

## Highlights from the 38<sup>th</sup> Annual San Antonio Breast Cancer Symposium



Photo Courtesy @ SABCS/Todd Buchanan 2015

The 38th Annual San Antonio Breast Cancer Symposium (SABCS) was held December 8-12, 2015. Since 1977, the Symposium's mission has been to provide state-of-the-art information on breast cancer research. From a one-day regional conference, the Symposium has grown to a five-day program attended by a broad international audience of academic and private researchers and physicians from over 90 countries.

The Symposium aims to achieve a balance of clinical, translational, and basic research, providing a forum for interaction, communication, and education for a broad spectrum of researchers, health professionals, and those with a special interest in breast cancer.

On the IBC specific front, there was no breaking news presented. We have listed all the important presentations related to IBC (see below). One finding is that HDAC type I inhibitor entinostat had favorable clinical activity in metastatic IBC. This is an interesting finding which needs further clinical trial development. From the non-IBC world, we were excited to hear that an immune checkpoint inhibitor has continued to show clinical activity with breast cancer. Further, the use of denosumab in ER+ newly diagnosed breast cancer, with aromatase inhibitor, reduces the disease recurrence. Lastly, a significant finding was that patients with residual disease after chemotherapy saw a reduced recurrence rate with the use of additional capecitabine. Clinical use of this knowledge will require additional studies and discussion among our group.

The Morgan Welch Inflammatory Breast Cancer Research Program and Clinic had a great turn out at SABCS. As a program, a total of ten abstracts were presented. Please join us in congratulating each program member on their recent presentations.

## 38<sup>th</sup> Annual San Antonio Breast Cancer Symposium Abstracts

### **High serum miR-19a levels correlated with favorable prognosis in patients with metastatic HER2+ breast cancer and might result from effective antibody-dependent cell-mediated cytotoxicity (ADCC) induced by trastuzumab and Th1-mediated antitumor immune response**

**Authors:** Anfossi S, Huo L, Woodward WA, Ueno NT, Valero V, Calin GA and Reuben JM

We found that patients with high levels of serum miR-19a had longer progression-free survival (PFS: 7.9 vs. 4.1 months;  $p=0.003$ ) and overall survival (OS: median not reached vs. 13.1 months;  $p<0.0001$ ) compared with patients with low levels. Furthermore, breast cancer tissue expressed higher levels of miR\_19a compared with normal adjacent tissue ( $p=0.048$ ). We hypothesized that high levels of serum miR-19a may result from an effective anti-tumor immune response, that consequently may be responsible for the better prognosis. Indeed, we found that in vitro antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells and induced by trastuzumab determined an increased release of miR-19a from killed breast cancer cells. Furthermore, type 1 T helper lymphocytes (Th1), the key regulators of antitumor cytotoxic T lymphocyte (CTL) activation, expressed and secreted high levels of miR-19a. Our results suggested that miR-19a may

potentially represent a novel serum biomarker to evaluate trastuzumab response and Th1-mediated anti-tumor immunity in patients with metastatic HER2+ breast cancer.

### **The histone deacetylase inhibitor entinostat enhances the efficacy of the MEK inhibitor pimasertib against aggressive types of breast cancer through Noxa-mediated myeloid cell leukemia 1 degradation**

**Authors:** *Torres-Adorno AM, Lee J, Kogawa T, Bartholomeusz C, Pitner MK, Ordentlich P, Lim B, Tripathy D and Ueno NT*

After recently demonstrating involvement of the histone deacetylase (HDAC) type I inhibitor entinostat on the regulation of apoptosis in IBC and TNBC cells, we were aiming to identify new combination-therapy candidates by genome-wide functional mRNA expression analysis after entinostat treatment. We observed that entinostat induced the activity of the cancer-associated ERK pathway, but also expression of Noxa, a pro-apoptotic member of the Bcl-2 family of apoptosis-regulating proteins, both of which are known to stabilize and degrade, respectively, Mcl-1, an anti-apoptotic Bcl-2 protein. Through our study, we discovered that entinostat, together with ERK pathway inhibition via the MEK inhibitor pimasertib, had a significant synergistic effect inducing apoptosis in both in vitro and in vivo models of TNBC and IBC through a mechanism involving induction of cell death by enhanced Noxa-mediated Mcl-1 degradation. These findings currently provide a novel preclinical rationale for developing a clinical trial based on HDAC and MEK pathway inhibition combination therapy for TNBC and IBC with high Mcl-1 expression.

