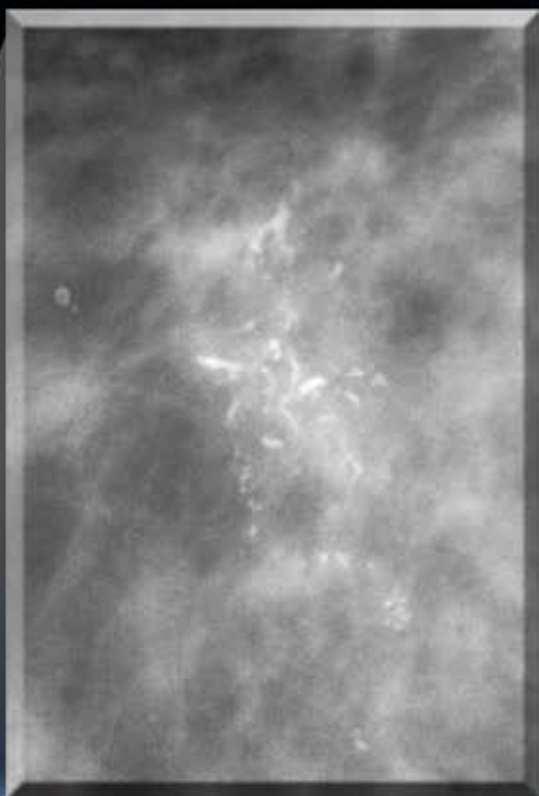


# **Ductal Cell Carcinoma *in Situ*: Strategies for Integrating Tumor Biology and Population Sciences**

**February 1-2, 2007**



**Crowne Plaza Union Square  
480 Sutter Street  
San Francisco, CA 94108**



**NATIONAL  
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**Division of Cancer Control and Population Sciences  
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**Summary Report**

# Ductal Carcinoma *in Situ*: Strategies for Integrating Tumor Biology and Population Sciences

## Summary Report

**THURSDAY, February 1, 2007**

### **Welcome and Overview of Purpose of Workshop**

Rachel Ballard-Barbash, M.D., M.P.H., Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD

Dr. Ballard-Barbash welcomed participants to the workshop and explained that the purpose of this workshop is to bring together investigators studying ductal carcinoma *in situ* (DCIS) in population-based samples as well as experts in disciplines such as basic science, clinical trials, and risk modeling. Because advances in technology and screening methods have resulted in increased diagnosis of DCIS, one of the goals of DCIS research is to predict prognostic factors for the disease. The goals of the workshop are to obtain a better understanding of the capacity to share data and collaborate on methods with respect to DCIS research; discuss the best markers for prognosis in population samples, particularly with respect to risk of occurrence and factors that affect prognosis; and identify limitations of the current setting and infrastructure for population-level DCIS research and develop strategies for overcoming these limitations. The participants reflect a wide array of expertise and various arenas related to DCIS research.

Dr. Ballard-Barbash then reviewed the agenda and informed participants what to expect during each session. Also, participants were assigned to various breakout groups according to their expertise.

The meeting planners have conceptualized a list of products they would like to see evolve from the meeting, and participants should provide input if there are additional ideas for meeting products that would be helpful. The expected meeting products are: a list of needs and resources to advance the research on identifying predictive measures of DCIS risk and prognosis; enhanced collaboration and increased potential for transdisciplinary research; brief reports/summaries of these research needs and potential resources, which will be distributed via the Web, listservs, and/or journal articles; and sharing of presentations on the National Cancer Institute's (NCI) Division of Cancer Control and Population Sciences (DCCPS) Web Site. Additionally, there is a list of available funding opportunities provided in the meeting notebooks. Dr. Ballard-Barbash acknowledged the planning committee, especially Ms. Emily Dowling, for their efforts.

### **Overview of Current Definition of Ductal Carcinoma *in Situ***

Donald L. Weaver, M.D., University of Vermont College of Medicine, Burlington, VT

During the 1890s, comedocarcinoma was defined as a clinically palpable mass with foci of dilated ducts containing necrosis that could be expressed from the cut section of the tumor and not dependent on a microscopic evaluation. At this time, surgical pathologists were surgeons interested in describing the tissue or cellular basis of disease through visual and manual inspection of removed tissue for purposes of surgical decisionmaking. By 1911, the cells of breast carcinoma were noted to look identical whether present in a duct or within the connective tissue, which led William MacCarty of the Mayo Clinic to ask, in 1913, whether it is necessary to identify penetration of the basement membrane to establish a diagnosis of carcinoma, because empirically patients did poorly whether the disease was in the duct or the connective tissue. In 1920, surgery and surgical pathology diverged into distinct specialties, and by the 1930s pathologists had established that intraductal carcinoma existed, but it was a subtype of typical, invasive carcinoma; the term carcinoma *in situ* was coined. In the 1950s, Stout and Haagensen defined intraductal carcinoma as a tumor with "at least 50 percent of tumor within ducts...infiltrating and fully

