

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 4th International Symposium on the Intraductal Approach to Breast Cancer in Santa Barbara, California, March 10-13, 2005. In attendance were more than 100 oncologists, epidemiologists, biostatisticians, surgeons, biochemists, pathologists, radiologists, and endocrinologists who are involved with research utilizing the intraductal approach.

Over a two-day period, prominent breast cancer researchers presented results of their studies in which they used the intraductal approach. Interwoven throughout each topic area were presentations by researchers interested in applying for pilot grant funding for new studies. The first day included sections titled: (a) Intraductal Approach: What We Have Learned From NAF, (b) Normal Breast: What Do We Know About Breast Fluid, (c) Ductal Lavage and Ductoscopy: The Intraductal Approach in the Clinic, and (d) High Risk Women: What Can the Intraductal Approach Tell Us? On the second day topics included: (a) Cellular Biomarkers in Ductal Fluid, (b) Proteomics and Other Markers in Ductal Fluid (c) Intraductal Therapy, and (d) and Ductal Fluid as a Marker for Prevention Research. In addition, a Public Panel was held on the second day to provide the community with an opportunity to learn more about ongoing intraductal research and the promise it holds for finding the answer for breast cancer. The schedule was structured to encourage attendees to discuss their ideas and share important research insights.

### **The Intraductal Approach: What We Have Learned From NAF**

#### **Some Pathophysiologic Perspectives on Breast Fluid Secretions**

Nicholas Petrakis, MD, professor emeritus at the University of California, San Francisco, delivered the first presentation. Dr. Petrakis discussed “Some Pathophysiologic Perspectives on Breast Fluid Secretions.”

Dr. Petrakis presented a brief overview of the research on breast fluid secretions obtained by nipple aspiration. This work began in the 17th century when it was suggested “breast secretions contained a corrosive substance that was damaging to the breast.” Three centuries later, in 1923, Geoffrey Keynes, MD, “brought a pathophysiologic perspective into thinking about breast secretions,” proposing “a dynamic between breast secretion and its reabsorption.”

Dr. Petrakis’s research, published in the *Journal of the National Cancer Institute* in 1975 (Apr;54(4):829-34), found an association between race, age, and menopausal status in the nipple aspirate fluid (NAF) of 606 non-lactating women. Additional studies Dr. Petrakis conducted in collaboration with Margaret Wensch, PhD, found that NAF production peaked between the ages of 35-40 and then dropped off as women aged. This suggests, he said, “that age-related decreases in the reabsorption of breast fluid may lead to prolonged exposure of the breast epithelium to chemical substances in NAF” that may lead to breast cancer.

Dr. Petrakis believes that by studying the biochemical components of NAF researchers will find markers that can be used to identify women at high risk for developing breast cancer. Research indicates “women who don’t yield fluid have the lowest risk of breast cancer and that women who do yield fluid have a twofold risk over women who don’t,” he said. Thus, he concluded, “Yielding fluid is a precursor of something going on in the breast.”

### **Is Presence of Nipple Aspirate Fluid and Cells Related to Subsequent Development of Breast Cancer? A 25-Year Prospective Study**

Gertrude Buehring, PhD, an associate professor in the Department of Virology at the University of California, Berkeley, School of Public Health. Dr. Buehring discussed “Is Presence of Nipple Aspirate Fluid and Cells Related to Subsequent Development of Breast Cancer? A 25-Year Prospective Study.”

Dr. Buehring’s study explored whether pathology is being missed in the ducts that do not produce fluid or in women who do not produce fluid. Previous studies had suggested that women who do not produce NAF are at lower risk of breast cancer than women who do.

The prospective observational cohort included 1605 asymptomatic women who donated NAF for research purposes between 1973-1976; information was obtained on 972 (61%) of these women. The study found “no elevated risk for women who had fluid but no cells,” said Buehring. “But there was, a significant relative risk of 1.57 for women who had fluid with epithelial cells.” For premenopausal women, the relative risk was 2.1. These numbers are similar to those Petrakis reported previously. Based on these findings, Dr. Buehring concluded, “NAF epithelial cells may be a general indicator of breast pathology and their presence is associated with a subsequent breast cancer risk.”

*\*\* Dr. Buehring’s research was supported by a grant from the Dr. Susan Love Research Foundation.*

### **Patient and Duct Characteristics Predicting Atypical Lavage Results**

David Euhus, MD, the Marilyn R. Corrigan Distinguished Chair in Breast Cancer Surgery at the Harold C. Simmons Comprehensive Cancer Center at the University of Texas Southwestern Medical Center, in Dallas, delivered the next presentation, “Patient and Duct Characteristics Predicting Atypical Lavage Results.”

Ductal lavage is currently recommended for risk-assessment for women who produce NAF and have a 5-year Gail Risk of 1.7 or greater. Dr. Euhus studied whether that criteria was useful, given the known problems with the Gail Model. “Although an increased Gail Risk does correlate statistically with increased breast cancer risk,” he said,

”it doesn’t discriminate well. More than half of women who get breast cancer have a low Gail score”—illustrating the need for a better method of determining who is at risk.

In this study, ductal lavage was performed on 125 women. All of the women’s NAF-producing ducts were cannulated; in addition, cannulation of one dry duct was attempted. Eighty percent of the patients had NAF, and an average of three ducts per patient were lavaged. Dr. Euhus found that the cytological atypia rate was identical for NAF-producing ducts and dry ducts, and that the atypia rate was similar for women who had a high Gail score and for those who had a low Gail score. In addition, atypia went down as women aged.

Based on these findings Dr. Euhus concluded, “NAF production is not associated with lavage atypia” and “five-year Gail risk over 1.7 percent does not predict lavage atypia.” He underscored that due to the small sample size a Type II error (the acceptance of the null hypothesis when the hypothesis is actually false) is a possibility.

In the discussion following this presentation it was noted that most women with atypia don’t get breast cancer. It was also noted that a similar study found that women who produced NAF were more likely to have atypia.

### **Lavage in NAF and Non-NAF Yielding Ducts**

Susan Love, MD, MBA, the Foundation’s president and medical director presented “Lavage in NAF and Non-NAF Yielding Ducts.”

The idea that ducts that produce NAF are the ones most likely to be abnormal is a primary assumption driving ductal lavage research. A second assumption has been that the breast with cancer would produce NAF with atypia and that the ducts with cancer would produce that NAF. But, said Dr. Love, recent studies have called these assumptions into question. A study by Carol Fabian’s group (Sharma et al, Breast Cancer Research and Treatment, 87:59-64,2004) of 113 women found cytologic atypia by random periareolar fine needle aspiration in 16 percent of women who did not express NAF. And a study by Kahn (Journal of the National Cancer Institute 96:1510-17,2004), found a high false negative rate when only the NAF ducts are studied in women with cancer or DCIS. This suggests that “using NAF as a marker of the most abnormal duct needs to be revisited.”

“We need to better understand why NAF is present in some ducts and not in others,” said Dr. Love. “Is it hormonally driven and a marker of higher estrogen levels? Are the ducts independent? Are those with NAF more likely to be abnormal? These are still things we need to learn.”

Dr. Love reported initial findings from the Foundation’s Normal Breast Study. This research was first presented at the 2004 San Antonio Breast Cancer Symposium. To date, 127 wet (NAF-producing) and dry ducts have been lavaged in 31 women who are not at

high risk for breast cancer. Fluid was analyzed for the presence of protein, epithelial cells, and macrophages. Fifty percent of the NAF producing ducts contained protein, as did 56 percent of the dry ducts. Epithelial cells were found in 38 percent of the NAF ducts and 27 percent of the dry ducts.

These findings raise the question, “What are we lavaging?” said Dr. Love. Are we lavaging large ducts, small ducts, rudimentary ducts, or are we actually perforating stromal tissue and in essence doing a fine needle aspiration? We don’t know.” To find out, Dr. Love concluded, “We need to be able to correlate the anatomy and the physiology. We’ve jumped ahead to molecular markers without knowing anatomy and physiology and until we know that it will be hard to interpret what the markers are telling us.”

