



5th International Symposium on the Intraductal Approach to Breast Cancer March 1-4, 2007 Santa Monica, California

The Dr. Susan Love Research Foundation is committed to advancing research and developing resources that explore the intraductal approach to the breast. As part of this effort, the Foundation hosted The 5th International Symposium on the Intraductal Approach to Breast Cancer in Santa Monica, California, March 1-4, 2007. In attendance were more than 120 oncologists, epidemiologists, biostatisticians, surgeons, biochemists, pathologists, radiologists, endocrinologists, and breast cancer advocates who are currently conducting, or are interested in, research utilizing the intraductal approach.

The four-day Symposium opened with a “Minisymposium on the Intracrinology of the Breast.” Over the next two days presentations addressed “Anatomy of the Breast,” “Ductoscopy and Imaging,” “Intraductal Therapy,” “NAF: The Optimal Approach to Screening?” and “Clinical Applications of NAF and Ductal Lavage.” On the fourth and final day of the Symposium, conference participants discussed “Are We Ready to Launch a Large NAF Screening Trial?”

Each topic area began with one or two longer presentations from experts in the field. This was followed by new research, some of which had been funded by the Foundation, and pilot grant proposals. The schedule was structured to encourage attendees to discuss their ideas and share important research insights, with the aim of building the field and moving it forward. Attendees also were able to observe demonstrations of ductoscopy and ductal lavage with ultrasound. A Public Panel provided the community with an opportunity to learn more about ongoing intraductal research and the promise it holds for eradicating breast cancer.

At the close of the Symposium, the Foundation awarded \$100,000 in pilot grants to 12 research studies.

Thursday, March 1

Minisymposium on the Intracrinology of the Breast

Intracrinology is a relatively new research field that focuses on hormones made by specific tissue that act on that tissue. Studies have found that the breast makes estrogen, prolactin, serotonin, and, possibly, progesterone for its own use. In addition, researchers have found increased hormone levels in nipple aspirate fluid and ductal lavage. The minisymposium provided an opportunity for attendees to discover common areas of interest in the intraductal and intracrinology approaches, with the aim of advancing both fields' efforts to prevent and treat breast cancer.



The Regulation and Inhibition of Estrogen Synthesis in Breast Cancer

Michael J. Reed, PhD, a professor in the Division of Medicine at Imperial College in London, UK, delivered the first presentation “The Regulation and Inhibition of Estrogen Synthesis in Breast Cancer.”

Many of the factors that influence breast cancer risk are estrogen related. For example, late menarche and early menopause decrease breast cancer risk whereas obesity and estrogen therapy increase it. (Dr. Reed also discussed two controversial risk factors: immunospression, which may decrease risk, and stress, which may increase it.)

The enzymes required for estrogen synthesis are present in normal and malignant breast tissue and contribute to the local synthesis of estrogens in this tissue. Dr. Reed’s group has identified a number of cytokins and growth factors, including IGF-1, IL-6 and TNF-alpha, that can stimulate the activity of these enzymes and that contribute to the high concentrations of estrogens detected in breast tumors. Dr. Reed suggested that these cytokins and growth factors might come from “macrophages and lymphocytes, which appear to make up about 50 percent of the material in a breast tumor.”

Along with estrogen synthesis, the pathway of estrogen metabolism also appears to influence breast cancer risk. As Dr. Reed explained, studies have suggested that 2-methoxyestradiol (2-MeOE2), which is a potent inhibitor of angiogenesis, might possibly be “the body’s natural anti-cancer metabolite.” This is significant, in part, because diet and body weight can affect 2-MeOE2 levels. For example, explained Dr. Reed, in women who are lean, E1 is ultimately converted into 2-methoxyestrogens; in contrast, in women who are overweight, E1 is converted into 16alpha-OH. Dr. Reed suggested that this difference, which results in overweight women having less 2-MeOE2, might explain why overweight women have a higher breast cancer risk.

Phase I and Phase II clinical trials evaluating the pharmaceutical form of 2-MeOE2 (Panzem) in patients with advanced and metastatic breast cancer are currently underway. Dr. Reed said that his team has shown that 2-MeOE2 can prevent cytokines from stimulating aromatase activity in breast fibroblasts (Purohit et al BBRC, 1999). This might mean, Dr. Reed said, that the anticancer effects of 2-MeOE2 “may result from its ability to prevent or reduce cytokine stimulated estrogen synthesis.”

Dr. Reed and others are currently studying a new class of drugs, called Selective Estrogen Enzyme Modulators (SEEMs), that function as estrogen synthesis inhibitors. These drugs could work in a number of different ways. For example, Dr. Reed explained, an HSD1 inhibitor might be able to disrupt the pathway by which estrone is converted into estradiol, whereas a steroid sulfatase (STS) inhibitor might be able to stop the conversion of estrone sulfate into estrone. Dr. Reed said that his group recently completed the first Phase I trial of an STS inhibitor (667 COUMATE) in postmenopausal women with advanced breast cancer. The trial found that the drug completely blocked STS activity and resulted in decreased estrogen and androstenediol levels (Stanway, Clinical Cancer Research, 2006). Additional studies are now underway.



In the future, concluded Dr. Reed, “we may find that these inhibitors or other novel approaches, when used alone, sequentially, or in combination with aromatase inhibitors, may improve the response rates to endocrine therapy.”

Estrogenic Enzymes in the Intracrinology of Human Breast Carcinoma

Hironobu Sasano, MD, PhD, a professor in the Department of Pathology at the Tokyo University School of Medicine, in Japan, followed with a discussion of “Estrogenic Enzymes in the Intracrinology of Human Breast Carcinoma.”

Dr. Sasano began by noting that postmenopausal women with breast cancer have serum estrogen levels similar to those of healthy postmenopausal women. Yet, he continued, studies have found that tumor tissue has higher estrogen levels than serum or urine, and that estrogen levels are higher in the adipose tissue around the tumor than in the adipose tissue in the abdomen, buttocks, or other areas. These findings, said Dr. Sasano, “not only raise the question of whether estrogen is stored by the tumor or produced by the tumor itself, but have implications for breast cancer treatment.”

Dr. Sasano discussed his use of laser capture microdissection in frozen tissue to extract RNA from stromal cells. He reported that aromatase is present in both isolated stromal cells and carcinoma cells. This is significant, Dr. Sasano explained, because “aromatase inhibitors suppress aromatase produced in cancer cells much more than they do aromatase produced in stromal cells.” Dr. Sasano said that he is currently trying to identify markers that can determine which tumors will respond to aromatase inhibitors. He is also exploring how aromatase is regulated in cancer cells. “The interaction that takes place between the cancer cells and the stromal cells is very important,” he said, “because cancer cells cannot survive on their own. They coexist with the stromal cells.”

In conclusion, said Dr. Sasano, by learning more about “the sources of estrogen in an individual patient’s tumor, we will be able to develop more individualized treatments” and improve breast cancer care.

Prolactin as an Autocrine/Paracrine Factor in Breast Cancer

Nira Ben-Jonathan, PhD, a professor in the Department of Cell Biology at the University of Cincinnati College of Medicine, Ohio, addressed “Prolactin as an Autocrine/Paracrine Factor in Breast Cancer.”

Dr. Ben-Jonathan began by noting that interactions between the glandular epithelium and stromal tissue are important both in normal breast development and in breast cancer. She then went on to discuss prolactin, which is a polypeptide hormone.

Prolactin has been found to affect the development of breast cancer in animals. But, Dr. Ben-Jonathan said, its involvement with breast cancer development in humans remains controversial, as studies have found little correlation between blood prolactin levels and human breast cancer risk. In addition, studies have found that suppressing pituitary prolactin has no effect on the disease. Yet, Dr. Ben-Jonathan said, tumor biopsies express prolactin and its receptor at higher levels than normal breast tissue does, raising the question of whether locally produced prolactin promotes breast cancer.



