignant transformation. Stiffening the ECM, or elevating mammary tissue tension, promotes malignancy, while inhibiting ECM remodeling prevents malignancy. They further observed that in high-risk women and women with the most aggressive tumors (Luminal B, triple-negative), the ECM is the stiffest. These findings introduced a new paradigm in breast cancer, implying that treatments to reduce ECM tension could constitute new therapeutic opportunities. Dr. Weaver is continuing her fundamental vision under a new FY12 Era of Hope Scholar Expansion Award. This project will build upon the prior findings to illustrate how tissue tension drives tumor aggression and will move the research into preclinical studies to test whether treatments to reduce tissue tension can "calm" the tumor sufficiently to improve therapy outcomes and inhibit cancer aggression and metastasis. By defining the role of tissue tension in breast tumor aggression and treatment, this work has the potential to be rapidly adopted into current clinical therapeutic regimes.

> "Advocates are an integral part of my group's research program. Not only do they keep my group members focused on relevant clinical goals, but they also motivate my trainees. They contribute on multiple levels, including advising and shaping research objectives, and they interact with and provide mentoring to my trainees. Our work, after all, is directed towards curing breast cancer, and without advocates it is far too easy to lose sight of why we are doing this work in the first place. Besides, they are wonderful colleagues and provide encouragement and support as a community."



Confocal immunofluorescence images of mammary epithelial cell colonies on 3D basement membrane gels of increasing stiffness (150-5000 Pa), showing colony morphology after 20 days. Top: β-catenin (green), β4 integrin (red) and nuclei (blue). Bottom: actin (green), laminin-5 (red) and nuclei (blue).

Investing in the next generation of innovators

Improving 3D Breast Imaging with a Stationary Digital Tomosynthesis System

Xin Qian, University of North Carolina at Chapel Hill

X-ray mammography is the most common detection tool for breast cancer. However, dense breast tissue presents a considerable challenge to two-dimensional (2D), traditional mammography. Recent technological developments have given rise to digital breast tomosynthesis (DBT), a three-dimensional (3D) imaging system that combines a series of images collected as the X-ray source circles the breast. But while 3D imaging makes it easier to distinguish breast tumors and dense breast tissue, long scanning times and limited spatial resolution due to motion blurring make current DBT scanners less than ideal.

Supported by an FY08 Postdoctoral Award and the mentorship of Dr. Otto Zhou, Dr. Xin Qian improved DBT-generated images by designing and building a stationary scanner. Dr. Qian achieved this by replacing the standard mammography X-ray tube on the Hologic, Inc. Selenia Dimensions 3D System – a device that is already approved by the U.S. Food and Drug Administration (FDA) for the screening for breast cancer – with a distributed carbon nanotube (CNT) X-ray source. The CNT source radiates X-rays from different angles without mechanical motion, thus reducing scanning time and minimizing motion blurring. The novel stationary system is currently being evaluated in a clinical trial in a performance comparison with 2D digital mammography in patients with known breast lesions.

The s-DBT system is part of a pilot clinical trial in the Department of Radiology at University of North Carolina-Chapel Hill Lineberger Comprehensive Cancer Center.

Inhibition of Invasive Breast Cancer by Programmed Necrosis

Leonardo Leon, University of California, Davis

A major challenge in developing new, more effective treatments for breast cancer recurrence and the emergence of metastatic lesions is finding unique drugs and targets that act independently of cell cycle to promote cancer cell death. Many anti-cancer therapeutics are specifically designed to target dividing cells, which limits their effectiveness toward cells that are dormant or proliferate slowly. Furthermore, cancer cells commonly activate anti-apoptotic pathways to promote their survival and resistance to conventional chemotherapies.

Amiloride, a potassium-sparing diuretic that has been used in the management of hypertension and heart failure, also exhibits anti-cancer characteristics via its ability to inhibit both cell cycle progression and tumor cell invasiveness. However, work in Dr. Kermit Carraway's lab has demonstrated that at high concentrations amiloride induces the death of breast cancer cells but not normal mammary epithelial cells, independent of cellular proliferation status. These observations suggest that higher potency derivatives of amiloride may be effective in targeting the poorly proliferative tumor cell population that is resistant to other therapeutic approaches.

Working in Dr. Carraway's lab and supported by funding from an FY09 Predoctoral Traineeship Award, Leonardo Leon determined that a cell-permeable amiloride derivative, achieved through the addition of a glycine benzoylate moiety to the core drug, results in more potent cytotoxicity toward breast cancer cells than the parent drug. This observation suggests that cytotoxic effects of amiloride and its derivatives may be due to the targeting of an intracellular component. More importantly, this amiloride derivative induces cell death via apoptosis-inducing factor translocation-mediated programmed necrosis rather than via apoptosis. These observations raise the possibility that such amiloride derivatives may be employed to attack tumor cells that are inherently resistant to apoptosis-inducing therapeutics. Thus, by virtue of their ability to attack poorly proliferative and apoptosis-resistant cells, cell-permeant amiloride derivatives could ultimately contribute to better therapeutic outcomes by suppressing tumor recurrence and metastatic outgrowth following conventional therapy.

Six1 Regulates VEGF-C, Lymphangiogenesis, and Lymphatic Breast Cancer Metastasis

Chu-An Wang, University of Colorado, Denver

Recent research suggests that the spread of metastatic breast cancer cells through lymph nodes does not occur merely by passive passage through lymphatic vessels, but also by the formation of new lymphatic vessels in or near the metastatic lesion. This generation of new lymphatic vessels – or lymphangiogenesis – is stimulated by the tumor itself and is promoted by the activation of several regulatory factors. One of these regulatory factors is thought to be Six1, a transcription factor that plays an important role in early development. While it is expressed at high levels during embryogenesis, it is largely undetectable in normal adult tissue. Recurrent expression of Six1, however, is seen in several types of cancer including breast cancer where expression levels are highest in metastatic tissue. Supported by an FY09 Predoctoral Traineeship Award and under the mentorship of Dr. Heide Ford, Dr. Chu-An Wang tested the hypothesis that Six1 promotes breast cancer-related lymphangiogenesis and lymphatic metastasis by inducing expression of VEGF-C, a critical lymphangiogenic regulator. Indeed, Dr. Wang found that overexpressing Six1 in breast cancer cells not only induced lymphatic metastasis, but also simultaneously induced VEGF-C expression. She found that Six1 was dependent on VEGF-C for some, but not all, of its metastatic-promoting effects, indicating that Six1 acts through additional, VEGF-C-independent pathways.

While studying the role of Six1 in lymphatic vessel formation and metastasis, Dr. Wang's work led to the discovery that a closely related transcription factor, Six2, may also play a causal role in metastatic breast cancer. While Six1 is implicated in the primary tumor growth and lymphangiogenesis, Six2 may be involved in the later stages of metastatic breast cancer. These findings provide the first mechanistic explanation behind VEGF-C's overexpression in breast cancer and suggest that patients with high levels of Six1 may be candidates for anti-VEGF-C therapy. Furthermore, the role of Six2 in late metastasis makes this protein a potential target to prevent or treat metastasis.

Pursuing innovative ideas and new technologies

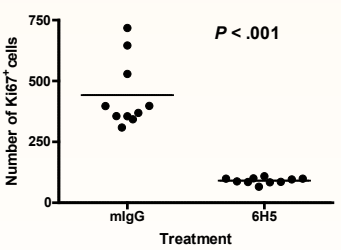
Innovative Strategies for Breast Cancer Immunotherapy

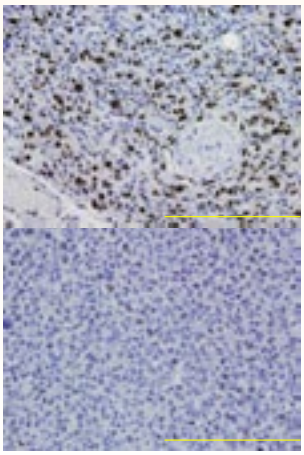
Feng Wang-Johanning, University of Texas MD Anderson Cancer Center

Developing new immunotherapies for breast cancer is limited in part by the need to identify target antigens, which are expressed in tumors. In this regard, human endogenous retroviruses (HERVs) represent one group of promising potential targets. HERVs are stretches of ancient virus deoxyribonucleic acid (DNA) that are believed to have integrated into the human genome long ago, and today may constitute up to 8% of the genome. While accumulated mutations over time have led to a loss of the infectivity of HERVs, HERV transcripts have been detected in a number of tumor tissues and tumor cell lines. Genetic loci encoding the HERV-K envelope (env) protein is thought to be silent in normal cells, but active in malignant cells. With funding from an FY05 Concept Award, Dr. Feng Wang-Johanning and colleagues attempted to exploit HERV-K env expression in breast cancer tumors by developing a conjugated antibody that targets HERV-K.

When tested in several breast cancer cell lines, the anti-HERV-K monoclonal antibody 6H5 inhibited the growth and proliferation of breast cancer cells but did not affect the proliferation of normal breast cells. Furthermore, when treated with 6H5, breast cancer cells were five times more likely to undergo apoptosis compared with normal cells. The cell death was dose-dependent, and greater amounts of HERV-K env protein expressed on the surface of malignant breast cancer cells correlated with more extensive apoptosis following 6H5 treatment.

To enhance its effectiveness at killing tumor cells, Dr. Wang-Johanning's group conjugated the 6H5 antibody to the toxin Gelonin. In a pilot study using mouse xenografts, mice treated with the conjugated antibody (6H5-rGel) exhibited significantly reduced tumor growth compared to vehicle-treated controls. The results of this study provided valuable information on the potential of this antibody-based therapeutic against HERV-K-positive breast cancer. In ongoing work, Dr. Wang-Johanning is continuing investigation of HERV-K with funding from an FY11 Impact Award, testing the hypothesis that HERV-K is a causative agent of breast cancer and a novel breast cancer biomarker. The Impact Award aims to develop HERV-K-specific cancer vaccines and adoptive T cell therapy.



