



## **The 3rd International Santa Barbara Symposium: The Intraductal Approach to Breast Cancer**

The Susan Love MD Breast Cancer 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 3rd International Santa Barbara Symposium on the Intraductal Approach to Breast Cancer on March 27–30, 2003. In attendance were more than 100 oncologists, epidemiologists, biostatisticians, surgeons, biochemists, pathologists, radiologists, and endocrinologists who are involved with research utilizing the intraductal approach.

Reverend Michelle Woodhouse, PhD, the Chair of the Foundation's Board of Directors, opened the Symposium by welcoming everyone to the conference and by noting that the intraductal approach to the breast "isn't a flicker; it's a flame" that will advance the detection, prevention, and treatment of breast cancer.

Reverend Woodhouse introduced the Foundation's President and Medical Director, Susan Love, MD. "This conference, dedicated to the intraductal approach to the breast, provides a unique opportunity for researchers throughout the world," said Dr. Love. "And as testament to these researchers and the importance of their work, we have grown each year in the amount of money we give out, the number of grant proposals we receive, and the amount of science we can present."

Over a two-day period, prominent breast cancer researchers presented results of their studies in which they used the intraductal approach. The last session of each day gave researchers interested in applying for pilot grant funding for new studies the chance to present their ideas to the entire group for review. The first day included sections titled: (a) Intraductal Approach: Path of an Idea Whose Time Has Finally Come, (b) Genetic Instability in Breast Duct Fluid, (c) Are There Early Markers of Malignancy in Breast Duct Fluid?, (d) Hormone Levels in Breast Duct Fluid: A Window on the Microenvironment?, and (e) Proposed Studies for Pilot Grants, Part 1. On the second day topics included: (a) Anatomy of the Breast Ductal System: Competing Views, (b) Does Cytology of Breast Duct Fluid Have Long-Term Follow-Up?, (c) Are There Clinical Applications for the Intraductal Approach?, (d) Panel Discussion: Ductal Lavage—Ready for the Clinic or a Research Tool?, and (e) Proposed Studies for Pilot Grants, Part 2. The schedule was structured to encourage attendees to discuss their ideas and share important research insights.

### **Intraductal Approach: Path of an Idea Whose Time Has Finally Come**

The Symposium's first speaker, Nicholas Petrakis, MD, professor emeritus at the University of California, San Francisco, is known by many as "the father of nipple aspirate fluid." His presentation on "Some Pioneers in Nipple Aspiration of Breast Fluid" began with a look back to 1838, the year both the first nipple aspirate cell theory was proposed and unstained colostrum was examined by microscope for the first time. Two years later, cytology began to be used for the detection of cancer cells in body fluid. But it would be nearly 75 years before the first report of microscopic detection of cancer cells in spontaneous nipple discharge was reported.



In the 1920s, Geoffrey Keynes, MD, proposed that a relationship existed between the pathology of chronic mastitis and the aberration of the normal balance of secretion and absorption of fluid within the breast. He noted, "I feel sure that much will be learned from the investigation of the fluid." His prediction, Dr. Petrakis said, was later supported by the work of George Papanicolaou, MD, who, in 1959, began using a breast pump to explore the exfoliative cytology of the mammary gland by extracting ductal fluid from women with and without breast cancer. Dr. Papanicolaou believed that understanding the cells found in ductal fluid would be critical to the diagnosis of cancer and other diseases of the breast.

Picking up on Papanicolaou's research, Otto Sartorius, MD, a surgeon who practiced in Santa Barbara in the 1960s, began detailed studies on nipple aspirate fluid (NAF), including experiments with ductal washings and contrast mammography. Drs. Sartorius and Petrakis began working together in the 1970s. Their research found an association between race, age, and menopausal status in the breast fluid secreted in non-lactating women. From 1974 to 1988, Dr. Petrakis was the principal investigator of a program-project grant from the National Cancer Institute entitled "Epidemiology and Natural History of Breast Cancer Using Nipple Aspirate Fluid." Through this research project more 7,000 women were studied, and numerous papers were published on the cytology, biochemistry, and epidemiology of NAF. Co-investigators included Eileen King, MD, and Margaret Wrensch, PhD. Early in these studies, Dr. King added to the understanding of NAF with the first detailed descriptive cytologic studies of the benign and abnormal cells found in NAF. Her careful cytologic studies provided the reliable cytologic basis for the later analyses.

Advancing the field further, Dr. Wrensch conducted the first risk analyses of a large cohort of women who were followed for a median of 20 years. These analyses demonstrated that women initially found to have epithelial atypia had an increased risk of breast cancer whereas non-yielders of NAF had the lowest risk. Concurrently, Dr. Petrakis began to explore the influence of pregnancy and lactation on serum and breast fluid estrogen levels and of other biochemical substances in NAF. More recently, in another important advance, Susan Love, MD, demonstrated that ductal lavage (DL) was better than nipple aspiration in retrieving a high number of epithelial cells and three times more sensitive in determining the presence of cellular atypia.

"Since I first met Otto Sartorius," said Dr. Petrakis, "I have believed that ductal lavage is one of the major tools for the study of the pathophysiology and diseases of the breast ductal system." Currently, nipple aspiration is important for screening asymptomatic women for cytologic atypia to identify those who are at increased risk of breast cancer. But, he concluded, "I believe that the discovery of new molecular risk markers, combined with new imaging techniques, may allow NAF to become the primary method of breast cancer screening and detection in high-risk women, while ductal lavage and endoscopy will be the follow-up procedures for finding premalignant lesions and early breast cancers and, in the near future, for the application of intraductal chemotherapy."

### **Genetic Instability in Breast Duct Fluid**

#### **Detection of Chromosomal Instability in Paired Breast Surgery and Ductal Lavage Specimens by Interphase Fluorescence In Situ Hybridization (FISH)**



The first presentation on genetic instability was by Bonnie King, PhD, an associate research scientist in the Department of Therapeutic Radiology at the Yale University School of Medicine. She addressed "Detection of Chromosomal Instability in Paired Breast Surgery and Ductal Lavage Specimens by Interphase Fluorescence In Situ Hybridization (FISH)." "Our current goal," said Dr. King, "is to develop markers that will help us detect and predict breast cancer in ductal lavage fluids."

Dr. King said the aim of her current research is to determine if genetic lesions do occur at the earliest stages of breast cancer and if these genetic lesions can be found in exfoliated ductal lavage cells. Between January 2001 and January 2002, she analyzed 39 paired cases of ductal lavage specimens and surgically excised breast lesions from women who were undergoing breast surgery for in situ and invasive breast cancer. She used both cytologic analysis and FISH analysis in chromosomes 1, 8, 11, and/or 17 to determine whether a correlation existed between cancer-associated abnormalities in the breast lesions and the exfoliated breast cells.

Dr. King found that abnormal cytology was detected in 7/15 (47 percent) of the lavage specimens in malignant cases and in 4/19 (21 percent) of the lavage specimens from benign cases, for a sensitivity and specificity of 47 percent and 79 percent, respectively. The interphase FISH analysis of the lavage samples revealed numeric changes in 10/14 (71 percent) of the specimens from malignant cases compared with 2/18 (11 percent) from benign cases, for a sensitivity and specificity of 71 percent and 89 percent, respectively.

She reported that in this study the FISH analysis had better sensitivity and specificity than the cytology did. "While this is not the definitive answer about whether to use cytology or FISH," Dr. King said, "it is proof of principle that we can detect cancer cells with genetic alterations in women with ductal lavage." In conclusion, said Dr. King, "FISH can clearly provide an adjunct to cytology for diagnosis." In addition, "we have validated ductal lavage cells as targets for marker development."

*\*\*Dr. King's research, supported in part by the Susan Love MD Breast Cancer Research Foundation, was published in Clinical Cancer Research, Vol. 9, 1509-1516, April 2003.*

### **Molecular Characterization of Mammary Ductal Epithelial Cells Using Interphase Fluorescence In Situ Hybridization (FISH)**

The second presentation on genetic instability was by Julian Kim, MD, a specialist in surgical oncology at the Cleveland Clinic Foundation. He discussed "Molecular Characterization of Mammary Ductal Epithelial Cells Using Interphase Fluorescence In Situ Hybridization (FISH)." Dr. Kim also wanted to determine whether cancer cells are missed when we rely on cytology alone to analyze ductal lavage. To explore this question further, Dr. Kim used FISH analysis of chromosomes 1, 8, 11, and 17 to look for losses or gains on cells obtained from ductal fluid. His aim was to test the hypothesis that characterization of chromosomal abnormalities by FISH could provide objective data to confirm cytological analyses. He went on to compare the sensitivity, specificity, and positive predictive values of cytology and FISH and to look at whether FISH could be performed on the ThinPrep slides used for ductal lavage specimens.

Dr. Kim reported that, to date, 25 paired samples have been analyzed by FISH. Of the slides that were interpreted as containing malignant cells by cytopathology, 4/4 were abnormal by FISH. Likewise, 6/6 of the slides that were found to be benign by cytology had no cells with chromosomal gains, while 5/6 had cells that were monosomic for chromosome 17. In the 15



slides that were interpreted as atypical, 4/15 demonstrated no chromosomal abnormalities while 5/15 had multiple chromosomal gains.

Dr. Kim found that FISH could easily be done on archival slides. Not only is this type of analysis feasible, he said, but we have shown that "combining both cytology and FISH could provide better sensitivity and specificity. The two methods are probably complementary, with FISH being able to detect cells that are chromosomally abnormal and thus confer risk." Dr. Kim also noted that there were nine cases of discordance between the tissue removed during surgery and the cytology and FISH analyses. This leads to the question, he said, of whether the cells obtained via ductal lavage "were from a papilloma or from somewhere else in the ductal system." Also, he said, "we need to understand what aneusomy means. But right now that is hard to determine when we have limited biopsy data."