malignant” (i.e., invasion did not have to be observed on the slide for a diagnosis). The problem at this time was that large tumors typically were undersampled, making it difficult to reliably exclude invasion. From the 1950s to the 1970s, there was a profound change in the evolution of DCIS, with the published incidence of node metastases decreasing from 29 percent to less than 1 percent, presumably as pathologists became better able to distinguish pure DCIS. In the 1970s, electron microscopy demonstrated that DCIS has cytoplasmic extensions through gaps in the basement membrane, and in the late 1970s to early 1980s, lower grade, noncomedo forms of DCIS were recognized widely. Finally, in the 1980s, DCIS was established as a distinct clinical-pathological entity. At this time came the realization that not all DCIS needed to be treated by mastectomy, and the concept of preneoplasia emerged.

The single most important event in diagnosing DCIS, however, was the introduction of mammography, with its ability to detect calcifications. The defining features of DCIS are: malignant cytologic features (monomorphic or pleomorphic); two duct cross-sections must be involved and/or the sum of the involved duct diameters must be greater than 2 mm (volume); all malignant cells must be confined to the basement membrane inside the duct; and single cells or angular clusters must not be present outside specialized stroma of the lobule. Additionally, DCIS can be graded as low (good prognosis) to high (poor prognosis) based on the nuclear grade and amount of necrosis. Because low-grade DCIS and atypical ductal hyperplasia (ADH) have similar cytology and genetic abnormalities, volume helps distinguish the two. In high-grade DCIS, the absence of basal myoepithelial layer indicates invasion.

Because of the change in definitions and perception, it is difficult to retrospectively examine DCIS in historical medical records. Additionally, grading schemes differ, margins may be recorded differently or not at all, and volume documentation is poor. Data are heterogeneous and of variable quality, and even with the introduction of College of American Pathologist (CAP) checklists, this trend probably will continue in the future. Because DCIS grades and cytology are similar to invasive cancer, the classification of DCIS may mirror that of invasive cancer. It is important to remember that there are only certain cells within the duct that can differentiate and lead to cancer. The progenitor cell concept, in which two lineage pathways exist, can be superimposed on the types of carcinomas that are seen in breast cancer and several categories of cancers emerge. At this point it is not known if the tumors come from dedifferentiated cells or aberrant differentiated progenitor cells.

### **Epidemiology of DCIS**

Karla Kerlikowske, M.D., University of California at San Francisco, San Francisco, CA

Once rare, DCIS now comprises 20 percent of all breast cancers, with an estimated 60,000 new cases diagnosed in 2006. Diagnosis of DCIS has increased as a result of screening mammography. Although there was a 500 percent increase of DCIS diagnosis from 1983 to 2003, the amount of cases being diagnosed each year has reached a plateau since the late 1990s. The increase in DCIS diagnosis has paralleled the increase in mammography machines since 1980, and 1 in 1,300 screening exams leads to a diagnosis of DCIS. Although the expectation is to observe a decrease in invasive cancer following increased screening capabilities, this has not been the case, which leads to the question whether DCIS is the premalignant lesion for invasive cancer or if it is a marker of subsequent invasive disease. Incidence of DCIS increases with age before decreasing again after the ages of 70 to 75. As incidence of DCIS is similar in white and black women, but white women have a higher incidence of invasive cancer, it is necessary to examine how much DCIS actually is a precursor to invasive cancer. The risk factors for DCIS are similar to that of invasive cancer and include a family history of breast cancer, nulliparity or late age at first birth, previous breast biopsy, and late age at menopause. No association with smoking, body mass index (BMI), alcohol consumption, or oral contraception was found. Hormone therapy, however, does significantly increase the risk for DCIS. Mammographic density, as measured by the Breast Imaging Reporting and Data System (BI-RADS) scale, displays an increased risk, similar to that of

invasive cancer, for women with dense versus fatty breasts. Carriers of oncogenes *BRCA1* and *BRCA2* also are at increased risk for DCIS.

DCIS is most commonly diagnosed on mammography, with 80 to 85 percent of cases being detected in this manner. Linear or multiple clusters of fine granular calcifications with a branching-type pattern are indicative of DCIS on mammogram; a core biopsy or needle localization and excisional biopsy are used for confirmation. NCI's Breast Cancer Surveillance Consortium has observed that sensitivity of mammography for DCIS is higher than that of invasive cancer, and although DCIS incidence increases with age, it does not increase at the rate of invasive cancer. Digital imaging is more sensitive than screen-film, and the use of both in screening increases the sensitivity synergistically.