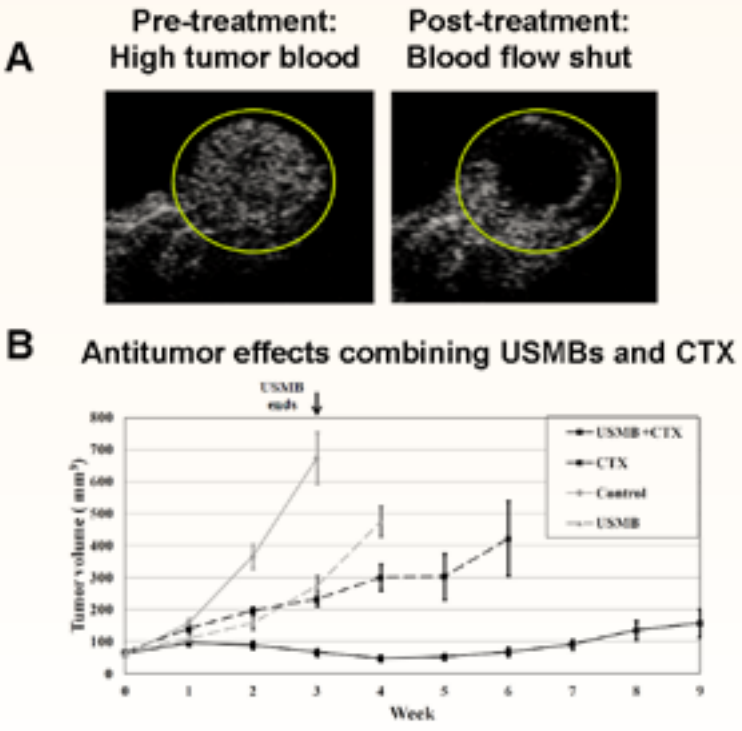


Potentiating Chemotherapy with the Antivascular Effects of Ultrasonically Stimulated Microbubbles

David Goertz, Sunnybrook Health Sciences Centre

Chemotherapeutic agents followed by surgery and radiotherapy are used to treat various primary breast tumors. Unfortunately, many tumors only partially respond to chemotherapy and some do not respond at all. Inadequate access of anticancer drugs to tumor cells is one reason for poor therapeutic responses. To address this problem, some researchers have turned to the use of therapeutic ultrasound with injected microbubbles. Microbubbles are commonly used in contrast-enhanced ultrasound imaging. Their small size – comparable to that of red blood cells – enables them to permeate the microvasculature that provides the tumor its blood supply. When stimulated with ultrasound, the microbubbles increase microvasculature permeability to improve local drug delivery.

Supported by an FY09 Idea Award, Dr. David Goertz and colleagues sought to take ultrasound-stimulated microbubbles (USMB) one step further, not merely by increasing endothelial cell permeability, but by causing microvasculature damage to slow blood flow specifically in tumors, which would increase the effectiveness of chemotherapy. Using a vascular-targeting approach, the group first optimized USMB conditions to shut down blood flow to tumors in breast cancer mouse models, which resulted in slowed tumor growth. Following this finding, they evaluated USMB in combination with two chemotherapeutic agents, docetaxel and paclitaxel. They discovered that combining USMB with these individual chemotherapies either before or after treatment resulted in decreased tumor growth in comparison to using the treatment alone. Furthermore, pairing USMB with metronomic cyclophosphamide (MCTX), a chemotherapy regimen that inhibits blood vessel growth, again enhanced antitumor effects when compared to the individual treatments alone. By boosting the tumor-specific lethality of chemotherapies, USMBs have the potential to increase the effectiveness of these treatments.



A) Ultrasound flow image of a tumor (green circle) before treatment (left) shows that there is blood flow throughout the tumor, and that post-treatment with USMBs (right) blood flow has been shut down (dark area). B) Untreated (control) tumors grow rapidly and individual treatments with either USMBs or metronomic cyclophospahmide slow tumor growth. The most profound anti-tumor effects are achieved by combining these two approaches (USMB+CTX).

XBP1, Unfolded Protein Response, and Endocrine Responsiveness

Robert Clarke, Georgetown University

Resistance to endocrine therapy remains a significant clinical obstacle in the treatment of breast cancer. Improved understanding of the mechanisms that regulate cell survival and cell death is central to the development of novel approaches to overcome resistance. The protein X-box binding protein-1 (XBP1) is an important component of the unfolded protein response (UPR), which mediates the balance between pro-survival and pro-death signals within the cell. Previous studies have demonstrated that XBP1 is associated with acquired endocrine resistance in breast cancer cells, and the spliced (active) form of XBP1 was found to be elevated in tumors that responded poorly to Tamoxifen. Dr. Robert Clarke received an FY07 Idea Award to explore the hypothesis that XBP1 is a key regulator of breast cancer cell fate, acting through the UPR and its downstream effectors.

Dr. Clarke demonstrated that XBP1 expression and UPR signaling are higher in anti-estrogen resistant cells, compared to anti-estrogen sensitive cells. Transient overexpression of XBP1 decreased cell sensitivity to the anti-estrogen drug ICI 182, 780 (also known as Faslodex or Fulvestrant), suggesting that XBP1 promotes anti-estrogen resistance. Dr. Clarke found that the knockdown of XBP1 led to a decrease in the downstream pro-survival protein Bcl2. Further, overexpression of active XBP1 in a mouse model of breast cancer resulted in an increase in tumor growth, supporting the pro-survival role of this protein. UPR induction in anti-estrogen sensitive cells was protective against ICI-induced cell death, confirming that the UPR pathway is important to the development of anti-estrogen resistance in breast cancer cells.

Dr. Clarke then identified a small molecule, NPPTA, that inhibits the activator of XBP1, thereby blocking UPR activity. He demonstrated that NPPTA inhibits the proliferation of breast cancer cells, but did not have an effect of the growth of normal mammary gland epithelial cells. Additionally, NPPTA treatment sensitized anti-estrogen resistant breast cancer cells to treatment with the anti-estrogen drug ICI. Building on these promising results, Dr. Clarke received an Idea Expansion Award in FY12 to perform preclinical optimization and validation experiments of the NPPTA analogs in breast cancer animal models.

Cellular stress results in initiation of the UPR that triggers release of GRP78 from Inositol-requiring enzyme 1α (IRE1α), one of the transmembrane endoplasmic reticulum sensor proteins. Release from GRP78 activates IRE1a and enables it to remove a 26 nucleotide fragment from within the unspliced form, i.e., Xbp1(U) mRNA, to produce the spliced form, i.e., Xbp1(S). Spliced XBP1 is a potent transcription factor and contributes to increased endocrine resistance in breast cancer cells. A small molecule inhibitor of IRE1 α, NPPTA, blocks the production of XBP1(S) and re-sensitizes breast cancer cells to antiestrogens.

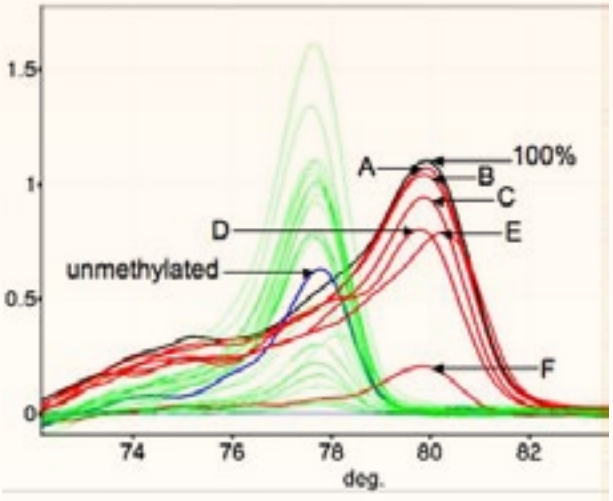
Predicting Breast Cancer Risk by Assaying BRCA1 Peripheral Blood Methylation

Alexander Dobrovic, Peter MacCallum Cancer Institute

Some women with a familial history of breast cancer have a mutation in the BRCA1 or BRCA2 gene that changes the DNA sequence, irreversibly inactivating the gene and resulting in an increased risk of developing breast cancer. Another type of modification occurs when a methyl group attaches to the BRCA gene DNA sequence and stably alters its structure without changing the sequence. This process, commonly called methylation, typically results in gene inactivation. It is unclear how DNA methylation of BRCA1 and BRCA2 varies among women who do not have a mutation in these genes, and whether variability in methylation could predict breast cancer risk. With BCRP funding from FY04 and FY05 Concept Awards, Dr. Alexander Dobrovic developed a test that is capable of measuring BRCA1 gene methylation in small, readily attainable blood samples. Utilizing this test, he proposed to compare methylation levels in women without breast cancer to those in women who developed breast cancer without BRCA1 or BRCA2 gene mutations.

The results from a pilot study of seven breast cancer patients who developed breast cancer at an early age (35 to 51 years), with no detectable BRCA1 or BRCA2 mutations, showed that DNA methylation was detected in the blood of three (42%) patients. Dr. Dobrovic's group showed that the tumor DNA from these three patients was heavily methylated on the BRCA1 gene. This suggested that the BRCA1 gene was silenced by methylation, and this silencing predisposed cells toward becoming cancerous.

In a 2011 publication from Dr. Dobrovic's lab, the team confirmed their initial findings through a larger study of 255 women diagnosed with early-onset breast cancer patients who also have no history of BRCA1 germline mutations. The results showed that BRCA1 methylation increased the risk for breast cancer 3.5-fold. This finding raises the possibility that BRCA1 methylation and gene silencing may represent an underlying alteration that causes some cases of familial breast cancer. These findings also suggest a new paradigm in which somatic alteration, in addition to germline mutations, can predispose individuals to breast cancer. Additionally, BRCA1 methylation may currently be an unrecognized cause of some BRCA1-associated cancers. Thus, screening for BRCA1 methylation in blood may be a relatively simple test to predict early-onset breast cancers.



Detection of methylated and unmethylated BRCA1 genes in the peripheral blood of an individual with breast cancer. The red curves represent methylated genes and the green curves represent unmethylated genes. Most normal individuals only have unmethylated genes.

Investigating Microenvironment Alterations in Breast Cancer Initiation and Progression

Dihua Yu, University of Texas MD Anderson Cancer Center

ErbB2, commonly known as Her-2, is a member of a family of genes that encode for growth factor receptors. Growth factors bind to these receptors to activate a cascade of genes that promote cell growth. While cell growth and proliferation are part of a normal and necessary process, aberrant gene amplification of the ERBB2 gene leads to excess ErbB2 protein, which can contribute to the growth of cancerous tumors. Indeed, ErbB2 is overexpressed in approximately 25% of metastatic breast cancers, and this overexpression is associated with poor patient survival. While this amount of overexpression is high, it is less than half the amount seen in pre-invasive, DCIS, 50% to 60% of which show ErbB2 overexpression. This paradox – the metastasis-promoting ErbB2 is more frequently overexpressed in noninvasive DCIS than in invasive breast cancer – has puzzled scientists and clinicians. Furthermore, attempts to resolve whether ErbB2 overexpression alone can promote progression from noninvasive DCIS to invasive breast cancer have yielded conflicting results.