Breast tumors develop primarily in the glandular/ductal compartment of the breast, and are fed by adipose (fat), hormones, and growth factors. Dr. Ben-Jonathan reported that her group has shown that the prolactin release rate is 10-fold higher in adipose (fatty) tissue than in glandular tissue. They also have shown that ovarian steroids affect the prolactin release in glandular tissue and adipose tissue in different ways. This suggests, said Dr. Ben-Jonathan, that fat tissue within the breast produces much more prolactin than glandular tissue.

Dr. Ben-Jonathan has demonstrated in a mouse model that a cancer's growth rate is accelerated when tumor cells produce large amounts of prolactin. She suggested this is because the prolactin-producing cells enhance the production of the survival factor bcl-2 protein. She also noted that prolactin appears to affect how tumors respond to chemotherapy treatments, as studies have shown that prolactin can reduce the effectiveness of several chemotherapy agents, including taxol and cisplatin.

In conclusion, said Dr. Ben-Jonathan, "analysis of prolactin in blood or in ductal lavage (if it is found to be present in breast fluid) or an assessment of prolactin in tumor biopsies" could be used to predict a breast tumor's response to specific chemotherapy treatments. Furthermore, "suppressing prolactin production or blocking its receptor in the breast could make tumors responsive" to specific chemotherapy agents.

Progesterone and the Breast

The fourth presentation on intracrinology was by Sandra Haslam, PhD, a professor of physiology and director of the Breast Cancer and the Environment Research Center at Michigan State University, East Lansing. Dr. Haslam discussed "Progesterone and the Breast."

Dr. Haslam pointed out that while progesterone is related to many of the known breast cancer risk factors, much remains to be learned about its role in breast cancer development. Studies have found that progesterone activity is mediated by two versions of the progesterone receptor, PRA and PRB. "PRA and PRB are structurally similar," explained Dr. Haslam, "but they have different functions in vitro and in vivo." It appears that PRB plays a critical role in lobule development and function, she said, whereas the functional role of PRA has yet to be determined.

Dr. Haslam said that her research on progesterone receptor isoform expression in the human breast and in breast cancer found a ratio of PRA: PRB of 1:1. However, in certain instances, this ratio was found to change after breast cancer occurs. For example, Dr. Haslam reported, mastectomy samples from women with BRCA mutations had a higher ratio of PRA to PRB, with PRB either reduced or absent. Furthermore, she said, in vitro studies showed that BRCA1 inhibits progesterone activity and that a BRCA1 deletion results in increased progesterone activity as well as an excess of PRA. Dr. Haslam hypothesized that this could be one mechanism by which BRCA mutations increase breast cancer risk.



Dr. Haslam also discussed the relationship between endocrine disruptors and progesterone. Most of the studies on endocrine disruptors, such as Bisphenol A, “have focused on their estrogenic activity,” she said. “Yet Bisphenol A also has been found to have independent progestogenic activity in the mouse uterus.” This, she continued, “raises the question: Are some of the detrimental effects of endocrine disruptors due to progesterone?”

In conclusion, said Dr. Haslam, “It’s possible that some of the effects that have been attributed to estrogen are actually mediated by the progesterone receptor.” This is why, she underscored, more research is needed on progesterone and the extent to which it is involved in the etiology of breast cancer.

The Local Endocrine Environment in the Breast and Cancer Risk

The next presentation was by Seema Khan, MD, a professor of surgery at Northwestern Feinberg School of Medicine in Chicago. Dr. Khan discussed “The Local Endocrine Environment in the Breast and Cancer Risk.”

Dr. Khan explained how breast cancer risk models, like the Gail model, have made it possible to assess which women, as a group, are at higher risk for developing breast cancer. However, she noted, there currently is no way to predict which individual women will go on to develop the disease. Dr. Khan believes that the intraductal approach could change this.

Dr. Kahn noted that studies have shown that estradiol concentrations are higher in breast tissue than in serum in postmenopausal women, and that estradiol is synthesized locally in the breast. Findings such as these, said Dr. Kahn, underscore the need to focus on hormones in the breast, not in serum. “If we can measure these hormones locally in the breast,” she said, “we might be able to sort out which women are at risk of certain subtypes of breast cancer.”

Nipple aspirate fluid (NAF) is important because it provides a window into the local endocrine environment. Dr. Kahn presented findings from her research into the relationship between NAF hormone levels and age and 5-year Gail risk. She reported that NAF estradiol and progesterone levels correlate with Gail risk in pre- and postmenopausal women. In addition, a relationship between DHEAS in NAF and Gail risk was seen. However, she said, there was no significant correlation between age and any of the NAF hormones.

Taken as a whole, said Dr. Khan, the findings from these and other studies, “provide the rationale for a prospective examination of the hypothesis that nipple fluid hormone levels are markers of breast cancer risk.” By conducting a prospective study, she noted, researchers would be able to look at whether women with higher levels of estradiol and its precursors are more likely to develop ER-positive breast cancer than ER-negative breast cancer. It would also allow researchers to investigate whether an individual woman’s breasts have different hormonal environments, and to learn more about which types of hormonal environments increase breast cancer risk. “The research to date has



provided tantalizing clues,” concluded Dr. Kahn. “Now, we need to study this in a prospective fashion.”

Hormones in Ductal Lavage Fluid in Normal Women

The next presentation by Dixie Mills, MD, the clinical research director for the Dr. Susan Love Research Foundation, addressed “Hormones in Ductal Lavage Fluid in Normal Women.”

Dr. Mills noted that although our knowledge of breast cancer has advanced, many basic questions about the anatomy of the breast still have not been answered. Surgeons approach the breast as an organ that can be easily divided into equal segments, like an orange, she explained. Yet studies have found that the breast ducts do not appear to be arranged radially in equal segments, raising the question of whether breast cancer surgery is as effective as it could be.

Dr. Mills discussed the research currently being conducted by the Dr. Susan Love Research Foundation on the anatomy of the breast and on the hormones that the breast synthesizes and makes on its own. This work had already advanced our understanding of the breast and ductal fluid. But, she underscored, there is much more to be learned. For example, she noted, the etiology of nipple aspirate fluid (NAF) is not yet fully understood. It is also not yet known, she said, if all the ducts that produce NAF are the same or what the non-NAF producing/dry ducts are doing.

The Foundation started the Normal Breast Study to answer these and other questions. But as the Normal Breast Study progressed, Dr. Mills said, it highlighted the need for a greater understanding of the breast itself. This led the Foundation to initiate the Correlation of Anatomy and Physiology (CAP) study. The CAP study is using ductoscopy and ultrasound to monitor the ductal lavage procedure and to identify any perforations that may occur as fluid is obtained. It also includes an analysis of ductal fluid for E1S, E2, P4, DHEAS, protein, and the presence or absence of epithelial cells and macrophages.

Dr. Mills said that the use of ultrasound and ductoscopy in the CAP study has demonstrated that perforations can occur during ductal lavage, which could affect how much fluid is obtained during the procedure. (The research team had an 11% perforation rate.)

Dr. Mills reported some of the preliminary findings from the fluid analyses. This research indicates that postmenopausal women appear to have higher levels of estradiol and progesterone and lower levels of estrone sulfate in their ductal fluid than premenopausal women do, which suggests that hormonal changes occur in the breast tissue itself after menopause. To date, she said, the research team has found little or no evidence that ducts from the same breast are more similar than are the ducts from different breasts. They also have found that ductal fluid obtained from different ducts from the same subject appears to be more similar than ductal fluid obtained from different subjects, except for estradiol and progesterone levels. This suggests, said Dr. Mills, that certain analytes are correlated within subjects while others vary from duct to duct, which would



support the theory that each duct is unique and makes its own hormones. It also suggests that NAF ducts may be physiologically different from non-NAF producing ducts.