### **Ductal Lavage and Magnetic Resonance Galactography in Women at High Inherited Breast Cancer Risk**

Allison Kurian, MD, a clinical instructor in the Division of Oncology at Stanford School of Medicine in Palo Alto, Calif., delivered the final presentation in the section. Dr. Kurian addressed “Ductal Lavage and Magnetic Resonance Galactography in Women at High Inherited Breast Cancer Risk.”

Every year, between 9000 and 18000 cases of breast cancer are diagnosed in women who have an inherited high risk for the disease. Many of these women carry a BRCA mutation. Studies have found that women with atypia on ductal lavage have an increased risk for breast cancer. Whether this is true for women who carry a BRCA mutation isn’t known.

Dr. Kurian’s pilot study enrolled 79 women at high-inherited risk for breast cancer. Ductal lavage was attempted in both wet and dry ducts in 79 women, 59 (75%) of whom had a BRCA mutation. Women who were found to have atypia on ductal lavage were invited to participate in a study of MR-galactography.

Ducts were successfully cannulated in 64 of 79 women (81%); 18 women (28.1%) had atypical cells, 15 of which were from dry ducts. The women with dry ducts (versus any NAF ducts) and the women who had atypia in these ducts were more likely to previously have had cancer or chemotherapy. To date, four women have had an MR-galactography exam to map the ductal system and localize the atypia. Dr. Kurian presented breast images from these four women.

*\*\* Dr. Kurian’s research was supported by a grant from the Dr. Susan Love Research Foundation.*

## **Normal Breast: What Do We Know About Breast Fluid**

### **Hormones in the Breast**

Robert Chatterton, PhD, a professor in the Departments of OB/GYN and Physiology at the Feinberg School of Medicine at Northwestern University, in Chicago, delivered the first presentation on the Normal Breast. Dr. Chatterton presented “Hormones in the Breast.”

Dr. Chatterton analyzed the hormonal status of the breast by evaluating NAF and ductal lavage fluid. Samples were fractionated for purification and to permit multiple analyses. Dr. Chatterton reported that when no proteins were found, there were also no significant levels of hormones present. This means, he said, “Proteins are an indicator of whether secretions that contain hormones have been obtained.”

Earlier, Dr. Petrakis raised the issue of whether the fluid in the breast is a stagnating pool. Dr. Chatterton noted that his study on changes during the menstrual cycle found that serum estradiol was twofold higher in the luteal phase than it was in the follicular phase, even though the amount of NAF did not increase. “So it could be,” he said, “that it is a stagnant pool of NAF but what is in it changes.”

Dr. Chatterton also has analyzed precursors of estradiol, EGF, and cathepsin D in the NAF of premenopausal women. These studies found that 83 percent of the variation in NAF estradiol could be predicted from concentrations of estrone sulfate, androstenedione, DHEA, DHEA sulfate, and progesterone in NAF. In contrast, serum and saliva estradiol were very weak predictors of NAF estradiol. In addition, cathepsin D, an estrogen response protein in NAF, was significantly associated with estradiol, estrone sulfate, SHEA, and DHEA sulfate. These findings indicate, said Dr. Chatterton, “hormone concentrations in NAF and ductal lavage fluid are good predictors of the estrogen and progesterone activity in the breast.”

*\*\* Dr. Chatterton’s research was supported by a grant from the Dr. Susan Love Research Foundation.*

### **Intraductal Insights into the Systemic Origin of Mammary Foam Cells, Tumor Desmoplasia, and Angiogenesis**

Sanford Barsky, MD, professor and chair of the Department of Pathology at Ohio State University College of Medicine and Public Health, spoke on “Intraductal Insights into the Systemic Origin of Mammary Foam Cells, Tumor Desmoplasia, and Angiogenesis.”

Intraductal foam cells are the most commonly found cells in nipple discharge, NAF, and ductal lavage fluid. Mammary fibroblasts and endothelial cells are the most commonly found cells in mammary stroma and are increased in breast cancer desmoplasia (stromal accumulation around the cancer) and angiogenesis. “The fact that foam cells not seen in

stroma,” said Dr. Barsky, “means their presence or absence may be a way to determine if the ductal lavage procedure is really in a duct and that a false entry into stroma of the breast has not occurred.”

Dr. Barsky explored the hypothesis that intraductal foam cells are macrophages—which come from monocytes, which come from the bone marrow—by placing a marker in the bone marrow of male GFP-transgenic C57 mice and then transferring that marrow into female mice. The mice were given hormones that made them pseudopregnant and enlarged their mammary fat pads. After the mice were euthanized and their mammary fat pad was removed they were found to have GFP-containing intraductal foam cells.

Next, Dr. Barsky studied the origin of the mammary gland stromal cells—the myofibroblasts and endothelial cells by taking bone marrow from GFP nude mice with green cells and putting it into recipient mice along with a human xenograft model of inflammatory breast cancer, MARY-X. The precursor cells in the cancers that developed were green, which indicated they were, at least in part, coming from the bone marrow.

Dr. Barsky then retrospectively examined breast cancer tissue from female patients who had received an allogenic bone marrow transplant for leukemia or lymphoma and later developed breast cancer. He found that a fraction of the foam cells, stromal myofibroblasts, and endothelial cells in the breast cancer tissue were of male donor origin.

Based on these findings, said Dr. Barsky, “cells come into the breast as part of breast cancer progression.” This means, “the paradigm for how breast cancer develops must reflect a contribution from both resident breast elements as well as systemic (bone marrow) ones.”

### **Analysis of Aromatase Expression in Intraductal Breast Fluid Cells**

Bonnie King, PhD, an associate research scientist in the Department of Therapeutic Radiology at the Yale University School of Medicine, addressed “Analysis of Aromatase Expression in Intraductal Breast Fluid Cells.”

Macrophages are found in normal and diseased breast tissue, are common in breast fluid, and can infiltrate breast tumors in large numbers. Dr. King’s preliminary studies to define the cell populations in ductal lavage fluid found extraordinarily high numbers of macrophages. “It’s not that we were surprised to see the macrophages,” she said. “What surprised us was the numbers we saw.” There were 15,680 epithelial cells but 29,200 macrophages per duct (median numbers).

Previous studies have shown that breast tumor-associated macrophages produce aromatase, which converts androgens into estrogen. Dr. King’s research looked at whether intraductal macrophages collected from benign breast ducts also express aromatase. In this study, ductal lavage was performed on 46 women; 10 of these were

scheduled to undergo breast surgery patients, 6 were high-risk asymptomatic women, and 30 were healthy asymptomatic women.

Dr. King found that 24/46 (52%) of the ductal lavage specimens contained macrophages that exhibited intense (3+) immunocytochemical staining. Further, all of the specimens classified as 3+ were collected from asymptomatic women. This suggests, Dr. King said, “a possible mechanism for the concentration of estrogen within the fluids bathing the ductal epithelial cells, from which most breast cancer originates.”

In the discussion that followed it was noted that enzyme activity would need to be shown in order to validate the findings. It was also noted that it was possible that there might be different levels of aromatase in different areas of the breast.

### **The Role of Estrogen Receptors in Breast Tissue and Breast Neoplasia**

Seema Khan, MD, an associate professor of surgery at the Northwestern University Feinberg School of Medicine, in Chicago, presented “The Role of Estrogen Receptors in Breast Tissue and Breast Neoplasia.”

More than 50 percent of breast cancer risk is attributable to estrogen exposure. To explore whether estrogen receptor (ER) expression is related to breast cancer risk, Dr. Khan conducted a case-control study comparing 68 premenopausal women and 101 postmenopausal women with breast cancer with 152 premenopausal women and 53 postmenopausal women undergoing benign breast biopsy. Dr. Khan found that women with breast cancer express higher levels of ER-alpha in normal breast epithelium than do women without breast cancer.

In addition, Dr. Khan said, studies by she and other researchers have found that estradiol levels were related to ER, LI, and Ki67; that there are differences in the number of ER-positive cells in various phases of the menstrual cycle, with the highest percentage (16.9) seen in women without breast cancer on days 6-9; that ER expression increases with BMI in HRT users; the breast epithelium of women on HRT had significantly higher ER expression than that of women not on HRT; and that ER expression is higher in proliferative lesions.