In work supported by an FY07 Idea Award, Dr. Dihua Yu showed that overexpression of ErbB2 alone was not sufficient to drive metastasis. Multiple changes are required for the transition from a normal to a malignant cell, including increased cell migration and decreased cell adhesion. Using an innovative 3D culture that replicates an in vivo breast-like environment more closely than conventional tissue cultures, Dr. Yu found that overexpression of ErbB2 and another protein, 14-3-3ζ, bring about changes prior to tumor invasion: Increased expression of ErbB2 promoted cell migration, while increased expression of 14-3-3ζ decreased cell adhesion. Thus, Dr. Yu's findings make 14-3-3ζ a promising target for future intervention. When Dr. Yu screened tumor samples from breast cancer patients, she found that 1 in 5 samples co-overexpressed both ErbB2 and 14-3-3ζ. Additionally, patients had comparatively greater survival when their tumors expressed neither ErbB2 or 14-3-3ζ, or simply expressed either one alone.

As a result of Dr. Yu's work, clinicians may be able to select high-risk DCIS patients for appropriate treatments at early stages of cancer development while sparing low-risk patients from aggressive treatments.

"Having breast cancer at such a young age put me at many disadvantages – including having limited knowledge and access to available treatments, education, and resources. But serving the BCRP through peer review has been a tremendous gift of empowerment for me."

Nikia Hammonds-Blakely
Susan G. Komen for the Cure

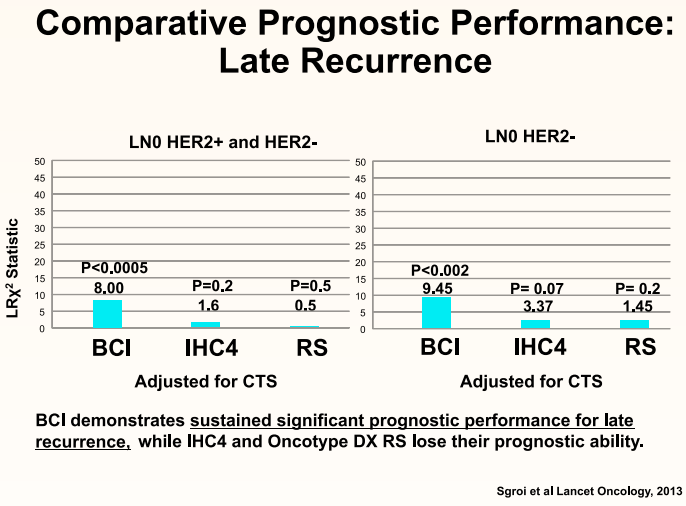
Stratifying Risk of Distant Recurrence in ER+ Breast Cancer

Dennis Sgroi, Massachusetts General Hospital

Approximately 70% of all breast cancers are estrogen-receptor positive (ER+). Women with ER+ breast cancer have been shown to have an increased risk of relapse for at least 15 years after their initial diagnosis. As a result, these women are often placed on extended adjuvant therapy. To identify women with an increased risk of relapse and metastasis, biomarkers are often used to guide treatment decisions.

With support from an FY03 Idea Award, Dr. Dennis Sgroi and his team validated previous findings that showed the high expression level ratio (termed the H/I ratio) of two proteins, HOXB13 and IL17BR, is a strong prognostic factor for ER+ node negative patients and is associated with relapse-free survival. His group also discovered a second panel of 5 genes whose expression levels correlated to tumor grade. Thus, Dr. Sgroi coined the term the Molecular Grade Index (MGI) for these markers. To evaluate the predictive value of using the combination of H/I and MGI in prognostics and treatment responses, Dr. Sgroi subsequently received an FY09 Idea Expansion Award. The primary objective of this study was to determine the prognostic power of H/I alone, MGI alone, and the two combined (termed the Breast Cancer Index, BCI) in a large clinical research setting.

Dr. Sgroi tested the BCI in tissues obtained from 665 women with ER+, node negative breast cancer, and compared the value of the results to two different marker assays that are currently available: one that measures the expression of 21 genes (Oncotype DX) and one that measures four key predictors: ER, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and Ki-67 (IHC4). Using predefined categories in a linear combination of the variables, Dr. Sgroi and colleagues showed that the risk of recurrence 10 years post-treatment increased linearly with increasing BCI values. In fact, this risk was significantly associated with both early (within 5 years) and late (5-10 years) recurrence when adjusted for clinical treatment score. All of the assays displayed significant predictive ability when looking at early recurrence. However, when looking at late recurrence in these women and factoring in the clinical treatment score, only the BCI was significantly predictive. Additionally, the data indicated two distinct risk groups, which potentially makes it easier to determine if a woman will have a high or low risk of recurrence. This information can potentially be used at the time of diagnosis and at the 5-year, disease-free follow-up milestone, to guide future decisions regarding maintenance therapy.



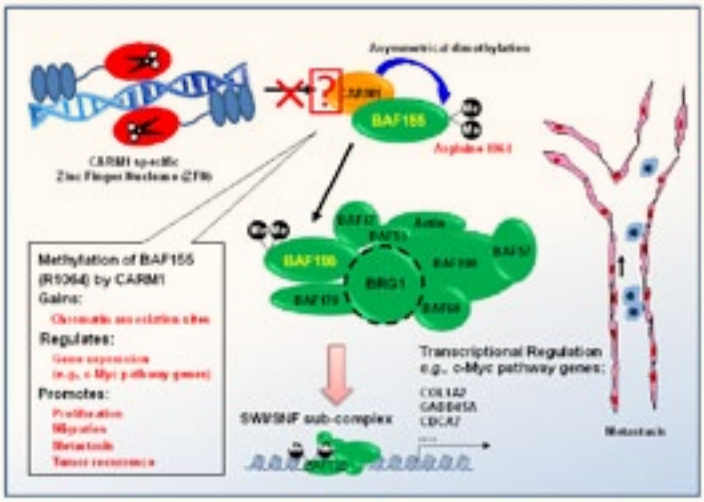
Graphic comparing the relative prognostic strength of BCI to IHC4 and Oncotype DX for late recurrence in all ER+ lymph node-negative patients as well as the clinically relevant low-stage ER+ lymph node-negative HER2-negative subset of patients.

Old Receptors, New Treatment Strategies for Breast Cancer

Wei Xu, University of Wisconsin, Madison

Estrogen supports growth of 70% of primary breast cancers. The biological effects of estrogen are mediated by two ER, ERα and ERβ. Dimerization, or the physical interaction between receptor subunits, is a prerequisite to downstream activation events; however, the biology of ER homo- and heterodimers (i.e., ERα/α, ERβ/β, and ERα/β) in physiological and pathological processes is poorly understood. In work supported by an FY10 Era of Hope Scholar Award, Dr. Wei Xu proposed that novel treatment strategies could be developed based on the function of different ER dimers in the context of breast cancer subtypes. She developed Bioluminescence Resonance Energy Transfer (BRET) assays to monitor ER dimerization in living cells and to identify compounds that selectively induce ERα/α, ERβ/β homodimers and ERα/β-heterodimers. Using the BRET assays, Dr. Xu has identified naturally occurring phytoestrogens that induce ERα/β heterodimer formation in cell-based assay and has begun pharmacokinetics experiments to optimize conditions for breast cancer prevention experiments in a rat model. Furthermore, she has shown that the environmental contaminants, polycyclic aromatic hydrocarbons (PAHs), selectively regulate ERβ activity and induce ERβ-involved dimers, suggesting that those compounds could affect breast cancer risk via regulation of the estrogenic pathways in humans.

In addition to the physical interaction between receptor subunits, Dr. Xu is investigating the function of an enzyme involved in epigenetic regulation, namely, co-activator-associated arginine methyltransferase 1 (CARM1), in breast cancer. Dr. Xu has knocked out CARM1 from several breast cancer cell lines. Strikingly, complete CARM1 knockout – but not partial knockdown to 10% – elicited drastic phenotypic and biochemical changes, emphasizing the need to completely knock out an epigenetic enzyme for functional studies. The CARM1 knockout cell lines enabled identification of novel CARM1 substrates, notably BAF155, a core subunit of the SWI/SNF chromatin remodeling complex. Methylation of BAF155 at a specific site was found to regulate breast cancer cell migration and metastasis, and to be a strong predictor for cancer recurrence. These studies uncover a novel mechanism by which methylated BAF155 acquires tumorigenic functions and presents new therapeutic targets for breast cancer.



Accelerating the Timeline for Validating Breast Cancer Targets

Josef Penninger, Institute of Molecular Biotechnology of the Austrian Academy of Sciences

As new technology identifies increasing numbers of candidate genes to target in breast cancer, there is a concurrent need to rapidly validate these. Dr. Josef Penninger has spent much of his career determining the effects of mutations on organismal development and disease. As a recipient of an FY11 Innovator Award, Dr. Penninger plans to utilize a number of screening tools in order to rapidly validate candidate genes and conduct the preclinical testing necessary to move to clinical trials for breast cancer. The Institute of Molecular Biotechnology, where Dr. Penninger is Scientific Director, is a home to the largest library of RNAi (specific gene silencers) for Drosophila fruit flies. This resource allows Dr. Penninger and his team to take an innovative approach to studying the function of breast cancer genes.

Fly genetics have been used in the past to study breast cancer. Expression of human HER2 in Drosophila, for example, uncovered the ability of HER2 to drive excess cell proliferation in epithelial tissues. Because screening in a fly system can be performed rapidly, genes first validated in the fly screen can move quickly to functional evaluation in cancer model systems. Under his Innovator award, Dr. Penninger will utilize the power of fly genetics to rapidly check the function of perhaps thousands of human candidate breast cancer genes involved in epithelial transformation and metastases. Candidate genes will move into an entirely novel tool that Dr. Penninger is developing under his award, haploid mouse embryonic stem cells, for rapid validation of gene functions. By using this novel stem cell system, which is made up of cells that carry only one copy of each gene instead of the two copies in mature cells, it is possible to more readily screen the effects of a given mutation that might otherwise be compensated for by a working gene copy. These cells can then be tested in model systems for tumorigenesis and metastasis. Moreover, any potential therapeutics targeting such mutations can be rapidly tested as well.

Already, Dr. Penninger has an initial target to screen. His lab previously identified RANKL, and its receptor RANK, as having a role in regulating progestin-mediated breast cancer, and he now plans to pursue this finding with the high-throughput screens. The results of these screens should lead to a better understanding of the role of RANKL/RANK in normal breast tissue, breast cancer and metastasis. As RANKL/RANK are involved in other diseases, therapeutics have already been developed to block this pathway that may be readily implemented to treat breast cancer. Dr. Penninger plans to make these haploid mouse ES cell mutant models available to other researchers as a resource. The ability to reduce the timeline to preclinical testing and to exploit drugs used in other diseases are exciting front lines in breast cancer research.

Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic

Jennifer Richer and Anthony Elias, University of Colorado Cancer Center

Dr. Jennifer Richer and Dr. Anthony Elias at the University of Colorado (CU) Cancer Center are discovering more about how androgens drive breast cancers. They are leading the charge toward proving the androgen receptor (AR) as an important breast cancer target. The collaboration started in 2001 when Dr. Elias, breast cancer program director at CU Cancer Center and professor of medical oncology at the CU School of Medicine, conveyed the clinical observation that certain ER+ breast cancers responded less well to anti-estrogen therapy to colleague Jennifer Richer, associate professor of pathology and co-director of the CU Cancer Center Tissue Processing and Procurement Core. Dr. Richer and Dr. Elias were convinced that, in these cases, something else was driving the cancer. With the support of an FY02 Clinical Bridge Award, Dr. Richer discovered that AR mRNA and protein expression are markedly decreased in estrogen receptor alpha positive (ER+) tumors that respond to neoadjuvant endocrine therapy, but remain steady or increased in tumors that fail to respond. These data led her to postulate that tumors that demonstrate resistance to anti-estrogen therapies may have switched from estrogen-driven growth to growth driven by androgens. Dr. Richer received an FY08 Idea Award to further investigate the role of AR in breast cancer and compare the anti-androgen bicalutamide, traditionally used to treat prostate cancer, to a novel anti-androgen, enzalutamide, in the early stages of development. Dr. Richer found that the new anti-androgen blocked not only androgen-mediated proliferation of breast cancer cells but also estrogen-mediated proliferation. The fact that this drug binds to AR, but not to ER, suggested that AR influences ER action through a novel, unidentified mechanism, and that this activity is abrogated by enzalutamide (a pure antagonist), but not by bicalutimide (a partial agonist).

Dr. Richer and Dr. Elias presented these preclinical findings in breast cancer to collaborators at Medivation, the company that developed enzalutamide. In April 2012, Medivation with Astellas Pharma, initiated a first-of-its-kind Phase I clinical trial at CU Cancer Center, Memorial Sloan Kettering, Karmanos Institute, and George Mason University using enzalutamide to treat advanced breast cancer patients who have not responded to standard of care therapies. Two months later, Medivation and Astellas announced enrollment of the first patient in a global Phase II clinical trial evaluating enzalutamide as a single agent for the treatment of advanced, AR-positive, triple-negative breast cancer (TNBC). The FDA approved enzalutamide in August 2012 for the treatment of castration-resistant prostate cancer. Around the same time, Dr. Richer and Dr. Elias submitted a collaborative application to the DoD BCRP Clinical Translational Research Award funding opportunity, together with colleagues at Memorial Sloan Kettering, Karmanos Institute, and George Mason University, proposing to validate the benefit of enzalutamide alone or in combination with breast cancer standard of care, and to incorporate findings into their own investigator-initiated trials with patients. This application was recommended for funding, and the new project, "Targeting Androgen Receptor in Breast Cancer: Enzalutamide as a Novel Breast Cancer Therapeutic," was awarded in August 2013.

The partnership between Dr. Richer and Dr. Elias is a model example of translational cancer research in action. Dr. Elias made an observation in the clinic, Dr. Richer explored the mechanisms in the laboratory, and now these investigators are taking the research back to the clinic, where it is hoped to have a direct impact on breast cancer patients.

Hybrid Nanotechnologies for Synergistic Therapies for Breast Cancer

Erkki Ruoslahti, Sanford-Burnham Medical Research Institute
Shiladitya Sengupta, Brigham and Women's Hospital
Roger Tsien, University of California, San Diego

Chemotherapeutic drugs are a vital part of the frontline attack on cancer. But, while many patients have been successfully treated with drugs, the benefits of chemotherapy are tempered by its significant drawbacks. In addition to cancer cells, chemotherapies also kill some normal cells, such as those found in the bone marrow, hair, and the lining of the digestive tract. The ideal treatment would be a selective drug that targets tumor cells and does not affect normal, healthy cells.

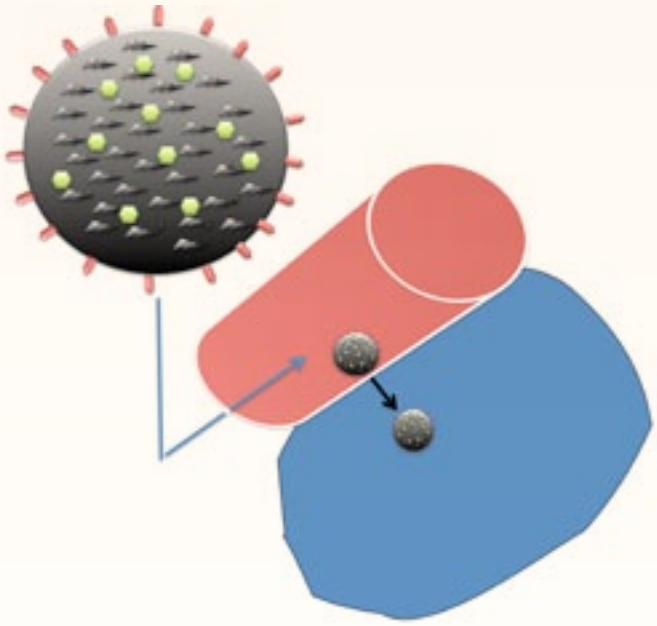
The BCRP's Collaborative Innovators Award brings together scientists across different fields of expertise to come up with new and innovative ways to treat breast cancer. In FY08, one such award went to a group of experts that included Dr. Erkki Ruoslahti, who specializes in blood vessel-based therapies; Dr. Shiladitya Sengupta, a nano-scale engineering expert; and Dr. Roger Tsien, renowned biochemist and recipient of the 2008 Nobel Prize in chemistry. This highly accomplished, interdisciplinary team of researchers proposed to use nanotechnology to make various "smart" chemotherapies that do more than serve as passive drug carriers. Their approaches employ nanomedicine and the unique expression of several molecules found inside tumors or tumor blood vessels. The research team has published a number of studies that demonstrate the promise of smart chemotherapies.

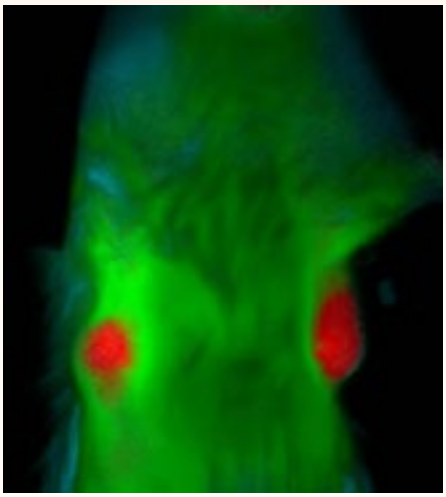
Cisplatin is a chemotherapeutic agent commonly used to treat malignancies in many types of cancers. Its use, however, is limited due to its severe toxicity, primarily to the kidney. Significant research effort has been focused on finding ways to boost cisplatin's destructive effects on tumors while limiting its effects on normal tissue. By taking advantage of the way cisplatin reacts with a new polymer material, Dr. Sengupta and his team created a cisplatin-polymer complex that assembles to form a nanoparticle that specifically enters cancer cells before the cisplatin drug is released. When tests were conducted in a breast cancer model, the platinum-bound nanoparticles were more effective at delaying tumor growth than cisplatin alone. Furthermore, systemic and kidney toxicities were also reduced with the nanoparticles. Drs. Sengupta and Ruoslahti have now made an advanced version of these nanoparticles by attaching iRGD to them for targeting to tumors. This work could one day lead to chemotherapy treatments in which higher doses are both more effective and safer for breast cancer patients.

Another major limitation to chemotherapeutic treatment is the physical nature of tumors, which prevents the penetration of drugs into tumor tissue. To improve the potency of drugs that reach tumor cells, researchers have been pursuing ways to increase the amount of drug internalized by the cells once it reaches its destination. Dr. Ruoslahti's lab created a peptide, called iRGD, containing two sequences. One sequence binds integrin receptors on the surface of tumor cells and the endothelial cells of the tumor vasculature, while the other sequence promotes the peptide's entry into the tumor cell. In recent work, a tumor-homing signal, called the NGR sequence, was added to the peptide. The NGR sequence binds to the CD31 protein that is

expressed in the endothelial cells of tumors. This smart tumor-homing and tissue-penetrating molecule, called iNGR, triggers the penetration of other molecules injected into the blood stream along with it. When the chemotherapeutic agent doxorubicin was administered simultaneously with iNGR in a mouse breast cancer model, higher amounts of doxorubicin accumulated within tumors, demonstrating greater effectiveness in slowing tumor growth than doxorubicin treatment alone. Importantly, enhancing doxorubicin's effectiveness occurred without increased side effects. Because it is not attached to the drug, iNGR could potentially be used to enhance the efficacy of other chemotherapeutics. Additionally, the sequences on iRGD and iNGR that give it its tumor-targeting and tissue-penetrating capabilities could be attached to other molecules to turn a passive delivery system into a smart one.

The ability of a surgeon to remove a tumor depends on his or her ability to differentiate tumor tissue from surrounding normal tissue. Dr. Tsien, recipient of the Nobel Prize for the discovery and development of green fluorescent protein, used extensively in medical research imaging, is now applying his expertise to improving the detection and treatment of cancer. Dr. Tsien's lab previously created "activatable cell-penetrating peptides" (ACPPs), polyarginine-based molecules that enter tumor cells much more than they enter normal cells. In work published in 2010, Dr. Tsien's lab attached the ACPPs with a fluorescent label and used them to guide surgery in a mouse model for breast cancer. The mice injected with ACPP showed a fivefold increase in tumor-free, long-term survival as compared with standard surgery. His team recently showed that even better results can be obtained by attaching two fluorescent labels simultaneously to the same ACPPs such that they respond in opposite directions when taken into tumors. Dr. Tsien's work with ACPPs shows that the value of nanoparticles is not limited to their ability to kill tumor cells directly—they can also be used to improve methods that are already in practice.