Treatment of DCIS is relatively aggressive, with the majority of patients undergoing lumpectomy or lumpectomy combined with radiotherapy. The number of patients undergoing mastectomy for DCIS is decreasing. Fifteen percent of women diagnosed with DCIS will have a subsequent invasive event within 10 years, and the percentage of women with DCIS dying of breast cancer is low. A Canadian screening mammography trial in women aged 50 to 59 indicated that although a mammogram in conjunction with a clinical breast examination significantly increased the number of DCIS cases diagnosed, the number of breast cancer deaths was not affected by the additional mammographic screening. Therefore, the dilemma with increased DCIS detection is that mammography cannot distinguish DCIS lesions associated with subsequent invasive cancer events from those without subsequent tumor events, and there are no criteria that can consistently risk stratify women with DCIS into the categories of those most likely to have a subsequent DCIS event, those likely to have an invasive event, and those likely to have no tumor event. In summary, DCIS is a relatively common diagnosis with a stable incidence during the last 5 years, and mammography is a strong risk factor for diagnosis.

### **Overview of Basic Biology: Precursors Through Invasive Disease and Future Directions**

Thea Tlsty, Ph.D., University of California at San Francisco, San Francisco, CA

Researchers are learning a lot about breast biology and how ductal structure grows during development. During the female cycle, changes occur on a monthly basis; histological changes are associated with breast cancer risk. The majority of women who undergo biopsies for benign lesions or DCIS are not at an increased risk for invasive breast cancer, and of those with increased risk, only a small proportion will develop disease. The identification of clinically relevant markers is needed to distinguish between those who will benefit from therapy and those who will not benefit from therapy. Relying on histology alone is not enough as dynamic processes cannot be observed, critical events cannot be identified, and therapeutics cannot be evaluated rapidly; therefore, *in vitro* models are preferable. Because DCIS occurs in small, sporadic lesions and early molecular changes are not fully understood, there is difficulty in studying the early steps of breast cancer. *In vitro* models could provide insight into molecular mechanisms and translate into clinical tools.

The laboratory has been studying a tissue culture model that allows cells to be studied in two and three dimensions. The researchers found a variant subpopulation of human mammary epithelial cells (HMEC) that have the ability to continue growing as a result of a silenced *p16* gene; *p16* is a cell cycle regulator and an important tumor cell suppressor gene that can be silenced as a result of hypermethylation of the promoter. The HMEC variants (vHMEC) provide a good opportunity to study the precursors of breast cancers. vHMEC cells are resistant to apoptosis, lack the ability to differentiate, and acquire genomic changes in a profound manner. The researchers concluded that the silencing of *p16* confers epigenetic plasticity. One out of 10,000 to 100,000 HMECs has a silenced *p16* gene. The researchers examined whether loss of *p16* is necessary and sufficient for allowing the expression of the variant phenotype and determined that it is.

The researchers used comparative genomic hybridization (CGH), a population-based analysis, to determine if breast cancer cells had a similar phenotype to the vHMEC cells and found that the cells appeared to be normal with no chromosomal changes. The changes seen in the individual cells in the karyotypic analysis were not occurring in a high enough frequency to be detected by CGH. Representational oligonucleotide microarray analysis then was used to analyze the chromosomal material and showed that, in culture, distinctive genetic changes occur that are the same as those in the human breast. Therefore, the silencing of *p16* produces chromosomal abnormalities that are the same as those in early breast cancer. These changes activate oncogenes and silence tumor suppressor genes. Data indicate that out of 20,948 genes, 517 display distinct expression profiles that differ between HMEC and vHMEC cells. The loss of *p16* in normal HMECs can lead to the activation of targeted DNA hypermethylation, which is very important in early breast cancer.

Epigenetic changes, the basis of differentiation, are heritable changes in gene expression that occur without changes in DNA sequence. Two major mechanisms of epigenetic remodeling are histone methylation and DNA methylation. Genetics (i.e., DNA sequence) can be thought of as the hardware of each individual cell, whereas epigenetics (i.e., DNA packaging) can be thought of as the software. Polycomb group (PcG) proteins, epigenetic regulators of cellular memory, are known to be overexpressed in many different types of cancer, especially breast cancer. Specifically, the laboratory studied two PcG proteins from polycomb repressor complex 2, SUZ12 and EZH2, which are known to be overexpressed in breast and prostate cancer. Data indicate that these two proteins are upregulated in the vHMEC cells as a result of reduced *p16* activity. The researchers then used restriction landmark genomic scaling to examine if *p16* was the only locus hypermethylated in vHMEC cells and determined that many genes involved in differentiation, including *HOXA9*, are hypermethylated and silenced. As a result, stem and progenitor cells are not able to differentiate properly. The researchers hypothesized that loss of *p16* expression is necessary for hypermethylation of *HOXA9*, and data indicate that the hypothesis is correct. The researchers also determined that PcG expression is sufficient for rapid DNA hypermethylation. Therefore, hypermethylation of *p16* and the increase of PcG proteins are necessary and sufficient for epigenetic remodeling, which affects differentiation and premalignant phenotypes. In 93 percent of tumor cells, *HOXA9* is not expressed. *HOXA9* is not expressed as a result of hypermethylation in approximately one-half of these cells.

