The Sixth International Symposium on the Intraductal Approach to Breast Cancer

The Dr. Susan Love Research Foundation is committed to advancing research and developing resources that explore the intraductal approach to the breast. As part of this effort, the Foundation hosted The 6th International Symposium on the Intraductal Approach to Breast Cancer in Santa Monica, California, February 19-21, 2009.

In attendance were more than more than 100 delegates from 14 countries, including clinicians, epidemiologists, pathologists, basic scientists, translational investigators, and breast cancer advocates. The program included talks by 34 invited speakers and 16 pilot grant applicants in sessions that addressed the etiology of breast cancer; biomarkers of risk in nipple aspirate fluid and ductal lavage; anatomy, ductoscopy, and breast imaging; and intraductal therapy. Delegates also had the opportunity to attend demonstrations of ductoscopy, sonoductography, and nipple aspirate fluid collection on live volunteers. A Public Panel provided the community with an opportunity to learn more about ongoing intraductal research and the promise it holds for finding a cure for breast cancer.

At the close of the Symposium, the Foundation awarded $84,000 in research pilot grants to support 8 research studies.

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 are used to collect fluid and exfoliated cells from the breast ducts, which can be examined for abnormalities. Studies that have followed women over many years have found that women who produce nipple aspirate fluid that contains atypical cells are at 2-5 times greater risk of going on to develop breast cancer than are women who do not produce any fluid at all.

Minisymposium on a Novel Etiology for Breast Cancer: Inflammation
The first session’s speakers discussed the role that inflammation, nipple aspirate fluid and macrophages (a type of white blood cell) may play in breast cancer development. Lisa Coussens, at the University of California, San Francisco, discussed her research, which is looking at how breast cancer develops in mice. Her findings suggest that the immune system plays a role in cancer development and confirmed epidemiological studies that had showed that women whose breast cancers contained macrophage tended to have a higher tumor grade and decreased survival. These findings could one day lead to new ways of determining which tumors are most likely to recur and to new cancer treatments.
Studies have shown that when cells that are in the lining of the breast duct get damaged it sends out messages that result in inflammatory proteins being delivered to the cell site. Chandice Covington, at Texas Tech University Health Services Center, in Lubbock, discussed one of these proteins, called C-Reactive (CRP). This protein is associated with cancer risk, and has been shown to be present at high levels in the blood of women with advanced breast cancer. Dr. Covington’s study showed that CRP levels in nipple aspirate fluid (NAF) were associated with a woman’s age at first pregnancy, how long it had been since she last breastfed, percentage of body fat, and body mass index (BMI, a measure of body fat based on height and weight). It also showed that CRP levels in NAF was related to breast cancer risk as predicted by the Gail model.

Several large epidemiological studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) are associated with lower breast cancer risk. Patricia Thompson, at the Arizona Cancer Center, in Tucson, presented data from her phase Ib dose study investigating the effects of sulindac, a non-selective NSAID on NAF biomarkers. In this study, 30 women who were considered high risk for developing breast cancer were randomized to receive either 150 mg or 300mg of sulindac daily for 6 weeks, and nipple aspiration was used to measure levels of sulindac in the breast fluid. Dr. Thompson found that sulindac and sulfide were detectable in 57.7% of the NAF samples, while sulfone was detectable in 11.6%.

Taken together, the findings from these three preliminary studies suggest that NSAIDs in general and COX inhibitors, in particular, may have a role in the prevention or early treatment of breast cancer.

**Biomarkers of Risk in Nipple Aspirate Fluid and Ductal Lavage**

Intraductual approaches have the potential to offer new methods of assessing an individual woman’s breast cancer risk. Cytology was the first method used to evaluate breast fluid for risk assessment. However, recent technological advancements have resulted in a growing field of research aimed at identifying biomarkers in breast fluid that could be used for risk assessment as well as determining the best techniques for finding these markers in NAF.

Viruses cause several major human cancers. Gertrude Buehring, of the University of California, Berkeley, School of Public Health, a prior Foundation pilot grant recipient, discussed her research exploring whether oncogenic viruses in NAF mammary epithelial cells can serve as biomarkers to identify women at high risk for breast cancer. She is using a method called in situ polymerase chain reaction (PCR) to detect chromosomes from bovine leukemia virus, Epstein-Barr virus, and human papilloma virus (HPV). Studies have shown that all three of these viruses are found more frequently in the breast tissue of women with breast cancer than they are in women with no breast cancer history.