A nanoparticle that carries two kinds of payload, a drug and an imaging agent. Such nanoparticles are known as "theranostic" because of their ability to deliver therapy and serve diagnostic functions at the same time. The outer surface of the nanoparticle is coated with a peptide (spikes on the sphere) that binds to a receptor on the vascular wall of tumor blood vessels (but not in normal vessels). The homing peptides cause the nanoparticles to burrow through the vascular wall and into the tumor tissue, resulting in selective accumulation of the nanoparticles in the tumor (blue), which increase the activity of the enclosed drug and enhances the contrast from the imaging agent.

Making breakthroughs in understanding breast cancer

Analysis of FAM83D, A Novel Oncogene in Breast Cancer

Mark Jackson, Case Western Reserve University

Normal breast epithelial cell growth is held in check by genetic "brakes" that keep them from proliferating. In contrast, cancer cells harbor a number of genetic alterations that disrupt their ability to keep cell growth in check. Supported by an FY07 Idea Award, Dr. Mark Jackson's laboratory developed a novel and highly efficient function-based genetic screen to find the genes permitting cancer cells to grow uncontrollably. Using this approach, they identified a family of uncharacterized genes, named Family with Sequence Similarity 83, that when overexpressed in normal cells cause the cells to behave like cancer cells. One of the proteins in this family, FAM83D, was found to significantly contribute to breast cancer cell growth, thereby functioning as a novel breast cancer oncogene.

With additional support from an FY09 Idea Award, Dr. Jackson was able to study the relationship between FAM83D and breast cancer cell growth. Dr. Jackson and his colleagues expressed high levels of FAM83D in breast cancer cells containing clinically relevant epidermal growth factor receptor amplifications or Ras signal transduction mutations. They found that two important cancer-associated "go signals," named MAPK and PI3K signaling, are turned on in these cells. They further demonstrated that when the level of FAM83D expression was experimentally decreased, the MAPK and PI3K cancer-associated pathways turn off and the cells stopped growing. These results indicated that FAM83D expression is required to initiate the growth factor and Ras signaling that leads to cancer cell growth. Dr. Jackson believes that FAM83D may represent a new target in the treatment of breast cancers containing aberrant activation of growth factor receptor pathways.

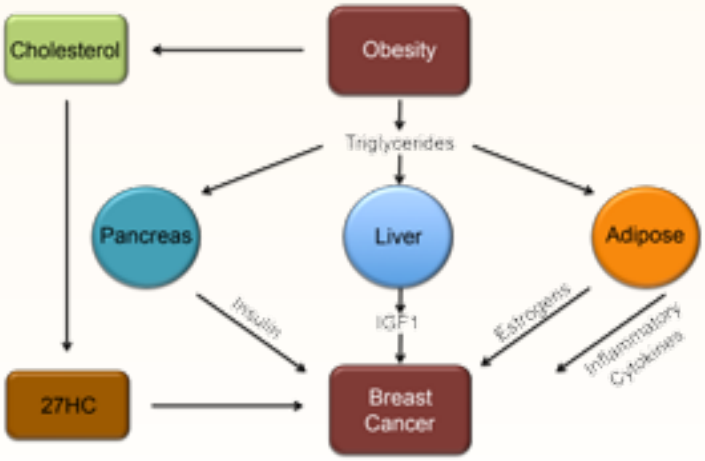
Mechanisms Behind Hypercholesterolemia-Associated Breast Cancer Risk and Progression

Donald McDonnell and Erik Nelson, Duke University Medical Center

Novel strategies for chemopreventive or lifestyle approaches to reducing breast cancer incidence are urgently needed. Several studies have shown a correlation between obesity and the development of breast cancer. Furthermore, breast cancer patients taking statins, which are drugs that inhibit cholesterol synthesis, demonstrate an increased time to recurrence. To decipher the mechanisms by which obesity and cholesterol affect breast cancer incidence and progression, Dr. Donald McDonnell conducted a research project supported by an FY09 Idea Award.

Dr. McDonnell's team found that 27-hydroxycholesterol (27HC), the main metabolite of cholesterol, induces breast cancer cell proliferation by activating the estrogen receptor (ER). Additionally, 27HC was found to activate those nuclear receptors that act as cholesterol sensors and help regulate cholesterol levels in the body, called liver X receptors (LXRs). This finding is one of the first to highlight a role for LXR in breast cancer. Using both genetic and pharmacological approaches, Dr. McDonnell demonstrated that elevation of circulating 27HC significantly increased tumor growth and metastasis in mouse models of breast cancer. These findings and other compelling data form the foundation of an FY12 Idea Expansion Award recently granted to Dr. McDonnell, thus enabling him to expand upon this innovative research.

Dr. Erik Nelson, a postdoctoral fellow in Dr. McDonnell's lab and the recipient of an FY08 BCRP Postdoctoral Fellowship Award, conducted studies that show 27HC promotes breast cancer through differential effects on ER and LXR. In contrast to the effects on the growth of primary tumors, the actions of 27HC on metastasis do not appear to involve ER, but instead occur through LXR activation. Moreover, gene expression analysis showed that 27HC modulates the levels of ER and LXR target genes, suggesting that the receptors may mediate not only the downstream activation of cancer-causing genes, but also the pathogenic effects of 27HC directly. Work conducted in the McDonnell lab also showed that macrophages, which express high levels of CYP27A1, a catalyst of 27HC production from cholesterol, promote tumor growth. These findings suggest that lowering total cholesterol may be a way to reduce breast cancer risk, and that interfering with 27HC production may be a useful strategy to prevent and/or treat breast cancer.



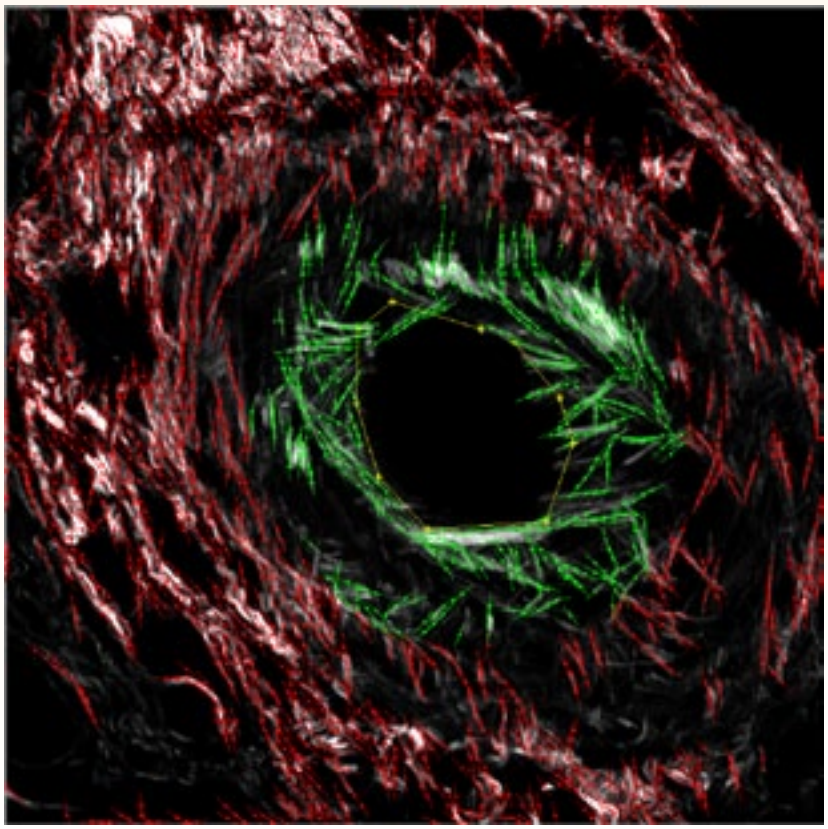
27HC is a biochemical link between hypercholesterolemia and breast cancer risk.

Tumor Microenvironment and Progression to Invasion after a Diagnosis of DCIS

Amy Trentham-Dietz, University of Wisconsin, Madison

DCIS accounts for more than 20% of all new breast cancer diagnoses and is clinically considered a precursor to invasive breast cancer. However, it is estimated that almost 70% of DCIS cases will never progress to invasive disease. Unfortunately, relatively little is known about the factors that regulate this progression. Therefore, it is critical to identify markers that delineate which cases carry a likelihood of cancer progression and warrant aggressive treatment, and which cases carry a low risk.

Dr. Amy Trentham-Dietz hypothesized that re-alignment of the ECM surrounding malignant cells plays a major role in the progression from DCIS to invasive breast cancer, and thus can be used as a marker to predict outcome. To test this hypothesis, Dr. Trentham-Dietz teamed with Drs. Patricia Keely and Andreas Friedl, both of whom have examined the process in other cancer types. Under an FY10 Idea Award, the team made use of archived tumor samples and follow-up patient data from Dr. Trentham-Dietz's cohort study of 267 DCIS patients who were recruited upon their diagnosis between 1995 and 1999. Collagen patterns were evaluated from archived tumor slides using state-of-the-art microscopy methods. Although final analysis of the data is ongoing, preliminary analysis suggests that certain collagen alignment patterns may predict increased likelihood for disease progression. Additionally, procedures established in this BCRP-funded project provided a basis for the newly established Vermont Population-based Research Optimizing Screening through Personalized Regimens Research Center, which is part of a consortium recently organized by the Applied Research Program within the Division of Cancer Control and Population Sciences at the National Institutes of Health. Findings from this work could potentially provide a way to make the critical distinction between those DCIS cases likely to progress to invasive disease and those that will likely remain stable even if left untreated.



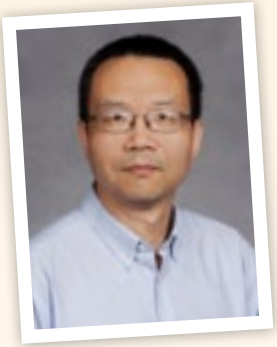
Stromal collagen alignment evaluated using second-harmonic generation microscopy of an H & E stained slide, with the stromal/DCIS lesion boundary (yellow), adjacent collagen fibers (green), and distant collagen fibers (red) rendered using the curvelet image transform and customized software. This program measures the angle of individual collagen fibers with respect to the boundary. The image is 750 um on a side.