In conclusion, said Dr. Mills, “the Normal Breast Study and the CAP study have opened up new areas of investigation and have made it evident that more research is needed.”

Effect of Tamoxifen Treatment on Hormones in the Breast

The minisymposium concluded with a presentation by Robert Chatterton, PhD, a professor of endocrinology in the Department of Obstetrics & Gynecology at Northwestern University Feinberg School of Medicine, Chicago. Dr. Chatterton discussed “Effect of Tamoxifen Treatment on Hormones in the Breast.”

Dr. Chatterton presented his research on the effect of tamoxifen on estrogen levels in ductal lavage fluid in pre- and postmenopausal women at high risk for developing breast cancer. For this study, ductal lavage was performed before tamoxifen treatment and then again 6-13 months later. The fluid was compared with fluid taken from high-risk women who chose not to take tamoxifen. In addition, Dr. Chatterton’s group also performed in vitro studies of breast cancer cell lines with titrated precursors.

Dr. Chatterton reported that the women taking tamoxifen had higher levels of estrogens and androgens in their ductal lavage fluid. His group also found that 4-hydroxytamoxifen (a form of the drug tamoxifen that is made by the body after the drug is taken) increases aromatization in breast tumor cells, whereas tamoxifen itself does not. Lastly, he said, it appeared that serum luteinizing hormone was not increased in women taking tamoxifen, and that tamoxifen and 4-hydroxytamoxifen were both much lower in ductal lavage fluid than in serum.

Dr. Chatterton noted that although tamoxifen has a long half-life in serum, the penetration into ductal lavage fluid was low. Even so, he said, the high correlation between tamoxifen and 4-hydroxytamoxifen and the higher concentration of 4-hydroxytamoxifen in ductal lavage fluid relative to serum suggests that conversion from tamoxifen to 4-hydroxytamoxifen occurs within the breast. Dr. Chatterton also pointed out that different estradiol levels were seen in the ductal fluids taken from pre- and postmenopausal women. This suggests, he said, “that future studies should look at the availability and effect of 4-Hydroxytamoxifen in premenopausal women.”

Friday, March 2

Anatomy of the Breast

The second day of the Symposium opened with a discussion on the anatomy of the breast. Virtually all of the presenters noted that little has been learned about the anatomy of the breast since Sir Astley Cooper’s seminal research in the mid-1800s. Yet, they underscored, a comprehensive understanding of breast anatomy is needed to advance breast cancer research and treatment strategies.



The Theory of The Sick Lobe: A New Concept in Understanding Breast Carcinogenesis

The session on the anatomy of the breast opened with a presentation by Tibor Tot, MD, PhD, a professor in the Department of Pathology at Central Hospital Falun and Uppsala University, in Sweden. Dr. Tot addressed “The Theory of the Sick Lobe: A New Concept in Breast Carcinogenesis.”

Dr. Tot has studied more than 6000 breast cancer cases. Based on this research, Dr. Tot said, it appears that when breast cancer occurs it is localized solely in one lobe in the breast. This finding has led Dr. Tot to hypothesize that some women are born with a lobe that contains a genetic instability, and that this instable lobe accumulates genetic changes over time that ultimately can “lead to malignant transformation of the epithelial cells.”

Dr. Tot also discussed some of the questions this hypothesis raises about breast cancer development. For example, he noted, it is widely believed that most malignant breast tumors originate in the epithelial cells of the lobules and that migration of malignant cells spreads the cancer to the ducts and other lobules. Alternatively, said Dr. Tot, it may actually be that cancer cells are found in various places in the ducts and lobules because the sick lobe contains many epithelial cells that can simultaneously become malignant.

Dr. Tot noted that his theory of the sick lobe also has implications for breast cancer surgery. Currently, he said, “surgeons imagine the breast as a birthday cake, and make resections by quadrants in a pyramid shape toward the nipple,” resulting in a triangular specimen. Dr. Tot argued that “this cake concept” cannot and does not account for the fact that lobes vary in origin, size, and shape. “Right now,” he said, “surgeons look for clean margins; they do not take out a whole lobe.” Yet when a woman has a local recurrence, it’s usually in the vicinity of where the tumor was removed. “If we were to think of breast cancer as a disease of the lobe,” Dr. Tot said, “and if we had a way to excise the whole lobe, we could prevent all of these recurrences.”

Thinking of breast cancer as a disease of a sick lobe also could alter approaches to breast cancer prevention. “Women with BRCA mutations often undergo bilateral prophylactic mastectomy,” noted Dr. Tot. But, he posited, “if it’s just the lobe and not the whole breast that is sick, isn’t it enough to just take out the sick lobe to eliminate breast cancer risk?”

In conclusion, Dr. Tot said, “I believe the sick lobe is the risk tissue in the breast, and that the ability to assess and treat the sick lobe could effectively prevent breast cancer.”

Towards a New Cartography of the Breast: The Astley Cooper Project

The second presentation on breast anatomy was by James Going, PhD, a senior lecturer in the Division of Cancer Sciences and Molecular Pathology at the University of Glasgow, Scotland. Dr. Going addressed “Towards a New Cartography of the Breast: The Astley Cooper Project.”



Dr. Going began by noting that the breast is comprised of many separate but closely packed lobes, each with one central duct in the nipple. However, he said, it is not yet fully understood how these lobes are organized within the breast.

Dr. Going discussed his “Astley Cooper Project,” a proposal for a research program that would “generate structural data about lobar organization of human breast tissue, with the ultimate goal of answering questions about and resolving current controversies surrounding the anatomy of the breast.” Dr. Going said the Project is intended to address questions such as: Are all lobes accessible to duct cannulation from the nipple? How many duct systems are there? Are there two distinct types of nipple ducts? And what is occurring in ducts that can be cannulated but appear to be going nowhere?

Dr. Going said that one of his primary aims is to convince other researchers in the breast cancer field of the need to advance our understanding of breast anatomy. Right now, he said, “anatomy seems old school, and it’s hard to get people interested. But you can’t understand the molecular biology of the breast if you don’t understand its anatomy. You need to put both together.”

Three-Dimensional Duct Anatomy in the Nipple

Jennifer Rusby, MD, of the Gillette Center for Breast Cancer at Massachusetts General Hospital, in Boston, presented “Three-Dimensional Duct Anatomy in the Nipple.”

Dr. Ruby began by noting how little is known about the anatomy of the nipple and by explaining how research on the nipple could improve ductal lavage and nipple-sparing mastectomy. She then described her team’s efforts to redress this problem by developing a three-dimensional reconstruction of the ducts in a nipple that could aid in both of these procedures.

To date, Dr. Rusby’s team has analyzed the relationship of the ducts to the nipple in 129 mastectomy specimens (Rusby, Breast Cancer Research and Treatment 2007). Dr. Rusby reported that they have found a median of 23 ducts in each nipple, with a central duct bundle narrowing to form a “waist” as the ducts enter the breast parenchyma. They have also found that beneath the skin most ducts are very narrow and then gradually become larger and deeper within the nipple. However, she said, they also found other ducts that terminate within 2cm of the nipple tip. Dr. Rusby said that she is now studying how these ducts differ from other ducts.

Dr. Rusby presented images of her team’s three-dimensional reconstruction of one nipple demonstrating 19 ducts arising from 15 orifices. Dr. Rusby noted that, as the reconstruction showed, many ducts share a few common openings on the nipple surface, which could explain the discrepancy between the number of ducts and the number of orifices. Interestingly, she added, neither duct diameter nor duct position predicts whether a duct system will terminate close to the nipple or pass deeper into the breast. In conclusion, Dr. Rusby said, the insights gained from this research will be useful for both the intraductal approach to breast cancer and surgical planning for nipple-sparing mastectomy.



