9th International Symposium on the Breast
Exploring the Human Breast: Employing New Technology
Conference Report

Dr. Susan Love Research Foundation is dedicated to achieving a future without breast cancer by engaging the public and the scientific communities in innovative and collaborative research, translating science to engage the public as informed partners, and inspiring novel research. As part of this effort, the Foundation hosts a biennial International Symposium on the Breast that brings together world-class researchers, clinicians, and advocates in an intimate think-tank environment to stimulate ideation, collaboration, and ultimately, breakthroughs that will end breast cancer.

The 9th International Symposium was held February 23 - February 24, 2017, in Santa Monica, California. More than 120 clinical researchers, epidemiologists, engineers, pathologists, basic scientists, translational investigators, and breast cancer advocates attended this year's conference, "Exploring the Human Breast: Employing New Technology."

Attendees utilized the unique forum to explore new theories and directions for studying the breast and breast cancer from multidisciplinary perspectives. In acknowledgment and support of their shared goals, many presenters discussed unpublished data that they hoped would spur or advance others' research. Many also forged new research partnerships.

"The goal of the symposium is to challenge you to think about things in new ways," said Dr. Susan Love during her opening remarks. No one left the event questioning whether it had achieved this goal.

Introduction

New technologies are leading researchers to pose new questions and pursue new avenues of research into causes and prevention of breast cancer. Over two days, Dr. Susan Love Research Foundation 9th International Symposium on the Breast provided valuable opportunities for pioneering researchers, clinicians, and breast cancer advocates to discuss and explore ways to use new insights gleaned from new technologies to advance our understanding of the breast. Each of the researchers who spoke at the Symposium demonstrated the importance of using a multidisciplinary approach to pursuing long unanswered questions about the biology of the breast and breast cancer.

New Insights Into Breast Cancer Risk Factors

Susan Clare, MD, PhD, research associate of surgery at Northwestern University Feinberg School of Medicine, opened the Symposium with the presentation, "Benign Breast Tissue as a 'Crystal Ball': Using Mutations to Predict Breast Cancer Risk."

Breast cancer incidence rates in the U.S. have changed little since 1975. This suggests that both new methods of breast cancer prevention are needed and currently available options—lifestyle modification, surgical intervention and/or chemoprevention—are inadequate. Dr. Clare discussed findings from a case-control study that analyzed tissue blocks from benign breast biopsies from 35 women, 24 of whom had had a breast cancer diagnosis within a year of their benign breast biopsy. Her team looked for genetic alterations in the benign breast tissue that would be predictive of the eventual development of breast cancer. The biopsy samples used in the study were not necessarily near where the tumor developed—or even from the same breast. Thus, any predictive genetic alterations her research identified would suggest the presence of a field cancerization—epigenetic or genetic—that encompasses both breasts and that occurs before the tumor is detected. The DNA sequencing analyses initially identified more than

50,000 nucleotide variants, either in splicing sites or exons. The team winnowed down these variants to 12 candidate variants across 360 genes. (*Journal of Clinical Oncology* 2016; 34:1872-1881)

These 12 were then cross-referenced with lists of known driver mutations. MST1 was found frequently; it drives a pathway involved with cell proliferation and organ growth. A non-negative matrix factorization identified two genes, NRAP and FRG2C. Noting that they had expected to find driver mutations, Dr. Clare concluded, "their absence suggests we are either on the wrong track or there is an early event that drives proliferation."

Keeping the focus on high-risk women, David Danforth, MS, MD, staff clinician at the National Institutes of Health, discussed "Characterization of Breast Ducts and Ductal Epithelium in Women at Risk for Breast Cancer." Dr. Danforth presented findings from a review of genomic changes in normal breast tissue (reduction mammoplasty specimens) published in *Breast Cancer: Basic and Clin Res. 10:109-146, 2016.* He reported that the genomic changes they found suggest the presence of a carcinogenic pathway in normal breast tissue that is initiated by estrogen or other carcinogens. The end result is a cancerized field of altered epithelial cells that can extend 5-10cm or possibly include the whole breast. Dr. Danforth and his colleagues have an ongoing clinical trial that uses ductal lavage and ductal endoscopy to characterize breast duct epithelium in women at normal and high risk for breast cancer. The study is using the improved ductal epithelial sampling method Dr. Danforth and his colleagues described in *Breast Cancer: Basic and Clin Res. 9:31-40, 2015,* which substitutes an angiocath for the standard catheter. Findings from analyses of 138 subjects suggest mild epithelial atypia may not be associated with increased breast cancer risk. Dr. Danforth said that this suggests atypical cells are are probably not a good prognostic factor. However, whether a relationship exists between mild epithelial atypia and breast cancer remains unclear. Dr. Danforth reported that he had recently received IRB approval for a whole exsome sequencing study of ductal fluid in women at high risk for breast cancer, which could provide more information on early carcinogenic changes.