Genome-Wide Discovery of Inherited Structural Mutations in Breast Cancer Families

Tomas Walsh and Mary-Claire King, University of Washington

The discovery of the BRCA1 and BRCA2 genes in the early 1990s had a major impact on clinical care, making it possible for individuals with a family history of breast or ovarian cancer to learn if they carry certain gene mutations that put them at significantly higher risk of developing these cancers. Subsequently, multiple genes in addition to BRCA1 and BRCA2 were also discovered to harbor mutations that significantly increase risk of breast cancer, including PALB2, CHEK2, BARD1, RAD51C, RAD51D, BRIP1, NBN, ATM, TP53, PTEN, STK11, and CDH1. Despite these major discoveries, it is estimated that 70% of families with multiple cases of breast cancer have no mutations in any known gene that predisposes them to the disease. Linkage analyses indicate multiple possible genomic locales for additional breast cancer genes. Dr. Tomas Walsh was awarded an FY08 Idea Award to identify large genomic mutations in new genes. In collaboration with Dr. Mary-Claire King, Dr. Walsh's research team identified and validated 133 rare mutations termed "copy-number variants," of which 7 were found to cause complete deletion or truncation of a gene and co-segregate with breast cancer. These discoveries led to development of a comprehensive test named "BROCA," which enables assessment of all known breast cancer genes and all mutation types in a single assay. Testing of this type is now becoming more widespread in academic and commercial settings, as Drs. Walsh and King freely share design details and bioinformatics scripts.

Recently awarded an FY12 BCRP Idea Expansion Award, the investigators are now expanding their research to include severely affected families suffering from unidentified mutations. Their hypothesis is that such families harbor individually rare alleles located in regions of the genome that do not directly code for genes. Using a technique called whole genome sequencing, they will evaluate 30 large, extended families severely affected with breast cancer, each of whom was previously evaluated comprehensively for known gene mutations. If successful, this study will lead to identification of regulatory mutations that may reveal new mutational mechanisms for breast cancer predisposition. Moreover, preventive management strategies may be extended to many families for whom the genes causing familial breast cancer are currently unknown.



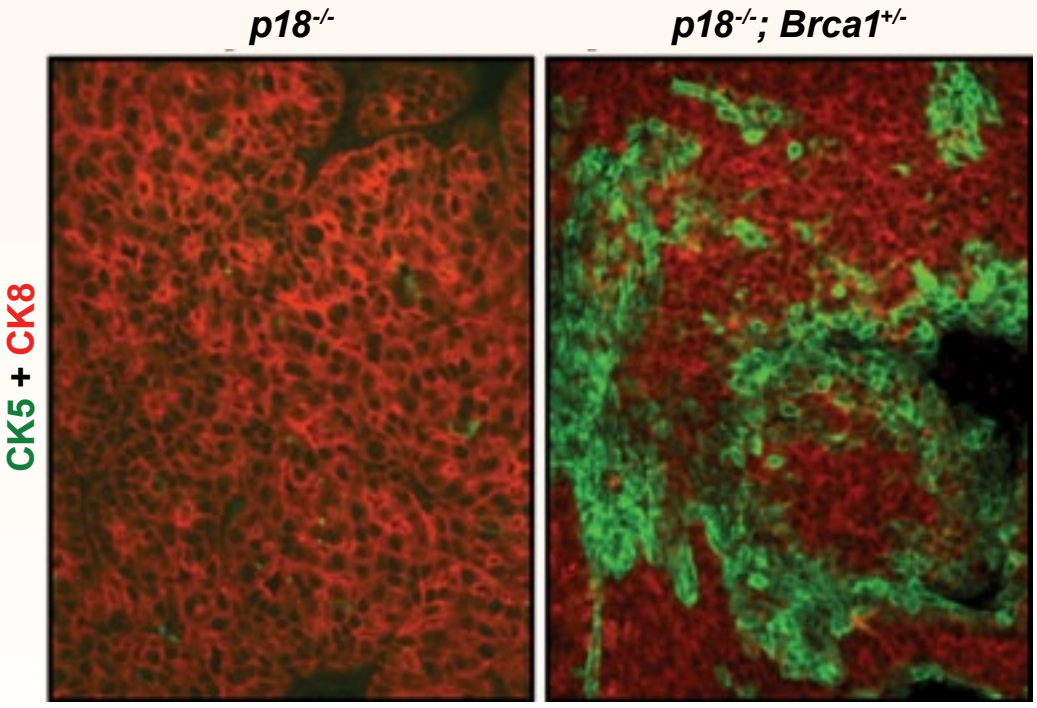
BRCA1 Controls Epithelial-Mesenchymal Transition and Tumorigenesis

Xin-Hai Pei, University of Miami Miller School of Medicine

Breast cancer is a heterogeneous condition – tumors are both pathologically distinct and diverse in response to treatment. While basal-like tumors account for a small portion (10% to 15%) of human breast cancers, they are more aggressive and respond poorly to treatment, and are thus associated with poor prognosis for patients. The underlying cellular pathways that lead to the development of basal-like tumors are not well understood. Studies have shown, however, that epithelial-mesenchymal transition (EMT) is important in the development of basal-like tumors. Dr. Xin-Hai Pei received an FY09 Idea Award to test the hypothesis that defects in BRCA1, a tumor suppressor gene associated with 50% of familial breast cancers, is critically involved in suppressing EMT and basal-like tumor development.

Dr. Pei discovered that when BRCA1 and p18, a gene involved in control of luminal stem cell proliferation, were co-mutated in a mouse model of breast cancer, luminal tumors were converted into aggressive basal-like tumors. These tumors also exhibited EMT, which enhanced their migration and invasiveness. In addition, Dr. Pei found that restoration of BRCA1 in cell culture resulted in suppression of EMT.

With a recently awarded FY12 Idea Expansion Award, Dr. Pei is continuing his work to determine whether BRCA1 can suppress EMT and prevent luminal tumors from converting into basal cells. This work has the potential to significantly improve our understanding of basal-like breast cancers, which, in turn, could translate into more effective targeted therapies in the clinic.



Conversion of luminal tumors into basal-like tumors by BRCA1 mutation in p18-deficient mice.

A Novel Mechanism Controlling the Oncogenic Accumulation of c-Myc Protein in Breast Cancer

Rosalie Sears, Oregon Health & Science University
Dale Christensen, Oncotide, Inc.

Normal breast cells become cancerous following the accumulation of mutations in genes that control cell fate, causing cells to enter a state of unchecked proliferation. One such gene encodes for the c-Myc protein. When c-Myc expression is elevated in mice mammary tissue, the animals develop invasive breast cancer. c-Myc is overexpressed in approximately 70% of human breast cancer biopsies, and its overexpression is associated with poor prognosis. Unfortunately, researchers have yet to be successful in developing drugs that directly target c-Myc.

For the vast majority of breast tumors, c-Myc overexpression appears to be brought about indirectly through the misregulation of other genes. Dr. Rosalie Sears and her team have employed a strategy that involves targeting these other genes, as well as the components of these indirect pathways that lead to increased stability of the c-Myc protein. Their hard work led to the elucidation of a novel signaling pathway that post-translationally controls accumulation of the c-Myc protein.

As the recipient of an FY06 Idea Award, Dr. Sears and colleagues made several important discoveries regarding the regulation of c-Myc in breast cancer cells. First, they found that c-Myc stability and oncogenic activity are regulated through phosphorylation of a key residue, Serine 62 (S62). Second, they determined that the tumor suppressor Protein Phosphatase 2A (PP2A) is responsible for dephosphorylating S62, which results in rapid elimination of the c-Myc protein in normal cells. Third, they determined that the overexpression of specific PP2A inhibitors, SET and CIP2A, cause c-Myc stabilization and overexpression in breast cancer cells. Thus, reactivation of PP2A could shut down c-Myc's ability to facilitate malignant cell growth. While confirming these findings, her collaborators at Oncotide, Inc. developed a novel drug that arrests SET's inhibition of PP2A, called OP449. It was determined that Myc levels are reduced after treatment with OP449, which, furthermore, kills breast cancer cells both in culture and after being transplanted into mice.

It is important to note that SET is overexpressed in approximately 60% of breast cancers, while CIP2A is reported to be overexpressed in 39% of breast cancers and correlates with poor outcome. Thus, Dr. Sears joined forces with Dr. Dale Christensen from Oncotide, Inc., and they were recently awarded an FY10 Idea Expansion Award. With this award, they will test the hypothesis that inactivation of PP2A through the overexpression of CIP2A or SET will result in c-Myc activation and subsequent development of breast cancer. The work performed by Drs. Sears and Christensen and their colleagues provides a new approach to reducing the oncogenic potential of breast cells.

Advancing Research on Metastasis

Attacking Breast Cancer: From the Lab to the Clinic

Alana Welm, Huntsman Cancer Institute, University of Utah

Mortality from breast cancer is almost always due to metastasis, the spread of cancer cells from the original tumor to other parts of the body. While early detection methods and treatment options are helping to reduce breast cancer progression to advanced stages, little remains known about the cellular processes that control metastasis. A major obstacle is the lack of robust models that reliably recapitulate the type of metastasis that is observed in human breast cancer. To address this, Dr. Alana Welm set out to humanize mouse models to closely mimic the true disease. With support from an FY07 Era of Hope Scholar Award, Dr. Welm took fresh patient-derived breast tumor samples and grafted them into the mouse mammary gland. These novel tumor grafts are remarkably representative of the original tumors, including their ability to metastasize. Using these and other novel models, Dr. Welm was able to identify and validate genes that promote breast tumor progression. Her lab discovered that macrophage stimulating protein, and its receptor Ron, are responsible for promoting metastasis through multiple mechanisms regulating tumor-host interactions. Inhibiting Ron halted metastatic spread, and Ron inhibitors were able to prevent metastatic outgrowth even after metastatic colonies had already been established. Dr. Welm's results suggested that Ron inhibitors may be a promising avenue to pursue in developing drugs to treat metastatic breast cancers.

In 2010, the first Ron inhibitor entered Phase I testing in cancer patients with advanced disease. Even though the success of this type of research has begun to reach the clinic, the ability to improve predictions of drug efficacy based on the biology of particular tumors are still needed to have the most impact. Therefore, Dr. Welm is expanding her vision under an FY11 Era of Hope Scholar Expansion Award where she will test another promising new Ron inhibitor for the ability to prevent breast tumor growth and/or metastasis using her high-fidelity patient-derived breast cancer explant models, with and without immune humanization. The outcomes of this work will hopefully determine the value of tumor grafts as predictors of therapeutic response and, ultimately, define which patient populations would benefit from Ron inhibitor therapy.

"I have lived for ten years with metastatic breast cancer because of the revolutionary work done by scientists to develop the drug Herceptin with the support of the DoD BCRP. The drug revolutionized clinical management of a very aggressive form of breast cancer. As a committed consumer reviewer, I am confident that new, innovative, high-risk/high-reward research, supported by the DoD BCRP, will uncover ways to prevent breast cancer from coming back years later as incurable metastatic disease. The DoD BCRP deserves strong backing because the program encourages smart scientists and researchers to think out of the box and tackle complex questions in order to save lives in the clinic."