*\*\*Dr. Kim's research was supported in part by the Susan Love MD Breast Cancer Research Foundation.*

### **Fiberoptic Ductoscopy and FISH Analysis of the Cells Obtained**

The final presentation on genetic instability was by Daigo Yamamoto, MD, of Kansai Medical University in Moriguchi, Osaka, Japan, who addressed "Fiberoptic Ductoscopy and FISH Analysis of the Cells Obtained." Dr. Yamamoto discussed his research comparing ductograms with fiberoptic ductoscopy (FDS) in 90 women who had nipple discharge. The ductal lavage samples were obtained from patients who had positive findings on ductography and/or FDS. These samples were then analyzed with cytology and FISH using probes for chromosomes 1, 11, and 17. Samples that showed aneusomy in at least one of the three chromosomes were diagnosed as positive.

Dr. Yamamoto reported that the histological examination revealed 54 benign lesions and 6 malignancies. For cytology, the sensitivity, specificity, and diagnostic accuracy were 33.3 percent, 88.9 percent, and 83.3 percent, respectively, and for FISH 100 percent, 100 percent, and 100 percent, respectively. This led Dr. Yamamoto to conclude that using FISH is useful for diagnostic accuracy, and that "combining the results of the two analyses would provide the best results."

### **Discussion: Genetic Instability**

The discussion that followed explored all three presentations. In addressing a question about the different levels of specificity and sensitivity that each of the researchers found, Dr. Kim noted that in his research "not all papilloma had aneusomy by FISH, and that of those women who had papilloma seen on biopsy, some had aneusomy and some did not. There is a lot of variability in patients who had papillomas," he continued, "and it's nice to have three different studies that don't necessarily agree. Many times you go to a conference and hear about one study, and that's it. These findings indicate that we need more validation of results with larger studies. I would ask those conducting large studies to consider looking at biomarkers in their trials. The research presented here shows the feasibility of doing this, but it doesn't provide any answers."

Another conference participant asked whether it was possible that an anatomical or artifact of the study design might have influenced the findings, since the patients who had lavage had already had a cancer diagnosis and were having the lavage before surgery. Was it not possible that since the cancer diagnosis was done first by fine needle aspiration or biopsy that the initial procedure might have blocked or disrupted the ducts?



"When you look at cancer through a ductoscope you can see that cancers involve many different ductal branches within the same ductal system," replied Dr. Kim. "Based on our own experience we think there are lots of questions about whether we can accurately obtain cells from cancers within the ductal system. Right now, it is clear that this work can generate more questions than answers. This field is in its infancy and we don't know a lot about retrieving cells from these cancers. You can be looking exactly at the cancer and take cells from the tip of the ductoscope and not get cytologically designated cancer cells."

Dr. Margaret Wensch, of the University of California, San Francisco, added that it was "inevitable that on the long road ahead there would be differences in sensitivity and specificity tests because we know that there is incredible variation in outcome even after cancer is detected." Dr. Wensch said she would encourage follow-up of the patients currently being studied to determine whether and how the FISH results correlate with their outcome.

Dr. Kim pointed out that researchers did not have to wait to do follow-up analyses. FISH analyses could be done on samples that were collected 20 years ago, which would allow for correlation with outcome. "This research," he said, "doesn't have to be prospective."

### **Are There Early Markers of Malignancy in Breast Duct Fluid?**

#### **Ductal Lavage for Serial Observation of Breast Epithelium in High-Risk Women**

The first presentation on early markers of malignancy, "Ductal Lavage for Serial Observation of Breast Epithelium in High-Risk Women" was by Seema Khan, MD, an associate professor of surgery at Northwestern University Medical School. "The appeal of ductal lavage for serial observation," said Dr. Khan, "is that we can go back into the same ductal tree." However, she added, "there is not yet any data on the ability to recannulate ducts on subsequent procedures or what the stability of biomarkers in control subjects is."

Dr. Khan recruited participants for her study from women who were considering tamoxifen intervention. She selected this group of women, she said, because "it is known that tamoxifen can prevent breast cancer, which means we could predict that some of the women would have changes in their breast epithelium due to tamoxifen use."

All of the women in the study underwent ductal lavage, and the findings were used to help them decide whether they wanted to go on tamoxifen. The women had a second ductal lavage six months later (or six months after they started tamoxifen). Dr. Khan found that she could typically get into at least one duct more than once. The cytology findings, however, were not consistent. In some instances, she said, the first cells lavaged were benign and then six months later mild atypia was present. In other instances, it was the opposite, with mild atypia present in the first lavage and benign cells found in the second. She also noted "that if you lavage more ducts you won't necessarily be able to get back into more ducts," but whether or not this is important is not yet known.

Dr. Khan reported that they found a trend toward acceptance of tamoxifen for women who had mild atypia on ductal lavage, but that this trend was not very strong and not statistically significant. Based on this study, she said, "cytologic findings do not appear to have an impact on women's choice of whether to use tamoxifen."



In the ensuing discussion Dr. Kahn was asked how she knew that she went back into the same duct. She explained that she created a nipple grid and also took a picture of the nipple with the probe in the duct. "But even with these measures," she said, "it was sometimes hard to be certain that we were in the same duct."

### **Mammary Duct Access for Prevention, Therapy, and Early Breast Cancer Detection**

Saraswati Sukumar, PhD, a professor of oncology at Johns Hopkins University School of Medicine, presented some of the most exciting research of the conference: "Mammary Duct Access for Prevention, Therapy, and Early Breast Cancer Detection." Dr. Sukumar explored whether the injection of cytotoxic agents through the teat of a rat could prevent breast cancer while confining its effects to the mammary epithelial cells alone. If this proved successful, she said, it could provide a prevention option for women who carry one of the BRCA genes, women with LCIS, or women who are candidates for bilateral prophylactic mastectomy.

She used a rat carcinogen-induced mammary carcinoma model to determine whether the drugs 4-hydroxytamoxifen and Doxil (liposomal preparation of doxorubicin), which are effective in the treatment and prevention of mammary cancer, could be used intraductally for cancer prevention. She reported that she found that both hydroxytamoxifen and Doxil did have an effect. In the Doxil experiment only 2 of the 40 pretreated ducts developed cancer compared to 60 of 200 untreated ones. There was no bone marrow suppression seen from the intraductal cytotoxic. And when the rats were ultimately sacrificed their ducts looked normal. This demonstrated, said Dr. Sukumar, that these ducts were protected against developing tumors. Additional studies looked at rats that already had developed tumors and were subsequently given intraductal Doxil. These showed reduction in the size of the tumors with no systemic effects.

This finding, she said, provided support for a human study that would introduce chemotherapy into the ducts prior to surgery, with the end point being a faster or greater change in tumor size. She also noted that "even drugs that are too toxic to be used systemically might be able to be used directly in the duct." What we don't know, she said, "is whether using the drugs in this way would have an additive or synergistic effect given that introduction through the ducts won't reach every part of the tumor."

In the discussion that followed questions were asked about the impact of tamoxifen and Doxil on the ducts. Dr. Sukumar said that the treated rats didn't develop any other growths or abnormalities in the ducts where the drugs were injected. Dr. Sukumar was also asked whether she thought it would make sense to deliver chemotherapy through the duct to a woman who had invasive cancer, since the cancer had already escaped from the ducts. She replied that she believed it might still be appropriate as it could help to reduce the tumor, which could lead to less extensive surgery. In addition, she said, giving chemotherapy through the ducts might also be appropriate for shrinking large DCIS (ductal carcinoma in situ) or other tumors prior to surgery, as this method would avoid the side effects that occur with typical chemotherapy delivery.

Dr. Sukumar also discussed her attempts to develop a panel of hypermethylated gene markers for breast cancer. She reported that she has had "good success at finding positive methylated markers from ducts that had tumors" and from using methylation as an adjunct to cytopathology. When asked about early changes seen in gene markers, Dr. Sukumar said that in at least two cases in which normal ducts surrounded DCIS or a tumor she could find markers in some of the





other ducts. "This suggests that that there is a field effect," she said, "which means that it is possible that some of these genes are methylated very early."

### **Carcinoembryonic Antigen (CEA) as a Marker of Breast Cancer Risk in NAF**

Carey Cullinane, MD, MPH, a surgical oncologist at the City of Hope National Medical Center, discussed "Carcinoembryonic Antigen (CEA) as a Marker for Breast Cancer Risk in NAF." Dr. Cullinane explained that although CEA is not produced by normal breast epithelium, it is present in both pre-invasive and invasive disease. This finding, she said, led her to look at whether a CEA monoclonal antibody she was using in her research was good at finding CEA in NAF. She began collecting NAF from women who had a demonstrated mammographic abnormality or a known BRCA mutation and who were scheduled to undergo biopsy or definitive surgical procedures. NAF was collected from both the breast with the abnormal findings and the contralateral breast.

Dr. Cullinane reported that NAF was collected in 83 percent of the patients, and that CEA was detected in the NAF of patients with invasive and noninvasive disease. In addition, CEA was detected in the NAF of patients who were at high risk for developing breast cancer but who did not have any pathological evidence of disease.

So far, she said, "there is a trend toward finding CEA, and though it is hard to say that the absolute level of CEA makes a difference, it did trend toward significance." She also found that in assessing the presence or absence of disease the difference between CEA levels in the right and left breast appeared to be more important than the absolute level, as there may be other reasons CEA is present.

### **Does Ductal Lavage Hold Risk Assessment and Diagnostic and Therapeutic Potential?**

Laura Esserman, MD, MBA, director of the University of California, San Francisco's Carol Franc Buck Breast Cancer Center, addressed "Does Ductal Lavage Hold Risk Assessment and Diagnostic and Therapeutic Potential?" Dr. Esserman began by discussing the need to find the optimal role for ductal lavage. She noted that "the bar is too high, with sensitivity and specificity having to be over 95 percent, for ductal lavage to be a screening tool." But as a risk assessment tool, she said, "it has clear advantages to what we already have."

