The Seventh International Symposium
on the Intraductal Approach to Breast Cancer

The Normal Human Breast: Building Our Understanding from Mice to Women

The Dr. Susan Love Research Foundation is committed to advancing research and developing resources that explore the normal breast. As part of this effort, the Foundation hosted The 7th International Symposium on the Intraductal Approach to Breast Cancer in Santa Monica, Calif., Feb. 23-26, 2011.

More than 100 clinicians, epidemiologists, pathologists, basic scientists, translational investigators, and breast cancer advocates from 11 countries attended this year’s conference, “The Normal Human Breast: Building Our Understanding from Mice to Women.”

The conference began with a pre-symposium workshop. This daylong program, “Crossing the Chasm from Animal Models to Women: Everything You Need to Know,” was focused on helping researchers move their research from animal models to humans. It addressed topics ranging from resources for tissue and recruitment to the role advocates can play in translational research and opportunities for funding. You can read the pre-symposium report at http://www.dslrf.org/pdfs/7thIntlSympPreSympWorkshop.pdf

The Symposium, which began the next day, was organized around three central topics: the biology of the breast, the physiology of the normal breast, and clinical applications of the intraductal approach. During the conference, attendees also has the opportunity to observe live demonstrations of nipple aspirate fluid collection, ductal lavage, and ductoscopy. A Public Panel allowed the community to hear highlights of the Symposium as well as learn more about how the intraductal approach is bringing us closer to ending breast cancer.

On the final day of the conference, the Dr. Susan Love Research Foundation awarded a total of $90,000 to support six multidisciplinary consortiums formed at the Symposium. Each consortium will use its planning grant to solidify its ideas and obtain initial data, with the aim of submitting a full proposal to the Avon Foundation for Women for additional funding.

Introduction
The human breast is composed of multiple ductal lobular systems that open onto the surface of the nipple. (It is through these openings that milk is delivered to a baby when a woman is
breastfeeding.) These ductal systems are lined with epithelial cells, where most breast cancers are thought to originate. Nipple aspiration and ductal lavage can be used to collect fluid and exfoliated cells from the breast ducts that can be examined for abnormalities. Studies have found that women who produce nipple aspirate fluid that contains atypical cells are at 2 to 5 times greater risk of going on to breast cancer than are women who do not produce any fluid at all.

Intraductal research has provided a foundation for new ways of thinking about what causes breast cancer and how to prevent it from occurring. This year’s Symposium included presentations and programs designed to foster communication and to encourage attendees to think about how their research interconnects with what is happening in other research fields. Dr. Susan Love underscored the importance of interdisciplinary research when she opened the conference. “It is only by transcending silos,” she said, “that we will be able to get the answers to fundamental questions about the underlying biology of the breast that are necessary to fully understand how breast cancer develops—and how to prevent it.”

**Biology of the Breast: Cancer Development**

Gabriel Dontu, MD, PhD, a clinical reader in mammary stem cell biology at King’s College London School of Medicine, in England, kicked off the morning session with her presentation, “Hierarchy of Cellular Differentiation in the Normal Breast Epithelium—Implications of Cancer. Dr. Dontu began by noting that the origin of the cells that express the estrogen receptor (ER) in normal and malignant breast epithelium is not known. She also discussed the role that stem cells play in both normal gland development and in the cancer stem cell model. It is not universally accepted that normal stem cells are the origin of cancer. However, if this hypothesis is correct, it has clear implications for breast cancer treatment and research. Dr. Dontu presented research on the stem cell marker Aldehyde dehydrogenase 1 family, member A1 (ALDH1A1). Using in vitro systems and mouse xenograft models, she has shown that ALDH and ER define a cellular hierarchy in the normal human mammary gland in which ALDH+, ER− stem cells can generate ER+ progenitor cells. Furthermore, the subset of ER+ tumors that are driven by an ER− stem cell have worse clinical outcome than the ER+ breast cancers that are not. These studies suggest that different subtypes of ER+ breast cancer may have different cellular origins and may require different targeted therapies.