Massimo Tommasino, of the International Agency for Research on Cancer, in Lyon, France, presented data from his study using PCR to evaluate HPV in ductal lavage fluid. His data showed that DNA from HPV was rarely present in the breast fluids of high-risk women, suggesting that a direct role of HPV in breast cancer is unlikely.
Paul J. van Diest, of the University Medical Center in Utrect, The Netherlands, discussed his ongoing prospective study in which high-risk women undergo annual standard screening along with nipple aspiration. He is using a procedure called quantitative multiplex methylation-specific PCR (QM-MSP) to identify which tumor suppressor genes that are known to play a role in breast cancer are methylated [or activated] in the NAF. “In five to ten years,” said Dr. van Diest, “some of these high-risk patients will either have an operation because they have breast cancer or decide to have a prophylactic mastectomy, which would permit us to compare [our] data with what is found in surgical tissue to determine if there is a correlation between the number of genes methylated and breast cancer risk.”

It is possible that proteomics, which involves studying and analyzing proteins, may have more valuable for assessing a woman’s breast cancer risk than as a breast cancer screening tool. Savitri Krishnamurthy, of the MD Anderson Cancer Center, in Houston, discussed the seven proteins her team is currently investigating. Her goal is to see if she can identify a protein signature in NAF that could be used for risk assessment.

Ercole Cavalieri, at the Eppley Institute for Research in Cancer and Allied Diseases, at the University of Nebraska Medical Center, in Omaha, discussed his research on DNA adducts (pieces of DNA that bond to cancer causing chemicals), which play a major role in cancer development. Estrogen-purine DNA adducts are shed from cells and end up in the blood and urine. Dr. Cavalieri’s studies have shown that high levels of estrogen-DNA adducts are associated with breast cancer risk, and he suggested that learning how to eliminate this DNA adduct formation could be a new target for breast cancer prevention and treatment.

Robert Chatterton, of the Feinberg School of Medicine at Northwestern University in Chicago, discussed his study investigating the endocrine environment of the breast, in which 47 premenopausal and 40 postmenopausal high-risk women had ductal lavage, nipple aspiration, and provided a blood sample. The women were offered tamoxifen as chemoprevention and a repeat ductal lavage six months later. The results showed that both the NAF and ductal lavage fluid contained a rich source of hormones and other markers of risk, but the findings are too preliminary to draw any conclusions.

Ferdinando Mannello, at the University “Carlo Bo,” Urbino, Italy, a prior pilot grant recipient, presented an update on his research study investigating the role oxidative stress plays in the breast microenvironment.

Catharina Svanborg, at the Lund University in Sweden, is studying HAMLET (human a-lactalbumin made lethal to tumor cells), a protein-lipid complex that is found in human milk, and that shows promise as a new anti-cancer agent because it can kill tumor cells and immature cells while leaving healthy cells alone.

Hypermethylated genes are promising powerful biomarkers of breast cancer detection and perhaps risk assessment. Studies have shown that tumor suppressor gene (TSG) methylation is identified more frequently in random periareolar fine needle aspiration
samples from women at high risk for breast cancer than in women at low risk. David Euhus, at the University of Texas Southwestern Medical Center, in Dallas, investigated whether the finding that would hold true for breast fluid samples obtained via ductal lavage. For this study, 514 samples obtained from 150 women were assessed cytologically and by a technique called Quantitative Multiplex Methylation-Specific PCR (QM-MSP). Dr. Euhus reported that the TSG methylation in the ductal lavage samples did not predict marked atypia. He also reported that both methylation and marked atypia were independently associated with highly cellular samples, Gail model risk, and a personal history of breast cancer, suggesting related, but independent, pathogenic pathways in breast epithelium.

Mary Jo Fackler, at the Johns Hopkins University School of Medicine, in Baltimore, is also using QM-MSP to identify potential biomarkers. Dr. Fackler presented findings from a pilot study that explored the potential of using ductoscopic washings along with QM-MSP to evaluate spontaneous nipple discharge. The data showed that QM-MSP is 3-fold more sensitive than cytology in identifying cancerous cells. She also discussed a prospective pilot study of 54 postmenopausal women who were receiving anastrozole (brand name Arimidex) as their sole adjuvant therapy. Thirty-three of the women had an optional contralateral biopsy at baseline and at 6 months after starting treatment. A comparison of these biopsy samples showed significant decreases in methylation for the genes known as TWIST1, RASSF1A, and RARbeta. Both findings suggest more prospective studies are necessary to evaluate the relationship between changes in methylation and breast cancer incidence.