Moving from the origin to the spread of breast cancer cells, Pepper Schedin, PhD, professor of cell, developmental and cancer biology at Oregon Health and Science University, discussed how mucosal biology and tissue involution cooperate to drive breast cancer metastasis. She presented unpublished data from her ongoing research focused on breast cancer in postpartum women. These findings, which were published after the meeting in *JCI Insight*, can be found [here](#).

Most invasive breast cancer begins in the breast ducts. The next speaker, Steffi Oesterreich, PhD, professor and vice-chair, department of pharmacology and chemical biology at the University of Pittsburgh Cancer Institute, shifted attention to invasive lobular breast cancer and research into its risk factors. Invasive lobular breast cancer accounts for an estimated for 10%-15% of all breast cancers, making it the 6th most common cancer in women. However, it is not studied as often as ductal breast cancer. Researchers have reported that lobular tumors tend to be larger in size, more difficult to detect, and have worse outcomes, but little else is known about its molecular, pathological, and clinical features. One study found late age at first pregnancy increases risk for lobular cancer while another showed a larger risk for this type of tumor in women who use menopausal hormones. There is also some evidence of tamoxifen resistance. The Cancer Genome Atlas characterization of lobular cancers shows that lobular tumors are more likely than ductal tumors to be ER+ and luminal A. They have more mutations in FOXA and fewer in GATA3. In addition, they are more likely to have PTEN inactivating alterations. Prolactin, which stimulates milk production, has been found to be associated with breast cancer risk, and lobular tumors express more prolactin than ductal ones. Dr. Oesterreich and her team are following up on this finding, looking for a lactation gene signature.

Moving from risk assessment to prevention, Oukseub Lee, PhD, research assistant professor at Northwestern University Feinberg School of Medicine, discussed the use of local transdermal therapy to prevent breast cancer in high-risk women or treat ductal carcinoma in situ (DCIS). Tamoxifen is a pro-drug that requires conversion in the liver. It is used for breast cancer prevention but it has side effects, including an increased risk for uterine cancer. A local transdermal therapy approach could offer prevention benefits

without the systemic side effects. Dr. Lee presented findings from a presurgical phase IIb trial that compared transdermal 4-OHT to oral tamoxifen in women with DCIS (*Clinical Cancer Research* 2014) which showed Ki-67 levels in DCIS decreased significantly in the patients in both the gel and oral tamoxifen groups. Based on these findings, Dr. Lee conducted in vivo preclinical studies using rat models (*Cancer Chemother Pharmacol, 2016).* This research required her to design special jackets for the rats that would cover the gel exposed area in the axillary mammary glands and prevent them from ingesting the transdermal treatment. She is now exploring whether it is safe to use a transdermal patch in women. Dr. Lee also presented research on androgen levels in breast tissue and the potential for using steroid hormone levels to assess breast cancer risk on behalf of her colleague Robert Chatterton, PhD.

Bacteria, Viruses and The Breast
Interest in the human microbiome has increased in recent years. This work has included research on the gut microbiota and the breast microbiome, each of which may possibly contribute to breast cancer risk. Researchers are also exploring whether viruses may cause or contribute to breast cancer development. To introduce attendees to this topic, Sarah Highlander, PhD, a professor at the J. Craig Venter Institute, provided an overview of the human microbiome in health and disease.