Zena Werb, PhD, a professor in the department of anatomy at the University of California, San Francisco, conducts research on how cells behave during mammary development and breast cancer tumor progression. She discussed her recent research on the trans-acting T-cell-specific transcription factor GATA-3. Her team found that GATA-3 is the most highly expressed transcription factor in the normal mammary epithelium. In addition, ductal carcinoma in situ (DCIS) that is well-differentiated, ER-positive, and collagen-positive tends to be GATA-3 high, whereas DCIS that is poorly differentiated, ER-negative, and collagen-negative tends to be GATA-3 low. Moreover, a tumor must lose GATA-3 in order to metastasize. Dr. Werb’s team has identified a number of genes that bind to GATA-3 downstream, and they have shown that when these genes are turned on by GATA-3 proteins in the extracellular microenvironment are affected and VEGF is inhibited. This means, she explained, “that GATA-3 is a master regulator for tumor dissemination that both directly and indirectly affects the tumor microenvironment. And because it is a differentiation gene, and not a tumor suppression gene, it makes you think
Pepper Schedin, PhD a professor of medical oncology at the University of Colorado School of Medicine, in Boulder, studies pregnancy-associated breast cancer (PABC). She began her presentation by noting that although PABC is typically thought of as occurring during or soon after pregnancy, it actually peaks at 5-7 years after pregnancy, with an increased risk extending to 10 years. Moreover, women diagnosed with breast cancer within five years of childbirth have a worse prognosis than young women who have not been pregnant or postmenopausal women. In rodent models, wound healing and inflammatory responses that are known to be tumor promotional have been found in the involuting mammary gland. Dr. Schedin and her colleagues believe that the physiologically normal microenvironment of the postpartum involuting gland is a window of risk for breast cancer as the ducts and lobules go through apoptosis prior to developing new structures to prepare for the next pregnancy. This is supported by their studies in human breast tissue that have shown that the microenvironment of the postpartum involuting breast in women is characterized by macrophage infiltration, increased levels of collagen and COX-2, and an architecture previously only associated with tumor progression. In conclusion, said Dr. Schedin, breast cancers that occur during pregnancy may be different than the breast cancers that develop in the postpartum period. Furthermore, postpartum gland involution is a promising window to target for prevention of postpartum breast cancer. For example, she said, studies her lab has conducted in animal models have shown that collagen deposition during involution and tumor promotion are both suppressed by NSAID treatment.

With the next speaker the topic progressed to observations from tumors in women. Tiber Tot, MD, PhD, a professor in the department of pathology at Central Hospital in Falun, Sweden, discussed his theory of the “sick lobe” (or sick duct). Dr. Tot explained that breast cancer should be thought of as a lobar disease, where the simultaneous or asynchronous, often multiple, in situ or invasive tumor foci originate in a single lobe (ductal system) of one breast. He suggested that the timing of malignant transformation of the progenitor cells within the sick lobe is genetically determined, and that when extensive DCIS is seen throughout a lobe it is not because the cells migrated but rather because they simultaneously transformed. He hypothesizes that the initial insult occurs in the breast stem cells of the embryo, and that when the breast develops at puberty these mutations are carried throughout the resulting lobe. This suggests that the risk of cancer extends to the whole lobe and can only be interrupted by elimination or destruction of the entire sick lobe. Approaching breast cancer as a sick lobe, he said, helps explain the earliest events in a tumor’s natural history. It also suggests that prognostic information from the breast ducts could potentially be used to screen for risk and to diagnose and treat DCIS and early-stage invasive cancer.

Much still remains unknown about the anatomy of the breast, and Sheldon Feldman, MD, the chief of breast surgery at Columbia University College of Physicians and Surgeons in New York City, discussed how the ability to perform breast anatomy mapping could influence breast cancer care. Surgeons typically make a circle around a mammographic abnormality or palpable tumor. But, said Dr. Feldman, clinical observations indicate that this surgical approach results in involved margins in up to 50 percent of cases, leading to 90 percent of recurrences occurring in the tumor bed and 25 percent of women who still have residual disease. This suggests that a method of ductal-lobular mapping is necessary to delineate the extent of disease. Dr. Feldman
and his team have been using autopsy and cadaver specimens and will soon begin using mastectomy specimens to investigate whether it is possible to create a map of the ductal-lobular units that a surgeon could use to perform anatomic resections and that would not interfere with subsequent pathology interpretation.

Melissa Troester, PhD, an assistant professor of epidemiology at the University of North Carolina, in Chapel Hill, discussed her Normal Breast Study, which is investigating whether non-neoplastic tissue that is adjacent to the tumor can predict outcome. Both tumor tissue and normal adjacent tissue, she explained, have many biological features in common with wound healing. However, normal adjacent tissue also shows considerable heterogeneity across patients. Her research using tissue samples from women with invasive breast cancer or DCIS identified two distinct classes of tissue, one of which showed gene expression consistent with epithelial-to-mesenchymal transition (EMT), a process that is activated in healing wounds. Roughly 40 percent of cancer-adjacent normal specimens showed activation of this signature, and the signature was independent of tumor subtype, with roughly 35-45 percent of tumors of each subtype expressing the EMT-like phenotype. Moreover, in the ER-positive patient group a strong association was seen between EMT-like adjacent normal tissue and overall survival. This suggests, said Dr. Troester, that responses in the cancer-adjacent normal microenvironment may have value as independent predictors of ER-positive patient outcome.