### **CD44v as a potential predictive biomarker for pathologic complete response in primary HER2+ breast cancer: Utility of adaptive response biopsy in preoperative therapy**

**Authors:** *Yamauchi T, Yamamura CK, Yamauchi H, Jinno H, Takahashi M, Kitagawa Y, Nakamura S, Lim B, Krishnamurthy S, Reuben JM, Liu D, Tripathy D, Zujewski JA, Chen H, Takebe N, Saya H and Ueno NT*

CD44v as a potential predictive biomarker for pathologic complete response in primary HER2+ breast cancer: Utility of adaptive response biopsy in preoperative therapy. This is a collaborative work between Japan and USA to develop novel cancer stem cells marker CD44v can predict the response to HER2 targeted therapy. The study shows that CD44v elimination by anti-HER2 may predict the pathological complete response of anti-HER2 therapy in HER2+ breast cancer. We are currently exploring if CD44v can be a therapeutic target.

### **Lipoproteins regulate the effects of macrophages and mesenchymal stem cells on radiation response of inflammatory breast cancer cells**

**Authors:** *Rahal OM, Wolfe AR, Larson RA, Ueno NT, Reuben JM and Woodward W*

The role of tumor microenvironment on the radiation response of IBC cells and whether this is regulated by lipoproteins has not been addressed in depth. In this study, we show that polarization of macrophages and MSCs into a "pro-tumor" M2-phenotype, upon co-culture with IBC cells, leads to radioresistance of IBC cells and this effect can be inhibited by high density lipoproteins (HDL). These data suggest that cells within tumor micro-environment such as macrophages and MSCs mediate radiation resistance of IBC and this can be reversed by HDL.

### **Women's triple-negative, first-line treatment: Improving outcomes in triple-negative breast cancer using molecular triaging and diagnostic imaging to guide neoadjuvant therapy**

**Authors:** *Mitri ZI, Ueno NT, Yang W, Valero V, Litton JK, Murthy RK, Ibrahim NK, Arun BK, Mittendorf EA, Hunt KK, Meric-Bernstam F, Thompson A, Piwnicka-Worms H, Tripathy D, Symmans F and Moulder-Thompson S*

This poster illustrates the neoadjuvant triple negative breast cancer triaging protocol that was launched at MD Anderson in November of 2015. This represents an unprecedented opportunity for a dynamic and real time translational platform to advance research in the field of triple negative breast cancer. In addition, this is an extremely attractive avenue for the evaluation of novel therapeutics for triple negative breast cancer in the neoadjuvant setting, with a go-no go decision obtained after enrolling a limited number of patients.

### **A single-center, open-label phase 1b study of entinostat, and lapatinib alone, and in combination with and trastuzumab in patients with HER2+ metastatic breast cancer after progression on trastuzumab**

**Authors:** *Lim B, Jackson S, Alvarez RH, Ibrahim NK, Willey JS, Murthy RK, Booser DJ, Giordano SH, Barcenas CH, Brewster A, Walters RS, Brown PH, Tripathy D, Valero V and Ueno NT*

Our in vitro and in vivo preclinical data showed that entinostat enhances the efficacy of lapatinib in HER2 positive (HER2+) breast cancer cells via FOXO3-mediated Bim1 expression, which resulted in enhanced apoptosis in HER2 targeted therapy (lapatinib and trastuzumab)-resistant breast cancer (IBC and non-IBC) cells [Lee et al.]. Based on these findings, we conducted a phase 1b trial of entinostat to determine the maximal tolerated dose (MTD) in combination with lapatinib alone and in combination with lapatinib and trastuzumab for metastatic HER2+ breast cancer patients (pts), who progressed on trastuzumab. MTD was reached at 12mg q 2wly entinostat, lapatinib 1000 mg daily and trastuzumab 8 mg/kg

followed by 6mg/kg q 3 wks. This combination was safe and had promising clinical efficacy in patients with trastuzumab-resistant metastatic HER2+ breast cancer including IBC, warranting further study.