Dr. Esserman also underscored the need for researchers to identify markers that can be used to detect when therapeutic intervention is necessary. "Currently most high-risk women don't take tamoxifen because of their fear of the side effects," she said. "Not only do women not like it, but the Gail Risk Model doesn't necessarily identify those women who we really do need to target. Women don't like what we are offering, and we don't have a good tool."

To help women with their decision-making, Dr. Esserman and her colleagues have created a "Prevention Decision Model for Patients" that allows women to compare their risk with the average risk. She has also developed a Cost-Benefit Model that illustrates the potential cost-effectiveness of ductal lavage. "Although NAF is more cost effective than ductal lavage," said Dr. Esserman, "it has low cellular yield. So, unlike ductal lavage, it doesn't benefit that many women because it is not a good risk stratifier." But what is really needed, Dr. Esserman concluded, "is a biomarker that will provide information on how to reduce risk. Once that is available, ductal lavage will be most cost effective."



### **NAF as a Potential Method for Early Breast Cancer Detection in BRCA1/2 Germline Mutation Carriers**

Gerald Gui, MD, of the Royal Marsden NHS Trust in London, England, presented "NAF as a Potential Method for Early Breast Cancer Detection in BRCA1/2 Germline Mutation Carriers." Dr. Gui described his research exploring whether NAF analysis is a complementary method of breast cancer detection in women who have been found to carry one of the BRCA genes. He is also looking at the feasibility of using ductal lavage in this group of women.

Dr. Gui and his group recruited 65 women who were either known to have a BRCA mutation or were members of a high-risk family. The women underwent NAF collection every three months, and the NAF was analyzed for breast cancer tumor markers, cytology, IHC for proliferation markers, p53 protein, tumor-associated macrophages, and molecular changes in BRCA1/2 copies using FISH. Ductal lavage was performed on a subset of these women at the end of the NAF study and analyzed using the same methods.

Dr. Gui reported that he collected NAF from 49 (75 percent) of the women (22 mutation carriers). In this group of women, 42/49 produced NAF samples adequate for cytologic analysis on at least one occasion. Four women developed breast cancer (3 invasive, 1 DCIS, and 1 had ADH [atypical ductal hyperplasia] at prophylactic mastectomy). In addition, 27 women (10 BRCA1, 3 BRCA2, and 14 "high risk") also had ductal lavage, with 24/27 of these women having a successful cannulation in at least one breast. In examining the cytology, Dr. Gui reported that median NAF epithelial cell count was 111 per sample. He also found that 8/42 (19 percent) women had mild atypia and that 6/42 (14 percent) had severe atypia on at least one occasion. One woman was diagnosed with breast cancer and five remain under close follow-up. In three other women with cancer, none demonstrated atypia in NAF. In contrast, the median ductal lavage epithelial cell count was 16,000 per duct, and when the lavage fluid was examined, 12/24 (50 percent) of the women were found to have mild atypia, 4/12 had no atypia in NAF, and 3/12 had severe atypia in NAF. Only one woman was found to have severe atypia on both lavage and NAF.

In conclusion, said Dr. Gui, although NAF can be used to repeatedly assess the intraductal environment, ductal lavage provides superior cellular samples. In addition, he said, cytology provides the most useful assessment of intraductal status, whereas the value of bioassays as surrogate markers is small.

*\*\*Dr. Gui's research was supported in part by the Susan Love MD Breast Cancer Research Foundation.*

### **Proteomic Screening and Identification of Novel Breast Cancers**

Helena Chang, MD, director of the Revlon/UCLA Breast Center at the University of California, Los Angeles, discussed "Proteomic Screening and Identification of Novel Breast Cancers." Dr. Chang described her use of surface-enhanced laser desorption/ionization (SELDI) to study 45 cancerous and 43 noncancerous nipple aspirates. She reported that she found 11 proteins in nipple aspirate that were significantly different in the cancerous and non-cancerous breasts. In addition, she selected a single peak of protein in cancerous nipple aspirate for protein identification by liquid





chromatography and tandem mass spectrometry (LC-MS/MS), which led to the identification of a novel cancerous protein.

Dr. Chang concluded that her data "suggests that breast cancer detection by SELDI screening on nipple aspirate is feasible and that breast cancer biomarkers can be individually identified by LC-MS/MS."

### **Detection of Potential Molecular Markers in Ductal Lavage Fluid and Cells from Breast Cancer Patients**

Yuriy Gusev, PhD, an assistant professor in the Department of Surgery at the University of Oklahoma School of Medicine, addressed "Detection of Potential Molecular Markers in Ductal Lavage Fluid and Cells from Breast Cancer Patients." Dr. Gusev discussed his pilot study, which is designed to assess the sensitivity, specificity, and accuracy of ductal lavage and endoscopy. He is also using the fluid, tissue, and cells obtained through this study to try to develop a molecular Pap smear for the breast. Toward that end, Dr. Gusev is testing detection methods such as genome and proteome profiling, immunohistochemistry (IHC), and digital image analysis. If this molecular Pap could be identified, he said, ductal lavage and endoscopy could be used to screen patients for early pre-malignant changes.

Dr. Gusev reported that he and his collaborators at a private biotech company had identified a potential serum marker (MT34) that was present in the serum of women identified as having breast cancer, but not in clinically determined healthy cancer-free women. They then used surface-enhanced laser desorption/ionization TOF-MS (SELDI MS) to identify putative markers for breast cancer. And in a clinical study of 78 women aged 40 or older, MT34 correctly identified 41/41 (100 percent) metastasis, late stage, and early stage cancers, and it ruled out 27/ 28 (96 percent) of the nonmalignant controls.

To develop a breast lavage test for global proteome profiling of breast cancer, Dr. Gusev analyzed human breast lavage samples. He reported that thousands of peptides were observed in the 500 to 2,000 mass range, and that the proteomics profiles were unique for each case. Next, using direct tryptic digest of the samples followed by MS/MS sequencing of the resultant peptides, they found that although many bands are in common from patient to patient, some differences do exist. They also found three proteins unique in these samples: immunoglobulin heavy chain, prolactin inducible protein, and human papillomavirus protein. Their goal, he said, is to exploit these differences to identify peptide/protein patterns unique to cancer patients.

### **Protein Expression Patterns in Ductal Fluid Samples from Women with Unilateral Carcinoma of the Breast**

The final presentation on early markers of malignancy was by Henry Kuerer, MD, PhD, an assistant professor of surgery at MD Anderson Cancer Center, who discussed "Protein Expression Patterns in Ductal Fluid Samples from Women with Unilateral Carcinoma of the Breast." Dr. Kuerer described his prospective pilot study in which he took NAF at the time of surgery from women with unilateral invasive breast cancer who had not previously had surgery behind the nipple. NAF was taken from both the cancerous breast and the contralateral breast, and the NAF protein expression profiles were analyzed with high-resolution SELDI proteomic analysis.



NAF could be obtained 80–90 percent of the time, Dr. Kuerer reported, and substantial qualitative differences were seen between the NAF protein expression patterns in the cancerous breast and those in the contralateral one. Further, Dr. Kuerer said, when they looked specifically at the HER2 (also sometimes referred to as HER-2 or Her-2/neu or erb-b2) molecule, they found a statistically significant difference in the extracellular material produced in the breast that had cancer.

### **Hormone Levels in Breast Duct Fluid: A Window on the Microenvironment?**

#### **Development of Assays for Analysis of Estradiol and Related Analytes in NAF**

The first presentation on hormone levels in breast duct fluid was by Angela Geiger, of Northwestern University School of Medicine, who discussed "Development of Assays for Analysis of Estradiol and Related Analytes in NAF." As Ms. Geiger explained, the median volume of NAF obtained from a breast is 11uL. Because estradiol measurements require most of this volume, she worked to develop a fractionation that would allow additional analytes to be measured. "Fractionation serves as a purification process," Ms. Geiger explained. "It allows for the separation of nine analytes into three fractions."

Ms. Geiger reported that after the fractionation procedure the estradiol samples measured averaged 1.1 percent of those assayed without fractionation. Thus, she concluded, "the purification process is necessary for accurate measurements of estradiol and the assays provide useful data for interpretation of hormonal attributes of the breast."

*\*\*Ms. Geiger's research was supported in part by a grant from the Susan Love MD Breast Cancer Research Foundation.*

#### **Nipple Aspirate Estrogen-Induced and Estrogen-Inhibited Proteins—Can They Give Us Information about the Breast?**

Anthony Howell, MD, of Christie Hospital NHS Trust at the University of Manchester, presented "Nipple Aspirate Estrogen-Induced and Estrogen-Inhibited Proteins—Can They Give Us Information about the Breast?" Dr. Howell explained that his research was designed to explore whether changes in estradiol around the breast cell could change what happens inside the duct. Specifically, he wanted to test the hypothesis that "there would be no increase in estradiol in patients with breast cancer but that the cells of these patients might be especially sensitive to estradiol and more sensitive to proteins."

To measure the responsiveness of the breast to hormonal changes, he studied the levels of two estrogen-stimulated proteins, p52 and cathepsin D (Cat D), and two estrogen-inhibited proteins, gross cystic disease fluid protein 15 (CP15) and apolipoprotein D (apo D), during the menstrual cycle, after treatment with the luteinizing hormone-releasing hormone (LHRH) agonist goserelin, and before and after menopause. What we wanted to know, Dr. Howell said, "was whether high levels were associated with risk."

Dr. Howell reported that following treatment with goserelin, median nipple secretion levels of p52 fell while levels of apo D and CP15 rose significantly. When they looked at the effect of menopause, he said, they found that "as expected, it resulted in a decline in Cat D and an increase in apo D." Treatment with hormone replacement therapy (HRT) resulted in a rise in p52 and a fall in apo D, but no changes were seen in the levels of cat D and CP15. Further, women who had mastalgia had higher levels of p52 and apo D. Because they found that women who had



higher levels of p52 and apo D had lower risk, Dr. Howell said, "we concluded that our hypothesis was wrong." Still, he noted, this was just a small study, and a larger study may find different results.