Neal Goldstein, MD, the director of surgical pathology at Clarient Laboratory in Aliso Viejo, Calif., discussed a subset of precursor lesions that his team has termed “monomorphic epithelial proliferations” (MEPs). “Most pathologists have been taught to ignore these,” said Dr. Goldstein, “but we believe they are precursor lesions for some atypical ductal hyperplasia (ADH) and DCIS. Morphologically MEP lesions are predominantly single-cell layered, slightly overcrowded, monomorphic, clonal-like luminal cell proliferations that often extend into the terminal duct lobular unit. They are also often seen with usual type hyperplasia. Dr. Goldstein discussed his research on tumor tissue that has been taken from women whose treatment included breast conserving therapy. His findings suggest that MEPs appear to be a pool of partially-transformed precursor lesions, less than ADH or DCIS, that can give rise to DCIS and invasive cancer. It is possible, he said, that radiation therapy may work to reduce the rate of ipsilateral recurrence because in addition to eradicating microscopic residual disease it eradicates these precursor lesions.

The next speaker, James Going, PhD, a senior lecturer at the Institute of Cancer Sciences at the University of Glasgow, Scotland, discussed why he believes it is time to abandon the lobular “creation myth” of breast cancer. It is widely accepted that breast cancer begins in the terminal duct lobular unit, he said. Yet, this belief is not justified by the available evidence and has been challenged by our current understanding of the molecular events that lead to cancer. In fact, Dr. Going said, extensive evidence supports the idea that it is genetic and epigenetic changes acquired over many generations of cell division that drive the abnormal behaviors seen in cancer cells, with successive waves of clonal expansion after each new growth-promoting mutation enabling progress towards a fully established tumor. Dr. Going said he believes that the Toker cell, or clear cell of nipple epidermis, might be able to identify risk, and he suggested that these duct-associated cells be further investigated.
Thea Tlsty, PhD, a professor of pathology at the University of California, San Francisco, spoke next about her research into the stromal markers that indicate which DCIS is going to recur locally and which will go on to become invasive. She also shared some early work on pluripotent cells that have been found in the breast ductal tissue. An interesting discussion ensued as to whether the MEPs, sick lobe, and breast stem cells represent different aspects of the same biological phenomena.

DCIS is described histologically as exhibiting three or four intraductal patterns: micropapillary, cribriform, solid, and comedo. In an attempt to understand how these patterns of growth evolve, Kerri-Ann Norton, BA, a graduate student at Rutgers, The State University of New Jersey, in New Brunswick, and her team developed a 2D mechanistic model of DCIS cell proliferation in a single duct. Extending this approach to 3D reconstructions from serially sectioned human breast cancer specimens allowed them to describe the aspects of proliferation that determine the intraductal pattern of growth. Preliminary findings show that these models can give some insight into the elements that dictate the 3D architecture of DCIS.

Breast cancer progression depends on functional interactions between epithelial cells (and/or progenitor cells) and the microenvironment. Hal Berman, MD, PhD, an assistant professor at the University of Toronto in Ontario, discussed the insights that can be gained by studying the physiography of the mammary gland. Physiography, or physical geography, is the study of complex natural phenomena spatially. Multidisciplinary studies involving mammary gland “Egan” sub-sectioning, pathological-radiological correlation including duct-system imaging (ductography), and spatially oriented in situ molecular studies demonstrate that breast cancer has a unique physiography. This approach supports the anatomical approaches of Drs. Tot, Goldstein, and Going. There is a generation of mammary tissue biorepositories with physiographic annotations, and Dr. Berman suggested this could provide a new approach to studying DCIS.

This first day of the Symposium closed with a debate on the topic: What is the unit of study: the breast or the duct? Seema Khan, MD, a professor in surgery-surgical oncology at Northwestern University Feinberg School of Medicine, in Chicago, took the position that the unit of study should be the breast. She argued that both breasts are a single epithelial field, and that the entire field is at risk. This idea is supported, she argued, by our understanding that there are systemic breast cancer risk factors; that contralateral risk is increased after a breast cancer diagnosis; and findings on breast imaging. This idea is also supported by the fact that cancer does not develop in the neighborhood of a prior site of atypia; that some NAF markers from both breasts correlate well; and that mammographic density appears to be symmetrical.

Susan Love, MD, president of the Dr. Susan Love Research Foundation, took the position that the unit of study is the duct. She discussed the research that has described the 6-9 ductal systems opening in the nipple that appear to be independent, and the evidence that suggests that cancer occurs in a single ductal system. She also argued that the studies that have shown that ducts have their own microenvironment, with differing levels of estrogen and metabolites, progesterone cytokines, epithelial cells, and macrophages in the ductal fluid, are further evidence that the duct should be the unit of study. Her findings support the concept of the sick lobe, and may set the stage for examination of ductal fluid for screening.
The Physiology of the Normal Breast

The second day of the Symposium focused on the physiology of the normal breast and the research utilizing NAF and ductal lavage fluid to advance our understanding of both the breast microenvironment and how breast cancer develops.