The human microbiome, "our second genome," contains 10 trillion bacterial cells. Microbes perform and regulate digestion and provide essential nutrients. They are also responsible for innate and adaptive immunity. Vast population shifts, called dysbiosis, can signal a change in an individual's health status. Studies have found that the microbiome changes over a person's lifetime and that different parts of the body have different microbiomes. There are three arms of bacterial induction of cancer: dysbiosis and inflammation, genotoxins, and metabolites. In a state of eubiosis, a diverse population of bacteria exist with a good balance of pro- and anti-inflammatory responses. In a dysbiosis state, there is less diversity, and there may be inflammation and immune dysfunction. Scientists are trying to develop bacterial therapies for cancer prevention or treatment that work by restoring the ecosystem of the microbiome.

Gertrude Buehring, PhD, a professor of virology at the University of California, Berkeley, shifted the focus from bacteria to viruses. She began by noting the causal relationships between viruses and cancers, such as human papillomavirus (HPV) and cervical, head and neck cancers; hepatitis B and liver cancer; and Epstein-Barr virus and nasopharyngeal cancer and certain lymphomas. Dr. Buehring discussed the retrovirus bovine leukemia virus (BLV), which is found in a large percentage of dairy cows and operations. A correlation exists between milk consumption and breast cancer incidence, and Dr. Buehring has tried for years to get funding to investigate whether BLV could cross species into humans and potentially explain this correlation. Her team has now conducted three case-control studies using in situ PCR to look for a link between BLV and breast cancer. To date, one has been published (*PLoS ONE* 10(9): e0134304, 2015). It suggested the relationship between BLV and breast cancer varies by race and ethnicity. Additional findings have been submitted for publication.

Returning to the microbiome, James Goedert, MD, from the Division of Cancer Epidemiology and Genetics at the National Cancer Institute, discussed gut microbiota and systemic estrogens and their association with breast density and breast cancer in postmenopausal women. Previous studies have found that not all women absorb the same amount of estrogen, and gut microbiota could potentially be contributing to the phenomena. Dr. Goedert and his team launched The Breast and Colon Health (BranCH) Study to investigate correlations between breast density on mammography, estrogens in urine, and diversity of the fecal microbiome. In 2015, they reported that postmenopausal women with breast cancer have altered composition and estrogen-independent low diversity of their gut microbiota. (*J Natl Cancer Inst* 2015; 107(8)). Additional findings have been submitted for publication.

Sabina Adhikary, PhD, a research associate at the Laboratory of Translational Immunology at the John Wayne Cancer Institute, is a member of a consortium that received a pilot grant at the 2015 International Symposium on the Breast. At this year's Symposium, she discussed their research on intraepithelial T cells in the breast tissue microenvironment.

Dr. Adhikary explained that intraepithelial cells are characterized by their expression of CD103 and referred to as "resident memory T cells." The CD103 these cells express interacts with E-cadherin, an adhesion molecule found on epithelial cells, allowing them to serve as "cellular guardians" that monitor the epithelial layer integrity. In cancers of epithelial origin, their presence has a positive correlation with survival. The consortium's pilot grant allowed Dr. Adhikary and her team to explore the hypothesis that CD8+ T cells in breast tissues express CD103 and promote anti-tumor response.

In 2014, Camilla Urbaniak, PhD, a member of the University of Western Ontario/Lawson Health Research Institute, published the first-ever study of the breast microbiome. Her research showed that the female breast contains a unique population of microbes relative to the rest of the body. At the Symposium, she discussed her research on the breast microbiome and metabolome and breast cancer risk. She presented findings from an analysis of bacteria found in tissue samples of 23 healthy women who had had surgery for breast reduction or enhancement.

Tina Hieken, MD, associate professor of surgery at the Mayo Clinic Rochester, also spoke about the breast tissue microbiome and breast cancer risk. In more than 70% of breast cancer cases, the etiology is unknown. Dr. Hieken is a member of the Mayo Premalignant Breast Disease Research Team, which studies breast cancer development, risk reduction and cancer prevention. This effort includes studying microbes, Dr. Hieken explained, because microbial cells carry out metabolic reactions that are not encoded in the human genome but are necessary for human health. Also, the strong evidence that microbial dysbiosis is implicated in GI cancers and lymphoma, suggests a potential role in breast cancer as well. A 2012 study found that the breast milk microbiome differed from the other microbiomes in the body. Her team's pilot study (*Sci Rep 2016; 6:30751*) on the microbiome in human breast tissue obtained from women with benign or malignant disease identified a distinct breast tissue microbiome that was distinct from that of overlying breast skin. The pilot study also found that the background breast microbiome was notably different in women with malignant disease compared to those with benign disease. Dr. Heiken's team is also studying the relationship between the breast microbiome and breast density.