These findings indicate, said Dr. Khan, “that when looking at biomarkers we need to differentiate between pre and postmenopausal women and account for menstrual cycle noise.”

### **Breast Epithelial Cell Membrane Transporters May Determine Exposure of Cells to Antioxidants and Carcinogens**

Karl Karnaky Jr, PhD, an associate professor in the Department of Cell Biology and Anatomy at the Medical University of South Carolina, Charleston, discussed “Breast

Epithelial Cell Membrane Transporters May Determine Exposure of Cells to Antioxidants and Carcinogens.”

Exocrine glands produce special secretions and excretory products and use special membrane transporters to move these molecules to the luminal space. Dr. Karnaky is studying the multidrug resistance associated protein transporter, called MRP2, in human breast epithelial cells. MRP2 is located in the apical membrane of exocrine gland epithelial cells, where it excretes toxic xenobiotics and endogenous molecules, including a wide variety of chemotherapeutic drugs and carcinogenic and antioxidant molecules found in the diet. In every exocrine gland in the body where this transport is found—except in the breast—there is a fluid flush in the epithelial lumen. “Breast ducts are the most unusual exocrine gland in the human body,” said Dr. Karnaky, “in that they may never be flushed. They are the only glands that may not go into the outside world--as tears do.”

Dr. Karnaky found that immunocytochemistry of isolated and tissue-cultured breast ductules and alveoli indicated that MRP2 is located predominantly in the apical region of luminal epithelial cells, where it may bioconcentrate toxic substances into sealed, non-flushed lumens. Dr. Karnaky believes that as the MRP2 transporter moves molecules from outside to inside the cell some of these molecules may get into the DNA and cause mutations while others might become bioconcentrated in the lumen. But the transporter can do positive things, too. For example, he said, “PhIP, a heterocyclic amine carcinogen that has been found in human breast milk, as well as other carcinogens, are transported into the lumen by MRP2,” he said. “But so are antioxidants and other protective molecules. So perhaps there is a way to use the MRP2 to transport chemotherapy agents as well.”

### **Biochemical Constituents and Ultrastructural Features of Human Nipple Aspirate Fluids and Milk**

Ferdinando Mannello, PhD, a professor in the Pathology Department at the Institute of Histology and Laboratory Analysis, in Urbino, Italy, presented “Biochemical Constituents and Ultrastructural Features of Human Nipple Aspirate Fluids and Milk.”

The human breast’s secretory activity balanced by reabsorption processes starts at puberty, continues into adult life and declines after menopause with the gland involution. The adult non-pregnant non-lactating breast secretes into the ductal system a fluid in which many exogenous and endogenous substances are retained, concentrated and metabolized by breast epithelium.

“Although the secretion mechanism of NAF is not well understood,” said Dr. Mannello, “the tight and gap junction structures seem to be responsible for the different biochemical and cellular composition of NAF.” Type I NAF, which is found in women who are healthy or who have benign breast disease, he explained, is characterized by proteins detected in breast cyst fluid and plasma. It contains epithelial cells with leaky tight

junctions. In contrast, Type II NAF, which is found in 70 percent of women with breast cancer, has a protein pattern similar to that found in breast milk. In addition, it contains clusters of epithelial cells with tightly sealed junctions. It also contains high concentrations of biologically active compounds that may make women more prone to developing breast cancer.

Dr. Mannello concluded that characterizing the biologically active substances or carcinogenic compounds in the two types of NAF may lead to a way to identify pre-malignant conditions in the breast.

In the discussion that followed this presentation it was noted that differentiating between the two types of NAF in future research may lead to new findings; that it would be interesting to know whether macrophages can get through the tight junctions in Type II NAF; whether women with Type II NAF always have tight junctions; and whether the junctions regulate the composition of NAF.

### **Ductal Lavage and Ductoscopy: The Intraductal Approach in the Clinic**

#### **Does Ductal Lavage Detect Known Cancer?**

Dr. Khan delivered the first presentation in the area of “Ductal Lavage and Ductoscopy: The Intraductal Approach in the Clinic.” She discussed “Does Ductal Lavage Detect Known Cancer.”

Dr. Khan investigated the association between ductal lavage cytologic findings and histologic findings in women undergoing mastectomy for known breast cancer or prophylaxis and in women undergoing a breast biopsy for suspicious mammographic microcalcifications.

In the first study, ductal lavage was performed in the operating room on 44 breasts from 39 women, 8 of whom were having a prophylactic mastectomy. Markedly atypical or malignant cytology was found in 5 breasts with cancer. In the second study, which included women who had microcalcifications, the histologic findings were 10 DCIS, 5 atypical hyperplasia, and 5 usual hyperplasia or fibrocystic change. In 8 of the DCIS lesions, 4 had no NAF, 1 had extravasation, and 3 were lavaged but the duct that produced NAF was not the duct where the DCIS was present.

In conclusion, said Dr. Khan, “in women with known cancer, ductal lavage cytology has a significant failure rate as a diagnostic test because there either was no NAF, the NAF came from non-cancer bearing ducts, or when the cancer-bearing duct was lavaged it did not have malignant cytology.” An additional concern, she said, is “the lack of reproducibility between cytopathologists of the diagnosis of mild atypia, which is the most frequent abnormal finding.” (The concordance was 40%.)

In the discussion that followed this presentation it was pointed out that a FISH analysis might be better than cytology. Dr. Khan said she is now conducting methylation assays on this data. It was also noted that NAF might not be present if the cancer has altered the breast duct. Dr. Khan emphasized the “need to separate risk issues from diagnostic issues” when discussing the utility of ductal lavage.

### **Ductal Lavage in Women with Breast Carcinoma: A Correlative Study**

Edie Brogi, MD, PhD, an assistant attending pathologist at the Memorial Sloan Kettering Cancer Center in New York, presented, “Ductal Lavage in Women with Breast Carcinoma: A Correlative Study.”

In this study, ductal lavage was performed on the cancerous breast of 26 women undergoing mastectomy and on the normal breast of four women undergoing a prophylactic mastectomy. Dr. Brogi found that four (14%) of the 29 ductal lavage samples that could be evaluated showed marked atypia; 10 (34%) showed mild atypia; and 15 (52%) were benign. Three cytopathologists independently reviewed all of the slides. There was complete agreement among the three in 31% (9/29) of the analyses and no agreement in 10% 3/29 cases. Dr.

Based on these findings, Dr. Brogi said, ductal lavage has a low sensitivity for carcinoma in situ in breasts that also contain invasive cancer. For this reason, “ductal lavage is not suitable for screening cancer, and not ready for clinical application, as the extent of disease may affect ductal lavage findings.”

### **Lessons Learned From Five Years of the Intra-Ductal Approach to Breast Cancer Treatment**

William Dooley, MD, a professor of surgical oncology and the director of The Institute for Breast Health at the University of Oklahoma, spoke on “Lessons Learned From Five Years of the Intra-Ductal Approach to Breast Cancer Treatment.”

Intraductal techniques are an integral part of Dr. Dooley’s patient management. Research conducted at his center on women at increased risk for breast cancer in which the mammographically and clinically normal contra-lateral breast was lavaged at the time of the surgical procedure found one occult malignancy and increased patient’s use of tamoxifen, indicating that using ductal lavage to screen or atypical hyperplasia can impact patient care.

Dr. Dooley has found that women with smaller tumors have more contralateral atypia. “Is this because the tumor burns itself out as it gets bigger,” he questioned. “Or is it that the tumor does something that impacts and then decreases the atypia? These are things we need to determine. He also noted that although “ADH seems to be the best target to be reversible by chemoprevention” he has found that “as tumor size increases, ADH seems to disappear in contralateral breast.”

Dr. Dooley began performing ductoscopy in order to better understand ductal lavage findings. Now, it is a routine part of his operative breast surgery. Performing ductoscopy has allowed him to get better margins and to detect nonobstructive lesions in the breast. It has also increased the rate of cancer detection when women present with bloody nipple discharge, and “brought the recognition that one-half of cancers are associated with retro-areola papilloma which is not the bleeding source.” He also noted that his annual hazard rate for local failure following a lumpectomy for early stage breast cancer has fallen from 0.67% to 0.08% due to his use of endoscopy.