Dr. Howell reported that they also studied nine women, none of whom had a BRCA mutation and all of whom had been pregnant at least once, to see how levels of p52 changed throughout the menstrual cycle. In this study, NAF was taken from both breasts in order to explore whether both breasts had similar p52 levels. Dr. Howell reported "that we could see peaks and troughs mid-cycle, but both breasts didn't always do the same thing." In conclusion, Dr. Howell said, "we see changes that we would predict to see following use of HRT or goserelin, and menopause. It is also possible that mastalgia may be due to increased sensitivity to estrogen."

### **NAF Estrogen Levels in High-Risk Women: Importance of Microenvironment and Rationale for Use of Aromatase Inhibitors for Chemoprevention in Women Receiving Hormone Replacement Therapy**

Carol Fabian, MD, a professor of medicine and director of the Breast Cancer Prevention Center at the University of Kansas Medical Center, addressed "NAF Estrogen Levels in High-Risk Women: Importance of Microenvironment and Rationale for Use of Aromatase Inhibitors for Chemoprevention in Women Receiving Hormone Replacement Therapy." Dr. Fabian explained that her research is exploring whether differences in the microenvironment of the breast could provide a partial explanation for an increased or decreased risk for developing breast cancer. She began by noting that, as research by Dr. Petrakis has shown, NAF estrogen levels substantially exceeded serum levels, except during pregnancy. Furthermore, there is no correlation between NAF and serum estrogen levels.

Our question, she explained, was whether it was possible to reduce breast estrogen levels and proliferation with aromatase inhibitors so that women at high risk for breast cancer could take HRT to reduce menopausal symptoms. This is a significant question because women continue to take HRT to combat menopausal symptoms, even though they know they are at higher risk for breast cancer.

Dr. Fabian reported that they have started a pilot study in which the aromatase inhibitor letrozole (brand name Femara) will be given to high-risk women who have been diagnosed with atypia and who are taking HRT. The primary end point was to assess the possibility of a response to letrozole. The secondary objective, she said, was to see if letrozole would reduce breast density. In addition, serum and NAF are being collected to determine what changes in estrogen levels took place, including serum bioavailable estradiol (E2).

Support for this pilot study came from research Dr. Fabian had previously done on women at high risk for breast cancer who produced NAF from multiple ducts in a single aspiration setting. In this study, she reported, no significant differences between NAF:serum E2 ratios were observed between pre- and postmenopausal women. In addition, they found that high NAF:serum E2 ratios were present in high-risk women regardless of menopausal status or the use of HRT. This supported the concept of using aromatase inhibitors as chemoprevention in women taking HRT for menopausal symptoms.

Dr. Fabian also discussed a second study she conducted with 145 high-risk women, median age 45. She reported that in these women NAF production did not differ based on menopausal status or HRT use. While more NAF producers than non-producers had atypia, the lack of NAF did not



exclude atypia. Further, there was high variability between ducts in the same woman. For example, the estrogen levels in the ductal fluid were different in different ducts. In addition, like Dr. Petrakis, they found that NAF production correlated with lactation. In conclusion, Dr. Fabian said, we have found that NAF is produced in 50 percent of high-risk postmenopausal women, and it is possible to assay estradiol and estrone in 80 percent of NAF producers. These findings, she added, underscore the role of the microenvironment in the promotion of breast cancer development.

During the discussion that followed, Dr. Fabian answered questions about the aromatase inhibitor study. She explained that using the aromatase inhibitor would allow a certain level of hormones in serum but reduce the amount of estradiol in NAF. This reduced level, she explained, would be a surrogate for what is happening in the breast "because estrogen in the breast is not the same as estrogen in the rest of the body."

### **Influence of Menopausal Status and Hormone Replacement Therapy on Estradiol and Related Compounds in Ductal Lavage Fluid**

Robert Chatterton, PhD, a professor in the Department of Reproductive Endocrinology and Infertility at Northwestern University School of Medicine, addressed the "Influence of Menopausal Status and Hormone Replacement Therapy on Estradiol and Related Compounds in Ductal Lavage Fluid." Dr. Chatterton discussed his preliminary data from a study of high-risk pre- and postmenopausal women. The objective of his research was to develop a method of measuring estradiol, potential precursors of estradiol, and products of estradiol actions in the breast. "The advantage of ductal lavage fluid is that it provides a fluid sample 6–10 times greater than the NAF sample," he said. "The disadvantage is that the concentrations of substances in the fluid are diluted by the lavage procedure."

The study looked at analytes in 29 premenopausal and 15 postmenopausal women. Dr. Chatterton reported that all analytes gave similar values in NAF and ductal lavage fluid when expressed per milligram of protein, except for the estradiol and DHEA (dehydroepiandrosterone), which were lower in the NAF. Because they hadn't recruited women before they had started taking HRT, they could not look at the effect of HRT. However, they were able to compare women who were taking HRT with women who were not. Dr. Chatterton reported that no significant differences in estradiol between pre- and postmenopausal women were seen. Further, like Dr. Petrakis, they found that HRT had no effect on estradiol. They also found that estrone sulfate, a major component of HRT, did not appear in the breast fluid and that cathepsin D appeared to be lower in women who were taking HRT. However, within subjects, there were significant correlations among the products measured. Estradiol correlated most strongly with DHEA sulfate, 0.87 percent; estrone sulfate, 0.74 percent; and EGF (epidermal growth factor), 0.58 percent. Further, using a stepwise ANOVA (ANalysis Of Variance between groups), 78 percent of the variance in estradiol could be estimated from androstenedione, estrone sulfate, DHEA, DHEA sulfate, and progesterone in all women.

These findings led Dr. Chatterton to conclude that there are homeostatic mechanisms within the breast for the maintenance of estradiol concentrations, even in the absence of an ovarian source of estradiol or in the presence of HRT. And he suggested that both hydrolysis of estrone sulfate and aromatization of androgen precursors could be involved in this process. He underscored that all of the women who took part in this study were at high risk for breast cancer, and that they will now be studying a population of normal, low-risk women to develop a normal baseline from which to measure the influence of high-risk factors on estrogen levels in the breast.



During the discussion that followed Dr. Chatterton was asked about the application of these findings to future research. He replied that he believed researchers should: attempt to obtain ductal lavage fluid from normal women as a baseline from which to measure responses to tamoxifen treatment in high-risk women; measure response proteins to tamoxifen in ductal lavage fluid; and, study aromatase inhibitor treatment by measuring ductal lavage fluid levels of estradiol, hypersensitivity responses, and accumulation of androgens in order to see whether these drugs decrease estradiol in the breast.

*\*\*Dr. Chatterton's research was supported in part by the Susan Love MD Breast Cancer Research Foundation.*

### **Analysis of Aromatase Expression in Intraductal Mammary Foam Cells**

The final presentation on hormone levels in breast duct fluid was by Nevine Ali, a senior at Yale University who is working with Dr. King at Yale University School of Medicine. She presented an "Analysis of Aromatase Expression in Intraductal Mammary Foam Cells." Ms. Ali explained that the high number of foam cells found in ductal lavage fluid generates questions about the role these cells play in normal breast physiology and breast tumor progression, especially because high numbers of foam cells are found in ducts with benign epithelial cells.

In the developing mammary gland, she said, macrophages can be found within and around the elongating ducts, which infers that they play a role in ductal growth. Further, during lactation they constitute a predominant cell type in milk. In addition, breast tumor progression is associated with high numbers of infiltrating macrophages. Thus, it is possible that these macrophages promote tumor growth through their ability to express aromatase and synthesize in situ estrogen to stimulate epithelial cell proliferation.

To explore this further, studies were initiated to measure aromatase expression in these cells. Our hypothesis, she said, "was that the intraductal macrophages would express aromatase, which would increase estrogen levels, and thus stimulate epithelial cell growth." She reported that preliminary results indicate that a subset of ductal lavage specimens contained foam cells that were found by IHC to contain the aromatase antibody; that aromatase-positive foam cells were present in ductal lavage specimens obtained from women with and without breast cancer; that foam cell aromatase expression varies among lavage specimens collected from separate ducts of the same woman; and that intraductal foam cells collected by ductal lavage are viable and can be maintained in cell culture, where they can be tested for estrogen production and their effect on cell proliferation.

### **Anatomy of the Breast Ductal System: Competing Views**

#### **Anatomy of the Breast Ducts: Controversy or Consensus**

The second day of the conference opened with a discussion on the anatomy of the breast ductal system. The first presenter was Susan Love, MD, MBA, who discussed, "Anatomy of the Breast Ducts: Controversy or Consensus." Dr. Love began by noting that the first studies of breast ductal anatomy were by Ashley Cooper in 1839, when he described many "straight tubes" behind the nipple but was only able to cannulate 6–8 milk duct orifices at the nipple. In the 1970s Dr. Otto Sartorius identified two types of orifices in the nipple: the slitlike ductal orifices and the oval



openings of the sebaceous glands. Thus, she said, "not all openings in the duct are milk ducts, and not all tubes behind the nipple are milk ducts, which means that some ducts we cannulate may actually be sebaceous glands."

Next, Dr. Love discussed the current discrepancy over how many ducts each nipple actually has. She noted that Dr. Michel Teboul, who looked at more than 600 women, concluded that there were 15-20 hollow ducts that diverge from the nipple, but said that there were only 6-9 openings in the nipple itself. She then described her own ductal anatomy studies, which began with the detailed mapping of the milk duct orifices of 219 women who were members of the La Leche League. She reported that they found that the median number of milk-duct orifices was 5, with a range of 1-12.

In order to describe the patterns of the ductal systems, analyses were then done of 1,312 archival ductograms in 470 women. This study found that the breast ducts emanate back toward the chest wall in a consistent pattern, a finding that was later confirmed through direct observation of the number and distribution of nipple duct orifices by in vitro sectioning and three-dimensional digital image analysis. This was confirmed using a transareolar dye injection technique in five cadaver breasts and 10 mastectomy specimens, allowing for the confirmation of the number and distribution of the ductal orifices by in vitro and in vivo transareolar dye injection.