### **Predicting the response of molecular targeting agents in triple-negative breast cancer cell lines by kinase activities**

**Authors:** Sato N, Wakabayashi M, Lee J, Lim B, Ueno NT and Ishihara H

This work discussed new diagnosis assay platform to determine functionalities/activities of the kinase molecules. While many therapeutic agents targeting kinases are being developed, however, the detection methods to predict the response of kinase inhibitors are not successfully developed to translate into the clinic. The objectives of this study are 1) Establishment of a new profiling system for molecular-targeted agents based on kinase enzymatic activity analysis, 2) Evaluation of the relationship between the relevant mutational status in two pathways and kinase enzymatic activity. Assay results show that relative activity of two relevant kinases in the signaling cascade could predict the cell lines that will not respond to molecular targeting agents against corresponding cascades. Study concept should be warranted in the clinical study with statistically sufficient number of patients.

### **Association between quantitative values of estrogen receptor expression level and pathological complete response in human epidermal growth factor 2-negative breast cancer: Should the clinical definition of triple-negative breast cancer be redefined?**

**Authors:** Fujii T, Kogawa T, Dong W, Moulder S, Litton JK, Tripathy D, Lim B, Shen Y and Ueno NT

The optimal ER cut-off on HER2-negative breast cancers to stratify patients into groups more or less likely to achieve pCR is still unclear. Previously guideline recommended 10% and currently it recommends 1%. Based on our analysis result of 1055 patients, percent ER in the 0-10% range does not correlate with pCR status and no ER cut-off was identified for stratification. Our conclusion is that in HER2-negative breast cancer, there is no difference of pCR rate after neoadjuvant chemotherapy regardless of ER value among patients with ER <10%.

### **Pilot study of prognostic utility of circulating tumor cells (CTCs) assessed by AdnaGen technology and clinical outcome of patients with stage III breast cancer who completed locoregional and systemic treatment**

**Authors:** Alvarez RH, Gao H, Ensor JE, Gomez HL, Ruiz-Garcia EB, Arce C, Sun H, Willey JS, Ueno NT, Valero V and Reuben JM

Detection of high number of CTCs (>5) before initiation of first-line therapy in patients with metastatic breast cancer is associated with shorter progression free survival and overall survival. The most widely used method is CellSearch (Veridex, Raritan, NJ). It relies on immunomagnetic capture of CTCs, using antibodies against the epithelial cell adhesion molecule (EpCAM). Although the US Food and Drug Administration approved CellSearch assay for clinical use. In addition to isolation and enumeration, a promising area of research is genomic CTCs characterization which entails phenotyping and molecular expression profiling of CTC subsets consisting of those of epithelial origin (CTC-Epi), others undergoing epithelial to mesenchymal transition (CTC-EMT), or expressing cancer stem cell-like phenotype (CTC-CSC; CD44+ CD24low, ALDH+), respectively. EMT is a molecular process to acquire the traits needed to execute the multiple steps of metastasis. Through the EMT process, epithelial cells lose cell-cell contacts and cell polarity, downregulate epithelial-associated genes, acquire mesenchymal gene expression and undergo major changes in their cytoskeleton. Currently, a CTC detection kit is available to detect CTCs expressing EMT-associated genes by semiquantitative RT-PCR (Adna EMT2/Stem Cell test). EMT will be detected by measuring EMT-inducing transcription factors such as TWIST1, SNAIL1, SLUG, ZEB1 and FOXC2) by RT-PCR. This study is a 7-year study (84 months). Pts will be classified as to the presence [negative (neg) vs. positive (pos)] of CTC and as to the expression of a biomarker (neg vs. pos). The primary endpoint of the study is breast cancer recurrence. Time to recurrence curves for the four breast cancer patient groups (neg/neg, neg/pos, pos/neg, or pos/pos) will be estimated using the Kaplan-Meier method and differences in the recurrence rates will be evaluated by the log-rank test at the end of the study (84 months). The confidence intervals for the quantiles of the recurrence distribution will be based on the sign test as described by Brookmeyer and Crowley.