Shirley Mertz
Metastatic Breast Cancer Network

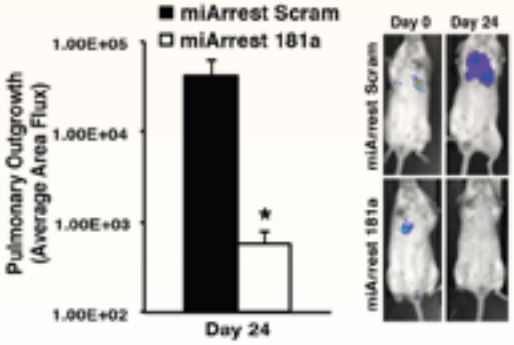
Chemotherapeutic Targeting of Fibulin-5 to Suppress Breast Cancer Invasion and Metastasis Stimulated by Transforming Growth Factor-β

William Schiemann and Molly Taylor, Case Western Reserve University

Despite improvements in breast cancer treatment strategies over the past several decades, 5-year survival rates for breast cancer patients with metastasis remain strikingly low (~25%), highlighting the need for a better understanding of the underlying biological events that lead to metastasis. Metastasis is a complex multistep process during which cancer cells must adapt to and survive in diverse microenvironments in order to disseminate and ultimately form secondary tumors in vital organ sites. The cytokine transforming growth factor-beta (TGF-β) is a protein that typically inhibits cancer formation by preventing uncontrolled cell proliferation and inducing cell death. However, mutations can convert TGF-β from a tumor suppressor to a tumor promoter. These mutations also permit TGF-β to stimulate breast cancer metastasis, the leading cause of breast cancer-related deaths, by promoting EMT. Uncovering the mechanisms that underlie the "TGF-β paradox" could lead to new pharmacological interventions designed to alleviate breast cancer.

With support from an FY08 Idea Award, Dr. William Schiemann utilized a 3D, organotypic cell culture system that simulates the tumor and metastatic microenvironments of breast cancer cells and tested for genetic abnormalities that contribute to breast cancer. Together with Molly Taylor, a graduate student in the lab supported by an FY09 Predoctoral Traineeship Award, Dr. Schiemann determined that a small Ribonucleic acid (RNA) molecule, called microRNA-181a (miR-181a), was upregulated by TGF-β in breast cancer cells, particularly in those from TNBCs, the most difficult to treat. Supporting a causal role for miR-181a in breast cancer, the investigators demonstrated that inhibiting miR-181a in a mouse model of breast cancer led to increased tumor cell death, decreased metastatic outgrowth in the lungs of these mice, and increased overall survival time.

MicroRNAs do not code for proteins but exercise control over RNAs that do. Through studies to determine the function of miR-181a and how it contributes to breast cancer, Dr. Schiemann and Ms. Taylor showed that miR-181a enhances the ability of TGF-β to stimulate breast cancer metastasis by inducing EMT programs and by promoting resistance to programmed cell death by downregulating the pro-apoptotic factor Bim. Elucidating the role of microRNAs in breast cancer represents an exciting new approach in the search for a breast cancer cure. With the identification of miR-181a as a mediator of the "TGF-β paradox," Dr. Schiemann has found a molecule with the potential to both shape therapy and provide a target for future therapeutics, and observes, "Importantly, examination of expression profiles of this microRNA in human patient samples indicates that high levels of expression of microRNA-181a predict for decreased survival in human patients with non-HER2 amplified breast tumors. Collectively, our findings implicate microRNA-181a as a novel diagnostic marker for metastatic progression and decreased survival as well as a potential therapeutic target to treat metastatic breast cancers."



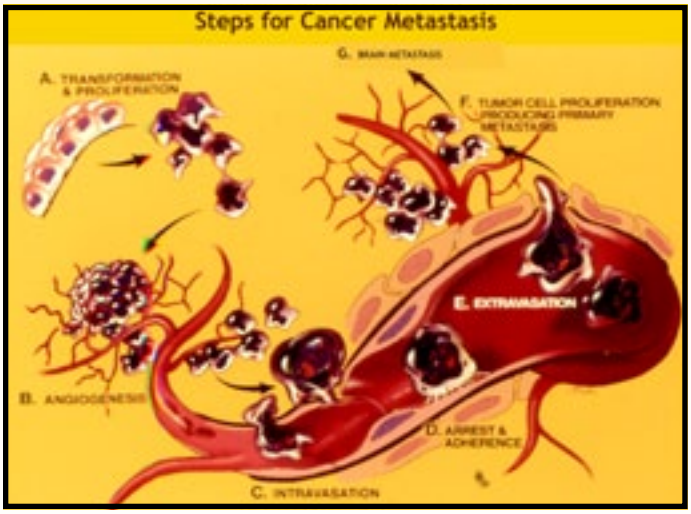
Heparanase Mechanisms in Brain Metastatic Breast Cancer

Dario Marchetti, Baylor College of Medicine

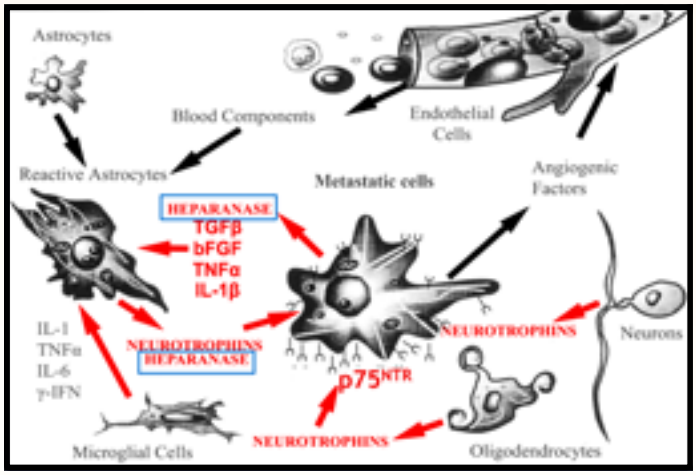
Patients with breast cancer that has metastasized to the brain, namely brain metastatic breast cancer (BMBC), have an exceptionally poor prognosis. Even when receiving the best treatments available, there is only a 20 percent chance that the patient will survive 1 year. Current approaches to treat BMBC include surgical resection and radiotherapy. Although BMBC is particularly associated with HER2-positive breast cancer, efficacies of targeted therapies such as the HER2 monoclonal antibody trastuzumab are limited due to their inability to cross the blood-brain barrier and access the brain compartment. Furthermore, those therapies that are able to cross the blood-brain barrier, such as the HER2/EGFR kinase inhibitor lapatinib, are only minimally effective against HER2-positive cerebral metastases.

Dr. Dario Marchetti was awarded an FY10 Idea Award to address BMBC treatment gaps. His team examined the potential of using heparanase as a novel target for BMBC treatment. Heparanase is an enzyme that degrades heparan sulfate molecules into shorter oligosaccharides possessing biological relevance. Increased heparanase activity typically activates the action of many cancer growth pathways and contributes to increased metastatic activity, notably to brain.

Using BMBC cells, Dr. Marchetti's team tested the effects of a modified heparan sulfate compound called SST0001, either alone or in combination with lapatinib. Results showed that SST0001 alone inhibited heparanase activity. When combined with lapatinib, SST0001 inhibited proliferation of lapatinib-resistant BMBC cells in vitro. Furthermore, the combination of SST0001 and lapatinib blocked tumor growth and brain metastasis in an animal model of breast cancer. Thus, the work accomplished by Dr. Marchetti and his team shows promise for this combined therapy to be utilized to treat BMBC, as well as the potential for SST0001 to be used as treatment for lapatinib-resistant cancers.



Mechanisms of Brain Metastasis



Heparanase Mechanisms in Brain Microenvironment

Targeting Cadherin-11 in Basal-Like, Hormone-Refractory Breast Cancer

Stephen Byers, Georgetown University

Although basal-like breast carcinomas constitute only 10% to 15% of known breast cancers, they are typically aggressive, resistant to hormonal therapy, and consequently associated with poor clinical outcomes. One molecule associated with invasive breast cancers is the cell-cell adhesion protein, E-cadherin. Normally, breast epithelial cells stop growing when they come in close contact with neighboring cells. Changes in cell-cell adhesion contribute to the ability of breast cancer cells to invade surrounding tissue during metastasis. A number of studies have linked decreased expression of E-cadherin to the EMT that is strongly associated with metastasis.

But E-cadherin loss, while important in cancer progression, may not be sufficient. Funded with an FY00 Idea Award and an FY06 Synergistic Idea Award, both from the BCRP, Dr. Stephen Byers and his colleagues showed that another adhesion molecule, cadherin-11, is also changed in breast cancers. However, unlike E-cadherin, it is not the decrease, but the increase of cadherin-11 expression that is associated with breast cancer. The increase is striking enough that compared to E-cadherin loss, elevated cadherin-11 actually provides an even more powerful predictor of tumor invasion and metastasis.

Continuing to build upon this work on cadherin-11 with funding from an FY09 BCRP Idea Expansion Award, Dr. Byers teamed with Dr. Milton Brown to explore the role of cadherin-11 in basal-type breast cancer. They found that when two splice variants of cadherin-11 – the full-length and truncated forms – are both expressed in the same cell, they cause the cell to exhibit metastatic, invasive behavior. Thus, Dr. Byers and colleagues determined that cadherin-11 was a potential therapeutic target for basal-like breast cancer.

The Byers lab further sought to identify compounds that inhibit cadherin-11 in order to treat basal-like breast cancer. In addition to the design and generation of molecules de novo, the team chose to screen drugs already approved by the FDA, in hopes of finding one that could be brought to the clinic quickly to treat breast cancer patients. Sivanesan Dakshanamurthy, a computational chemist at Georgetown, created a program that predicted how drugs would interact with potential molecular targets. Interestingly, he found that the arthritis drug Celebrex was among the top drugs with the potential to bind and inhibit cadherin-11.

The finding was published only last year, and it is still too soon to know how effective Celebrex might be in treating basal-like breast cancers, but Dr. Byers is optimistic about his lab's approach: "The prospect of using a drug developed for arthritis in treating breast cancer is very powerful," Dr. Byers said. "The support of the DoD BCRP was crucial to the success of this work as it allowed us to explore a hypothesis that was high risk and high reward."

Eliminating Late Recurrence to Eradicate Breast Cancer

Jayanta Debnath and Jennifer Rudnick, University of California, San Francisco

Recurrence remains a major challenge in the fight against breast cancer. Following initial treatment, cells can lie dormant for years or even decades before emerging as cancerous once again. Moreover, recurrent cancers are typically resistant to treatment and are a high risk for metastasis, making late recurrence an indicator for poor prognosis. Any strategy with potential to eradicate breast cancer needs to either eliminate these dormant cells or prevent them from reawakening as new cancers. With funding from an FY10 Era of Hope Scholar Award, Dr. Jayanta Debnath and colleagues are exploring the link between the reawakening of late recurrent cells and an evolutionarily conserved pathway on which all cells rely to survive.