Dr. Love concluded that in over 99 percent of the cases studied, the nipples contained from 5–9 ductal orifices arranged in a central group and a peripheral group. The ducts emanating from the central group were directed toward the chest wall, while the peripheral ones were more radial. Further, each nipple orifice communicates with separate non-anastomosing ductal systems that extend to the deep recesses of the ductal lobular units, allowing for cannulation of a specific duct. These findings indicate, she said, that "if you look at a piece of tissue and see two ductal pieces you cannot assume that they are from the same ductal system just because they are right next to each other."

### **Three-Dimensional Anatomy of Complete Duct Systems in a Human Breast**

James Going, MB, PhD, of the Department of Pathology at the University of Glasgow in Scotland, addressed "Three-Dimensional Anatomy of Complete Duct Systems in a Human Breast." Dr. Going explained that an understanding of ductal anatomy is necessary to understand breast development, the origins of breast neoplasia, and the spread of carcinoma in situ. "You can't understand where breast cancer comes from," he said, "if you don't understand what is normal." And "you can't treat in situ if you don't have a map of the highways and byways that would allow the disease to spread." This is why he decided to reconstruct a breast and to examine in three dimensions the spatial arrangement of all major ducts and their branches from nipple to periphery. The breast he used was that of a 19-year-old woman, and was obtained at necropsy.

Dr. Going found that the subgross method was effective for creating a three-dimensional image of the breast. This method entails taking fixed tissue, 2–3mm in thickness, and staining in with haematoxylin and clearing it in methyl salicylate. "This allows us to see a great deal of detail in the ductal system of the breast," he said, "and if we stacked the slices together we could see exactly where a duct goes." Through this process, ten complete duct systems were successfully traced. "To some extent," Dr. Going said, "we found a radial structure but there was not geometrical regularity." Further, he reported, "the duct branches don't intertwine, but they do adapt their shapes to one another. They are not simple wedges."





Dr. Going said they also tried to measure the volume that each duct contributes and plotted that information on a pie chart. They found that there were three ducts that contributed up to 50 percent of the breast volume, and that there were other ducts that were very small. If you added them up, he said, "we had 14 ducts, with 6 ducts that did nothing. I'm not sure if these were sebaceous glands, but they didn't contribute to the volume of the breast." In conclusion, said Dr. Going, this study confirmed that there are discrete duct territories that are variable in size, in shape, in the length of the central unbranched ducts, and in the configuration of the boundaries between adjacent duct systems. In addition, anastomosis between ductal systems was not identified in this particular breast.

Dr. Going then presented, for the first time, a three-dimensional construction of a nipple. For this procedure, he took the mastectomy nipple, divided it into 6–8 sections, and looked at 7mm of nipple from the surface to the duct bundle. Digital modeling of the 27 main tubular structures in a mastectomy nipple showed only seven openings to the skin surface; the rest appeared to originate from skin appendages. Also, all 20 duct systems in an autopsy breast were visualized completely and their volumes measured in 2mm subgross slices. Eight ducts contributed only 1.6 percent of total breast volume. Conversely, one duct system alone contributed 23 percent, three contributed 50.3 percent, and six contributed 75 percent of breast volume. Whether the small rudimentary ducts are really ducts or some other structure, Dr. Going said, is not known.

### **Advantages of Ductal Echography**

Michel Teboul, MD, head of the Baglivi Senologic Center in Rome, ended the section on the anatomy of the breast ductal system with his presentation, "Advantages of Ductal Echography." Dr. Teboul noted that it is not possible to assess that a breast has been adequately investigated if its ductolobular structures have not been observed. He also noted that the deficiencies in the current methods of breast imagery have regularly been confirmed by ductal echography (DE), which has proven to be capable of revealing non-radiovisible epithelial lesions and elucidating misleading mammographic signs.

Next, Dr. Teboul explained that the introduction of high-quality fully digitalized equipment in 2000 significantly increased DE performance. In turn, the DE display of internal structures "became rapid and obvious," allowing for the observation of abnormalities in less time. Further, as the visual evaluation of lesions improved, needle aspiration was often bypassed and surgical biopsies were recommended and preformed with a high rate of reliability. Because of this, Dr. Teboul concluded, physicians should consider performing both DE and mammography to improve the evaluation of early lesions.

### **Does Cytology of Breast Duct Fluid Have Long-Term Follow-Up?**

#### **Breast Cancer Risk in Women with Abnormal Cytology in Nipple Aspirates of Breast Fluid**

Margaret Wrensch, PhD, a professor of epidemiology and biostatistics at the University of California, San Francisco, began the session on long-term follow-up on breast duct fluid with her presentation, "Breast Cancer Risk in Women with Abnormal Cytology in Nipple Aspirates of Breast Fluid." Dr. Wrensch explained that her previous research found that women with abnormal cytology in breast fluid obtained by nipple aspiration had an increased relative risk (RR) of breast cancer compared with women from whom breast fluid was not obtained and with women whose



fluid had normal cytology. Her current study extends the follow-up in the original study group of 4,046 women and presents the first follow-up for a second group of 3,627 women.

For these studies, nipple aspirate fluid was collected from women in the San Francisco Bay Area from 1972–1991 and classified according to the most severe epithelial cytology seen in the fluid specimens. Breast cancer incidence was then determined through March 1999. Dr. Wrensch reported that the results from the newly followed women independently confirmed the previous findings that women with abnormal cytology in nipple aspirates of breast fluid have an increased risk of breast cancer.

### **Evaluation of Risk Modification Using Long-Term Follow-Up from Ductal Lavage Patients**

The final presentation on long-term follow-up was given by Catherine Carpenter, PhD, a postdoctoral fellow at the USC/Norris Breast Cancer Research Training Program. She addressed the "Evaluation of Risk Modification Using Long-Term Follow-Up from Ductal Lavage Patients." Dr. Carpenter conducted a retrospective cohort study of 56 ductal lavage patients who had been seen by Dr. Otto Sartorius. Dr. Carpenter noted that "with a follow-up of 20 years, this data represents the longest follow-up to date of lavage patients." She reported that they found that the overall age-adjusted Poisson rate-ratio of developing breast cancer associated with hyperplasia was 1.29 and with atypical hyperplasia it was 2.32. Further, it was determined that women with atypical cells were at 2.4 times greater risk of developing breast cancer than women who had no fluid, normal, or fibrocystic cells.

In the discussion that followed Dr. Wrensch noted that "while these samples were collected in different ways, there are still similar results, so I think that there is something real going on. And I think it is more diluted than it really is because there is probably misclassification in all of the categories." Dr. Wrensch then discussed the need for pathologists to talk to each other about cytology of breast duct fluid. "Some presentations are combining moderate and marked atypia," she noted, "and since more cells are obtained through ductal lavage it is increasing the sensitivity and the ability to detect atypia."

A cytopathologist from the University of Kansas commented that pathologists "are trained to diagnose specific lesions, but risk assessment is a different animal." She went on to say that intra-observer comparisons among cytopathologists show that there is reproducibility but that strict morphologic criteria are needed.

### **Are There Clinical Applications for the Intraductal Approach?**

#### **Lessons Learned from the First Three Years of Clinical Use of the Ductal Approach to Breast Cancer**

The first presentation on clinical applications of the intraductal approach was by William Dooley, MD, the G. Rainey Williams Professor of Surgical Breast Oncology at the University of Oklahoma Institute for Breast Health, and director of the Division of Surgical Oncology. He addressed "Lessons Learned from the First Three Years of Clinical Use of the Ductal Approach to Breast Cancer." Dr. Dooley said that his center has adopted and routinely uses ductal lavage on high-risk patients. The first series he studied was in 2001, when he lavaged the contralateral breast of women undergoing breast surgery in the operating room. In doing so, he found that 25 percent of the women had atypia in the contralateral breast, and that the incidence of atypia was highest in women 40–49 years of age, "a decade before the incidence peak of breast cancer."



He reported that through the ductal lavage procedure one occult malignancy that ended up being DCIS was found in the contralateral breast. In addition, he found that "tamoxifen use increased by 16 percent in women with small, Stage I disease, or DCIS, and that among women who were concerned about tamoxifen as chemoprevention knowing that they had atypia on the other side seemed to help with their tamoxifen decision."

These findings led him to question the frequency of atypia in the contralateral breast. To explore this further, Dr. Dooley studied lavage samples from a series of 161 high-risk patients who were undergoing diagnostic or therapeutic surgery between March 2001–August 2002. He reported that 22 percent of the 27 women who were being treated for DCIS had atypia in the contralateral breast. "When we looked closer," he said, "we saw that there was less atypia in the women with high-grade DCIS and more in the women with low-grade DCIS."

Among the women with invasive cancer, he said, the incidence of contralateral atypia fell as tumor size increased. In addition, 57 percent of the 7 women undergoing nipple exploration had contralateral atypia while 29 percent of the 31 women undergoing excision of core biopsy sites due to atypical hyperplasia had contralateral atypia. Dr. Dooley then discussed his interpretation of these findings. "I speculate and I predict that if atypical hyperplasia is associated with elevated risk," he said, "that that is true only for 10 years, and then the risk goes down. I think that if a woman has atypia it either burns itself out or turns into cancer."

Dr. Dooley also does routine operative breast endoscopy; to date, he has performed this procedure on 349 patients. He reported that "72 percent of the time I could scope and get to the target lesions. This allows me to see the ductal system in real time while operating and to determine who had DCIS from radiographic imaging." In addition, he said, "it has helped me to get negative margins. There is a steep learning curve, but lots of future potential." Dr. Dooley emphasized that while ductal lavage is a screening test for atypical hyperplasia, "we don't yet know what percent of atypical hyperplasia it identifies and what percent it misses. So we need to be careful as we go forward because a woman can have a negative lavage and still have cancer."