Researchers tested the hypothesis that cells with the certain characteristics are the preclonal precursors to the clonal evolution of cancer. Another marker of interest in addition to *p16* and *HOXA9* genes is the COX-2 protein, which is expressed in vHMEC but not HMEC cells. COX-2 stimulates angiogenesis, proliferation, and invasion and inhibits apoptosis. The malignant phenotype expressed by tumor cells includes angiogenesis, proliferation, invasion, and loss of apoptosis. Overexpression of COX-2 induces this phenotype in tumor and vHMEC cells, whereas loss of COX-2 in vHMEC cells induces the loss of this phenotype. Next, the laboratory investigated the clinical relevance of these findings. Using normal human tissue to map the hypermethylation foci in tissue, the researchers determined that the markers from the vHMEC cells co-localize in the tissue and that normal tissue contains isolated foci with *p16* hypermethylation, telomeric dysfunction, and the expression changes discussed above. These lesions are associated with increased cancer risk and appear clinically relevant.

Some groups have proposed that breast cancer may be influenced by aberrant ductal structure. There is some evidence that there is clonal expansion in cells and that clonal evolution drives neoplastic progression. In cells with clonal expansion, hypermethylation occurs. This phenotype is well recognized in premalignant diseases, and the same phenotypic changes occur in the vHMEC cells under investigation. To address the clinical problem that there are no criteria available to consistently identify the women with DCIS that are most likely to progress to invasive cancer, collaborators investigated which markers identified in the above research could help distinguish which DCIS lesions would move toward progressive cancer and which would not. Preliminary data indicate that although COX-2 by itself does

not have the ability to stratify risk, when combined with Ki67, a molecule that indicates tumor cell growth rates, risk can be stratified. High expression of COX-2 and Ki67 appear to significantly increase the risk of progression.

In conclusion, the silencing of *p16* appears to provide cells with premalignant properties; cells with these properties exist in healthy individuals and probably serve as precursors to cancer; and selected expression changes allow the identification of DCIS patients who are at risk for a subsequent tumor event.

### Group Discussion

*Panelists:* Donald L. Weaver, M.D., University of Vermont College of Medicine, Burlington, VT; Karla Kerlikowske, M.D., and Thea Tlsty, Ph.D., University of California at San Francisco, San Francisco, CA

Dr. Stuart Schnitt asked Dr. Tlsty about the prevalence of variant cells in normal breast tissue, including how prevalent they are, how prevalence varies from case to case, and if there is an association between various breast cancer risk factors and prevalence of variant breast cells. Dr. Tlsty responded that this is a question that she would love to have answered, but a large piece of tissue is needed. Currently, the laboratory is using mastectomy samples to attempt to answer these questions. The foci are seen in no greater or less frequency in *BRCA1* and *BRCA2* individuals, and complete analysis to answer these questions is ongoing.

A participant asked Dr. Kerlikowske how many invasive cancers had DCIS with the invasive cancer. He asked for clarification if the sensitivities that were less for invasive cancer meant that the sensitivities were less for precursors that were not detected mammographically. He asked what phenotype the tumors tended to display (e.g., ER positive vs. ER negative, methylated vs. nonmethylated). Dr. Kerlikowske responded that the sensitivity of DCIS indicated was pure DCIS, so there was no accompanying invasive cancer. In the invasive cancer cases, there may have been some accompanying DCIS, but in tumor registries the higher tumor is the one that is recognized and recorded, and that generally is the invasive cancer. Just because the DCIS is adjacent, however, does not necessarily indicate that it is the precursor.

Dr. Saraswati Sukumar asked if *HOXA9* staining also was performed in DCIS tissues. Dr. Tlsty responded that it was not done because DCIS tissue is precious as a result of the small size of the lesions. She is hoping to collaborate with someone with a repository of DCIS tissue so that she can explore this avenue. Dr. Sukumar asked about two *HOX* genes that were not presented in the expression profiling results. Dr. Tlsty replied that all three *HOX* genes are silenced, but because of time constraints her presentation focused on *HOXA9* as an example of a gene that is targeted for DNA methylation. As a result of a paper about methylated *HOXA5* previously published by Dr. Sukumar, Dr. Tlsty's laboratory investigated this gene in the vHMEC cells and found that it is not methylated.