Autophagy, a genetically controlled process through which a cell "eats itself," is a process conserved in all eukaryotic cells. Genetic studies show that autophagy preserves genomic stability and limits cancer initiation. On the other hand, in established tumors, those same capabilities act to support survival and tumor progression. There is currently great interest in autophagy as a possible target against breast cancer due to the fact that inhibiting autophagy in tumor cells could be a way to halt cancer.

Using a 3D organotypic culture model, Dr. Debnath's group uncovered two opposing, context-dependent functions for autophagy in breast cancer cells. On the one hand, in breast tumors driven by the PI3K pathway, autophagy restricts proliferation and cells maintain a quiescent state. On the other hand, autophagy promotes invasive behavior in tumors where the Ras/MAPK pathway is hyper-activated.

Dr. Debnath's group discovered that the crucial factor that determined whether autophagy inhibition liberated proliferation or caused cell death was whether or not it was in direct contact with the ECM. Cells that came in contact with the ECM continued to grow following autophagy inhibition, while cells located centrally within the tumor and deprived of ECM contact underwent cell death following autophagy inhibition. The context-dependent and differential functions of autophagy learned in this study could lead to a more refined treatment approach that is more effective at killing tumor cells, while causing fewer side effects.

Pursuing related work, a member of Dr. Debnath's lab, Dr. Jennifer Rudnick, won an FY12 Postdoctoral Fellowship Award to explore the possible role of autophagy in survival of the "tumor stroma" – the complex network of cells surrounding and nourishing tumors to promote tumor cell growth, survival, invasion, and metastasis. Remarkably, autophagy inhibitors are currently in clinical trials for late-stage breast cancer. Dr. Rudnick plans to study the effects of autophagy inhibition in the tumor stroma and, comparing their effects on tumor cells, will determine whether these therapies work in unison or antagonistically in the treatment of breast cancer.

Research on the Horizon

Jennifer Richer and Anthony Elias, University of Colorado, Denver

FY12 Clinical Translational Research Award

Conducting clinical studies to validate the AR as a novel target for breast cancer therapeutics

Rebecca Cook, Vanderbilt University Medical Center

FY12 Postdoctoral Fellowship Award

Targeting the immune system's natural response to cell death to improve therapeutic response in breast cancer

David Spiegel, Yale University

FY12 Era of Hope Scholar Award

Developing "molecular fingerprinting" as a platform to identify personalized therapeutic regimens

Timothy Chan, Memorial Sloan Kettering Cancer Center

FY12 Era of Hope Scholar Award

Targeting master regulators of the breast cancer metastasis transcriptome

Thomas Westbrook, Baylor College of Medicine

FY12 Era of Hope Scholar Award

Tackling triple negative breast cancer by targeting tumor suppressor networks that regulate tyrosine kinases

Richard Steinman, University of Pittsburgh

FY12 Idea Award

Real-time visualization and manipulation of the metastatic trajectory of breast cancer cells to better understand the tumor microenvironment

Weian Zhao, University of California, Irvine

FY12 Idea Award

Exploiting mesenchymal stem cells as mechanoresponsive sensors and vehicles for drug delivery to target breast cancer metastases

Kevin Williams, North Carolina Central University

FY12 Idea Award

Defining a new approach to treating inflammatory breast cancer through targeting of GLI1

Nira Ben-Jonathan, University of Cincinnati

FY12 Idea Award

Exploring therapeutic and imaging applications of dopamine receptor agonists in breast cancer

Yoannis Imbert-Fernandez, University of Louisville

FY12 Postdoctoral Fellowship Award

Delineating the mechanisms of glucose utilization by estradiol in breast cancer

Yi Li, Baylor College of Medicine

FY12 Idea Expansion Award

Repositioning the drug pimozide for breast cancer prevention

Ruben Rene Gonzalez-Perez, Morehouse School of Medicine

FY12 Idea Award

Targeting breast cancer stem cells in TNBC

Robin Fuchs-Young and John Lightfoot, Texas A&M University

FY12 Idea Expansion Award

Reprogramming the effects of early high-sugar/high-fat diets on breast cancer risk

Brunhilde Felding-Habermann and Gary Siuzdak, Scripps Research Institute

FY12 Idea Award

Metabolomic imaging of early breast cancer brain metastasis to identify targets for prevention

Valerie Weaver, University of California, San Francisco

FY12 Era of Hope Scholar Expansion Award

Defining how tissue tension regulates breast tumor aggressiveness, and testing whether reducing tissue tension represents a new therapeutic opportunity

Stephanie Yazinski, Massachusetts General Hospital

FY12 Postdoctoral Fellowship Award

Deciphering Novel Mechanisms of PARP Inhibitor Resistance in BRCA1-Deficient Breast Cancer

Paolo Serafini, University of Miami School of Medicine

FY12 Idea Award

Silencing Chemokine Signaling in Tumor-Conditioned Myeloid Cells for the Treatment of Breast Cancer

Vadivel Ganapathy, Georgia Health Sciences University

FY12 Idea Award

Defining Homocysteine as an Oncometabolite in Breast Cancer

In the Clinical Pipeline

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.

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.

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 demonstrated that MBI may be used to monitor patients' response to chemotherapy. Currently, two FDA-approved MBI units are commercially available.

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 granulocyte/macrophage colony-stimulating factor (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.

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.

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.

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.

GM-CSF-Secreting Vaccine

Leisha Emens

Dr. Emens developed a therapeutic GM-CSF-secreting breast cancer vaccine to be used in combination with standard cancer therapies. Her preclinical data provided the basis for a clinical trial that tested vaccine-cyclophosphamide-trastuzumab combination therapy in women with Stage IV metastatic HER2+ breast cancer. Clinical benefit, defined as complete or partial response to treatment (tumor shrinkage) or stabilization of disease (halted growth or spread), was present at 35% after one year. Early analysis showed that overall survival was 40 months, a significant improvement over the historical overall survival of 12 to 24 months for similar patients who received trastuzumab alone. Dr. Emens is continuing clinical trials on this vaccine in a larger breast cancer study, as well as expanding to similar immune-based strategies in other gynecological malignancies.

HER2 Peptide-Based Vaccine

Mary (Nora) L. Disis

Dr. Disis developed a vaccine that, when concurrently administered with trastuzumab strongly elicits an immune response to the growth factor receptor HER2, generating long-term tumor-specific immunity. The HER2 intercellular domain (ICD) peptide-based vaccine is designed to treat breast cancer by stimulating the immune destruction of remaining cancer cells after primary cancer therapy. The HER2 ICD peptide vaccine was evaluated in a Phase II clinical trial in Stage III and Stage IV HER2+ breast cancer patients concurrently receiving trastuzumab. Results of the trial indicated considerable improvements in relapse-free survival, as well as minimal toxicity and prolonged, robust, antigen-specific immune responses. The vaccine has been licensed commercially for further investigation.

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 and Edmiston of 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 prevent progression to invasive breast cancer.

PD0332991 (Palbociclib)

Dennis Slamon

Preclinical research supported by the BCRP led to the identification of cyclin-dependent kinases (CDKs) as a target for ER+ breast cancer and the discovery that ER+ breast cancer cells are sensitive to a CDK inhibitor, PD-0332991. These and other findings provided the basis for Phase I and Phase II clinical trials, supported by Pfizer, in which PD-0332991 in combination with the aromatase inhibitor letrozole demonstrated an increase in median progression-free survival. These results prompted "Breakthrough Therapy" status by the FDA and Pfizer's 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-0332291 can be administered to patients who will benefit most from its use.

TRC105 Antibody

Ben Seon

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

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

Making an Impact

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.

BreastCancerTrials.org

Laura Esserman

Breast cancer patients can benefit from objective information about clinical trials. The process of identifying an appropriate clinical trial by performing independent research is challenging. BCRP funding contributed to the development of an online resource (BreastCancerTrials.org) that educates patients about breast cancer clinical trials and matches them with appropriate trials.

Expression Arrest™ shRNA Libraries

Gregory Hannon and Stephen Elledge

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

Herceptin®

Dennis Slamon

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

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, now provides a range of risk assessment, screening, and preventive services to individuals who have a family history of breast or ovarian cancer.

OncoVue®

Eldon Jupe

Risk-association studies funded by the BCRP formed the foundation for a breast cancer risk assessment test that is now commercially available. 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. SNPs can help determine the likelihood that someone will develop a particular disease. 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 30 breast care centers in the U.S.

PTEN Tumor Suppressor Gene

Michael Wigler

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

Sentinel Lymph Node Biopsy

Douglas Reintgen and 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 multi-center clinical trials that validated lymph node mapping and sentinel lymph node biopsy as an accurate method to predict the presence of disease in the axillary lymph nodes.

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 early-stage ER+ breast cancer. The focus of the ATLAS trial was to examine whether 10 years of adjuvant tamoxifen confers greater benefit overall than 5 years of adjuvant tamoxifen. The clinical trial was initiated in 1996 and completed randomized accrual in 2005. Women with ER+ early stage breast cancer who had completed 5 years of adjuvant tamoxifen were randomized to either continue for another 5 years or to stop the treatment. Preliminary analysis indicated that recurrence rate was lower among those who continued tamoxifen treatment. ATLAS is currently in the follow-up phase until 2015.

3D 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 ECM 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.

Skp2 Oncogene

Michele Pagano

Skp2 and p27 are genes involved in the regulation of the cell cycle. The BCRP supported the establishment of Skp2 as an oncogene that is overexpressed in human breast tumors. High Skp2 expression correlating with destabilization of p27 was found to be associated with poor prognosis in breast cancer patients. These findings contributed to the practice of Skp2/p27 immunohistochemical analysis as a prognostic test performed in clinical pathology laboratories.

BrainMetsBC.org

Patricia Steeg

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

PALB2

Bing Xia

BCRP funding contributed to 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 and division. 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 who tested negative for BRCA1 and BRCA mutations.

Digital Mammography and Breast Tomosynthesis

Laurie Fajardo and 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 multi-center clinical validation of digital mammography. The study demonstrated that digital mammography is superior to film mammography in detecting breast cancer in women with moderate to marked dense breast tissue, leading to a change in clinical practice. The BCRP also supported the development and clinical evaluation of DBT. 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.

For more information, please visit
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