Delphine Lee, MD, PhD, of the Los Angeles Biomedical Research Institute, presented the final talk on the breast microbiome, homing in on the microbiome of the breast ducts. Dr. Lee began by discussing the bacteria found in breast milk. She noted that the milk microbiome often reflects the bacteria seen in the mother's skin. Previously, nipple aspirate fluid (NAF) has be used to study the microbes in the breast ducts, where breast cancer develops. One NAF study (*Sci Rep, 2016*) identified differences in bacteria found in healthy women and women with a history of breast cancer. Dr. Lee is currently conducting a study analyzing bacteria found in ductal lavage fluid. These findings are expected to provide new information on the breast microbiome.

Dr. Lee also summarized the findings from a 2015 Symposium pilot grant conducted by a consortium that included Peter Sieling, PhD, of the John Wayne Cancer Institute. The study was designed to determine whether intraductal delivery of a TLR5 agonist, flagellin, induces an inflammatory response that is protective against progressive breast cancer. Flagellin was selected because it had previously been shown to inhibit breast cancer cells. The pilot study found that the tumors were larger in the animals that received intraductal TLR5 than in those who were untreated.

The Mechanics of the Breast

Fatemeh Hassanipour, PhD, an associate professor of mechanical engineering at the University of Texas at Dallas, opened the second day of the Symposium with a presentation on the biomechanics of human lactation. Dr. Hassanipour explained that her personal experience with breastfeeding led her to become interested in engineering aspects of the breast. Engineers have studied how the body transports fluids, but sheno comprehensive analysis of bio-fluid transport in the human breast had ever been done. So, she decided to pursue the question herself. Dr. Hassanipour's team uses commercial fluid dynamics simulation to model the vacuum pressure, jaw movement and jaw pressure of a baby on the breast and the mechanisms the baby uses to extract milk from the nipple. They also have developed ways to measure the amount of pressure the baby puts on the breast

surface and the properties of the breast milk. This information could potentially be used to build a breast pump that mimics natural human suctioning.

Keeping the focus on engineering, Maria Kallergi, DEA, MS, PhD, a member of the department of biomedical engineering at the Technological Educational Institute of Athens, discussed a pilot study conducted with Dr. Susan Love Research Foundation and the Jet Propulsion Laboratory in Pasadena, California, that used automated breast ultrasound and computer aided detection to generate ductal patterns of the normal breast. Their goal was to improve upon the images published in 1840 by Sir Astley Paston Cooper in *On Anatomy of the Breast*. Dr. Kallergi reported that their imaging protocol made it difficult to identify the ductal patterns. They intend to address these deficits and develop new protocols.

Nicola Natsis, BA, MD candidate at University of California, San Diego School of Medicine, also wanted to go beyond Cooper's wax models. She presented a proof of concept study that used cadaveric breast tissue to create 3D ductal images that can be used to learn more about DCIS and the anatomy of the breast.

In the next presentation, Ann Ramsdell, PhD, an associate professor of cell biology and anatomy at University of South Carolina School of Medicine, provided evidence of differences between the left and right breast that could contribute to breast cancer development. The mammary glands develop from tissues that are molecularly left/right asymmetric, and Dr. Ramsdell's lab found differences in the genes in the stroma on the left and right sides. This is significant, she noted, because the stroma affects tumor progression. The hypothesis that microenvironment right/left stroma differences will impact tumor development continues to drive her lab's research.

New Treatment Approaches
The intraductal approach to the breast provides new avenues for preventing and treating DCIS. Patrick Sinko, PhD, RPh, professor of pharmaceutics and drug delivery at Rutgers University, discussed molecular approaches for targeting intraductal therapy for DCIS. His research aims to identify vehicles for intraductal drug delivery that will maintain the ductal

structures and improve drug retention inside the duct. His team also is trying to identify a drug that can penetrate the duct's first layer of epithelial cells to the deeper layer stem cells. Nanoscale drug carriers could potentially be used for this purpose.