Combining mouse genetics with tissue recombination techniques, Cathrin Briskin, MD, a molecular oncologist at the Swiss Institute for Experimental Cancer Research at the Ecole Polytechnique Fédérale de Lausanne, in Switzerland, recently established that the female reproductive hormones—estrogens, progesterone, and prolactin—act sequentially on the mammary epithelium to trigger distinct developmental steps. Her research also showed that hormones impinge directly on a subset of luminal mammary epithelial cells that act as sensor cells, translating and amplifying systemic signals into local stimuli. Dr. Briskin hypothesized that cell proliferation occurs in two waves, and that receptor activator of nuclear factor kappa-B ligand (RANKL) is a key mediator of the second wave of progesterone-induced proliferation, which involves mostly hormone receptor negative cells.

Eva Gordon, PhD, a research consultant with the Dr. Susan Love Research Foundation, presented data from the Foundation’s exploratory study investigating the physiology of the normal breast. Dr. Gordon noted that while the physiology of breast milk and the molecular transport systems within the lactating breast have been extensively studied, little is known about how these systems function in the non-lactating breast. To study drug transport in the non-lactating breast, healthy parous and nulliparous women (women who have had children and women who have not) were given cimetidine and caffeine orally and then their ductal fluid was collected periodically over 10 hours. This study demonstrated that neither caffeine nor cimetidine were transported into ductal fluid in the same way as they were into milk, indicating that the physiology of these transport systems differed. This suggests that carcinogens found in breast milk may not be representative of the exposures the non-lactating breast experiences.

Changes in the inflammatory microenvironment of breast ducts may make the duct more hospitable to cancer cells. These changes may also be an early indicator of future neoplasia. Brian Ruffell, PhD, a postdoctoral scholar at the University of California, San Francisco, presented findings from his research using real-time PCR to measure inflammatory gene expression of immune mediators in NAF and ductal lavage fluid. These studies showed that the NAF and ductal lavage fluid from the same volunteer had distinct expression profiles, and that unique expression signatures were seen in each of the four ducts analyzed. This suggests, he said, “that it will be unlikely to discover a gene signature that might be predictive from individual ducts.” Other studies that investigated whether it was possible to identify upregulated inflammatory genes within NAF found some variation between the cancerous and contralateral breast, but many high-quality NAF samples will need to be studied to determine if a pattern exists. Lastly, Dr. Ruffell presented data from his research using flow cytometric analysis to study the leukocyte composition of human breast tumors. These studies identified an increased percentage of macrophages, myeloid-lineage leukocytes, and functionally active CD8+ T cells present following chemotherapy, which suggests, said Dr. Ruffell, that immune markers might be predictive of recurrence.
Most of the body’s prolactin is made by the pituitary gland, but it is also produced and used locally by mammary epithelial cells—both normal and cancerous. Studies have found that women who have prolactin levels in the upper quartile of normal range have an increased breast cancer risk. Ameae Walker, PhD, a professor of biomedical sciences at the University of California, Riverside, presented data from her laboratory studies in normal human tissue and mice which showed that most prolactin receptors are located on the luminal side of the ductal epithelium, and that a proportion of these receptors are present at any time on the luminal surface. Using a highly sensitive assay her team developed to study ductal fluid prolactin, Dr. Walker discovered that the fat portion of milk, specifically the milk fat globule membrane, contains a high affinity PRL-binding moiety. She is now investigating whether the levels of this binding complex are predictive of breast cancer risk, and whether the amount of binding complex is higher in women who have had a full-term pregnancy.

In the final presentation, Ferdinando Mannello, DSc, PhD, an associate professor of molecular pathology at the University “Carlo Bo” in Urbino, Italy, presented data from his Dr. Susan Love Research Foundation-funded pilot grant that used NAF to explore the balance between protein oxidation and anti-oxidant enzyme activity and the relationship between iron-binding proteins and inflammation. This work showed that protein oxidation occurs in NAF, and that significantly higher protein carbonyl concentration was found in the NAF of women with breast cancer and pre-malignant conditions than in the NAF of healthy women. It also showed that median levels of Superoxide Dismutase-1 were higher in the NAF of women who had cancer than in the NAF of healthy women. In addition, the mean level of C-reactive protein was significantly higher in the NAF of women who had cancer. These data suggest that C-reactive protein levels in NAF along with a disruption of iron homeostasis may help to identify women at higher risk of developing breast cancer.