"In my opinion," he concluded, "we are talking about two different groups of disease. Once you get an initiating event for atypia and you get classic ADH (antidiuretic hormone), then angiogenesis is associated and you can see red lesions in the terminal ducts. That seems to be associated with high-grade DCIS and those lesions tend to be headed toward high-grade invasive cancer. The other atypia tends to be in bigger ducts, is not red, and is more likely to be low-grade DCIS. And the fact that you can't have both in the same duct at the same time means that we may be able to separate atypia differently in the future using ductal lavage and endoscopy."

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

Edward Sauter, MD, an associate professor of surgery and director of the Breast Cancer Prevention and Early Detection Laboratory at the University of Missouri-Columbia, addressed "Nipple Aspirate Fluid: Possible Clinical Applications." Dr. Sauter's laboratory studies biomarkers of breast cancer prevention, early detection, and response to treatment using non- and minimally invasive techniques. Over the past eight years, his lab has recruited more than 900 women for translational research studies. In these studies NAF was collected using a modified breast pump "because it is the simplest, lowest cost, and lowest tech method." In addition, said Dr. Sauter,



"NAF may have its own strengths. It may even be the best and possibly the first screening test. The problem is that while it has good specificity, it does not have good sensitivity in and of itself."

Dr. Sauter is currently analyzing NAF for extracellular markers and, to date, about 30 markers have been studied. Specifically, he said, "we have found that prostate specific antigen (PSA) is in the breast in high levels and that low levels are indicative of invasive breast cancer." In addition, they found that PSA levels are high in premenopausal women. "This led us to do another study," he said, "which suggested that PSA is under the stimulation of progesterone, and that lower rates of PSA in these women are associated with higher odds of cancer." Dr. Sauter also reported that "NAF PSA is inversely associated with breast cancer."

Dr. Sauter's lab also found that insulin-like growth factor binding protein type 3 (IGFBP-3) was associated with breast cancer, with the higher levels of IGFBP-3 found in women with breast cancer. In addition, an inverse association was seen between PSA and IGFBP-3, with low PSA being correlated with high IGFBP. Lastly, when the lab explored basic fibroblast growth factor (bFGF), they found that NAF bFGF is higher in breasts that have cancer in them than it is in normal breasts.

### **The Impact of Dietary Change on Breast Nipple Aspirate Fluid**

Zora Djuric, PhD, an associate professor in the Department of Internal Medicine at the Karmanos Cancer Institute at Wayne State University Detroit, addressed "The Impact of Dietary Change on Breast Nipple Aspirate Fluid." Dr. Djuric began by discussing the Nutrition and Breast Health Study, which enrolled 122 women between the ages of 21–50. All of the women were healthy, premenopausal nonsmokers who had a family history of breast cancer in at least one primary relative. The women were all taught how to express and collect NAF by themselves in their homes. To study the biochemical effects of a low-fat and/or high fruit-vegetable diet, the women were randomized to one of four diets: control, high fruit-vegetable, low-fat, or combination low-fat/high fruit-vegetable. NAF was collected by the women at the start of the study and then 6 and 12 months later.

Dr. Djuric reported that protein and cholesterol levels did not change with time in any group, but that levels of 8-isoprostane were affected by fat intake, with decreases occurring in both the low-fat and combination arms. Mean retinol, total carotenoid, and total tocopherol levels in NAF were slightly increased at 12 months in the control group, while in the low-fat arm mean levels of 9 different carotenoids and retinol decreased after intervention. Dr. Djuric also reported that the carotenoid levels in NAF were similar to that in plasma, but that the tocopherol levels were higher in NAF than in plasma. "Although a low-fat diet can decrease carotenoids in NAF," said Dr. Djuric, "if that is accompanied by an increase in fruit and vegetables then the carotenoids can increase."

In the ensuing discussion the role that diet plays in breast cancer was addressed. Dr. Victor Vogel noted that, "based on the available data, there appears to be very little impact of dietary change on breast cancer incidence or outcome." In response, Dr. Wrensch pointed out that European studies have found an association and that it may be that no such change is seen in studies conducted in the United States because the differences between groups in this country are small.



### **Angiogenesis and the Intraductal Approach to Breast Cancer Detection**

Mai Nguyen Brooks, MD, an associate professor in the Department of Surgical Oncology at the University of California, Los Angeles, discussed "Angiogenesis and the Intraductal Approach to Breast Cancer Detection." Dr. Brooks explained that a cancer that does not have new blood vessels cannot grow beyond 1mm in size, which is why it metastasizes. In her research, she has observed that the levels of bFGF (basic fibroblast growth factor, a major angiogenic factor) in NAF are significantly elevated in breasts with cancer compared with breasts that do not have cancer.

"This remains to be validated with large numbers of women, and we need to compare this to the current methods of detection," said Dr. Brooks. "It's nice that the bFGF is higher in women with cancer, but we need to see if it can add anything to the way that we practice medicine." If it can be validated, she said, then it could provide a simple, fast, inexpensive means of detecting women with breast cancer.

Dr. Brooks also discussed another angiogenesis-related molecule, the EG-1-gene, which is expressed in epithelial cells. "This gene appears to be significantly higher when there is cancer," she said, "and this may have an impact on breast cancer since breast cancer is epithelial cell-derived." Identifying this molecule, she added, would not replace current screening methods. But it might possibly be used to detect those women who are high risk, to monitor women, or during chemoprevention.

### **Breast Malignancy Is Found Frequently in Patients with Pathologic Nipple Discharge: Careful Clinical Evaluation, Ductography, and Accurate Surgical Excision Are Essential for Diagnosis**

Margaret Lawler, MD, a clinical instructor of surgery at the Faulkner Breast Center at the Tufts University School of Medicine, addressed "Breast Malignancy Is Found Frequently in Patients with Pathologic Nipple Discharge: Careful Clinical Evaluation, Ductography, and Accurate Surgical Excision Are Essential for Diagnosis." Dr. Lawler explained that at her center patients with nipple discharge are assessed clinically for features of pathologic discharge. If the finding is positive, patients undergo a ductogram and the duct washings are sent for cytologic evaluation. Since 1994, she has performed 300 consecutive duct excisions. In all of these cases, she said, mammography or physical examination had not indicated an abnormality was present.

Cancer was found in 18 percent of the women on review of surgical excision histology while a proliferate lesion was found in 85 percent of the cases. In addition, 70 percent of the excisions were found to contain benign papillomas, while in 9 percent of the cases the discharge was attributed to some sort of inflammation. In a subset of patients, cytology from the duct washings was compared to the histologic findings on excision. Of the 27 malignancies found in this group, 3 were identified as malignant by cytology, 21 were read as atypical, and 3 were negative. Based on these findings, Dr. Lawler concluded that "a high percentage of patients with pathologic nipple discharge have an associated breast carcinoma" and that, because of this, "the clinical features of discharge must be thoroughly assessed." And, she noted, "ductography can help ensure that the right area of the duct is excised."



## **Of Mice, Macrophages, and Mammary Glands**

Sanford Barsky, MD, a professor of pathology and laboratory medicine at the University of California, Los Angeles, addressed "Of Mice, Macrophages, and Mammary Glands." Dr. Barsky explained that intraductal foam cells are the most numerous type of cell found in spontaneous nipple discharge, nipple aspirate fluid, and ductal lavage. Yet the origin and significance of these cells is unclear. "Cytologists consider these cells a nuisance," said Dr. Barsky, "because they hide the epithelial cells, which is the *raison d'être* for ductal lavage."

Dr. Barsky then discussed what is known about macrophages. He noted, for example, that intraductal foam cells increase in pregnancy and other conditions of ductal ectasia and that they frequently surround DCIS and other intraductal proliferations. He added that studies have found that foam cells are clearly of macrophage lineage and terminally differentiated, and that other variants of these foam cells exist. Because macrophages only are observed intraductally and because their appearance resembles lactating and vacuolated epithelial cells, "people assumed foam cells came from the ductal lining epithelium," said Dr. Barsky. "But our data confirm that these cells are not of epithelial cell origin." And that led to the question, he said of "where do these cells come from?"

Dr. Barsky believed that the foam cells were bone marrow-derived cells, and set out to test this hypothesis. This research included two mice studies in which marrow was harvested from wild type C57 black mice and green fluorescent protein (GFP)-transgenic black mice and put into lethally irradiated C57 mice. This process, Dr. Barsky explained, allowed them to identify where the cells of donor origin were in these mice. Next, the mice were made pseudo-pregnant with injections of progesterone in order to increase the number of foam cells that would develop in the breast duct. Finally, the fat pads of the mice were excised and examined.

"A pregnant mouse has lots of foamy macrophages," Dr. Barsky explained, "and when we looked at these cells we could see that they had the GFP label, which suggests they were coming from the donor marrow." Thus, he concluded, "These cells originate in the bone marrow and are brought to the breast. They only become foam cells once they enter the intraductal lumen."

Dr. Barsky is now conducting studies designed to determine if what is true in mice is also true in humans. He has just initiated a study in which NAF and DL fluid will be taken from women who had male bone marrow transplants. He will use the fluid retrieved to test for the presence of Y chromosome and donor/recipient microsatellite polymorphic markers and to compare the intraductal macrophages with buccal epithelial cells to see if the foam cells are male in origin.

## **Molecular Field Mapping and Ductal Lavage Assessment in Chinese Women**

The final lecture on clinical applications for the intraductal approach, "Molecular Field Mapping and Ductal Lavage Assessment in Chinese Women," was presented by Jian Yu Rao, MD, an assistant professor in the Department of Pathology and Laboratory Medicine at the University of California, Los Angeles. Dr. Rao explained that the goal of his research was to determine if lavage could be performed in Chinese women and if they could duplicate the findings seen in the United States in China. More specifically, he said, they wanted to see if there were markers that could be identified in the lavage fluid.





As part of a series of studies to explore this question, Dr. Rao performed ductal lavage on mastectomy specimens, and then cut the breast into three pieces for microdissection. He reported his preliminary findings which indicated that in one of the five breasts examined changes in the noncancerous duct could be observed. Next, he said, they will study lavage prior to mastectomy to see if the findings will differ.