In the next presentation, Mark Haynes, of the University of Southern California, discussed his team's research on focused microwave thermal therapy with integrated real-time monitoring for breast imaging. Thermal therapy is high intensity focused ultrasound. It currently has other medical applications, such as ablation without surgery and treatment of hypothermia. Dr. Haynes and his team have developed a focused microwave thermal therapy system for the targeted treatment of breast cancer and a real-time microwave thermal imaging method for the monitoring of thermal therapies (*Biomedical Engineering, IEEE Transactions on*, vol.61, no.6, pp.1787,1797, June 2014). They are now investigating whether these systems can be combined to monitor the effect of the treatment on breast tumors.

Many women around the world do not have access to high-quality breast cancer diagnostic tools. As a result, women in remote areas often present with late-stage breast cancer and have limited treatment options. Mary Jo Fackler, a research associate at the Sidney Kimmel Cancer Center at Johns Hopkins, discussed efforts to develop an automated gene test that could be used for rapid breast cancer diagnosis in remote areas of the world. DNA methylation is a type of epigenetic alteration that is widespread in tumors. Epigenetic alterations don't change DNA sequence; they influence DNA function and expression. Because methylation occurs early in tumor development, it can be an early cancer marker. Dr. Fackler and her team want to identify a set of methylated gene markers that can distinguish between benign and malignant breast disease with high sensitivity and high specificity. The assay would be performed on tissue biopsies from women who had had a positive ultrasound test. The technology—a type of cartridge—is already being used in remote areas to quickly screen for HPV and HIV. It is not yet known if the technology can be used to perform QSMP analyses for methylated genes.

The microenvironment that surrounds a cancer cell contributes to its growth and development. Thea Tlsty, PhD, director of the Center for Translational Research in the Molecular Genetics of Cancer at the University of California, San Francisco, discussed her research on the stroma and breast cancer development. The stroma, she explained, is "dominant and dynamic" and tells the epithelial cells what to do. Dr. Tlsty previously reported that carcinoma-associated fibroblasts (CAFs) create an environment more conducive to the spread of premalignant and malignant mammary epithelial cells, whereas reduction mammoplasty fibroblasts constrain cancer cell growth. More recent studies identified a relationship between the transmembrane receptor CD36 and angiogenesis, but it is not clear if it is a correlation or a mechanistic link. These studies also show CD36 is greatly repressed in all stromal cells within tumor tissues, compared to normal adjacent tissues, which suggests fibroblasts with low CD36 promote an environment of tumor growth. Dr. Tlsty's team is conducting a clinical trial investigating whether a tissue-selective estrogen complex (TSEC) upregulates CD36 and downregulates tumor growth when it is given before surgery to women with DCIS.

The focus remained on DCIS as Piyush Gupta, PhD, an assistant professor of biology at the Massachusetts Institute of Technology, Whitehead Institute, discussed the genetic basis of in situ tumor progression. Mammography screening has led to an increased incidence of DCIS. However, epidemiologic studies show that more than half of the DCIS found on mammography would not be life-threatening if it were not treated. Learning which DCIS is more likely to progress to breast cancer could help women avoid unnecessary treatment. Dr. Gupta's team's genetic analyses identified the transcriptional regulator SMARCE1, which is required for extracellular matrix invasion, as a key driver of the progression of DCIS into an invasive tumor. Their studies also showed SMARCE1 is required for metastasis in vivo. Dr. Gupta's team is continuing to explore whether SMARCE1 expression could be used to identify poor prognosis DCIS as well as early-stage tumors with a high propensity to metastasize.

In the following presentation, Dipali Sharma, MD, PhD, associate professor of oncology at the Sidney Kimmel Cancer Center at Johns Hopkins University of Medicine, discussed the

relationship between obesity and cancer risk. An estimated one out of every five cancer deaths in the U.S. is linked to obesity. A number of studies have found that overweight patients have a higher incidence of recurrence and a significantly worse overall survival. Studies have also found that among women with early-stage ER-negative tumors, those with a higher body weight have a five times greater risk of dying compared to those with a lower weight. There is also evidence that at the molecular level an obesity-related gene signature correlates with poor survival. Dr. Sharma's own research is exploring ways to use bioactive strategies to prevent biodirectional crosstalk between breast cells and resident adipoycytes. This type of intervention may be able to disrupt the link between obesity and breast cancer.