Dr. Peggy Porter asked if there was a contribution of other mechanisms of silencing *p16* or if all silencing is a result of methylation pathways. Dr. Tlsty responded that point mutations, loss of heterozygosity, deletions, and a variety of other mechanisms can silence *p16*.

Dr. Roland Holland stated that one possible area of investigation is to determine in what percent of invasive cancer can intraductal lesions be detected. In one examination of 100 invasive cancer cases, it was found that intraductal cancer was present in 98. It may not be a precursor to invasive disease but the presence is compelling.

Dr. Sukumar asked why, if *p16* methylation/silencing is an essential, critical step for the cell to become immortalized, frequent methylation is not seen in the ultimate lesion in DCIS or invasive cancer. Dr. Tlsty responded that the silencing of *p16* does not confer immortality. There are various ways to inactivate the Rb pathway, including cyclin D, *p16*, CDK4, Rb, and E2F. It is known that virtually every tumor has inactivated some component of the Rb pathway, so one area of investigation, in the cells that

do not have methylated *p16*, is if there is an abrogation of the pathway in an upstream or downstream member of the pathway.

Dr. Steven Katz asked the panel if in the next 5 years the research would be advanced enough that watchful waiting would be a feasible approach to DCIS. Dr. Kerlikowske replied that if better predictors of women that will experience a subsequent invasive event (vs. progression) are developed, then this would be a feasible plan. Despite the fact that DCIS may be found adjacent to invasive cancer, it does not mean that it is the precursor. The goal of longitudinal studies is to determine if invasive cancer or DCIS is the precursor or predictive. It is possible that the invasive cancer came first. Dr. Tlsty stated that some of the markers discussed in her presentation are very viable as clinically relevant markers and may lead in the direction of watchful waiting. Dr. Weaver added that the progression to a watchful waiting paradigm is desirable.

## **DCIS RESEARCH: CURRENT CHALLENGES AND FUTURE DIRECTIONS**

### **Problems in the Diagnosis of Ductal Carcinoma *in Situ***

Stuart Schnitt, M.D., Beth Israel Deaconess Medical Center, Boston, MA

Although the diagnosis of DCIS is straightforward in many cases, there can be many histological differences. Histologically, invasive cribriform carcinoma, pleomorphic lobular carcinoma *in situ*, collagenous spherulosis, and lymphovascular invasion are similar to DCIS; therefore, the diagnosis of DCIS is not always straightforward. In one study of 818 locally diagnosed cases of DCIS, on central review, 6.2 percent were not actually DCIS, but invasive cancer or ADH. In another prospective, randomized trial that compared fine needle aspiration and core needle biopsies, 596 patients underwent surgical biopsy following the needle biopsy, and the specimens were reviewed locally and centrally. When the local and central reviews were compared, pathologists agreed on a diagnosis of DCIS in 114 of 123 (93%) DCIS cases, with the local pathologists underdiagnosing and overdiagnosing an equal amount of cases. In a third case-control study of 606 DCIS cases, 9.6 percent of locally diagnosed cases were found not to be DCIS on central review; again, there was both under- and overdiagnoses by the local pathologists. These studies illustrate the central problem in diagnosing DCIS: the heterogeneity of the disease that allows it to be easily confused with a variety of other diseases, such as ADH, microinvasive carcinoma, invasive carcinoma, lymphatic invasion, lobular carcinoma *in situ* (LCIS), and other intraductal lesions. The three diseases that cause the most clinical problems are ADH, LCIS, and microinvasive cancer.

The distinction of ADH from DCIS may be made via qualitative features (e.g., architecture, cytology) or quantitative features (e.g., size, extent), and the use of standardized diagnostic criteria can be useful in fostering observer agreement in the diagnosis of equivocal cases. In one study, in the absence of standardized criteria, there was no complete pathologist agreement of diagnosis in any of the samples, and only 18 percent of the samples had the agreement of all but one pathologist. With standardization, however, 58 percent of the samples had complete pathologist agreement, and 71 percent had all except one pathologist agreeing on diagnosis. The major obstacle to differentiating between ADH and low-grade DCIS is that there are no qualitative histologic features, singly or in combination, that permit the reliable distinction between the two diseases in all cases. Also, there are no biomarkers to distinguish between the two. Additionally, because ADH is a term used to describe two different types of lesions, the question is if the clinical implications of these two lesions are the same. There are arguments for and against the continued attempt to distinguish ADH from low-grade DCIS; however, follow-up studies have documented clinically important differences between ADH and low-grade DCIS that are considered in formulating patient management. Therefore, given that there are documented, clinically important differences between ADH and fully developed low-grade DCIS, they should be considered distinct with regard to patient management.