“We are beginning to have the tools to unlock the answers to this disease,” he concluded. “It seems to me that combining ductal lavage with ductoscopy should allow us to be able to find a subgroup of women with invasive disease that can be treated in a different way.”

### **Mammary Ductoscopy: Lessons Learned**

Julian Kim, MD, a surgical oncologist at the Cleveland Clinic Foundation, discussed “Mammary Ductoscopy: Lessons Learned.”

Dr. Kim has studied ductoscopy for nearly six years. He has explored the feasibility of ductoscopy in human mastectomy specimens, the use of ductoscopy in women with pathologic nipple discharge, and the effectiveness of ductoscopy in women undergoing partial mastectomy. He has found that ductoscopy is able to identify more abnormalities than ductography. He also discovered that the procedure is less likely to be successful if hyperplasia or cancer is present. (Dietz JR. Surgery. 2002. Oct; 132(4) 582-7.)

Data will soon be published from his comparative analysis of minimally invasive microductectomy versus major duct excision in patients with pathological nipple discharge. This study is comprised of 95 women who underwent microductectomy and 140 who had a major duct excision. Dr. Kim found that 3% (3/95) of the women who had microductectomy women were diagnosed with DCIS and that no patients were diagnosed with invasive carcinoma. In contrast, 8% 12/140 of the women who had major duct excision were diagnosed with either DCIS or invasive cancer, a statistically significant increase. Based on these findings, said Dr. Kim, “in patients with pathologic nipple discharge undergoing surgery, microductectomy appears to result in a diagnosis of fewer breast cancers than major duct excision.”

In summary, said Dr. Kim, “ductoscopy is feasible and safe and it allows us to identify intraductal lesions in patients with pathologic nipple discharge not seen by ductography. But, to date, it has not been demonstrated that there is any added patient benefit from ductoscopy for women undergoing partial mastectomy in terms of improved margin control.”

In the discussion that followed Dr. Khan said that her group had a paper forthcoming in the Annals of Surgery on her research that compared ductoscopy-guided and

conventional surgical excision in women with spontaneous nipple discharge. This study found that there were more significant lesions classified as ADH when women had ductoscopy. The finding was not statistically significant but did trend in that direction. (Ann Surg. 2005 Apr;241(4):575-581.)

### **First Series of 26 Patients with Diagnostic Breast Micro-Ductoscopy for Direct Visualization of Intraductal Lesions**

Volker Jacobs, MD, an ob/gyn at the Technical University, Munich, Germany, presented “First Series of 26 Patients with Diagnostic Breast Micro-Ductoscopy for Direct Visualization of Intraductal Lesions.”

Standard radiological or ultrasound exams of breast duct lesions only offer indirect information about the lesion. In contrast, micro-ductoscopy allows direct visualization of the lesion, which may help in the decision to perform or avoid exploratory breast tissue resection.

In October 2003, Dr. Jacobs began performing micro-ductoscopy with one of the smallest ductoscopes available. (0.55 mm external diameter) To date, he has performed the procedure on 26 patients with a suspicious and/or bloody nipple discharge. He found that 23/26 (88.5%) of the patients had benign problems, such as papillomas, fibroadenomas or ectasia, while one had DCIS and one had invasive breast cancer. Dr. Jacobs showed video images obtained during this procedure of normal duct walls, DCIS, and intraductal papilloma.

In conclusion, said Dr. Jacobs, “micro-ductoscopy is feasible and safe and a promising new technique.” And while “it is still experimental and we need more studies and more experience to distinguish benign from malignant lesions,” micro-ductoscopy highlights why “we need more visual experience” to fully understand how breast cancer begins.

### **Light for a Lightning Diagnosis**

Mohammed Keshtgar, MD, PhD, a senior lecturer in surgery at University College London, addressed “Light for a Lightning Diagnosis.”

Elastic scattering spectroscopy (ESS) is an optical technique that can detect cellular and subcellular changes that occur in malignancy. When the procedure is performed light is transmitted through an optical fiber that is placed on the tissue being examined. A second fiber transmits elastically scattered light to a computer that analyzes the spectra and compares it to a reference database of matched spectra to provide a histological diagnosis, creating the potential for instant ductoscopic diagnosis.

To date, Dr. Keshtgar has analyzed 144 breast tissue specimens, ranging from normal breast tissue to invasive cancer. Optical biopsy had a sensitivity of 93.7 percent and a specificity of 92.3 percent in differentiating benign from cancer. Dr. Keshtgar has also

conducted an intraoperative diagnosis of the sentinel node histological status. To date, he has analyzed 782 spectra from 139 nodes, 53 of which contained cancer. In this setting, optical biopsy had a sensitivity of 84 percent and a specificity of 91 percent.

Dr. Keshtgar's goal is to develop a procedure that would have 100% specificity and sensitivity. He is currently developing more training sets for the computer, attempting to refine the spectral analysis, and developing a probe that can deliver light and obtain spectra at the same time.

In the discussion that followed Dr. Keshtgar's presentation it was pointed out it is not only necessary to be able to differentiate normal tissue from cancerous tissue, but to identify changes along this continuum, such as atypia. Dr. Keshtgar said that with the right training sets the computer could be taught to identify these differences.

### **High-Risk Women: What Can the Intraductal Approach Tell Us?**

#### **Breast Cancer Risk Assessment and Ductal Lavage in Practice**

The first presentation on "High Risk Women: What Can the Intraductal Approach Tell Us" was by Freya Schnabel, MD, an associate professor of Clinical Surgery at Columbia University College of Physicians & Surgeons, in New York, and medical director of the college's Women At Risk program. Dr. Schnabel discussed "Breast Cancer Risk Assessment and Ductal Lavage in Practice."

Surveillance programs, chemoprevention, and prophylactic surgery are all options for high-risk women. But before a woman can determine the method that is right for her, she needs to assess her current risk. The Gail Model and the Claus model are commonly used epidemiologic risk-assessment models, but both have limitations. Genetic testing is an option as well. But what is needed, said Dr. Schnabel, "is a biomarker that is biologically and statistically associated with cancer development, present in a reasonable proportion of at-risk individuals, obtained by minimally invasive techniques, and reversible with prevention techniques." Cellular atypia, which can be identified through ductal lavage, may meet these criteria.

Dr. Schnabel uses ductal lavage to seek out atypical hyperplasia in high-risk women to help them manage their risk. Between July 2001 and July 2004, 57 high-risk women were lavaged through Dr. Schnabel's high-risk program. Dr. Schnabel found that the women who received ductal lavage used the information to help them determine how to manage their breast cancer risk. Six patients with atypia began taking tamoxifen for chemoprevention, three initiated intensive surveillance with MRI, four had genetic testing, and two with BRCA mutations had a prophylactic salpingo-oophorectomy.

The advantage of ductal lavage is that it "is patient-specific, cell-based, and minimally invasive," concluded Dr. Schnabel. "The limitation is that there is an uncertain negative predictive value and a variation of cytology interpretations by pathologists."

## **Ductal Lavage in Women with Germline BRCA 1/2 Mutations**

Gillian Mitchell, MD, PhD, an oncologist at the Peter MacCullum Cancer Center in Melbourne, Australia, presented “Ductal Lavage in Women with Germline BRCA 1/2 Mutations.”

Although the number of women who carry a BRCA mutation is small, they are of significant concern because their lifetime risk for developing breast cancer is between 40 and 85 percent.

Dr. Mitchell’s study aims to identify a potential biomarker of breast cancer risk in BRCA1/2 carriers. Her group has recruited 54 women with BRCA1/2 mutations to undergo nipple aspiration, ductal lavage, and blood sampling every six months for three years, with a 10-year follow-up for breast cancer development. Similar studies will take place in the UK and the US. The fluid collected will be analyzed for cytology, loss of heterozygosity (LOH) at the BRCA1/2 loci, methylation frequency of candidate genes, and proteomic profile.