A second pilot study enrolled 21 women in China and 19 Chinese women in the United States. Dr. Rao reported that this study not only demonstrated the feasibility of conducting ductal lavage but found "that about 30 percent of women showed cytological atypia." Next, they want to look more closely for markers. "The problem," he said, "is that we don't know the meaning of atypical cytology, and this means we need more studies to follow up on findings."

*\*\*Dr. Rao's research was supported in part by a grant from the Susan Love MD Breast Cancer Research Foundation.*

### **Panel Discussion: Ductal Lavage - Ready for the Clinic or a Research Tool?**

Panelists:

- William Dooley, MD, Professor of Surgical Oncology, University of Oklahoma
- Seema Khan, MD, Associate Professor of Surgery, Northwestern University Medical School
- Ragsbert Phillips, MD, Metro Surgical Associates, Lithonia, Georgia
- Victor Vogel, MD, Professor of Medicine and Director, Comprehensive Breast Program, University of Pittsburgh

Dr. Khan discussed the analysis of ductal lavage and the "troublesome category" of atypia, the difficulty differentiating between benign, mild, and marked atypia, and the impact this had on her own study. "We had some reviewers in our study who thought the atypia was mild and other reviewers who thought it was benign," she said. And while there were six samples the reviewers did agree on, "the most discordance between the two readers was in the category of mild atypia, with the transition between mild atypia and benign the most difficult. This is a problem in the clinic when we think about adding ductal lavage to what women will use to help them decide what they want to do."

Dr. Vogel noted that it has not been determined what effect the presence of atypical hyperplasia would have on the Gail Risk Model for breast cancer. But if atypia is collected on ductal lavage and we can put it into the Gail Model and see if it is meaningful, he said, that could help women. Right now, Dr. Vogel said, when he sees women who have atypia all he can do is tell them how tamoxifen benefits women who are at increased risk, not how much atypia increases their risk. "That is why I agonize whether to use ductal lavage as a risk stratification tool," he said.

"There are Type 1 and Type 2 errors that are involved with introducing new strategies," Dr. Vogel continued. "If you are an early adapter," he said, "you can commit a Type 1 error, and begin using a test or treatment and then later find out that it is not beneficial, such as what we've seen happen with high-dose chemotherapy. Or you can commit a Type 2 error, which is to reject the strategy. But then we might be rejecting an effective strategy, which is like what occurred when



tamoxifen was not used in premenopausal women because it was not believed that it would work. I don't know that either error is a worse sin, but there are problems you encounter whichever way you go."

To address these unresolved questions, Dr. Vogel instituted a Risk Assessment Working Group. The group is currently addressing questions such as: What is the sensitivity and specificity of detecting atypia with ductal lavage? If there is atypia in the breast, how good is ductal lavage at finding it? Are there false positives? Is lavage atypia different than NAF atypia? Is cytology atypia as accurate a predictive marker as the newer molecular approaches? And, do we need to wait for complete validation studies before using lavage-detected atypia to stratify risk and make intervention recommendations? To answer these questions, he said, "we will need to follow women with atypia to get the data we need."

Dr. Phillips is a clinician with a community-based practice outside Atlanta, Georgia, that serves as a referral source for women in the metro-Atlanta area who are diagnosed with breast cancer. She sees about 1,200 new patients a year, and has a high volume of high-risk patients. Dr. Phillips said she has conducted ductal lavage on about 140 women. Of those, about 55 percent had ductal lavage because they were deemed high-risk on the Gail Risk Model, had cancer in their other breast, or had pathological nipple discharge (spontaneous bloody or clear nipple discharge, typically from a single duct). Dr. Phillips reported that 33 percent of these women were found to have atypia. She is having the women who had a benign result followed, and they will have a repeat ductal lavage. Less clear, she said, was how to use the information when women had atypical cytology or what she would do if a woman had mild atypia on a repeat ductal lavage.

Dr. Phillips said that she had three patients with mild atypia on cytology who at surgery had extensive DCIS. "This indicates," she said, "that the atypia seen on ductal lavage often times does not equate pathologically to the disease that we see in the breast. So from where I'm standing, when I see mild atypia I believe that I need to further evaluate the breast."

In the ensuing discussion the following question was raised: What should a physician do if they have a patient who is premenopausal, in her early 40s, and who had ductal lavage because she has breast cancer in her other breast and the findings on cytology are mild atypia? I've been doing ductoscopy, but what do you do if ductoscopy doesn't show anything? Can we truly ignore doing an excision on patients who have atypical cytology?

Dr. Dooley responded that if the patient had marked atypia he "would do a radiographic witch-hunt and then scope the patient to find and biopsy a lesion." But if it were mild atypia, he said, "I wouldn't do that because you could intervene with chemoprevention." But, he added, "I would re-lavage at six months and a year, and if it doesn't clear up, then I would do a whole witch-hunt again."

Dr. Vogel said that if the woman was ER-negative he would be more anxious than if she were ER-positive. Dr. Ragsbert said that she would be aggressive in trying to prove that the mild atypia was not due to early cancer but due to some other cause. Dr. Khan added that it was important to intellectually separate out the reasons for offering women ductal lavage outside of a study. "If we all agree the reason for lavaging outside of a study is risk assessment," she said, "I don't know of any data that finding atypia in the contralateral breast further increases a woman's risk. If those data existed then there would be rationale to lavage the contralateral breast. But lacking those data we don't offer contralateral lavage to women who have had breast cancer and the only



women we have lavaged have been those in our tamoxifen study in whom we are looking for surrogate end points."

Another participant asked: If tamoxifen is given for five years as chemoprevention, and the woman begins taking it at 37, what do you do when she turns 42? Further, what have you noticed about the psychology of patients at high risk? And when you inform them that they have atypia, how do they cope while under surveillance?

In response, it was noted that adjuvant studies indicate that the effect of tamoxifen extends beyond five years. It was also noted that very few women actually choose tamoxifen, and that when women age or go through menopause sometimes this atypia goes away on its own.

Dr. Khan said that "if we are thinking only about risk assessment, fine needle aspiration [FNA] is good to evaluate atypia." The problem with ductal lavage, she said, "is that it has muddied the waters in terms of risk assessment. The idea is that it might detect early cancer. But we don't know the reproducibility of ductal lavage and we need to remember that when making recommendations about how to evaluate ductal lavage findings." Dr. Dooley pointed out that "ductal lavage was never a cancer screening test. It was meant to be a screening test for atypia, which is known to present a high relative risk for breast cancer."

Another participant noted that some physicians were anxious about doing ductal lavage because if mild atypia was found you would feel like you need to do everything under the sky out of fear that the woman would develop breast cancer in five years and sue. Dr. Love responded by saying, "I think that if you find atypical hyperplasia on biopsy you wouldn't do anything, but you would tell the woman that she is at higher risk. So I don't understand why finding mild atypia means we have to go searching for a lesion. With ductal lavage we are not diagnosing cancer, we are just looking at what's in the ducts."

Another doctor thought that this was an educational problem. "When I started doing this with FNA [fine needle aspiration]," he said, "it was a disaster. Patients did not understand what atypia means. So now, before I allow someone to have FNA they must undergo prevention consultation, and that makes a big difference. We go over what precancerous disease is, so when we tell them they have atypical cells, they don't freak out. Also before we actually do the procedure, we have the woman develop a plan of what she will do depending on what we find."

Fran Visco, president of the National Breast Cancer Coalition, noted, "We don't know how to prevent breast cancer and yet the term that keeps being used is prevention. The P1 trial showed that there was a short-term risk reduction, but we don't yet know mortality in that trial. That's why we are concerned about the interventions we are discussing for these high-risk women."

### **Pilot Grants**

The Susan Love MD Breast Cancer Research Foundation had money to distribute as pilot grants at the 2003 Symposium. The Foundation typically provides pilot grants in the range of \$10,000–\$20,000. The Peer Review Committee recommends the grants to be funded, based on the scientific merit and relevance to the Susan Love MD Breast Cancer Research 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 a new process. Rather than submit a lengthy application, applicants for pilot grants only submit a one-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, 19 researchers presented proposals. At the close of the Symposium, the Foundation provided a total of \$151,760 to support the pilot work of 10 researchers utilizing the intraductal approach for a wide range of projects.

The 2003 recipients of these pilot grants were:

**\*Cellular and Molecular Evaluation of the Intraduct Environment in the Assessment and Screening of Women Carrying BRCA1 and BRCA2 Mutations**  
**Gerald Gui, MD, Consultant Surgeon, Academic Surgery (Breast Unit)**  
**Royal Marsden NHS Trust, London**

**This study was awarded a \$20,000 research grant from the Foundation.**

This study will enroll 50 women with a known BRCA1 or BRCA2 mutation or a family history of more than three members with breast and/or ovarian cancer. The negative control group will consist of 10 volunteer women from BRCA1 and BRCA2 families who have tested negative; the positive control group will include 10 women from BRCA1 and BRCA 2 families who have established breast cancer. All of the women will undergo ductal lavage every six months for two years.

The ductal fluid will be subject to cytologic analysis; immunocytochemistry for ER, PR, p53, and PSA; molecular analysis including gene methylation specific PCR and gene expression assays; biochemical studies; and tumor marker content. The aim of the study is to determine the prevalence of cytologic atypia, genes, and biological markers; the temporal changes of these variables within individual women and their relationship to subsequent cancer development; and potential biomarkers of malignancy. This could potentially improve the sensitivity of breast cancer screening, decision-making, scheduling of risk-reduction surgery, and investigation of chemoprevention agents through intermediate markers.

**\*Detection of Breast Cancer by Proteomic Analysis of Ductal Lavage Fluid Using SELDI-Mass Spectrometry**  
**Jinong Li, PhD, Department of Pathology**  
**Johns Hopkins University School of Medicine, Baltimore**

**This study received a \$20,000 research grant from the Foundation.**

SELDI is an affinity-based mass spectrometry method in which proteins are selectively absorbed to a chemically modified surface on ProteinChip Arrays. Non-specific bound proteins are removed by washing with a buffer solution. This technology has been used to detect several disease-associated proteins in complex biological specimens, and NAF has been shown to be compatible with this technology.