Microinvasive carcinoma is defined as the extension of cancer cells beyond the basement membrane with no focus more than 0.1 cm in greatest dimension. The high-grade/comedo histology; extent (e.g., size, number of involved ducts); and periductal lymphoid infiltrates are features of DCIS that are associated with microinvasion. Because DCIS cells may have areas that mimic invasion, such as duct branching, involvement of lobules and benign sclerosing lesions, distortion of involved spaces, and tangential sectioning among others, DCIS often is overdiagnosed as microinvasive cancer. Histologic distinction between mimics of invasion and real invasion is not always possible. Additionally, because microinvasive foci may be overlooked or not sampled, underdiagnosis is common as well. In aggregate, however, there are no clear differences between DCIS and microinvasive cancer with regard to disease-free or overall survival; therefore, patients with large areas of DCIS with and without microinvasion should be managed similarly.

DCIS and LCIS, however, are managed differently. DCIS is seen as a precursor and treated with complete eradication and margin evaluation, whereas LCIS is seen as a risk factor and observed (with or without tamoxifen) with no margin evaluation. Because there can be overlap in distribution within ductal-lobular system (i.e., DCIS can involve identifiable lobules and LCIS can involve ducts) and some LCIS lesions have features more commonly associated with DCIS and vice versa, problems occur when attempting to distinguish the two disease entities. E-cadherin staining, however, may be of help in problematic cases. Currently, the most appropriate management of patients with histologically ambiguous *in situ* lesions is unknown.

### **Overview of Treatment**

Monica Morrow, M.D., Fox Chase Cancer Center, Philadelphia, PA

The standard treatments in DCIS management are mastectomy, excision with radiotherapy, and excision alone; tamoxifen may or may not be used in conjunction with any of these therapies. Each of these treatments has approximately the same overall survival rate of 97 percent. Treatment is predicated on the fact that the presence of invasive cancer cannot be excluded reliably without complete excision of the lesion. Complete excision is a major operation that is itself an overtreatment in most cases; however, approximately 10 percent of DCIS diagnosed by core biopsy is later diagnosed as invasive cancer on removal of the entire mass.

Prevention of local recurrence is a major goal in the treatment of DCIS and is an appropriate endpoint, as 70 percent of women surveyed indicate that risk of recurrence is the greatest factor influencing their treatment decisions. Three prospective, randomized trials of radiotherapy treatment for DCIS have indicated that radiotherapy significantly reduces local recurrence, but the subsets of patients who do not benefit from radiotherapy have not been determined. One major question that remains is if a large enough surgical operation is performed on the breast, can radiotherapy be avoided? Retrospective studies indicate that local recurrence is not reduced when radiotherapy is used with excision. Prospective studies have attempted to duplicate this finding; a Dana-Farber study using wide excision alone was not able to duplicate the results of the retrospective study. The Intergroup Study E5194, which stratified patients on the basis of low- to intermediate-grade versus high-grade DCIS, found that after 5-year followup, excision-only patients diagnosed with low- to intermediate-grade DCIS had approximately a 6.1 percent recurrence rate. Patients diagnosed with high-grade DCIS were harder to recruit into the study, as radiotherapy is generally considered desirable in these cases. Results showed that the excision-only, high-grade DCIS patients had a higher recurrence rate. The European Organisation for Research and Treatment of Cancer (EORTC) randomized phase III trial 10853 found that patients of all subgroups (age, method of detection, architecture, margins, and histologic type) benefited from radiotherapy combined with local excision. In predicting recurrence after excision and radiotherapy, time to local recurrence is the most important factor.

Mastectomy is indicated in the treatment of DCIS when the disease is too extensive to resect with a good cosmetic outcome, when there is an inability to achieve negative margins, and when radiotherapy is contraindicated in a high-risk patient. Because DCIS lacks the ability to metastasize, axillary surgery only is indicated because of the risk of unsampled invasive carcinoma. The risk of axillary recurrence in DCIS is extremely low, and sentinel node biopsy in DCIS cases is contraindicated. DCIS, however, is a marker for increased risk of invasive carcinoma in both breasts, and the 15-year risk of new cancer in a previously diagnosed DCIS patient is the same as the 5-year risk of local DCIS recurrence. The use of tamoxifen to treat DCIS has met with mixed results, but studies indicate that estrogen receptor (ER)-positive DCIS is more susceptible to tamoxifen than ER-negative DCIS. Additionally, a trial in the United Kingdom indicated that tamoxifen given in conjunction with excision does not reduce the risk of recurrence when compared to excision alone. The biggest reduction in risk of recurrence is in patients treated with excision and radiotherapy.