To date, 5 cases of mild atypia, 4 cases of severe cytologic atypia and 4 breast cancers have been identified in the 54 women. (Not all have been lavaged yet.) Initial studies found that LOH was reproducibly detected in 13 percent of ducts from women with a BRCA1 mutation and 16 percent of ducts from women with a BRCA2 mutation. However, a greater proportion of ducts (~45%) showed LOH that was not reproducible between experiments.

Based on these findings, Dr. Mitchell concluded, “Atypia is poorly correlated with established breast cancer.” Further, “concerns remain regarding the quality of DNA recovered from ductal lavage samples and its suitability for use in PCR-based experiments.”

## **Preliminary UK Experience of a Comprehensive Ductal Program in BRCA1 and BRCA2 Gene Mutation-Carrying Families**

Imogen Locke, MD, a clinical research fellow in cancer genetics at the Royal Marsden Hospital Institute of Cancer Research, in the UK, discussed “Preliminary UK Experience of a Comprehensive Ductal Program in BRCA1 and BRCA2 gene Mutation-Carrying Families.”

Dr. Locke collected nipple aspirate fluid (NAF) from 47 women who come from families with pathogenic BRCA1 or BRCA2 mutations. Thirty-six of the women had a BRCA1/2 mutation; 11 were non-carriers. NAF was obtained from 37 percent of the BRCA1 carriers, 47 percent of the BRCA2 carriers, and 36 percent of the non-carriers. At least one breast duct was cannulated in 17 women, 16 of whom produced NAF. Through

cytology, one BRCA1 carrier was found to have mild atypia in one breast. Another BRCA1 carrier who did not produce NAF was found to have invasive carcinoma during a prophylactic mastectomy. An LOH analysis was performed to assess the methylation status of the DNA; Cyclin D2 and RARB genes were largely unmethylated in both carriers and controls. And the proteomic analysis found that the profiles from unaffected left and right breasts were similar.

Based on these findings, Dr. Locke concluded, “If ductal lavage is only used on women who produce NAF, its utility as a risk-assessment tool may be limited in women from BRCA families.” Following this assessment, the research team altered its protocol to include lavage of non-NAF producing ducts.

*\*\* Dr. Locke’s research was supported by a grant from the Dr. Susan Love Research Foundation.*

### **Nipple Aspirate Fluid Cytology and the Gail Model**

Jeffery Tice, MD, an assistant adjunct professor of medicine at the University of California, San Francisco, addressed “Nipple Aspirate Fluid Cytology and the Gail Model.”

The Gail model does not accurately determine who will develop breast cancer. Dr. Tice’s research explored whether the addition of NAF cytology results improve the ability of the Gail model to predict breast cancer.

Dr. Tice used data from Wrensch’s study, a prospective observational cohort of 6904 asymptomatic women in which breast cancer cases were identified through follow-up with the women and linkage to cancer registries. During 14.6 years of follow-up, 400 women were diagnosed with breast cancer; 940 (14%) women were diagnosed with hyperplasia; and 109 (1.6%) women were diagnosed with atypical hyperplasia.

Adding NAF cytology results to the Gail model significantly improved the model’s ability to predict breast cancer, particularly in higher risk women. However, said Dr. Tice, “the concordance statistic for the Gail model is 0.62, and adding the NAF cytology results only brings that up to 0.64.” Thus, Dr. Tice said, “a better biomarker” is still needed.

### **Pilot Study to Determine the Incidence of Cellular Atypia in African-American Women at High Risk for Breast Cancer**

Katherine Lee, MD, a surgeon at the Cleveland Clinic, discussed “Pilot Study to Determine the Incidence of Cellular Atypia in African-American Women at High Risk for Breast Cancer.”

African-American women have a 17 percent lower incidence rate of breast cancer than Caucasian women, but they have the highest mortality rate of all races. Young African-American women (40 and younger) have a higher incidence rate of breast cancer than do Caucasian women. African-American women also are more likely to be diagnosed at a later stage of disease and have a lower 5-year survival rate.

The Gail model is the most common tool used to determine breast cancer risk. Previous research by Dr. Lee's group that reviewed Gail scores in women newly diagnosed with breast cancer found that African-American had lower estimated Gail scores than Caucasian women did. "This means that if the Gail model is used to estimate risk," said Dr. Lee, "that there will be African-American women who are not eligible for chemoprevention."

Dr. Lee's study explored whether ductal lavage findings would make more women eligible for chemoprevention. The study looked at the incidence of cellular atypia in the contralateral breast of women who had been diagnosed with breast cancer or DCIS. To date, the study has enrolled 21 African-American women. Twelve women were lavaged successfully; nine had no NAF or the duct could not be cannulated. In seven cases the results were benign; in six atypia was present (five mild, one marked).

Dr. Lee noted that, while this study was small, it has found a higher incidence of atypia than a study on high-risk women published in JNCI. (J Natl Cancer Inst 2001; 93: 1624-1632) "We have an atypia rate of 42 percent, which is higher than the atypia rate of 24 percent in that study. This lends support to idea that cellular atypia may provide additional information for African-American women not seen as high risk based on their Gail score."

*\*\* Dr. Lee's research was supported by a grant from the Dr. Susan Love Research Foundation.*

### **Panel Discussion**

The first day of the symposium ended with the panel discussion "Ductal Lavage and Ductoscopy: Clinical Practice or Clinical Trials?" The panel included Victor Vogel, MD, William Dooley, MD, Seema Khan, MD, and Julian Kim, MD. It was moderated by Susan Love, MD, MBA.

### **Clinical Practice**

Dr. Vogel opened the discussion, explaining why he supports the use of ductal lavage in the clinic. "Yes, we need better models to predict an individual patient's risk," he said, "but even though we don't know all we need to about atypia from ductal lavage, we know that atypia doubles in general any breast cancer risk."

Dr. Vogel argued that atypia found on ductal lavage should be treated the same as that found during a biopsy. "Atypia is atypia," he said. "We shouldn't argue about its

significance when found on ductal lavage. The real problem is that not all women with breast cancer have it and only small number with it get breast cancer.”

And he concluded that the information obtained from ductal lavage should be used in clinical practice. “Until we see ductal lavage atypia does not mean the same as atypia found in other way, we must consider it. What is harm of waiting for a clinical trial before using ductal lavage in the clinic? We could make a Type I error, and that means not doing it is bigger harm--not from a scientific perspective but from a public health perspective.”

Next, Dr. Dooley explained why he supports the use of ductoscopy in the clinic. He noted that Dr. Kim reported that he had found a cancer that hadn't been seen on mammography when performing endoscopy during a lumpectomy. And he noted that the same thing has occurred in his own series of endoscopies. “I collected all my patient data,” he said, “and the recurrence rate without endoscopy is 67 percent.” In contrast, he said, when he has performed a lumpectomy with endoscopy the “annual hazard rate for locoregional recurrence has decreased eightfold. Is that not evidence that it is better to do endoscopy-guided lumpectomy?”

### **Clinical Trial**

Dr. Khan argued against the use of ductal lavage in the clinic. “We don't know what fraction of women who have ductal lavage will have abnormal cytology,” she said. “We don't know the significance of mild atypia. And intraobserver variability is high, even among experienced cytopathologists—and this is significant because ductal lavage is being promoted as something that can help women in their risk assessment.”

For example, she said, “what would we do with a woman who has severely atypical ductal lavage cytology? It is difficult to convey what this means, because we can't say how much this increases risk. Also more atypia is found through ductal lavage than with FNA or NAF. So if we're going to counsel women, we should use methods that have been validated so that we can tell women how the findings impact their risk more precisely. And that can't be done with ductal lavage until a long-term validation study has been completed.”

Next, Dr. Kim explained why he does not think mammary ductoscopy should be the standard of care. “My feeling is that it should only be the standard of care,” he said, “if there is demonstrated benefit to the individual patient, based upon peer-reviewed published data.” Currently, he said, “there is not a lot of published evidence that a scope will find a lot of cancer. Dr. Kim encouraged Dr. Dooley to publish his data. “I think you are rally on to something,” he said. “But if learning curve is huge how practical is this? Is this conclusive enough?”