Inside the Functional Breast—Ultrasound Imaging

Donna Ramsay Geddes, PhD, a research associate at the University of Western Australia, in Perth, closed the session on breast anatomy. She addressed “Inside the Functional Breast—Ultrasound Imaging.”

Dr. Geddes began by noting that the most comprehensive study of the anatomy of the lactating breast was carried out by Astley Cooper in 1840, and that little has been learned since then. To advance the field, Dr. Geddes and her team are using ultrasound to investigate the structures of the lactating breast, to assess the size and number of ducts at the base of the nipple, and to trace the ducts into the breast.

To date, Dr. Geddes said, her research team has studied 21 women who had breastfed between one and six months, and they have found an average of nine milk ducts, with a range of 4-18—fewer main milk ducts than most textbooks describe. Dr. Geddes described these milk ducts, which branch in an erratic, complex fashion, as relatively small (2mm), superficial, and compressible.

Dr. Geddes’ group also found that the lactating breast is composed predominantly of glandular tissue, and that the milk ducts increase in diameter at milk injection. Based on these findings, said Dr. Geddes, it appears that the main function of the ducts is to transport milk, not to store it. In conclusion, said Dr. Geddes, this research on the lactating breast advances our understanding of breast anatomy and may be useful in treating women with ductal problems and in carrying out intraductal research.

This Symposium session included a presentation by one pilot grant applicant.

Ductoscopy and Imaging

Ductoscopy, which involves inserting a microendoscope into the ductal opening of the nipple, is an emerging technique. Research studies and clinical trials on its use in the intraoperative setting in patients with benign or malignant conditions are currently being conducted.

Ductoscopy—Where We Stand

The session on ductoscopy and imaging opened with a presentation by William Dooley, MD, a professor of surgical oncology at the University of Oklahoma Health Sciences Center, in Oklahoma City. Dr. Dooley, a recognized leader in this field, addressed “Ductoscopy—Where We Stand.”

Dr. Dooley discussed his use of ductoscopy in his clinical practice and what it has taught him about breast anatomy and breast cancer.

In January 2000, Dr. Dooley began routine scoping of all of his lumpectomy patients in whom ductal orifices could be identified. As of February 2005, he reported, he had conducted routine operative breast endoscopy on 689 women. In early 2000, he also began using ductoscopy to examine the ducts of women who presented with bloody nipple discharge. In 2002, Dr. Dooley published a 20-month study of 27 of these patients



(ASO 2002). He reported that in 26 of the 27 women he was able to find the bleeding lesion. In addition, 2 of the 27 women were found to have cancer, while one-third had papillomas associated with ADH or cancer.

More recently, Dr. Dooley said, he has begun performing office-based ductoscopy in women with nipple discharge. He has now conducted office-based ductoscopy on 88 women. Most of these women were high risk, as determined by the Gail model, or had a prior breast cancer history. To date, he reported, 82 percent of these patients had abnormal findings endoscopically.

Dr. Dooley also discussed his use of routine operative breast endoscopy during breast cancer surgery and the dramatic difference it has made in his rate of local failure. "I am almost ready to say," he announced, "that local failure from residual disease in the ducts can virtually be eliminated if we can improve our mapping of the ductal system so that we can remove the affected ductal system." It is not possible to make a diagnosis with ductoscopy alone, he said. But using ductoscopy has shown us "we need to be sure we are taking out the ductal system when treating patients with breast cancer."

AutoFlourescence Ductoscopy for Semi-Quantitative Visual Differentiation of Benign Non-Benign Lesions

Volker Jacobs, MD, an ob/gyn at the Technical University, in Munich, Germany, presented "AutoFlourescence Ductoscopy for Semi-Quantitative Visual Differentiation of Benign Non-Benign Lesions."

In Germany, breast endoscopy is a gynecologic procedure performed by ob/gyns. Recently, Dr. Jacobs said, German ob/gyns have begun performing interventional ductoscopy and autofluorescence ductoscopy. Interventional ductoscopy uses a ductoscope with mini-forceps and a cytology brush or basket to remove papillomas, Dr. Jacobs said, while autofluorescence ductoscopy (AFD) is a new technique that can help distinguish benign from malignant lesions.

AFD involves a two-step imaging process. When autofluorescence is used, the healthy tissue, which has strong autofluorescence, appears red, Dr. Jacobs explained, "whereas the pathological tissue, which has weak or no autofluorescence, appears white." To make the pathological tissue visible, a picture inversion is performed, making the pathological tissue appear red or violet and the healthy tissue appear white.

Dr. Jacobs' group performed ductoscopy combined with AFD intraoperatively prior to lumpectomy in a series of four patients with one-sided suspicious nipple discharge to evaluate the usefulness of AFD as an additional diagnostic tool. Dr. Jacobs reported that they found that AFD was not only minimally invasive, but aided in breast cancer diagnoses. He will soon launch a controlled trial to determine if AFD is equal to or better than standard imaging techniques.

This Symposium session closed with presentations by four pilot grant applicants.



Saturday , March 3, 2007

Intraductal Therapy

The second day of the Symposium opened with presentations that addressed the research assessing the feasibility and safety of administering anti-tumor agents intraductally.

Preclinical Evaluation of Anti-Tumor Agents Administered by the Intraductal Route

Saraswati Sukumar, PhD, a professor of oncology and pathology at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins School of Medicine, in Baltimore, and a leader in the intraductal field, opened the session on intraductal therapy. She discussed "Intraductal Administration of Anti-Tumor Agents for Therapy and Prevention."

Dr. Sukumar discussed her research exploring whether intraductal injections of cytotoxic agents can effectively prevent cancer from occurring. "Given that more than 95 percent of breast cancers begin in the epithelial cells lining the milk duct system," said Dr. Sukumar, "ablation of the mammary epithelium with cytotoxic agents should prevent tumor formation." In addition, she said, "the effects of these agents should be confined to the mammary epithelial cells alone."

If this approach proves effective, Dr. Sukumar continued, it could provide a new form of breast cancer prevention for high-risk women. This would include women with a BRCA mutation, a family history of breast cancer, LCIS, or who, for other reasons, are candidates for a prophylactic mastectomy.

Dr. Sukumar's team has conducted a series of studies using rat and mouse models. One study was performed on Her-2/neu transgenic mice that had developed spontaneous tumors. In this study, half of the mice were given Doxil intraductally and the other half received it intravenously. Dr. Sukumar reported that the tumor response was better in the mice that received Doxil intraductally. However, she said, as the tumors grew larger, the response became poorer.

A second study explored whether intraductal treatment could prevent tumors from developing. In this study, mice were given repeated injections of Doxil intraductally or intravenously starting at age 20 weeks. Dr. Sukumar reported that all 10 of the mice treated with Doxil intraductally were living without tumors at the 16th week, whereas 9 of the 10 mice treated with Doxil intravenously developed tumors by 16 weeks.

Dr. Sukumar reported that the intraductal Doxil not only kept tumors from developing but also changed the mammary gland architecture during the time treatment was given. However, after the course of treatment ended, she said, "the mammary gland began to build itself up again." Yet, she noted, it never again looked exactly the same as an untreated mammary gland.

Dr. Sukumar's group has also studied the effectiveness of, and side effects associated with, the intraductal use of other forms of chemotherapy. One of these studies tested



carboplatin, methotrexate, 5-fluorouracil (5FU) and Doxil. Dr. Sukumar reported that this study found that 5FU resulted in hair thinning and anemia. In addition, a small number of tumors formed when carboplatin or methotrexate was used, but not when Doxil or 5FU was given.