Gerald Gui, at the Royal Marsden Hospital, in London, presented data from his study comparing measurements of methylation-specific PCR in ductal lavage fluid, breast cancer tissue, adjacent normal breast tissue, and blood in women with early breast cancer. The study showed a positive correlation between tumor tissue and ductal lavage in the cancerous breast (n=24). Methylation was also significantly higher in ductal lavage fluid from the cancerous breast when compared with adjacent normal tissue and ductal lavage taken from the noncancerous breast.

Bassem Haddad, at Georgetown University in Washington, DC, discussed his research aimed at identifying a panel of biomarkers that could improve the diagnostic value of ductal fluid in detecting pre-malignant breast lesions and early stages of breast cancer in high-risk women. Edward Sauter, at the University of North Dakota School of Medicine, in Grand Forks, discussed his use of two intraductal approaches, ductoscopy and nipple aspiration, to assess response to both pharmacologic and nutritional chemopreventive interventions.

Catherine Carpenter, at the University of California, Los Angeles, a prior pilot grant recipient, provided an update on her research investigating the impact of diet and exercise on biomarkers in breast ductal fluid in overweight postmenopausal women. To date, five previously sedentary overweight women have completed the study.

The Gail Risk model, which is widely used to assess breast cancer risk, was developed based on data from Caucasian women and has been found to be less accurate in
assessing risk in women of other ethnicities. Prior pilot grant recipient Lisa Bailey, at the Alta Bates Summit Medical Center in Oakland, Calif., provided an update on her research exploring the feasibility of offering an intraductal approach for risk assessment to African American, Asian, and Hispanic women.

Another prior pilot grant recipient, Atilla Soran, at the University of Pittsburgh Medical Center, presented preliminary research, which is looking at antibodies in nipple aspirate fluid in breast cancer patients. He suggested that more sensitive techniques for antibody detection might be needed to identify the low antibody levels in the premalignant lesions, which could boost the usefulness of NAF as the source for this diagnostic assay.

These presentations demonstrated the different types of strategies researchers are using to identify biomarkers associated with genetic, epigenetic, and proteomic abnormalities in breast fluid. These reports add to the growing body of published studies that have found that biomarkers appear to be more sensitive than cytologic atypia in predicting breast cancer risk.

Anatomy, Ductoscopy, and Imaging of the Breast
Intraductal approaches have been used to advance our understanding of the anatomy of the breast. They have provided evidence that the breast ducts do not form an even, radial pattern. They also have shown us that the breast contains both central ducts that go directly back to the chest wall and peripheral ducts that drape over this central group. This knowledge of the anatomy of the human breast will guide future breast cancer research. As James Going, at the University of Glasgow, Scotland, a prior pilot grant recipient, underscored in his presentation, “Anatomy seems old-fashioned, but a house without a good foundation is a rickety structure and we need to understand that foundation, no matter how good the biology.”

Dr. Going presented findings from his research investigating what he calls “the paradox of nipple anatomy.” As he noted, 5-9 ostia can be observed in the lactating breast, and a few of these ostia can be cannulated. Yet 20-30 ducts are observed when the nipple is transected, which raises the question, “What are these ducts doing?” Dr. Going reported that he found no evidence for his previous two-duct-type hypothesis, but that he did find evidence that appears to point to ostium sharing—several ducts converging in a common opening in the nipple.

Gerald Gui, at the Royal Marsden Hospital, London, reported findings from his study investigating the anatomical association between fluid yielding ducts and breast cancer location. This study of 40 patients undergoing mastectomy for breast cancer, used ductal lavage followed by infusion of the mastectomy specimens with a colored resin. It showed that the duct system in the cancer affected segment of the breast was connected to a fluid-yielding duct in 58% of cases, a similar finding to previous studies. Dr. Gui said this suggested that cytology studies would likely be limited by duct accessibility whereas protein studies, which could demonstrate a field effect, would not.
Sheldon Feldman, at Columbia University in New York City, provided a history and overview of the field of mammary ductoscopy. The ability to see ductal pathology and monitor response to therapy in real time along with technological improvements, including better visualization and an interventional approach, are moving the field forward. Current challenges include the absence of an established CPT code and a lack of data from clinical trials evaluating the effectiveness of ductoscopy. Even so, said Dr. Feldman, “We are on the threshold of the future.”

Fatih Balci, at the Ankara State Hospital, in Turkey, presented images and data from his study evaluating the therapeutic value of endoscopic papillectomy in patients with pathologic nipple discharge (PND). Breast ductoscopy was performed on 178 of 213 patients with PND enrolled in the study. All visualized papillomas were removed endoscopically with a scope with a grasping basket. (The papillomas were removed through an opening in the nipple.) Dr. Balci reported that his team identified 34 papillomas and 5 cases of DCIS, and that there was a 100% correlation between ductoscopy findings and histopathology. These findings, said Dr. Balci, confirm that ductoscopic papillectomy “is a safe and easy procedure to remove intraductal papillomas from the breast that avoids unnecessary resection of breast tissue.”