### **Clinical Practice Rebuttal**

Dr. Dooley argued that sentinel node biopsy had a huge learning curve as well, but that it has now become widely used. Additionally, he said, “we would not be arguing about atypia and what it means on ductal lavage if we could scope every patient with atypia, get

tissue through the scope and get a pathologic answer to advance our understanding.” This is similar to what occurred with MRI, he noted. “We used a technique that was marginal but showed promise. And it’s the same with ductoscopy.”

Dr. Vogel noted that ductal lavage is not being used to determine if someone will get cancer right away, but over a multi-year span of risk. He also underscored the need for a better consensus among pathologists in identifying atypia. If you look at risk reduction strategies, no matter the intervention, Dr. Vogel said, “you always will have a certain number needed to treat to have a benefit, unless there is a marker that correlates 100 percent of the time and intervention that lowers risk 100 percent of the time. So we shouldn’t ask lavage atypia or any other risk factor to perform what is possible beyond epidemiology. And that’s why I won’t wait for a clinical trial—although such trials should take place—to start using lavage atypia now to help manage risk.

### **Clinical Trial Rebuttal**

Dr. Khan argued that the fact that there is no consensus “reflects a lack of data and in this vacuum of data it’s hard to tell women how to make decisions.” Doctors with expertise in this area might be able to explain the ramifications of the cytology, she said, but not all doctors currently have this knowledge. “We heard today of women getting prophylactic mastectomy after getting cytology on ductal lavage,” she said, “so I believe it verges on irresponsibility to use this in clinical practice where women will make irreversible decisions based on these findings.”

Dr. Kim argued that there is not convincing data that shows that identifying ADH at time of breast cancer surgery and removing it will improve relative risk. He again urged Dr. Dooley to publish his data. And he said that this discussion has led him to think about re-opening and refining his ductoscopy study because “the only way to advance the field and to see clearly what role ductoscopy will have in the future is to test it.”

## **Cellular Biomarkers in Ductal Fluid**

### **Cellular Biomarkers for Ductal Lavage Samples**

The second day of the Symposium began with a discussion of Cellular Biomarkers in Ductal Fluid. The first presentation was by Jian Yu Rao, MD, an assistant professor in the Departments of Pathology and Lab Medicine at the University of California, Los Angeles, School of Medicine. Dr. Rao discussed “Cellular Biomarkers for Ductal Lavage Samples.”

Conventional cytomorphological analysis of ductal lavage is very subjective. Biomarker-based analysis has the potential to be more reliable and provide more accurate information for breast cancer risk assessment. Dr. Rao previously found that cellular biomarkers including DNA 5c exceeding rate (DNA 5cER) and G-actin when measured on materials obtained through fine needle aspiration with Quantitative Fluorescence Image Analysis might be markers for breast cancer risk. He presented data from his

research that explored whether these markers could also be measured on ductal lavage slides.

Dr. Rao conducted a two-part study. In the first phase, 34 women were enrolled. In the second phase 17 women were enrolled. In phase 1, 24/34 women had adequate ductal lavage samples to perform a biomarker analysis. For phase 2, the validation study, 12/17 women did.

Dr. Rao found that more than one-third of the ductal lavage samples collected from women who did not have cancer contained atypical cytology. This study indicated a cut-off threshold for DNA5cER at 10.5 had 92 percent specificity and 90 percent sensitivity for cancer while a cut-off threshold for G-action at 200 had 84 percent specificity and 100 percent sensitivity. The validation study found that combining DNA5cer and G-actin resulted in 100 percent sensitivity and 100 percent specificity for cancer. Based on these findings, Dr. Rao concluded that a biomarker analysis provides better separation between cancer and non-cancer than cytology and that incorporating a third marker of histiocytes (CD68) or epithelial cells (eg keratin) may produce more accurate biomarker measurement.

*\*\* Dr. Rao's research was supported by a grant from the Dr. Susan Love Research Foundation.*

### **Detection of LOH and Mitochondrial DNA Alterations in Ductal Lavage and Nipple Aspirate Fluids from High-Risk Patients**

Luciane Cavalli, MD, an assistant professor in the Oncology Department at Georgetown University's Lombardi Comprehensive Cancer Center, in Washington, DC, discussed "Detection of LOH and Mitochondrial DNA Alterations in Ductal Lavage and Nipple Aspirate Fluids from High-Risk Patients."

Dr. Cavalli's group is working to identify molecular markers that can augment mammography for the early detection of breast tumors in high-risk women. They have developed a method to isolate and evaluate free DNA from ductal lavage fluid.

As proof of principle, the team examined the free DNA for LOH, using two dinucleotide microsatellite markers, one for the BRCA1 gene (intrinsic marker D17S855) and one for the FHIT gene (intrinsic marker D3S1300) and for mitochondrial DNA mutations at the D310 marker. Mitochondrial analyses were possible on all of the samples. In two patients, a mutation was identified.

Based on these findings Dr. Cavalli concluded, "it is feasible to use free DNA isolated from ductal lavage fluid and NAF to conduct molecular arrays and to detect LOH at relevant markers of mtDNA mutations. Further, the technique preserves the total cellular content of the ductal lavage fluid for cytology analysis.

## **Cytomorphology As A Breast Cancer Risk Predictor: Experience with FNAB, Nipple Fluid Aspiration, and Ductal Lavage**

Shala Masood, MD, professor and associate chair in the Department of Pathology at the University of Florida Health Sciences, in Jacksonville, discussed “Cytomorphology As A Breast Cancer Risk Predictor: Experience with FNAB, Nipple Fluid Aspiration, and Ductal Lavage.”

Dr. Masood developed the “Masood Cytology Index,” which provides a method for differentiating cells that are normal, atypia, proliferative, or suspicious for cancer. She noted that previous research has identified a 4-5 fold risk of breast cancer in women with proliferative breast disease with atypia, and that the risk is similar regardless of whether cells were obtained through breast duct fluid or fine needle aspiration. And, she pointed out, a similar risk is seen when biopsy tissue is analyzed (histopathology). “Even so, said Dr. Masood, “the idea of using cytology has not been widely accepted or practiced and we have a long way to go to gain consensus on how to use cytology in clinical practice.”

Dr. Masood noted that a study by Dr. Dooley that compared ductal lavage to nipple aspiration found that cellular yield is greater and that more cellular atypia is detected through ductal lavage. (JNCI. 2001;93;1624-32.) But, she said, more work needs to be done to understand what is relevant. Part of the problem is the term atypia. “This can cover everything from fibrocystic changes to DCIS,” she said, “The category is too broad and does not differentiate enough.”

Dr. Masood showed a series of slides that demonstrated the close similarity between how papillary lesions, DCIS, and ADH appear on FNA biopsy and on ductal lavage.

In conclusion, said Dr. Masood, “It is possible to use cytomorphology as a breast cancer risk predictor, but further studies are required to better define the criteria of prognostically relevant atypia in ductal lavage.” In addition, “it is essential to integrate morphology with biology, contemporary breast imaging, and innovative surgical procedures for early breast cancer and detection.”

### **Nipple Aspirate Fluid: Possible Clinical Applications**

Edward Sauter, MD, an associate professor of surgery and vice chair of research at the University of Missouri Health Care Cancer Center, in Columbia, presented “Nipple Aspirate Fluid: Possible Clinical Applications.”

Dr. Sauter’s laboratory focuses its research on biomarkers of breast cancer prevention, early detection and response to treatment. Over the past 10 years, more than 1500 women have taken part on their NAF studies. His group has looked at cytology, proteins (alone or in combination) and DNA (nuclear, mitochondrial, and methylated) as possible candidates for NAF biomarkers for breast cancer detection.

NAF cytology is “a specific but not sensitive indicator of breast cancer.” This is why, he said, “other markers that can enhance sensitivity” are needed. Our goal, he explained, “Is to find a way to reduce the number of mastectomies for residual breast cancer that reveal nothing is there.”

This effort has included the development of a reexcision algorithm. It has also included studies that have found prostate-specific antigen (PSA), insulin-like growth factor binding protein type 3 (IGFBP-3) and basic fibroblast growth factor (bFGF) to be predictive of breast cancer. “Combining race, NAF, bFGF, and PSA can predict cancer 83 percent of the time,” Sauter said. In addition, their exploration of DNA methylation and NAF, which investigated 22 matched breast cancer tissues and matched NAF, found hypermethylation in 22/22 breast cancer tissue and 18/22 NAF specimens but in no normal tissue or NAF specimens. The study also found that three proteins—GCDFP-15, alpha-1-acid glycoprotein, and apolipoprotein D—found in NAF are overexpressed by 2-D PAGE when breast cancer is present.