### **IPC-366 cell line, a canine inflammatory breast cancer (IBC), as a good model for in vitro studies on human IBC research**

**Authors:** Caceres S, Peña L, Lacerda L, Illera MJ Jose, Monsalbe B, de Andres PJ Jimena, Larson RA, Gao H, Debeb BG, Woodward WA, Montaña AV, Reuben JM and Illera JC Carlos

Inflammatory breast cancer (IBC) is an aggressive type of cancer with poor survival in women. Canine IBC is clinically and histopathologically very similar to human IBC and has been proposed as a good surrogate model for study the human disease. Recently a triple negative canine IBC epithelial cell line, IPC-366 with many characteristics of the human IBC cell line SUM149, has been established. The aim of this study was to validate IPC-366 as a good model for IBC research in terms of stem cell markers expression by flow cytometry, protein production by western blot and their capacity to form tumors in vivo in SCID mice in adherent (2D) an non-adherent (3D, mammospheres) culture conditions. Our results revealed that the canine IBC cell



line IPC-366 is capable of forming long-term mammospheres with a grape-like morphology. IPC-366 2D and 3D exhibited fast growth in vivo having differences in histology tumor sections. Stem cell marker expressions showed that IPC-366 in adherent and non-adherent conditions has mesenchymal-like characteristics that could be due to its aggressive and angiogenic phenotype. Epithelial-to-mesenchymal transition (EMT) markers expression, such as E-cadherin and N-cadherin, was higher in 2D than in 3D cultures, revealing that the loss of their expression is an important characteristic for forming mammospheres. In spite of this, scanning electron microscopy showed that mammospheres are formed mainly by cohesive cells and flattered cells resembling endothelial cells (attributable to vasculogenic mimicry phenomenon). These results were consistent with those found in SUM149 under the same conditions. This work is of significance because there are currently very few cell lines to study human IBC. As such, we believe that the IPC-366 cell line provides a useful vehicle to conduct basic tumor biology studies on IBC and aggressive metastatic cancer in general, but also it will be helpful for the development of potential therapeutic agents and for future interspecies comparative new therapeutic strategies against IBC/IMC.

## Workshop Develops New Collaborations Within MD Anderson Cancer Center

Each year, the MD Anderson Morgan Welch Inflammatory Breast Cancer Research Program and Clinic holds a workshop at the end of the year. At these annual workshops, Program Leaders discuss what was accomplished during the past year, and focus on what we hope to accomplish in the year to come. This year, our workshop theme was encouraging new collaborations within MD Anderson. To do this, we used the Research-Match Workshop guideline that has been used throughout the institution.

Research-Match is a workshop designed around the concept of speed dating. The end result of the Research-Match Workshop is to give a seed grant award to the group(s) with the highest quality, and most novel idea. Participants are split into three groups and given a number, letter or Greek symbol. Each round begins with group members giving their 'research elevator pitch' (a 20-30 second description of their personal research goals). After this initial introduction, each group discusses potential novel concepts and ideas that may lead to the eradication of inflammatory breast cancer.

After all rounds have been completed, participants may reconvene with those who they are interested in writing a letter of intent with. The letters of intent, with sketches of the proposed study (scientific/clinical question being investigated, hypothesis and methods), are presented, and a panel of program members votes for five LOIs to submit a full proposal for the chance to receive a seed grant.

The five LOIs that were asked to submit a full proposal from our group were:

### **Proximity Extension Assay**

Hui Gao, Sr. Research Scientist, Hematopathology  
Gitanjali Jayachandran, Sr. Research Scientist, Hematopathology

### **Gold Nano-Particles and IBC**

Pankaj Singh, Instructor, Experimental Radiation Oncology  
Shanta Bhattarai, Instructor, Experimental Radiation Oncology  
Kelsey Boitnott Mathieu, Instructor, Imaging Physics

### **EPA / EPHA2**

Angie Torres-Adorno, Graduate Student, Breast Medical Oncology  
Shanta Bhattarai, Instructor, Experimental Radiation Oncology  
Rajagopal Appavu, Postdoctoral Fellow, Experimental Radiation Oncology

### **siRNA Targeting T1G1 Delivered by Nanoparticle**

Xiaoping Wang, Research Scientist, Breast Medical Oncology  
Rajagopal Appavu, Postdoctoral Fellow, Experimental Radiation Oncology  
Jun Zhao, Instructor, Cancer Systems Imaging

### **NK Cells to Predict the Treatment Outcome in IBC**

Gitanjali Jayachandran, Sr. Research Scientist, Hematopathology  
Hui Gao, Sr. Research Scientist, Hematopathology

## Recent Awards and Grants:

**Angie Torres-Adorno**, Graduate Student with Breast Medical Oncology, and **Omar Rahal, PhD**, Postdoctoral Fellow with Experimental Radiation Oncology, are the winners of the Fourth Annual Zeta Tau Alpha Houston Alumnae Association Fellowship in Inflammatory Breast Cancer. The Zeta Tau Alpha Foundation has established an endowment through the Morgan Welch IBC Program to fund a fellowship in inflammatory breast cancer (IBC) research. This year, the Program will award two travel grants recognizing efforts with exceptional quality of IBC research and high impact (or potential impact) for our IBC patients. Angie and Omar have both been invited to present their winning abstracts at the San Antonio Breast Cancer Symposium next month in San Antonio, Texas.