This second day of the Symposium closed with a debate on the topic: What is the physiology of ductal fluid/NAF? Edward Sauter, MD, a professor of surgery at The University of North Dakota School of Medicine and Health Sciences, in Grand Fork, took the position that NAF is representative of ductal fluid and the breast epithelium Dr. Sauter explained that the components of both NAF and ductal fluid are synthesized by the breast and secreted into the duct lumen, diffuse from the blood or are transported into the duct lumen. Dr. Sauter argued that the different technologies that have been used to analyze NAF are still in their infancy, and that more research is needed to determine the best approach for studying NAF fluid. Even so, he concluded, the data show that NAF represents ductal fluid and that NAF, which is more accessible than ductal fluid, holds great promise as a noninvasive approach to assessing the breast.

Robert Chatterton, PhD, a professor of obstetrics and gynecology at Northwestern University Feinberg School of Medicine, in Chicago, took the position that NAF is not the same as ductal fluid and that it represents a pathological condition. He discussed the evidence supporting an association between biomarkers in NAF and breast cancer using data from studies conducted by others that showed a lack of correlation among NAF, serum, and saliva as well as his own data that showed a lack of correlation between NAF and serum estradiol during the menstrual cycle. Dr. Chatterton also suggested that progesterone may be an important stimulus for breast cancer
growth, noting that NAF progesterone typically follows serum concentrations during the menstrual cycle, and that sporadic increases in NAF progesterone may indicate inflammatory or other processes that could increase breast cancer risk.

**Clinical Applications of the Intraductal Approach**

The third day of the Symposium focused on the clinical applications of the intraductal approach. These presentations included clinical observations, epidemiology studies, interesting pilot studies, and new applications of the intraductal approach. Particularly interesting were the reports from previous pilot grants funded by the Dr. Susan Love Research Foundation.

The first speaker, Hong Ling, MD, an attending physician in the Department of Breast Surgery Cancer Hospital at Fudan University in Shanghai, China, and a pilot grant recipient, studied the pH value of NAF to explore her hypothesis that the necrosis and anaerobic metabolism of cancer cells results in calcifications in the duct. Dr. Ling found that the pH value of NAF taken from 76 primary breast cancer patients prior to surgery did not differ between the cancerous and noncancerous breast. However, she did find that patients with calcifications were more likely to have nipple discharge in the contralateral breast.

Mammography is less effective in dense breast tissue. A diagnostic tool that used NAF to identify occult breast disease would be beneficial to many women, including those with dense breast tissue. Barbara Urban, MD, the High Risk Program Coordinator at the Anne Arundel Medical Center in Annapolis, Maryland, presented data from a small pilot study funded by the Foundation that used a non-invasive nipple aspirate testing device [HALOTM (Neomatrix)] to identify breast cancer protein biomarkers that could be used to identify occult breast tumors. Dr. Urban reported that cytologic evaluation of the cellular portion of the NAF followed by proteomic evaluation of the supernatant portion using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) could differentiate between cancerous and noncancerous NAF protein signatures with high statistical confidence. She will now validate this work in a larger population.

Marion Lee, PhD, MPH, a professor of epidemiology at the University of California, San Francisco, presented data from an Avon-funded feasibility study conducted with the Dr. Susan Love Research Foundation of an inexpensive, home test using NAF to assess breast cancer risk in premenopausal women. The test uses a nipple cap, which can collect self-expressed NAF. The fluid is collected on filter paper and then tested for three protein markers that have been found to be increased in NAF from cancer patients, bFGF, C reactive protein, and EGF. The pilot study enrolled 1002 women at three hospitals in China, and Dr. Lee reported that it identified 243 women who expressed NAF, three of whom tested positive on the breast fluid test. If the biomarkers used in this study can be validated prospectively, said Dr. Lee, this could represent a new approach to identifying which premenopausal women need more aggressive screening.

Paul Van Diest, MD, PhD, a professor of pathology at the University Medical Center Utrecht in The Netherlands, presented data from his research exploring the use of methylation analysis of nipple fluid for breast cancer screening. Promoter methylation is an early marker of malignancy, and Dr. Diest is currently conducting a prospective validation study in breast cancer patients to
compare promoter methylation patterns by quantitative multiplex methylation-specific PCR (QM-MSP) in nipple aspirates and breast cancer tissue. He is also conducting a long-term prospective study in which nipple fluid will be obtained annually from over 200 high-risk women for 10 years and assessed for methylation patterns by QM-MSP. In both of these studies, prior to NAF collection, the women receive intranasal oxytocin, a drug typically used to induce breast feeding that Dr. Diest has found increases the percentage of women who produce NAF. Dr. Diest’s initial results show that the cumulative methylation in nipple fluid of unaffected breasts from high-risk women is more than twice as high as that seen in healthy controls. In addition, the cumulative methylation in nipple fluid from the unaffected breast from women with previous contralateral breast cancer is eight times higher than the healthy controls. These initial findings, said Dr. Diest, indicate that methylation analysis of NAF obtained under oxytocin support may be useful for early breast cancer detection.