William Dooley, at the University of Oklahoma, discussed current techniques and uses for ductoscopy. In his clinical practice, Dr. Dooley routinely uses ductoscopy to manage early invasive cancer. As a result, he said, the breast cancer recurrence rate (in the cancerous breast) in his patients is very low. In the discussion that followed, Dr. Dooley noted that surgeons rely on radiation to improve local control, but that ductoscopy could do the same. “If we can get good enough at doing endoscopically directed lumpectomy,” he said, “we would not need to perform radiation unless there was lymphovascular invasion.” One essential area needed to advance the field of ductoscopy will be carefully planned prospective multi-center studies to address the sensitivity, specificity, false negative and false positive rates of ductoscopy in accurately identifying cancerous versus non-cancerous tissue.

Wai-ka Hung, at Kwong Hospital in Kowloon, Hong Kong, discussed the role of mammary ductoscopy in breast surgery. He noted that for the field to move forward good endoscopic-pathologic correlation was necessary. This, in turn, could spare women unnecessary surgery, as it would allow for therapeutic mammary ductoscopy and removal or ablation of a papilloma. In addition, if it could be determined that there was no ductal involvement in the areola, ductoscopy might also be used to better select appropriate candidates for nipple-sparing mastectomy.

Debra Strick, at the University of California, Los Angeles, a prior pilot grant recipient, presented findings from her research on intraductal micromagnetic resonance imaging and spectroscopy. Dr. Strick developed an intraductal radiofrequency microcoil that can reduce the volume of diseased tissue to a cubic-centimeter, allowing MRI spectroscopy, which could distinguish between benign and malignant tissue, and therefore be used for early diagnosis. This microcoil could potentially be used with ductoscopy and conventional MRI to reduce false-positives or for an MRI-guided biopsy.
Intraductal Therapy: Background, Current and Future
The session on intraductal therapy highlighted studies investigating preclinical testing and early phase human clinical trials.

Dixie Mills, of the Dr. Susan Love Research Foundation in Santa Monica, Calif., presented data from a study exploring the physiology of the resting, or non-lactating, breast. A total of 14 women underwent blood collection, nipple aspiration, and ductal lavage five times over 12 hours. After baseline testing, subjects were given 200 mg of caffeine (NoDoz) and 200 mg of cimetidine (Tagamet). Previous studies have shown that in lactating women caffeine passively diffuses into milk rapidly and reflects blood levels. This study found that in the resting breast, caffeine levels peaked at 6 hours or later. Cimetidine has previously been found to be concentrated in the milk of lactating women, but was not detected in the ductal fluid obtained from the resting breast. These findings reinforce the need for more studies on the resting breast as its properties are clearly different than the lactating breast.

Saraswati Sukumar, at Johns Hopkins University in Baltimore, presented new data from her preclinical studies with pegylated liposomal doxorubicin (PLD) in rat and mouse models of breast cancer. Dr. Sukumar reported that in addition to preventing tumor formation for three months or more, PLD appeared to stunt the growth of the mammary glands in mice. Following up on this finding, she looked at the response of the treated mammary gland to a new pregnancy. She reported that that pups were unable to get sufficient milk from the treated duct, suggesting a compromised response in the mammary gland to pregnancy hormones. In addition, pregnancy did not increase the incidence of tumors in PLD treated mice. She also noted that their studies have found that mice receiving intraductal treatment have fewer stem cells than other mice, which has resulted in a new line of investigation.

Moving into the clinic setting, Vered Stearns, at Johns Hopkins School of Medicine, in Baltimore, presented data from a phase I feasibility study of PLD in women awaiting mastectomy. Participants underwent nipple aspiration and ductal cannulation using a dose escalation scheme. Blue dye was injected into the treated duct just prior to mastectomy and tissue was obtained for pharmacokinetic and biomarker analysis. Dr. Stearns reported that doxil was present in the region at both the 5mg and 10 mg dose, evidence that it can leave the duct. No changes were seen in the stroma surrounding the ducts, however a dose-dependent effect was seen in plasma and in breast tissue. Noting that there is no data on how IV doxil affects breast tissue, Dr. Stearns said her next study would compare the breast tissue of women who receive IV doxil prior to surgery with that of women receiving intraductal doxil.