Because patients are confused about the fundamental nature of DCIS, the perception of risk of recurrence and death as a result of DCIS is the same as these perceptions in invasive cancer. In a population-based study from the Detroit and Los Angeles Surveillance Epidemiology and End Result (SEER) Registries, nearly 70 percent of patients chose breast conserving surgery versus 30 percent who chose mastectomy. Of the patients choosing breast conserving surgery, 71 percent chose radiotherapy in conjunction. Those patients with the highest risk of recurrence chose mastectomy, and surgeons' recommendations in these cases were appropriate. Patients concerned with the risk of radiation also chose mastectomy.

Reliable predictors of biologic behavior, progression to invasive cancer, and local recurrence are needed to resolve the DCIS dilemma. Additionally, better methods to communicate risks and benefits of treatment choices are needed. Physicians should frame discussions with their patients in terms of complete diagnosis and the prevention of invasive cancer.

### ***Predictors of DCIS Recurrence and Risk of Invasive Breast Cancer in Women With DCIS***

#### **Overview of State-of-the-Field and Current Challenges**

Lawrence Solin, M.D., F.A.C.R., University of Pennsylvania, Philadelphia, PA

Currently, the predictors for local recurrence are based on patient and tumor characteristics and are not adequate for treatment decisions; therefore, there is a need for new biologically based predictors for tailored treatments. Risk factors for local recurrence come from many areas, including patient characteristics (e.g., age), tumor characteristics (e.g., size, margins, pathology), biology (e.g., receptor status), and treatment (e.g., surgery, radiation, tamoxifen, hormones). Tailoring the treatment to these risk factors is an important paradigm in successfully treating DCIS. Three trials have investigated the use of radiotherapy for treatment of DCIS, and radiotherapy appears to lower the risk of recurrence in all patient subgroups. Although there is a dramatic benefit to radiotherapy, doctors as yet do not know the individual patients that will benefit the most and those who will not benefit at all. Because DCIS has approximately a 97-98 percent survival rate, the endpoint to be considered for successful treatment is local recurrence. Unfortunately, the parameters to predict recurrence are crude, and the selection of patients for treatment is based on these crude parameters; the tools are not available to assess which patients can receive more tailored treatments.

Factors that predict for local recurrence are patient-, tumor-, and treatment-driven. The tissue blocks from the E5194 study have been collected and will be analyzed to examine molecular predictors of local recurrence. Another lesson learned from the E5194 study is that followup data need to be examined at 10 years out or more, as early (5-year) results can be misleading. The results regarding the usefulness of using tamoxifen to treat ER-positive DCIS still are preliminary, but tamoxifen may not be as useful as was once thought. Within 5 years, molecular and genomic factors will allow for tailoring. It has been determined that the following biologic factors alone are not correlated with local recurrence: ER,

progesterone receptor (PR), p53, *HER-2/neu*, Ki-67, p21, and *BCL-2*. One study showed that the combination of specific factors is more important than single factors in predicting recurrence. Another study found that the combination of ER+/PR-/HER2+ had the highest level of recurrence compared to all other combinations of biologic markers.

A preliminary model, called *Adjuvant! for DCIS*, is available online and analyzes the global picture to estimate the outcomes of DCIS patients who have undergone breast conserving surgery, providing input into decisions for further treatment. This program may be helpful in dealing with the current challenges of treating DCIS, which include developing predictive factors to tailor treatment for individual patients and adding biologically based factors to current models based on patient and tumor factors for tailored treatment.

### **Overview of Methodologic Issues; Evaluating Predictors in Population Sciences**

James Dignam, Ph.D., University of Chicago, Chicago, IL

Some of the attributes of DCIS that are potentially important in prognostic marker studies include its increase in prevalence as a result of screening, its heterogeneity with respect to various features, its excellent survival prognosis, and the fact that it confers considerable excess risk for invasive breast cancer and breast cancer mortality. DCIS presents a clinical opportunity for early intervention to avoid invasive breast cancer. To take advantage of this opportunity, it is necessary to understand the heterogeneity of DCIS presentation and its characteristics. A prognostic marker is defined as a characteristic associated with prognosis or outcome, usually in terms of relative hazard of failure, whereas a predictive marker is defined as a characteristic that is associated with and predicts treatment response. Therefore, a predictive marker is a prognostic marker that exerts prognostic influence differentially according to treatment, offers the opportunity for prospective intervention, and may lend insight about biological aspects of the disease. An example is ER content on breast tumors. ER is a prognostic marker in that ER-negative tumors are associated with a greater failure hazard, and it is a predictive marker for response to anti-estrogen drugs, as virtually no response is noted in patients with ER-negative tumors, whereas those with ER-positive tumors do respond.