In summary, said Dr. Sauter, “NAF biomarkers have been found that are associated with breast cancer, but no biomarker is sufficiently sensitive and specific to detect breast cancer.”

### **Proteomics & Other Markers in Ductal Fluid**

#### **The Nipple Aspirate Fluid Proteome: A Comparison of the Cancer-Bearing and Healthy Contralateral Breast**

Sascha Dua, MBBS, of the Academic Department of Surgery at Royal Marsden Hospital Foundation Trust, in London, discussed “The Nipple Aspirate Fluid Proteome: A Comparison of the Cancer-Bearing and Healthy Contralateral Breast.”

To test the feasibility of the intraductal approach in their clinical setting, Dr. Dua’s group collected samples of NAF from both breasts of 50 women undergoing surgery. Surface enhanced laser desorption coupled with time-of-flight mass spectrometry (SELDI-TOF/MS) was used to evaluate the consistency and reproducibility of proteomic data and to define specific phenotypic subgroups within the sample population.

Following univariate analysis, six peaks were found to have statistical significance in discriminating between NAF from the cancer-bearing breasts and the healthy breasts. These peaks were then further analyzed with classification and regression trees, artificial neural networks, and genetic algorithms. None of the models was good at differentiating between the breast with cancer and the healthy breast.

Based on these findings, said Dr. Dua, “SELDI can be reproducible, and holds promise as a method of identifying potential biomarkers, but there is huge potential for inter-institutional variation.”

## **Significant Differences in Nipple Aspirate Fluid Protein Expression Between Healthy Women and Those With Breast Cancer Demonstrated by Time-of-Flight Mass Spectrometry**

Timothy Pawlik, MD, MPH, a clinical fellow at the University of Texas MD Anderson Cancer Center, in Houston, presented “Significant Differences in Nipple Aspirate Fluid Protein Expression Between Healthy Women and Those With Breast Cancer Demonstrated by Time-of-Flight Mass Spectrometry.”

Proteomic profiling technologies, such as SELDI-TOF/MS, may be able to identify tumor markers in biological fluids. Dr. Pawlik studied whether differences exist in protein expression patterns in NAF taken from the cancerous and noncancerous breasts of women with unilateral breast cancer and the breasts of healthy volunteers. Samples were obtained from 23 women with stage I or II unilateral invasive breast cancer and ten healthy volunteers.

The proteomic data matrix showed 463 distinct peaks. No differences in protein expression were identified in women with breast cancer between the breast with cancer and the contralateral healthy breast. Seventeen peaks were overexpressed in the cancer-bearing breasts compared to the breasts of healthy volunteers. Two peaks were overexpressed in the healthy breasts of women with cancer compared to the breasts of healthy volunteers and one peak was underexpressed in women with breast cancer.

In conclusion, said Dr. Pawlik “SELDI-TOF/MS can detect unique peak profiles and it is able to identify differences in the phenotypic proteomic profile of NAF samples obtained from women with early-stage breast cancer and healthy women. It is still necessary, however, to identify the actual proteins and to prospectively validate these findings.”

## **Intraductal Fluid Cytology: Emerging Insights In The Use of Ancillary Tests**

Savitri Krishnamurthy, MD, an associate professor of pathology at the University of Texas MD Anderson Cancer Center, in Houston, delivered the next presentation, “Intraductal Fluid Cytology: Emerging Insights In The Use of Ancillary Tests.”

The cytological categorization of epithelial cells obtained from NAF and ductal lavage is largely subjective. Dr. Krishnamurthy’s group investigated whether molecular tests such as fluorescence in situ hybridization (FISH) and methylation-specific polymerase chain reaction (PCR) can be adjuncts to conventional cytology studies.

FISH was used to detect aneusomy in chromosomes 1, 8, 11, and 17. Methylation-specific PCR was used to detect methylated alleles of specific genes, including RASSF1A, Twist, Hin1, CyclinD2, RAR, Er alpha, APC1, BRCA1, BRCA 2, and p16. All of the benign and four of the five cases of mild atypia were disomic. Aneusomy for at least one chromosome was noted in all the cases of malignant and marked atypia and in

one case of mild atypia. In addition, all of the malignant and three of the four cases of marked atypia had methylated alleles of one or more genes, while all cases of mild atypia had negative results.

In conclusion, said Dr. Krishnamurthy, molecular tests such as FISH and methylation-specific PCR can be used to “fine tune cytology diagnoses and overcome subjectivity in interpretation of these specimens as well as create further categorization of mild atypia, which would aid in the decision-making for therapeutic intervention.”

### **The QM-MSP Method of Analyzing Hypermethylated Genes in Ductal Cells**

Sara Sukumar, PhD, a professor of oncology and pathology at Johns Hopkins Oncology Center, in Baltimore, discussed “The QM-MSP Method of Analyzing Hypermethylated Genes in Ductal Cells.”

Dr. Sukumar is studying ways to develop methods for risk assessment and detection and to determine if methylated genes can be used from free DNA or cells.

Hypermethylated genes are an excellent marker for early detection of cancer. The MSP-gel based assay is a qualitative analysis of gene methylation and is subjective. What is needed, said Dr. Sukumar, is a quantitative and objective assay that can derive clear cut-off points to distinguish between tumor, high risk, and normal.

The Multiplex Q-MSP Method (QM-MSP) is able to show cumulative methylation differences between normal, benign, and malignant with just four genes with 86 percent accuracy. A pilot study conducted by Dr. Dooley found that this method was able to use fluid taken during endoscopy to differentiate between malignant and benign samples. A second study conducted by Drs. Krishnamurthy and Kuerer found this method could also detect tumor cells obtained from NAF. Dr Sukumar is now trying to improve the technique to detect signals from methylated and unmethylated genes and to tailor methylation to different ethnic populations.

In conclusion, said r. Sukumar, QM-MSP is “highly specific and highly sensitive and can simultaneously assess multiple genes at the same time.”

### **Panel on Technical Aspects of the Collection and Analysis of Ductal Fluid**

This section closed with a panel on the Technical Aspects of the Collection and Analysis of Ductal Fluid, which permitted symposium attendees to ask questions about ways they could improve their own work with ductal fluid.

## **Intraductal Therapy**

### **Intraductal Approach to Prevention and Therapy of Breast Cancer**

Sara Sukumar, PhD, gave the first presentation on Intraductal Therapy. She discussed, “Intraductal Approach to Prevention and Therapy of Breast Cancer.”

Over 95 percent of breast cancer begins in the epithelial cells lining the breast duct. Dr. Sukumar explored whether treating the ductal epithelium of rats with cytotoxic agents could prevent breast cancer and if the effects of these agents would be confined solely to the mammary epithelial cells.

Dr. Sukumar studied two animal models for breast cancer: a carcinogen induced rat mammary tumor model-MNU and the HER2/transgenic mouse mammary tumor model. Tamoxifen and Doxil were studied in the first model; Doxil was given alone in the second. Dr. Sukumar found that both in the treatment and prevention settings, Doxil administered intraductally achieved longer tumor free survival and lower tumor incidence compared to intravenous drug in the HER2/neu transgenic mouse model.

Dr. Sukumar also studied the mammary glands that had been injected with Doxil. She found that the end buds were no longer present and that the mammary glands looked completely empty. More research is needed, she said, “to determine how long they will remain denuded and if it is possible to have a breast with no ducts at all.”

In summary, said Dr. Sukumar, “the idea that we could use inject cytotoxic agents into the breast this way for prevention or treatment has gained validity and has translational potential.”

In the discussion following this presentation Dr. Sukumar explained that injecting Doxil into the ductal epithelium caused no toxicity in the mice; that this type of intervention could be done at any time; and that, at least in mice, it does not appear to change the size, texture, or structure of the breast.