Susan Love, of the Dr. Susan Love Research Foundation in Santa Monica, Calif., presented data from a Phase I feasibility study conducted in Beijing, China. One of two drugs, PLD or carboplatin, were used intraductally 2-7 days prior to mastectomy, at 3 dose levels, with the highest dose approximating the clinical intravenous dose. Dr. Love reported that PLD stayed in the duct longer and showed a lower serum dosage. At the highest doses, women receiving PLD reported tenderness and erethyma, whereas the highest doses of carboplatin resulted in mild nausea and vomiting, a sign that the drug
had gotten into the bloodstream. Dr. Love also reported that pathological examination showed the drugs were widely distributed through the ductal systems reaching the terminal duct lobular units. In conclusion, said Dr. Love, this shows us that this approach is feasible and safe.

Jianyu Rao, of the David Geffen School of Medicine at the University of California, Los Angeles, described the histopathological changes observed in the study Dr. Love presented. The pathology was not performed to study the tumor but to assess ductal epithelial cell changes and inflammatory reaction seen in ducts and surrounding tissue in association with duct treatment. Dr. Rao reported that carboplatin resulted in a dose-response increase of both in inflammation and ductal epithelial cell changes. In contrast, PLD resulted in no severe inflammatory changes at any dose, but there was a significant increase of epithelial response. Dr. Rao said this suggests that short-term intraductal treatment may induce some degree of epithelial changes and some inflammatory response, while the long-term effect remains to be determined.

Ellen Mahoney, at St. Joseph Hospital in Eureka, Calif., presented data from her pilot preoperative study testing the effects of PLD delivered through the affected duct of 30 women with DCIS diagnosed by core needle biopsy awaiting surgery. To date, six patients have undergone treatment. In those who were treated successfully with PLD, the pathology has revealed reactive and reparative changes to the duct. If this proves effective, said Dr. Mahoney, we could develop “a chemical mastectomy for post-breastfeeding women that would eradicate the stem cells and epithelial cells inside the breast duct, thus eliminating the possibility for breast cancer to occur.”

Taken together, the intraductal approach for potential prevention and therapy of breast cancer has been born and is growing as more researchers begin to study and answer the critical questions needed to advance this exciting emerging field.

**Pilot Grants**

The Dr. Susan Love Research Foundation utilizes a unique grant review mechanism to distribute pilot grants at the Symposium. Applicants submit one-page abstracts and responsive proposals are selected for presentation at the meeting. A multidisciplinary peer review committee, comprised of basic scientists, breast cancer activists, and physicians, evaluates both the abstracts and the presentations. At the Symposium, 16 researchers presented proposals. At the close of the Symposium, the Foundation announced that it would be providing $84,000 to support 8 innovative research projects utilizing the intraductal approach.

The 2009 recipients were: Robert Chatterton, PhD, and Seema Khan, PhD (Northwestern University), ‘Investigation of factors regulating estrogen uptake and retention by the breast’; Hong Ling, MD (Cancer Institute, Fudan University), ‘Comparison of PH value in nipple aspiration fluid from ductal carcinoma with or without calcification’; Ferdinando Mannello, PhD, and Gaetana Tonti, PhD (University of Urbino ‘Carlo Bo’), ‘Iron-driven inflammation in the breast microenvironment: assessment of iron-overload in breast cancer development’; Atilla Soran, MD (University of Pittsburgh Medical Center), ‘Autoflourescence ductoscopy for the early detection of breast cancer’;
Barbara Urban, MD, and Lorriaine Tafra, MD (Anne Arundel Breast Center), ‘Pilot study to identify breast cancer protein biomarkers in nipple aspirate fluid’; Ameae Walker, PhD (University of California, Riverside), ‘A prolactin binding compound in breast ductal fluids’;
Daniel Wreschner, MD (Tel-Aviv University), ‘Monoclonal antibodies and peptides targeting the MUC1 alpha/beta junction of the ablation of human cancer cells via the intraductal approach’; Gang Zeng, PhD (University of California, Los Angeles), ‘A multiplex approach for detecting antibodies in the nipple fluid of breast cancer patients’.

Conclusion
The 6th International Symposium on the Intraductal Approach to Breast Cancer highlighted the advances that have made in intraductal research and the potential the intraductal approach has to change clinical practice. Delegates in attendance expressed an interest in designing collaborative studies that can provide more data on the effectiveness of ductoscopy and identify new biomarkers in breast fluid. An update on these projects, along with findings from this year’s pilot grant projects, will be presented in 2011 at the 7th International Symposium on the Intraductal Approach to Breast Cancer.

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