Dr. Sukumar is continuing to investigate the effects of intraductal therapy. For example, she said, her team has begun introducing pregnancy after treatment to assess what impact intraductal chemotherapy has on the mammary gland and to determine whether the gland can still produce milk after it has been treated.

Effect of Perductal Paclitaxel Exposure on the Development of MNU-Induced Mammary Carcinoma in Female S-D Rats

Daigo Yamamoto, MD, of the Department of Breast Surgery at Kansai Medical University in Japan, presented, "Effect of Perductal Paclitaxel Exposure on the Development of MNU-Induced Mammary Carcinoma in Female S-D Rats."

Dr. Yamamoto discussed a study his team had conducted on the impact of intraductal paclitaxel in a rat model. The first step of the study involved injecting S-D rats with MNU for cancer. Next, the team used a fiberscope to see whether cancer had developed. Then, all the rats with cancer were divided into four groups: untreated, low-dose injection through the duct, high-dose injection through the duct, and high-dose intraperitoneal injection.

Dr. Yamamoto reported that the rats that received the paclitaxel through the duct had a lower tumor burden. Furthermore, paclitaxel did not produce any toxic side effects. Based on these findings, concluded Dr. Yamamoto, "the intraductal approach may be useful for early breast cancer treatment."

The Feasibility and Safety of Intraductal Pegylated Liposomal Doxorubicin (PLD, Doxil) in Women with Breast Cancer Awaiting a Mastectomy

The session on intraductal therapy closed with a presentation by Regina Brown, MD, an oncologist at the Cancer Center of the Rockies in Fort Collins, Colorado, who addressed "The Feasibility and Safety of Intraductal Pegylated Liposomal Doxorubicin (PLD, Doxil) in Women with Breast Cancer Awaiting a Mastectomy."

Dr. Brown discussed her study investigating whether local infusion of a standard or novel agent can eradicate pre-invasive disease or prevent breast cancer. To date, her study has enrolled four women with DCIS or breast cancer who are awaiting mastectomy. The first three women enrolled were dose level 0; these women received the vehicle, D5W. The fourth woman received 2mg of Doxil.

Dr. Brown explained that the vehicle/drug was administered after a ductogram was used to localize a ductal system. The duct was later identified in the mastectomy specimen by infusing blue dye into the duct. Dr. Brown reported that in the one woman who received Doxil, the mastectomy specimen showed changes to the duct "consistent with the clinical history of ductography and intraductal injection of anti-neoplastic agents."



Based on these findings, concluded Dr. Brown, “it is feasible to introduce chemotherapy into a duct.” The study will continue to enroll women to determine the safety and maximum tolerated dose of intraductal Doxil.

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

NAF: The Optimal Approach to Screening

The next session of the Symposium explored nipple aspirate fluid (NAF) and its role in breast cancer screening. NAF provides a window into the microenvironment of the breast and the breast ducts. The presenters discussed the importance of this microenvironment and the work being done to look for biomarkers in NAF that could be used to assess an individual woman’s breast cancer risk.

History of NAF Analysis for Cancer Detection and Risk Stratification

Edward Sauter, MD, PhD, an associate professor of surgery and vice chair of research at the University of Missouri Health Care Cancer Center, in Columbia, opened this session with the presentation “History of NAF Analysis for Cancer Detection and Risk Stratification.”

Dr. Sauter provided an overview of the history of research on breast fluid. The first person to discuss breast fluid was Sir Astley Cooper, who began studying breast anatomy in the 1800s. The field remained dormant until the 1950s, when Dr. George Papanicolaou, who developed the Pap test for cervical cancer, turned his attention to breast fluid. Dr. Papanicolaou began to conduct research on breast fluid, and, in 1958, he published findings from the first-ever large study evaluating nipple aspirate fluid.

In the 1970s, Dr. Nicholas Petrakis, professor emeritus in the Department of Epidemiology and Biostatistics at the University of California, San Francisco, began working with Dr. Otto Sartorius, a breast cancer surgeon and researcher who had become known as the man who wanted to develop a “Pap” test to detect breast cancer. Their work advanced the field further.

In 1983, Dr. Sartorius founded The Santa Barbara Breast Cancer Institute, and developed a research program that utilized ductography and nipple aspiration. Dr. Sartorius died in 1994. The following year, the Institute’s board of directors selected Dr. Susan Love to become its new medical director. In 2000, the Institute’s name was changed to The Susan Love MD Breast Cancer Research Foundation. In 2004, it was renamed the Dr. Susan Love Research Foundation.

Dr. Sauter closed his presentation with a short review of the advances made in analyses of NAF cytology and the candidate NAF biomarkers that have been studied.

Nipple Aspirate Fluid: Scope of Molecular and Proteomic Studies –MDACC Experience

The next presentation was by Savitri Krishnamurthy, MD, an associate professor of pathology at the University of Texas MD Anderson Cancer Center, in Houston, who



addressed “Nipple Aspirate Fluid: Scope of Molecular and Proteomic Studies –MDACC Experience.”

Dr. Krishnamurthy discussed her cytomorphological, molecular, and proteomic studies of NAF collected from the breasts of healthy volunteers and from women with breast cancer. Dr. Krishnamurthy reported that these studies have found that the foam cells in NAF appear to be macrophage in origin. However, she said, it is unclear whether these foam cells come from the tumor site or from normal tissue.

Dr. Krishnamurthy’s study also examined correlations between the cytologic findings in NAF and the histologic findings in breast tissue. This research showed, she said, that cells that appear benign to the naked eye might actually contain methylated genes. This indicates, she said, that studies of gene methylation “have a role to play in further identification of cells in NAF in differentiating truly benign and atypical cells.”

Dr. Krishnamurthy reported that her team’s proteomic SELDI-TOF analyses found no significant differences in NAF taken from the cancerous breast and NAF taken from the contralateral breast. Differences were seen, however, when healthy women were compared with women with breast cancer. In addition, she said, an association was found between protein expression patterns and the number of positive axillary lymph nodes, indicating that unique expression patterns may be associated with the extent of disease.

In conclusion, Dr. Krishnamurthy said, “Our studies of cellular and protein components of NAF support the potential for the discovery of biomarkers that may be useful for breast cancer detection and monitoring, targeted therapy of breast cancer, and risk identification.”

Biomarker Discovery in Breast Fluids—Validation of BC5 and Its Protein Identification

The third presentation in this session was by Jinong Li, PhD, from the Center for Biomarker Discovery at Johns Hopkins University in Baltimore, MD, who addressed “Biomarker Discovery in Breast Fluids—Validation of BC5 and Its Protein Identification.”

Dr. Li discussed his research team’s discovery of a 4.7KD peptide marker (BC5) in the ductal fluid of women with breast cancer that was not present in the ductal fluid of healthy women. Following this discovery, the team performed a validation study on prospectively collected breast fluid from women with and without breast cancer.

This study, Dr. Li explained, involved bilateral nipple aspiration on 31 women with unilateral breast cancer or atypical ductal hyperplasia (ADH) and on 32 women with no apparent breast disease. Dr. Li reported that elevated expression of BC5 was observed on mass spectrometry in NAF from the affected breasts of 4 women with invasive cancer, 1 patient with DCIS, and 1 with ADH. None of the healthy women had breast fluid that contained BC5. Neither did the unaffected breasts of the cancer patients.



Dr. Li also reported that peptide finger printing and subsequent tandem mass fragmentation identified BC5 as a 41/42-aa C-terminal of alpha1-antitrypsin (AAT). He said that C-41/42 might represent a specific marker for breast cancer, but that the effect of AAT, which is present in various molecular forms with distinct functions, on tumor growth and progression is not known.