## Recent Awards and Grants, continued:

**Bisrat Debeb, PhD**, Assistant Professor with Experimental Radiation Oncology, has been awarded an NCI U54 seed grant or U54 feasibility grant titled, "LCN2 in inflammatory breast cancer metastasis". The U54 is a grant awarded from NCI to MD Anderson Cancer Center to establish a comprehensive, long-term, partnership with the University of Puerto Rico Cancer Center. The ultimate goal of this partnership is to reduce the cancer health disparities.

**Geoffrey Bartholomeusz, PhD**, Associate Professor with Experimental Therapeutics, was awarded the AVON Foundation for Women grant titled, "Development of an Ex-vivo tumor model of inflammatory breast cancer for drug testing". This is a collaboration among Dr. Bedrich Eckardt, Dr. James Reuben, Dr. Naoto Ueno and Dr. Geoffrey Bartholomeusz. The goal of this project is to develop a new technology for a drug screen, using an ex-vivo culture system that mimics PDX or human setting.

## Quarterly Oral Presentations

### **Development of a microfluidic system for the isolation and molecular characterization of circulating tumor cells**

Kelsey Boitnott Mathieu, PhD  
Instructor, Imaging Physics

### **Integrated Meeting – Providing updates on clinical trials and lab studies**

Naoto Ueno, MD, PhD  
Professor, Breast Medical Oncology

### **Updates on patient-derived xenograft (PDX) models of IBC at the Morgan Welch Inflammatory Breast Cancer Research Program**

Bedrich Eckhardt, PhD  
Instructor, Breast Medical Oncology

## Recent Publications

### **Simvastatin prevents triple-negative breast cancer metastasis in pre-clinical models through regulation of FOXO3a**

Wendy Woodward, MD, PhD  
View article here: <http://1.usa.gov/1RncD4q>

### **Inflammatory breast cancer: unique biological and therapeutic considerations**

Wendy Woodward, MD, PhD  
View article here: <http://bit.ly/20rAOBE>

### **Comprehensive two and three-dimensional RNAi screening identified PI3K inhibition as a complement to MEK inhibitor AS70326 for combination treatment of triple-negative breast cancer**

Arvind Uppore Kukkillaya, PhD  
View article here: <http://bit.ly/1OuESrI>

## Current Clinical Trials Open for New Patient Enrollment

**2006-1072 IBC Registry**

**2013-0007 Phase II study of denosumab to define the role of bone related biomarkers in patients with breast cancer and bone metastasis**

**2013-0139 Phase IB trial of two folate binding protein peptide vaccine (E39 and J65) in breast and ovarian cancer patients**

**2013-0436 Combination immunotherapy with Herceptin and HER2 vaccine E75 in low and intermediate HER2 expressing breast cancer patients to prevent recurrence**

**2014-0464 A phase II study of BIBF-1120 (Nintedanib) for patients with HER2 normal metastatic inflammatory breast cancer**

**2014-0533 A phase II study of anti-PD1 (MK-3475) therapy in patients with metastatic inflammatory breast cancer who have received prior chemotherapy with clinical response**



## Current Lab Studies

- PA12-0097 Prognostic utility of CTCs assessed by adnagen technology and clinical outcome of patients with stage III breast cancer**
- PA12-0728 Expansion and characterization of tumor-infiltrating and tumor-associated T cells from primary and metastatic triple-negative breast cancer and inflammatory breast cancer**
- PA12-0860 Assessing feasibility of sentinel lymph node increase dissection in IBC**
- PA14-0772 Derivation of Pt derived xenograft tumor models from isolated CTC from breast cancer pts( IBC/TNBC)**
- PA14-0778 Gene profiles in androgen receptor-positive CTC in patients with metastatic breast cancer**

If you are interested in learning more about our clinical trials, or lab studies, please email the Morgan Welch Inflammatory Breast Cancer Research Program and Clinic directly at [ibcp@mdanderson.org](mailto:ibcp@mdanderson.org). We are happy to provide general information and eligibility guidelines for our clinical trials and lab studies.