### **Pilot Study: Safety of Intraductal Doxil for Prevention of Breast Cancer**

Ellen Mahoney, MD, a breast surgeon in Arcata, Calif., presented “Pilot Study: Safety of Intraductal Doxil for Prevention of Breast Cancer.”

The pilot study was designed to show the feasibility and safety of intraductal liposomal doxorubicin in a 60-year-old woman with a history of breast cancer who has been scheduled for a prophylactic mastectomy of her normal breast. If this procedure is proved effective as breast cancer prevention, said Dr. Mahoney, it could be thought of as a “chemical mastectomy. The patient will have her breast and it will appear normal, but she

will never be able to get breast cancer because she no longer has the epithelial cells from which breast cancer arises.”

Fluoroscopy was used to monitor the intraductal location of 10cc of Doxil, which was instilled in a lateral duct. Serum Doxil levels were monitored at 4, 24, and 48 hrs; they were zero. A baseline mammogram was taken before the procedure and a follow-up mammogram will be done right before the mastectomy.

This study “fits well into the historical paradigm of modern surgical developments,” said Dr. Mahoney, and it lays the groundwork for future studies designed to define the best drugs and drug combinations to use and the best time to perform the procedure. Studies should continue, said Dr. Mahoney, in prophylactic mastectomy patients, in the contralateral breast of breast cancer patients, and in high-risk patients, such as women with BRCA1/2 mutations.

### **Pilot Trial Assessing the Feasibility of Intra-Ductal Delivery of Epirubicin (Epi)-Containing Nanoparticles (NP) via InDuct Breast Microcatheter (IDBM)**

Robert Goulet, MD, an associate professor of surgery and medical director of the Breast Care and Research Center at the Indiana Cancer Pavilion, in Indianapolis, presented “Pilot Trial Assessing the Feasibility of Intra-Ductal Delivery of Epirubicin (Epi)-Containing Nanoparticles (NP) via InDuct Breast Microcatheter (IDBM).”

Dr. Goulet explored whether IDBM could be used to deliver chemotherapy to a diseased breast duct. He used Epirubicin Hydrochloride, which was encapsulated into a biodegradable nanoparticle (NP) and then suspended in saline for injection.

Nineteen women consented to have the procedure performed prior to their mastectomies. After the mastectomy was performed and the tumor removed for pathologic assessment, the IDBM was used to inject the epi-containing NP into a breast duct. Of the 13 ducts that were frozen and examined, intact NP was seen in the terminal lobules in 8 specimens.

In summary, said Dr. Goulet, “it is technically feasible to use the IDBM to cannulate the breast ducts and infuse a substance throughout a specific ductal network. The next step should be in vivo Phase I trials.

### **Ductal Fluid as a Marker for Prevention Research**

#### **COX-2 Inhibitors for Breast Cancer Prevention**

Banu Arun, MD, an associate professor of medicine in the Breast Medical Oncology Department and co-medical director of Clinical Cancer Genetics at the University of Texas MD Anderson Cancer Center, in Houston, discussed “COX-2 Inhibitors for Breast Cancer Prevention.”

Researchers are currently exploring the use of the COX-2 inhibitor celecoxib for cancer prevention. COX-2 is believed to contribute to cancer development and progression by inhibiting apoptosis, stimulating angiogenesis, immunosuppression, and enhancing metastasis.

Dr. Arun's group is conducting a pilot phase II prevention trial that is exploring whether changes in proliferation and apoptosis are induced by celecoxib in the breast tissue of high-risk women.

Thirty-seven women have enrolled in the study; 32 have completed it. Dr. Arun reported data from 25 women. All of the women underwent baseline FNA and ductal lavage and then took 400 mg of celecoxib for six months. They then returned for a repeat FNA and ductal lavage. Average ER expression prior to treatment was 30.8 percent; after treatment it was 21.8 percent, a statistically significant finding.

In conclusion, said Dr. Arun, "because ER expression is a marker of proliferation, this finding confirms celecoxib's antiproliferative properties." Dr. Arun is currently conducting an analysis of other markers of proliferation and apoptosis.

### **Consensus Statement Discussion**

The second day of the conference closed with a dialogue moderated by Julian Kim, MD, "Discussion of Consensus Statement: The Status of the Intraductal Approach and the Areas of Needed Research.

### **Pilot Grants**

The Dr. Susan Love Research Foundation distributed pilot grants at the 2005 Symposium. A Peer Review Committee recommends the grants to be funded, based on the scientific merit and relevance to the Foundation's mission and goals—exploiting intraductal access to better understand, prevent, and treat breast cancer.

In an effort to expedite the funding, the Foundation has developed an innovative grant review process. Rather than submit a lengthy application, applicants for pilot grants only submit a 1-page abstract of the proposal that they want to present for funding at the conference. A multidisciplinary Peer Review Committee composed of basic scientists, breast cancer activists, and surgeons listens to the presentations at the Symposium, reviews the grant proposals, and decides who will receive the pilot grant awards and the amount of each award. This year, 11 researchers presented proposals. At the close of the Symposium, the Foundation provided a total of \$85,000 to support the pilot work of seven promising researchers utilizing the intraductal approach for a wide range of projects.

**The 2005 recipients of these pilot grants were:**

**Expression of BP1 in Ductal Lavage Samples from Women at Risk for Breast Cancer**

Patricia Berg, PhD, Associate Professor, Department of Biochemistry and Molecular Biology, George Washington University Medical Center, Washington, DC.

This study was awarded a \$10,000 research grant from the Foundation.

**Laser Capture Microdissection and Real Time Polymerase Chain Reaction to Assess Breast Biomarker Gene Expression Following Random Periareolar Fine Needle Aspiration and Ductal Lavage**

Brian K. Petroff, DVM, PhD, Assistant Professor, Department of Internal Medicine, Breast Cancer Prevention Center, University of Kansas Medical Center, Kansas City, Kansas.

This study was awarded a \$10,000 research grant from the Foundation.

**Bio-Molecular Characterization and Cytometric Evaluation of Nipple Aspirate Fluids to Identify Bio-Markers of Breast Cancer**

Ferdinando Mannello, PhD, Associate Professor, Institute of Histology and Laboratory Analysis, University "Carlo Bo" of Urbino, Urbino, Italy.

This study was awarded a \$10,000 research grant from the Foundation.

**Intraductal Nano-Polymer Drug Delivery for Breast Cancer Prevention and Therapy**

Scott L. Kominsky, PhD, Assistant Professor, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

This study was awarded a \$10,000 research grant from the Foundation.

**The Feasibility and Safety of Intraductal Administration of Pegylated Liposomal Doxorubicin (Doxil) in Women**

Regina Brown, MD, Medical Oncology Fellow, Hematology and Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

This study was awarded a \$10,000 research grant from the Foundation.

**Is Persistent Nipple Aspirate Fluid in Women on Tamoxifen Prognostic for Adverse Breast Events?**

Edna K. Valdes, MD, Fellow, Surgical Oncology, Beth Israel Medical Center, New York, New York.

This study was awarded a \$ 5,000 research grant from the Foundation.

**Short-Term Effects of Soy on Estrogens and Breast Cell Proliferation in Nipple Aspirate Fluid**

Gertraud Maskarinec, MD, PhD, Associate Professor, Cancer Research Center of Hawaii, University of Hawaii, Honolulu.

This study was awarded a \$ 10,000 research grant from the Foundation.

**Additional Pilot Grants Presented**

**Menstrual Variation in NAF Biomarkers of Healthy Women at Population Risk for Developing Breast Cancer**

Gerald Gui, MS, FRCS, Royal Marsden NHS Foundation Trust, London, UK.

**Mechanism of Pregnancy Protection Against Breast Cancer**

Pentti Siiteri, PhD, University of California, San Francisco.

**Proteomic Analysis of NAF and DL Cell Lysates by Capillary Isoelectric Focusing Coupled with High Sensitivity of MALDI-TOF MS Enzymatic Digests**

Mark Hayes, PhD, Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona.

**A New Method of Fluorescence-Ductoscopy for In Vivo Detection of Intraductal Malignant Cells in the Breast**

Volker Jacobs, MD, Technical University Munich, Munich, Germany