Dr. Li is now developing specific assays for each molecular variant that can assess various forms of AAT in breast fluid, test the cocktail effect of AAT on tumor growth and metastasis, and capture C-41/42 in the serum of women with breast cancer.

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

Methylation Markers for Early Detection of Breast Cancer

Mary Jo Fackler, PhD, an assistant professor of oncology at the Sidney Kimmel Cancer Center at Johns Hopkins School of Medicine in Baltimore, followed with the presentation "Methylation Markers for Early Detection of Breast Cancer."

Dr. Fackler discussed her efforts to develop ancillary methylation tests for breast cancer that could be used in the clinical setting along with cytology for cell analysis.

Dr. Fackler explained that methylation is a heritable DNA modification that occurs without a change in DNA sequence, and that gene promoter hypermethylation is one of the earliest and most frequent changes that occurs in tumor cells. When this methylation occurs in tumor suppressor genes, Dr. Fackler said, it prevents gene expression, which permits tumor growth. In addition, "multigene methylation is common in breast cancer and appears very early in the development of breast cancer."

Dr. Fackler noted that one of the problems facing researchers using ductal lavage, fine needle aspiration, and nipple aspirate fluid is that these procedures typically only produce small amounts of cellular material, which makes performing tests on these fluids challenging. To redress this problem, Dr. Fackler and her team have developed a novel method called quantitative multiplex methylation-specific PCR (QM-MSP) that can detect gene methylation in a panel of genes when only small amount of cellular material are present (Fackler, Clinical Cancer Research 2006).

Dr. Fackler reported that a comparison of histology, cytology, and QM-MSP using a 9-gene marker panel found that cumulative methylation is low in high-risk women, but significantly higher in women with cancer. Her team also found a 100 percent correlation with QM-MSP when cytology was positive (marked atypia or malignant) on ductal lavage.

The challenge for the future, concluded Dr. Fackler, "is to develop an approach to applying QM-MSP to cellular samples collected from regions of the breast under transition towards breast cancer" and to determine which "ideal method of sampling the breast will provide an accurate representation of breast health."



Cytoskeletal Actin Remodeling as a Target for Cancer Biomarker Development

JianYu Rao, MD, an associate professor of pathology/epidemiology at the David Geffen School of Medicine at the University of California, Los Angeles, discussed “Cytoskeletal Actin Remodeling as a Target for Cancer Biomarker Development.”

As Dr. Rao explained, cytoskeletal actin is a major structural and functional protein in the cell. In addition, actin remodeling plays an essential role in regulating many different aspects of cancer cell phenotypes.

Dr. Rao’s research has analyzed whether expression profiles of proteins associated with actin remodeling can provide useful molecular makers for early detection and progression prediction of breast cancer. His team has also studied whether signaling pathways associated with actin remodeling may provide a potential target for anti-cancer drug development.

Dr. Rao reported that this research indicates that actin remodeling in cancer cell progression may be a dynamic process that might assist in gauging a cancer’s development. “Altered actin polymerization, as reflected by shifting of G versus F-actin, appears to be a marker for cancer detection and progression,” concluded Dr. Rao. “In addition, actin binding and signaling proteins may be a more specific marker than actin polymerization,” while actin remodeling might be a novel target for anti-cancer drug development and a marker for monitoring how a patient is responding to therapy.

This Symposium session included one presentation by a pilot grant applicant.

Clinical Applications of NAF and Ductal Lavage

Studies have found that women with NAF that contains epithelial cells are at higher risk for breast cancer than women who do not produce NAF at all. In this session, researchers discussed what we have learned from studies on diet, hormones, and other factors that affect NAF and how this information could be used in the clinical setting to assess breast cancer risk.

Nipple Aspirate Fluid Cytology and Subsequent Breast Cancer Risk

Gertrude Buehring, PhD, an associate professor in the Department of Virology at the University of California, Berkeley, School of Public Health, opened this session with the presentation “Nipple Aspirate Fluid Cytology and Subsequent Breast Cancer Risk.”

Dr. Buehring reported findings from her 25-year follow-up study of a cohort of Bay Area women that assessed the relationship between NAF cytology and subsequent breast cancer risk. Dr. Buehring discussed the original cohort and the work that went into locating the women for her follow-up study. She reported that her team was able to locate 972 out of the 1605 women originally studied. Significantly, the located group was representative of the eligible cohort for established breast cancer risk factors.

Dr. Buehring reported that the follow-up study found that women with NAF that did not contain epithelial cells were not more likely to develop breast cancer than were women



with no NAF. However, she said, women with NAF containing epithelial cells were significantly more likely to develop breast cancer than were women with no NAF. Dr. Buehring reported that they also found, “that at 22-23 years after sampling, the rate of breast cancer climbs steeply, giving us a good time course of breast cancer development.”

In conclusion, Dr. Buehring said, “NAF has proved to be a useful screening test for triaging women into different categories. No fluid, is no increased risk, while the presence of epithelial cells or atypical cells, means a woman is likely to be at higher risk.”

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

Abnormal Cytology in NAF and Subsequent Breast Cancer Risk

Kimberly Baltzell, PhD, an assistant adjunct professor in the School of Nursing at the University of California, San Francisco, discussed “Abnormal Cytology in NAF and Subsequent Breast Cancer Risk.”

Dr. Baltzell discussed her analysis of NAF cytology and subsequent breast cancer risk in a subset of the 2480 women seen by Dr. Otto Sartorius in his Santa Barbara, California, breast clinic between 1970 and 1990. Dr. Baltzell explained that this group of 946 women represented an aggregate of two sub-groups: women with questionnaire data and women with NAF visits beginning in 1988.

Overall, Dr. Baltzell reported, 10% (93) of the 946 women in this subset developed breast cancer during the follow-up period. Specifically, she said, breast cancer incidence was 9% (63 of 714) in women from whom no fluid could be obtained, compared with 12% (11 of 89) in women with normal epithelial cells and 28% (17 of 124) in women with hyperplasia/atypia.

In conclusion, Dr. Baltzell said, it is notable that these findings support previous findings by Wrensch (1992, 2001) and Buehring (2006) that women with epithelial cells in NAF are at higher risk for developing breast cancer than are women with no NAF or NAF without epithelial cells.

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

The Relation of Soy Intake and Isoflavone Levels in Nipple Aspirate Fluid

The third presentation on clinical applications of NAF and ductal lavage was by Gertraud Maskarinec, MD, PhD, an associate professor at the Cancer Research Center of Hawaii at the University of Hawaii. Dr. Maskarinec discussed “The Relation of Soy Intake and Isoflavone Levels in Nipple Aspirate Fluid.”

Dr. Maskarinec began by noting that studies have suggested that isoflavone intake may be protective against breast cancer by having a direct effect on breast tissue. One way



to detect whether isoflavones induce changes in the breast, she said, is by measuring the level of isoflavones found in nipple aspirate fluid.

Dr. Maskarinec described a pilot study she conducted to assess whether consuming two servings of soymilk per day (25mg per serving) affected isoflavone concentrations and estradiol and estrone levels in NAF or the cytologic characteristics of epithelial breast cells. To measure whether changes occurred, NAF and urine samples were collected at the start of the study and after one month of soy consumption.

Eleven women were enrolled in the study. Dr. Maskarinec reported that no significant change in NAF volume or estradiol levels during one month of soy in these 11 women. She also reported that the isoflavonoid levels in NAF were 10 times lower than in plasma, but were closely related to the levels of isoflavanoids in urine and plasma. This suggests, Dr. Maskarinec said, that isoflavanoids are present in breast fluid and may act directly on breast tissue.

After completing the pilot study, Dr. Maskarinec and her team launched a second study called the BEAN2 Study (Breast, Estrogen, and Nutrition). This randomized cross-over study will enroll 120 women, and will measure and compare estrogen levels in NAF, blood, and urine in women consuming two daily servings of soy.