The researchers propose that proteomic analysis of ductal lavage fluid from the cancer-bearing and non-cancer-bearing contralateral breast may reveal a protein pattern that is associated with breast cancer. In this study, about 200 ductal lavage fluid samples from 50 women with breast cancer enrolled in an ongoing clinical trial will be tested. The specific aims of this study are to optimize the current protocol of sample processing and SELDI analysis so it will be adequate for ductal lavage fluid and to identify breast cancer associated protein patterns by comparing protein profiles of ductal lavage fluid from the cancer-bearing ducts and the normal ducts.

**\*Intraductal Approach for Breast Cancer Risk Monitoring in Contralateral Breast in Patient Receiving Mastectomy in China**

**Jian Yu Rao, MD, Assistant Professor, Department of Pathology and Laboratory Medicine  
University of California, Los Angeles**

**This study was awarded a \$20,000 research grant from the Foundation.**

In China, women diagnosed with breast cancer often undergo mastectomy as the definitive method of therapy because of the difficulty in obtaining appropriate follow-up care. Yet the contralateral breast still carries a higher risk for developing cancer (RR=10), even if no detectable tumor mass is present at the time of surgery. The researchers hypothesized that intraductal lavage of the contralateral breast at the time of surgery could provide a means for risk evaluation and monitoring of the remaining breast. To test this hypothesis, this pilot study will enroll 50 women admitted to the Cancer Hospital with newly diagnosed breast cancer who are undergoing a unilateral modified or radical mastectomy and who have no tumor mass in the contralateral breast. Ductal lavage will be performed on both breasts, and patients will be followed every six months for one year, with follow-up evaluations that include repeat lavage, mammography, ultrasound, and clinical examination.

**\*Early Detection of Breast Cancer by Methylation Profiling of DNA from Ductal Fluids  
Zunde Wang, PhD, Director, Genomics Division**

**EpiGenX Pharmaceuticals, Santa Barbara**

**This study was awarded a \$20,000 research grant from the Foundation.**

Abnormal DNA methylation changes, mostly at promoter CpG islands of genes, are important events in tumorigenesis and breast cancer progression. Abnormal DNA methylation changes have been demonstrated in ductal lavage fluids of breast cancer patients and show promise for early detection. However, the limited amounts of DNA available from ductal fluids and the small number of genes capable of being analyzed with current methods are major impediments to the development of methylation markers for breast cancer diagnosis and treatment monitoring.

This study will test a new microarray-based technology called DMS2 (Direct Microarray-Based Screening of Differentially Methylated CpG Sites) that was developed by EpiGenX and that allows for the quantitative analysis of methylation patterns of hundreds to tens of thousands of genes with very small amounts of input DNA. Analysis of DNA samples from ductal lavage fluids and nipple aspirate fluids of breast cancer patients at various stages will be conducted to define the potential application of DNA methylation analysis for early diagnosis of breast cancer.



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**\*Development of an Intraductal Cell and Gene Therapy Approach for Treatment of Early Stage Breast Cancer**

**Michael Lewis, PhD, Assistant Professor  
Breast Center—Baylor College of Medicine, Houston**

**This study was awarded a \$19,500 research grant from the Foundation.**

The unique biology of the normal breast presents the opportunity to fuse cell and gene therapy techniques in a way that circumvents many of these technical limitations for the treatment of early stage breast cancer. In principle, it should be possible to obtain a patient's own breast cells, genetically modify them to perform a therapeutic function, and reintroduce those cells into the affected regions of the breast via intraductal methods. To explore this possibility, the researchers will conduct a "proof of principle" study in a mouse model that could be adapted for use in women.

To simulate normal human breast epithelium, the study will use genetically "tagged" mammary epithelium isolated from mice that express the enhanced green fluorescent protein (EGFP). These cells can be cultured and genetically modified and reintroduced to an intact host mammary gland. Then, a reporter gene, red fluorescent protein (RFP), will be introduced into the EGFP primary mammary epithelial cells.

Next, RFP-transfected, EGFP-tagged epithelial cells will be reintroduced into an intact mammary gland that expresses a third genetic tag, enhanced cyan fluorescent protein (ECFP). Immediately thereafter, a dissociation technique will be used to allow reintroduced and endogenous cells to mix. The efficiency with which the "therapeutic" gene was delivered will be judged. The study will also evaluate the ability of reintroduced cells to integrate into host mammary tissue and the efficiency with which reintroduced cells can be expected to express "therapeutic" protein in a living animal.

**\*Proteomic Analysis for Discovery of Breast Cancer Biomarker from Breast Ductal Fluids**

**Chao-Cheng Wang, PhD, Post-Doctoral Fellow, Pharmacology  
Purdue-UAB Botanical Center for Age-Related Disease  
University of Alabama at Birmingham**

**This study was awarded an \$18,900 research grant from the Foundation.**

The overall goal of this project is to identify proteins/peptides responsible for, or associated with, development of breast cancer at different stages of tumor development using innovative microscale technologies. To accomplish this goal, the researchers will develop microscale devices for size-based fractionation of proteins in breast duct fluid (BDF) that allow for further proteomic analysis and the identification of peptides/proteins that are differently expressed in BDF collected from various stages of breast cancer. The BDF samples will be fractionated into two portions according to molecular weights. A microscale device will be developed to fractionate proteins/peptides in microliters of BDF without significant sample dilution. In addition, 2D-electrophoresis with two different dyes will be used to differentially label proteins normal and cancer samples. Then, very high-resolution and high-sensitivity Fourier transformation cyclotron resonance mass spectrometry will be used for protein/peptide identification. The information gained from this study has the potential to create a knowledge base for providing not only a breast cancer diagnosis, but also an accurate clinical prognosis. It may also lead to the definition of therapeutic targets and mechanism(s) of breast cancer prevention.





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**\*Clinical and Radiological Correlations of Breast Lobe Anatomy Using an Intraductal Approach**

**Phillip Carson, MD, Assistant Professor of Surgery  
Royal Darwin Hospital and Flinders University NT Clinical School,  
Darwin, Australia**

**This study was awarded a \$17,360 research grant from the Foundation.**

This study aims to develop a reproducible method of identifying and cannulating all breast ducts at the nipple and to demonstrate the lobar anatomy of the breast by radiological and cast modeling techniques. Mastectomy specimens will be cannulated at the nipple, sequentially injected with radiographic contrast, and X-rayed by computed tomography (CT) before being processed for routine histology. Then, a three-dimensional reconstruction of the imaged ductal systems will be produced. In addition, several specimens obtained during the course of a prophylactic mastectomy will be injected with latex casting material and be subjected to extensive histological and corrosive casting techniques to check the completeness of the radiological images.

**\*Identification of Ductal Atypia with MRI Galactography**

**Anne-Renee Hartman, MD, Co-Director, Stanford Breast Cancer Genetics Clinic  
Stanford University, Palo Alto, California**

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

The purpose of this study is to define the normal anatomy of the breast ductal system through magnetic resonance imagery (MRI) galactography and to localize atypia not identified by mammography and MRI. This study will test the hypothesis that atypia identified on ductal lavage seen in the context of a normal contrast-enhanced MRI can be localized using MRI galactography, which provides a 3-D image of the breast. The long-term objective of the study is to localize atypia and to determine if severe atypia has a characteristic appearance on MRI. The study will recruit women at high risk for developing breast cancer who are enrolled in Stanford's comprehensive screening protocol. These women, who are being monitored for atypia with MRI and ductal lavage, will have Gd-DTPA injected in the duct that was just lavaged. MRI will then be performed and the duct that was lavaged will be visualized and mapped.

**\*Determining Sensitivity, Specificity, and Proteomic Profiles of Ductal Lavage Cells in Women Undergoing Breast Biopsy or Breast Cancer Surgery**

**Victor Vogel, MD, Professor of Medicine and Director of the Comprehensive Breast Program at the University of Pittsburgh**

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

For ductal lavage to gain validity as a risk assessment tool, and to evaluate its potential as a diagnostic aid, sensitivity and specificity must be defined.

This study will include women who are undergoing core needle biopsy to evaluate either a palpable or mammographic abnormality or having breast cancer surgery. Ductal lavage will be performed on these women prior to biopsy, surgery, fine needle aspiration biopsy, core biopsy, or excisional biopsy. The cytology will be evaluated and compared with the breast biopsy that will serve as the diagnostic standard. The sensitivity of ductal lavage will be the proportion of women



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who have positive ductal lavage findings among those who have a positive biopsy (invasive ductal or lobular carcinoma or DCIS). The specificity will be the proportion of women with negative ductal lavage findings among those women whose biopsies are benign.

**\*Matrix Metalloproteinase 9 Expression and Secretion in Healthy Mammary Ducts: Risk Factor for Breast Cancer?**

**Sandra Gaston, MD, Instructor in Surgery  
Harvard Medical School, Boston**

**This study was awarded a \$1,000 research grant from the Foundation.**

Altered expression of matrix metalloproteinases (MMPs) has been associated with invasive cancer in many different malignancies, including breast and prostate cancer. Our current working hypothesis is that men with a high set point for constitutive prostatic MMP-9 expression are predisposed to biologically aggressive prostate cancer. This research study investigates the question of whether healthy women have a constitutive MMP-9 set point in the secretory epithelium of healthy mammary ducts. This hypothesis will be tested by measuring MMP-9 and MMP-2 in breast nipple aspirates fluid and ductal lavage, using techniques developed in the Gaston laboratory for analysis of minimal fluid volumes. The focus will be on collecting fluid from women with no evidence of disease in at least one breast, noting patient age, parity, menopausal status, and family history of breast cancer.

**Closing**

Following the awards announcement, Dr. Love brought the 3rd International Santa Barbara Symposium on the Intraductal Approach to Breast Cancer to a close.

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