A previous study suggested that dietary soy increased atypia of cells in NAF as well as NAF volume in premenopausal women. Shannon Conroy, MD, MPH, a postdoctoral fellow at the University of Hawaii Cancer Center, in Honolulu, presented data from her research on the effects of soy on NAF in 96 premenopausal women between 18 and 50 years of age who had no history of breast cancer and a low baseline soy intake. She reported that her study showed that moderate amounts of soy did not lead to increased breast tissue activity as assessed by NAF volume or estrogen levels in NAF.

There is increasing interest in a potential viral etiology of breast cancer. Building on the research that has found various strains of HPV to be present in breast tumors, Fatih Balchi, MD, a general surgeon at the Numune Training and Research Hospital in Ankara, Turkey, investigated whether HPV type 16 is present in tissue obtained from women who had a papilloma extracted with ductoscopy or microductectomy. In these histopathologic samples, real time PCR was used to identify HPV-DNA and genotyping was performed by pyrosequencing. Dr. Balci reported that his research showed that HPV was not found in these intraductal breast papillomas.

Yong Soon Chun, MD, PhD, a postdoctoral fellow of oncology working with Saraswati Sukumar, PhD, a professor of oncology and pathology at The Johns Hopkins University School of Medicine Sidney Kimmel Comprehensive Cancer Center in Baltimore, presented data from their group’s preclinical studies examining the safety and long-term consequences of intraductal instillation of pegylated liposomal doxorubicin (PLD, Doxil) in mouse models. These studies, which were extensions of Dr. Sukumar’s previous research on intraductal PLD, found a significant reduction in the outgrowth potential of the transplanted cells in cleared fat pads from intraductal PLD-treated mammary glands compared with control mice. This supported the hypothesis that PLD reduced normal mammary stem cell function, resulting in compromised milk production.

In another experiment, all the ducts were treated at the same time, which was a more dose-dense exposure for the mouse as a whole. The glands were harvested periodically and transplanted into cleared fat pads to look for the presence of stem cells that could produce new glands. Dr. Chun reported that stem cells were found in the glands of the doxil treated mice in almost equal number to the control. Of more concern, the doxil treated mammary glands that had been harvested at 33 weeks and transplanted resulted in tumors in all of the recipient mice. These
tumors were about 20mm in size and on pathology represented fibrosarcomas or very poorly differentiated mammary tumors. In addition, the doxil treated SVBN mice at 33 weeks showed areas of atypical ductal hyperplasia and foci of DCIS. Although there have been no instances of induced malignancy in the neoadjuvant human studies using this drug, this data suggests that PLD may not be a good choice for intraductal therapy. Dr. Chun’s team also investigated the utility of a proven plant chemopreventive, curcumin, in nanoparticle formulation (NanoCurc) given intraductally, and this data showed that small quantities of intraductal NanoCurc was as effective as mega doses of native curcumin given orally in reducing the number of carcinogen-induced rat mammary tumors.

Based on the initial preclinical data and two human safety trials conducted in women undergoing mastectomies, Ellen Mahoney, MD, a breast surgeon at St. Joseph’s Hospital in Eureka, Calif., and the Dr. Susan Love Research Foundation launched a study to test the safety and feasibility of giving neoadjuvant intraductal Doxil to women with DCIS. To date, reported Dr. Mahoney, of the 14 women enrolled in the study, one was not treated due to technical difficulties with the ductogram; three sustained perforated ducts and were not treated; and in two women the ducts could not be cannulated. Five received the full dose of drug into the correct duct; one had a smaller dose in an adjacent duct; and one received a placebo dose of saline. In these 7 subjects the treatment was very well-tolerated, with one complaint of local pain and one case of erythema of the skin. In addition, pathology results have shown some decrease in mitotic activity, stromal effects, fat necrosis and inflammation. This trial was halted after these 14 women were enrolled based on the adverse events seen from Dr. Sukumar’s lab.