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

Nutritional Predictors of Cellular Nipple Aspirate Fluid

The next presentation was by Zora Djuric, PhD, a research professor in the Department of Family Medicine at the University of Michigan Comprehensive Cancer Center. Dr. Djuric addressed "Nutritional Predictors of Cellular Nipple Aspirate Fluid."

Dr. Djuric discussed findings from her study that explored whether the presence of epithelial cells in NAF was associated with nutrient intake among 71 healthy premenopausal women who had participated in the Nutrition and Breast Health Study.

Dr. Djuric reported that the probability of finding epithelial cells in NAF progressively increased with increasing total fat intake. Her team also found that the probability of detecting epithelial cells in NAF decreased with increasing plasma levels of lutein, which is found in high levels in dark green vegetables, and alpha-carotene, which is found in high levels in dark orange vegetables. Based on these findings, Dr. Djuric concluded, "if cellularity in NAF is indeed a risk factor for breast cancer, these results support role of nutritional factors in breast cancer."

Dr. Djuric recently launched a new study, called the Benign Breast Markers Study, which will examine cellular morphology and NAF composition in women who have had proliferative breast disease previously diagnosed by biopsy.



Bio-molecular Characterization of Nipple Aspirate Fluids to Identify Biomarkers of Breast Cancer

The next speaker, Ferdinando Mannello, PhD, a researcher at the Institute of Histology and Laboratory Analysis at the University “Carlo Bo” Urbino, Italy, addressed “Bio-molecular Characterization of Nipple Aspirate Fluids to Identify Biomarkers of Breast Cancer.”

Dr. Mannello discussed his research on biochemical and molecular markers in NAF associated with breast cancer. Dr. Mannello reported that NAF from women with breast cancer contains “abnormal” epithelial cells. These cells, he explained, “are ultrastructurally identified as biosynthetically active apocrine cells that show interdigitated cytoplasmic protrusions on the cell surface and several zonulae occludens.” In contrast, he reported, NAF from healthy women contains epithelial cells that are “infrequently clustered and linked mainly by zonulae adherents.” This could explain, Dr. Mannello said, the different levels of a variety of compounds found in NAF and why NAF may mirror the breast microenvironment.

Dr. Mannello also reported that his team has identified “high levels and specific isoforms of proteolytic enzymes that may enhance the detachment of epithelial cells from the basal membrane” into the NAF of women with breast cancer. The team also found that NAF from women with breast cancer contains the expression of erythropoietin, which may be a biomarker for breast cancer progression. Lastly, Dr. Mannello reported, his team found high concentrations of homocysteine in the NAF of women with breast cancer, which, he said, suggests that “this amino acid may trigger early molecular transformation of breast ductal epithelial cells.”

In conclusion, said Dr. Manello, these findings advance our understanding of NAF and could lead to the identification of useful biomarkers in NAF for diagnosing women at higher risk for breast cancer.

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

Gene Methylation in Ducal Fluid from BRCA1 and BR CA2 Carriers as a Predictor of Breast Cancer Risk

The next presentation on clinical applications of NAF and ductal lavage was by Gillian Mitchell, MD, an oncologist at the Peter MacCallum Caner Center, Melbourne, Australia. Dr. Mitchell discussed “Gene Methylation in Ducal Fluid from BRCA1 and BRCA2 Carriers as a Predictor of Breast Cancer Risk.”

Dr. Mitchell began by explaining that hypermethylation of tumor suppressor genes is believed to precede the phenotypic changes associated with breast cancer. Studying the epithelial cells, she said, provides a way to evaluate these changes.

Dr. Mitchell reported findings from a study she conducted of 34 women with breast cancer who were known to carry a BRCA1 or BRCA2 mutation. These women



underwent ductal lavage every six months for up to three years, resulting in a total of 173 samples from 98 ducts in 56 breasts. Dr. Mitchell and her team analyzed the ductal fluid for methylation in the promoter region of p16, RASSF1A, RARbeta, and twist. They found methylation of p16 in ductal fluid from 21 of the breasts. Additionally, she said, methylation of p16 was both strongly associated with a known BRCA1 mutation and more common in women who had a history of contralateral breast cancer.

Although further research is needed to validate these findings, “it is possible,” Dr. Mitchell said, “that p16 methylation may represent a further feature of women with a history of breast cancer who carry a BRCA1 mutation and that p16 methylation may be predictive of breast cancer development.”

LOH and Gene Promoter Hypermethylation in Ductal Lavage Fluid From Healthy BRCA Gene Mutation Carriers and Controls

Dominique Twelves, MRCS, a surgeon at the Royal Marsden Hospital in London, UK, presented “LOH and Gene Promoter Hypermethylation in Ductal Lavage Fluid From Healthy BRCA Gene Mutation Carriers and Controls.” (Dr. Twelves presented for Gerald Gui, MD, who was unable to attend.)

Dr. Twelves discussed research conducted by Dr. Gui and his group that explored whether healthy BRCA mutation carriers are more likely than women who do not carry a BRCA mutation to have an increased frequency of aberrant gene promoter hypermethylation or loss of heterozygosity (LOH) in ductal lavage fluid.

The LOH analysis was of “free” DNA extracted from the ductal lavage samples and involved 33 samples from 17 healthy women of known BRCA status. Dr. Twelves reported that Dr. Gui and his group found an LOH rate of 36.4% - 56.3% at the BRCA1 locus and 45%-61.5% at the BRCA2 locus. In contrast, she said, the LOH rate at the APC control locus was 18.5%. Although the LOH results were consistent between separately extracted aliquots of DNA for 14/21 samples, “the inter-aliquot reproducibility for the marker of the BRCA1 locus was only 66.7 percent,” said Dr. Twelves, “while the intra-aliquot reproducibility was 90 percent. This finding, she said, “raises questions about the use of LOH as a risk assessment tool.”

The hypermethylation study evaluated 51 ductal lavage samples from 24 women of known BRCA status (5 were controls). Dr. Twelves reported that 8 of 19 BRCA mutation carriers had at least 1 hypermethylated gene in a four-gene panel (RARbeta, HIN-1, Twist, and Cyclin D2), whereas none of the 5 controls did. HIN-1 was the most frequently methylated gene, while CyclinD2 was the least. A comparison of these findings with the cytology results, found that 2 BRCA mutations carriers with hypermethylation also had asymptomatic atypia.

In conclusion, said Dr. Twelves, “methylation is a frequent event in the breasts of apparently healthy BRCA mutation carriers, however more research is needed to evaluate the specificity and predictive value of hypermethylation in women with a known BRCA mutation.”



Laser Capture Microdissection and Real Time PCR to Assess Breast Biomarker Gene Expression in Paired Archival Specimens Collected by Random Periareolar Fine Needle Aspiration (RPFNA) and Ductal Lavage (DL)

The next presentation was by Brian Petroff, DVM, PhD, an assistant professor in the Breast Cancer Prevention Center at the University of Kansas Medical Center. Dr. Petroff discussed “Laser Capture Microdissection and Real Time PCR to Assess Breast Biomarker Gene Expression in Paired Archival Specimens Collected by Random Periareolar Fine Needle Aspiration and Ductal Lavage.”

Dr. Petroff began by explaining how risk biomarkers are used in prevention to predict and measure response. Currently, he noted, morphology remains the only breast tissue biomarker validated in prospective trials. However, he said, it is biologically plausible that molecular markers could be used in addition to cytomorphology to predict and/or assess response but the number of markers is limited by sample size.