Mina Bissell, PhD, distinguished scientist at the Lawrence Berkeley National Laboratory, has spent 40 years studying the basement membrane and extracellular matrix in normal and cancerous breast tissue, expanding our understanding of the role the microenvironment plays in breast cancer development. Dr. Bissell reviewed the impact the microenvironment has on oncogenes and their ability to drive tumor formation. She also discussed her current research on the important role laminin plays in sustaining the structure of the mammary gland. Laminin, which regulates p53, keeps cells from becoming malignant, even if they have oncogenic mutations. Dr. Bissell concluded by emphasizing more would be learned about cancer treatment and drug resistance pathways if studies were done in 3D instead of 2D.

The next speaker, Cathy Moleans, PhD, of the University Medical Center Utrecht discussed biomarker discovery in nipple aspirate fluid (NAF). Members of the Utrecht Group have attended many past symposia and Dr. Molean updated attendees on their work to identify biomarkers in nipple aspirate fluid (NAF) for early breast cancer diagnosis. Dr. Molean's group began by analyzing DNA promoter hypermethylation, comparing methylation in NAF in healthy volunteers and breast cancer patients (*Oncotarget*. 2016 Apr 26;7(17):24778-91). They then developed a new assay with higher sensitivity to study microRNAs in NAF. MicroRNAs are of unique interest because they have an established role in carcinogenesis and could potentially be targeted with anti-cancer drugs. Dr. Molean reported that although

the assay is able to identify dysregulated microRNAs in NAF further optimization is necessary for improved identification of early cancers.

As a basic researcher, Amy Brock, PhD, PharmD, assistant professor in cellular and biomolecular engineering at the University of Texas at Austin, studies the dynamics of cell state transitions. Probing the transitions cancer cells undergo led Dr. Brock to investigate whether it was possible to "reprogram" cancer cells to steer them toward more benign states. Dr. Brock used computer modeling to analyze longitudinal transcriptome data from transgenic mice mammary glands to predict novel key mediator genes that could be tested in cell culture models with the ultimate goal of developing an intraductal RNAi Therapy.

Keeping the focus on the intraductal approach, Sara Sukumar, professor of pharmacology and molecular sciences at Johns Hopkins University School of Medicine, discussed her efforts to identify drugs that could be used intraductally to prevent breast cancer or to treat DCIS. Dr. Sukumar has been conducting research on intraductal treatments for breast cancer prevention since 1995. Her team's research led to a clinical trial of intraductal Doxil that ended early after mice on the chemotherapy drug developed cancer. Dr. Sukumar then turned her attention to anti-estrogens, with initial studies that focused on 4-hydroxy tamoxifen. Her team is now investigating intraductal use of the anti-estrogen fulvestrant (Faslodex). This drug is not currently used to treat DCIS or for breast cancer risk reduction, primarily because it must be injected intramuscularly. Atossa Genetics, Inc, is currently enrolling patients in a phase II pharmacokinetics study of pre-surgical intramuscular and intraductal fulvestrant in women with invasive breast cancer or DCIS undergoing mastectomy or lumpectomy.

Gerald Gui, MD, a breast surgeon at The Royal Marsden NHS Foundation Trust, followed, discussing his randomized clinical trial of duct endoscopy. Dr. Gui began performing ductoscopy in 2001 in patients who had a single duct nipple discharge that was persistent, spontaneous, and blood stained but had no abnormalities on mammography. Dr. Gui shared video from ductoscopy procedures that showed normal ducts, papillomas (the most

common identifiable source of nipple discharge), microcalcifications, features associated with DCIS and major duct excisions.

The Symposium's final presentation was by Faith Balci, MD, of Acibadem University, in affiliation with Columbia University. Dr. Balci presented data from his consortium's 2015 Symposium pilot grant feasibility study investigating real-time microscopic imaging of breast ducts by ductoscopy integrated optical coherence tomography (OCT). OCT is used in cardiology to look at blood vessels from the inside using light instead of sound (as an ultrasound does) to create three-dimensional images. OCT may be able to identify early changes in the breast duct before ductal carcinoma in situ (DCIS) or cancer forms. The goal of this research is to develop a way for patients to avoid having to have surgery to investigate the cause of pathological nipple discharge.