Patrick Sinko, PhD, associate vice president for research at Rutgers, The State University of New Jersey, in New Brunswick, discussed the impact nanotechnology could have on intraductal drug delivery and imaging. Currently, most drugs and imaging moieties are small molecules that exhibit poor retention in breast ducts and diffuse into the systemic circulation. Dr. Sinko explained that using macromolecular carriers or biomaterials for intraductal delivery could keep the drugs from moving out of the breast ducts. Dr. Sinko described his research using biomaterials such as poly(ethylene glycol) (PEG) as a platform for intraductal delivery and imaging of DCIS. His team has also designed, fabricated, and evaluated several PEG hydrogels containing passively-entrapped drugs and dyes and nanocarrier-based hydrogels in vivo in rats. Dr. Sinko reported that these proof-of-principle studies have shown that molecular size influences the retention in the duct, and that duct retention increases with increasing molecular size of the nanocarriers. In addition, non-degradable and degradable PEG hydrogels administered intraductally in rats was found to remain in the duct for 30-60 days. Dr. Sinko’s team intends to continue to pursue the development of nanocarriers that will have the ability to remain inside the duct long enough to allow for prolonged drug exposure.

Spectral imaging is the application of spatially resolved spectroscopic analyses to macroscopic or microscopic images. Autofluorescence spectra can characterize variations in tissues harboring histologically proven dysplasia or in situ cancers, and it is currently used to detect premalignant and early skin, gynecological, and tracheobronchial tree cancers. Atilla Soran, MD, MPH, a professor of surgery at Magee Women’s Hospital at the University of Pittsburgh Medical Center, is using ductoscopy images from invasive breast cancer, DCIS, and normal breasts to develop a library of images that can be used to teach a computer to identify the spectral signature of a
breast lesion. The findings from his pilot study suggest that spectral analysis may be a useful, minimally invasive diagnostic tool for differentiating benign from malignant or premalignant breast lesions.

Autofluorescence is currently used during colorectal cancer screening to identify adenomas and polyps in the colon. In the Symposium’s final presentation Alexandre Douplik, PhD, a professor at the Friedrich-Alexander University in Erlangen, Germany, discussed his research exploring the technical feasibility of using autofluorescence along with ductoscopy to examine the breast ducts in the \textit{ex vivo} setting. His studies also assessed whether there are distinct changes in the tissue autofluorescence images of malignant and benign tissues. If so, these could potentially facilitate visualization of lesions that are not seen under conventional white light ductoscopy. His findings suggest that the current imaging algorithm for visualizing tumor tissue against the normal tissue background, although developed and optimized for other organs, appears to be able to differentiate between intraductal breast tumor and normal breast ductal tissue.

\textbf{Pilot Grants: Challenge to Collaborate}

During the Symposium participants were challenged to form multidisciplinary teams of clinicians, basic scientists, and advocates to develop proposals that would tackle key questions that would advance our understanding the anatomy and physiology of the breast and its relations to cancer. On the final day of the Symposium, nine multispecialty and multinational teams presented their concepts, and, that evening, at the Foundation’s Award Dinner, a total of $90,000 was awarded to the six best proposals. Each consortium will use its planning grant to solidify its ideas and obtain initial data, with the aim of submitting a full proposal to the Avon Foundation for Women for additional funding.

The six consortiums that received planning grant awards are:

\textbf{Inflammation Changes Over Time in Obese and Normal Weight Women: Insights into organ specific vs. systemic changes over time}

\textit{PI}: Edward Sauter, MD, Associate Dean for Research and Professor of Surgery
The University of North Dakota School of Medicine & Health Sciences, Grand Forks;
Ferdinando Mannello, Dsc, PhD, Associate Professor of Molecular Pathology, Chair of Biology and Clinical Research Unit University “Carlo Bo,” Urbino, Italy; Tara Locher, Advocate.

Studies have shown that inflammation is a process that is critical to the development and progression of breast cancer It is also known that chronic inflammation is a hallmark of obesity, and that ovarian hormones influence the expression of proteins involved in multiple pathways. To advance our understanding of the anatomy and physiology of the breast, this consortium will explore the hypothesis that inflammation marker expression is higher in the breast than in the circulation; higher in obese women than non-obese women; and varies more through the menstrual cycle of premenopausal women than over a 30-day period in postmenopausal women. This will be done by analyzing blood samples along with NAF that has been collected every three days from the same breast ducts (one from each breast) throughout one menstrual cycle, or for 30 days, from 40 healthy women, half premenopausal and half postmenopausal, and half obese and half not obese.
Do NAF Yielders Differ From Non-NAF Yielders in Ways that Affect Breast Cancer Risk?

PI: Seema Khan, MD, Professor of Surgery-Surgical Oncology, Northwestern University Feinberg School of Medicine, Chicago, IL; Robert Chatterton, PhD, Professor, Northwestern University Feinberg School of Medicine; Vered Stearns, MD, Assistant Professor of Oncology, Johns Hopkins School of Medicine, Baltimore, MD; Sara Sukumar, PhD, Co-director of the Breast Cancer Program in the Department of Oncology and Professor of Oncology and Pathology, Johns Hopkins School of Medicine.