Dr. Petroff investigated whether the use of real time PCR for RPFNA/DL samples would allow quantitation of expression of multiple genes from the small number of cells obtained through these procedures. Dr. Petroff used laser capture microdissection to capture the cells, amplified RNA before real time PCR was performed, and designed real time assays that could be used on RNA/cDNA molecules. Dr. Petroff reported that archived breast epithelial cells harvested by RPFNA and followed by laser capture yield RNA/cDNA of sufficient quantity and quality for PCR analysis following one round of linear amplification. He also reported that equivalent paired DL specimens generally yielded lower amounts of lower quality RNA/cDNA, and that RNA yield and/or stability appeared to be greater for fixed and archived RPFNA than DL specimens. The poor cDNA from DL, he said, “may reflect initial RNA quality, less stability with storage and/or need for optimization of protocol for DL specimens.”

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

Nipple Aspiration Fluid Yield and Risk Reduction Therapies

Bonnie King, PhD, of the Department of Biological Sciences at Quinnipac University in Hamden, CT, discussed “Nipple Aspiration Fluid Yield and Risk Reduction Therapies.”

Dr. King began by noting that a previous multicenter trial found that NAF-yielding ducts were observed in 84 percent of the women studied. However, in a post-trial series of similar high-risk women, only 36 percent of the women had NAF-yielding ducts. Why the discrepancy? Dr. King said that the second series differed in that it included more women with BRCA mutations, many of who had undergone bilateral oophorectomy and/or were using SERMs. This suggests, said Dr. King, that “women on risk reduction therapies or who have had an oophorectomy are less likely to have NAF.” It may also mean, Dr. King said, that “NAF reflects estrogen-driven breast cancer progression.” But, Dr. King added, there are also a “number of paradoxes.” For example, the majority of BRCA1 tumors are ER-negative, yet oophorectomy and SERMS reduce breast cancer risk in these women.



Still, Dr. King concluded, it is possible that “endocrine mechanisms associated with risk-reducing therapies may be related to patterns of diminished breast fluid production.” This could mean, said Dr. King, that NAF may be an indicator of endogenous reproductive hormones implicated in breast cancer development. It might also mean that, for high-risk women, NAF may provide additional information about a woman’s risk profile or serve as an endpoint in intervention trials.

This Symposium session included six presentations by pilot grant applicants.

Sunday, March 4

Are We Ready to Launch a Large NAF Screening Trial?

The conference concluded with a group discussion that explored “Are We Ready to Launch a Large NAF Screening Trial?”

Dr. Love opened the discussion by describing the need for a breast cancer risk assessment test that could be used in places where mammography is not readily accessible. “NAF is the perfect way to assess risk,” she said. “We need to think about not what would work here, but what would work in other places in the world that don’t have the access to mammography that women here have.”

The discussion then focused on what markers in NAF might be incorporated into a screening test that could be used to find high-risk women in resource poor settings. It was noted that whatever markers were selected would need to be assayed easily. It was also noted that if a protein marker were used, a cut-off point would be needed to differentiate between normal levels and higher risk (like with the PSA test.) Conference participants’ suggestions for markers included estradiol, apolipoprotein D, vitamin D binding protein, mammoglobin, carbohydrate markers, and GCP15.

Dr. Love suggested that a test that it would be fine to have a test that was highly sensitive but not necessarily highly specific, since the goal was to develop a first-level screening program that could assess who needed additional services. She also discussed the possibility of working with a Chinese research group to evaluate a NAF screening test in conjunction with a new mammography-screening program now getting underway in China.

Conference participants noted that if the study was carried out in a country with a homogenous population, like China, it could not be assumed that the NAF test would be applicable to women in other countries, since NAF may vary by ethnicity. It was also noted that the test would need to be validated in order to avoid the problems that have plagued the PSA test. Some participants thought it was premature to use a panel, since none of the markers suggested have been validated. Others felt the China study provided an amazing opportunity to move the field forward.



Pilot Grants

The Dr. Susan Love Research Foundation knows that we are not the only ones who have exciting new ideas. For that reason, an important part of our mission is to encourage others to think about the intraductal approach to breast cancer. At each Symposium we offer pilot grants that can help researchers get the preliminary data they need to apply for a larger government or private foundation grant. As this report shows, many of our previous grantees presented their data at our 2007 Symposium. In addition, at least two of our 2005 grantees have parlayed their pilot grant into funding for a larger more definitive study.

The Foundation has implemented an innovative mechanism to distribute these pilot grants. Applicants submit a one-page abstract of their proposal. At the Symposium, a multidisciplinary Peer Review Committee composed of basic scientists, breast cancer activists, and surgeons listens to the presentations and gives advice to the applicants on how to improve their projects. The Committee then meets to review the grant proposals, to decide who will receive the pilot grant awards, and to determine the amount of each award. The grants are announced at the end of the Symposium. This year, the Foundation gave out \$100,000 in research grants to support the pilot work of 12 promising researchers utilizing the intraductal approach.

The 2007 recipients of these pilot grants were:

Oncogenic Viruses in Nipple Aspirate Fluid: Biomarkers for Breast Cancer Risk Assessment?

Kimberly Baltzell, RN, PhD, Assistant Adjunct Professor, University of California, San Francisco.

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

Impact of Diet and Exercise Intervention on Breast Ductal Fluid Among Overweight to Obese Postmenopausal Women

Catherine Carpenter, PhD, Adjunct Assistant Professor, University of California, Los Angeles.

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

Electronic Ductal Lavage as a Non-Invasive Technique to Identify Proliferative Changes and Altered Potassium Permeability Within the Ductal System of the Breast

Richard Davies, MD, Chairman, Department of Surgery, Hackensack University Medical Center, and University of Medicine and Dentistry, New Jersey.

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

Resolving Paradoxes of Human Nipple Duct Anatomy

James Going, PhD, Senior Lecturer and Consultant Pathologist, University of Glasgow Division of Cancer Science and Molecular Pathology, Scotland, UK.

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



An Intraductal Approach to Breast Cancer Risk Assessment and Screening in a Diverse Population

Jon Greif, DO, Surgeon, Carol Ann Read Breast Health Center, Alta Bates Summit Medical Center, Oakland, California.

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

Ductoscopic Biopsy and Ductoscopy Navigated Minimal Invasive Therapy of Intraductal Breast Lesions

Michael Hunerbein, MD, PhD, Department of Surgery and Surgical Oncology, Charite Campus Buch and Helios Hospital, Universitätsmedizin Berlin, Germany.

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

Searching New Biomarkers in Nipple Aspirate Fluid: Peroxidation Status and Adhesive Molecules to Early Identify Breast Cancer

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

This study was awarded a \$7,500 research grant from the Foundation.

MMP-1 and CEACAM6 Expression in Ductal Lavage Cells to Predict Breast Cancer Development

Indira Poola, PhD, Research Professor, Biochemistry and Molecular Biology, Howard University College of Medicine, Washington, DC.

This study was awarded a \$15,000 research grant from the Foundation.

Elastic Scattering Spectroscopy and Photodynamic Therapy for Diagnosis and Treatment of Ductoscopic Abnormalities.

Santosh Kumar Somasundaram Research Fellow, Royal Free and University College Medical School, University College London, UK.

This study was awarded a \$7,500 research grant from the Foundation.

Evaluation of Anti-Cyclin B1 Antibodies in Nipple Aspirate Fluid

Atilla Soran, MD, Professor of Surgery, Magee Women's Hospital of University of Pittsburgh Medical Center, Pittsburg, PA.

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

Intraductal Micro Magnetic Resonance Imaging and Spectroscopy

Debra Strick, Doctoral Candidate, University of California, Los Angeles.

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

Effects of Flaxseed on Nipple Aspirate Fluid Levels of Biomarkers of Breast Cancer Risk

Susan Sturgeon, PhD, Associate Professor, University of Massachusetts Amherst School of Public Health and Health Sciences.

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