It is still not known why some women yield NAF while others do not. This consortium will explore the secretory phenotype and investigate whether and how breast tumors that develop in NAF yielders differ from those that occur in non-yielders. They will begin by creating matched sets of NAF yielders and non-yielders who have been diagnosed with breast cancer by using data and tissue previously collected for a case-control study of NAF steroids at Northwestern University. Then, they will investigate whether there is a relationship between the ABCC genotype and NAF production; identify SNPs of prolactin and oxytocin that affect milk production; and compare tumor sub-types, methylation profiles, and microRNA expression in NAF yielders and non-yielders. The consortium will use its findings to select more specific targets to study in a larger cohort.

Investigating the Anatomy of the Breast and Premalignant Disease Through the Ductoscope

PI: Paul van Diest, MD, PhD, Adjunct Professor of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine; Alexandre Douplik, PhD, Head of Medical Engineering Photonics Group, Friedrich-Alexander Erlangen-Nuremberg University, Germany; Susan Love, MD, Dr. Susan Love Research Foundation, Santa Monica, Calif.; Sherry Goldman, RN, Advocate.

Key questions about the anatomy of the ductal system have yet to be answered. It is still not known, for example, what the structures behind the nipple really are, if the breast duct is clonal, what autoflourescence represents pathologically, and whether the area of premalignancy is a patch, a segment, or a duct. This consortium will advance our understanding of these critical questions by using mastectomy specimens to identify the best way to distinguish the structures behind the nipple; and test three different techniques to biopsy the wall of the duct through ductoscopy in women.

This data will establish the foundation for a larger grant that will sample and analyze areas of autoflourescence and a normal area in 100 healthy women, 100 high-risk women, and 100 women with breast cancer.

3-D Lymphatic Anatomy of the Mammary Lobe

PI: Elizabeth Peralta, MD, Department of Surgery, Southern Illinois University School of Medicine; Kerri-Ann Norton, Fellow, Computational Biology and Molecular Biophysics, Rutgers University, New Brunswick, New Jersey; James Going, PhD, Senior Lecturer, Institute
Only 30% of ductal carcinoma in situ (DCIS) cases will progress to invasive disease, but there is currently no way to differentiate these from the ones that will not progress. This consortium will use physiography, the spatial study of complex physical phenomena, to study how precursor lesions progress to invasion and to determine whether lymph-angiogenesis both precedes invasion and is increased around not only DCIS but also the precursor lesions. This will be done with fresh mastectomy specimens that have been sliced into 3mm sections and utilize lymphatic vessel immunohistochemistry, 2-photon microscopy, and stereoscopic imaging techniques. The consortium will also investigate whether 3D reconstructions of the lymphatic system of precursor lesions from pathology specimens can be modeled mathematically, which would provide additional information about the pathogenesis of DCIS recurrence and/or progression.

**HPV Typ**

Since HPV was first identified in 1949 more than 100 different types of HPV have been characterized. HPV is known to cause cervical, anal, and vaginal cancers as well as oral cancers in the tongue, throat, and tonsils. High-risk HPV types 16, 18, and 33 have also been seen in a subset of human breast cancers. HPV causes cancer after the HPV virus DNA becomes integrated into the host cell. (It can take 10-30 years for the cancer to develop after the integration occurs.) Laboratory studies have found that HPV 16 and HPV 18 oncogenes can change normal human breast cells into cancer cells in vitro. It also is known that the HPV E6 protein helps degrade p53 while the HPV E7 protein binds to pRb, both of which play a role in cancer development. Inactivation of pRb has been shown to lead to the upregulation of the p16 protein, and that upregulation is seen in HPV-positive tumors. Laboratory studies also have shown that the E6/E7 proteins of HPV type 16 can convert breast cell lines into cancer cells. This is accompanied by an upregulation of Id 1, which is an important regulator of breast metastasis. This consortium believes that previous researchers have had mixed results finding HPV in breast tissue because they did not use techniques that could recognize HPV after it has integrated into the cell. They intend to overcome this problem by looking for signs of DNA integration by measuring HPV-16 E2/E6 ratio. If HPV is found in breast tumors, this work could lead to the development of a novel approach—ID-1 antisense retroviruses—to treat breast cancer.
Dr. Thea Tlsty’s laboratory has identified rare human cells with extensive plasticity. This consortium will explore where these cells reside in the ductal structure; whether these cells are differentially distributed in small and large lobes; and if these cells are found in greater abundance in irradiated tissue or involuting tissue. To investigate these questions, consortium members will visualize and quantify these cells in situ in the context of the anatomy of the normal and diseased mammary gland.