Sample size calculations for prognostic markers resemble those in study designs for treatment effects, with some important exceptions: (1) The relative frequency of different levels of the marker cannot be manipulated or controlled. (2) The strength of the relative hazard imparted needs to be larger than for treatments to be of interest and/or utility. (3) The marker may be correlated with multiple other known prognostic markers. The challenge in prognostic marker studies is determining how large the sample size must be to be useful. Because a redundant marker is undesirable, an inflated sample size is necessary. For predictive markers, the situation is more challenging. The detection of interaction effects is equivalent to the comparison of treated versus untreated within levels of a prognostic marker and the comparison of the size of the treatment effect between levels of the prognostic marker, (i.e., the differential treatment effect). It can be shown that the sample size needed for studying prognostic markers, under some favorable assumptions, is approximately four times larger than that needed to detect a main effect of the same magnitude. As some interaction effects are large, such studies are not infeasible. Targeted clinical trials, where patients most likely to benefit are preselected, often can be much smaller than trials with more general eligibility. Similarly, if a treatment essentially is null in one group and effective in another, then the interaction effect may be detected.

The advent of modern biotechnology tools (e.g., microarrays) requires use of statistical methods previously little-used in marker studies, as well as the development of novel methods. These studies, however, tend to be more involved in the discovery of candidate markers than testing for clinical utility; once a candidate marker is developed, regardless of origin, analysis from that point forward largely resembles traditional prognostic/predictive marker problems. In these situations, a marker variable may

come in the form of a prognostic score or index that is a function of several other variables, and statistical principles for model building in general need to be followed to develop these scores. Independent validation data are critical, as there may be several equally reasonable scoring algorithms and the choice of which to use may be less important than validating one objectively. Additionally, reproducibility of the assay also is critical; the scoring algorithm must be portable to other settings to have utility. Numerous time-to-event endpoints are of interest in DCIS (e.g., ipsilateral recurrence, invasive contralateral tumor, breast cancer death), so it is important to determine which are the most important. Competing risks also need to be considered.

Data sources for DCIS studies may include SEER Registries, single institution or single health care system cohorts, and randomized clinical trial databases. Each source has its strengths and weaknesses. Randomized trials have the advantage of uniformity of stage at diagnosis and cohort entry criteria, randomized treatment assignment, uniform treatment per a specific protocol, and rigorous followup and outcome ascertainment; however, there is limited patient diversity and a need for centralized pathology to assure common definitions. Observational cohorts provide a diversity of patients and disease presentations, ancillary data, and control over pathology information. Drawbacks of observational cohorts include the treatment selection effects and the validity of the predictive markers in question, differences in institutional or regional pathology definitions, and loss to followup.

A number of commentaries and guideline articles regarding the development and use of prognostic markers in breast cancer, motivated by the need for high-level evidence to recommend markers for clinical use, have been published. These include “Statistical Aspects of Prognostic Factor Studies in Oncology” (Simon and Altman, *Br J Cancer*, 1994;69:979-985); “Statistical Issues in Tumor Marker Studies” (Pajak, et al., *Arch Pathol Lab Med* 2000;124:1011-1015); “College of American Pathologists Conference XXXV: Solid Tumor Prognostic Factors—Which, How and So What?” (Hammond, et al., *Arch Pathol Lab Med* 2000;124:958-965); and “Reporting Recommendations for Tumor Marker Prognostic Studies” (McShane, et al., *J Clin Oncol* 2005;23:9067-9072). The CAP article (Hammond, et al.) recommended that: (1) clinical trials should be designed specifically to test whether a factor has prognostic value; (2) the prognostic factor question must be prioritized by multidisciplinary groups; (3) journals should adopt publication guidelines for reporting results; (4) a consensus must be made about the ranking of prognostic factors; and (5) research into multivariate analysis techniques should be continued. To bring prognostic and predictive markers to full potential, researchers, editors, and those funding studies should collectively implement recommendations for quality improvements in designing, conducting, and reporting marker studies.

### **Predicting Local Invasive Recurrence After Conservative Treatment**

Melvin Silverstein, M.D., The USC/Norris Comprehensive Cancer Center, Los Angeles, CA

At 12 years followup, DCIS local recurrence rates are 32 percent for excision alone, 16 percent for excision plus radiotherapy, and 1 percent for mastectomy. The University of Southern California (USC)/Van Nuys Prospective Database contains data from single-piece excisions with oncoplastic resection; mini-excisions of the biopsy cavity wall are not included. All samples are color-coded or inked, serially sectioned, completely and sequentially embedded, and size-estimated serially with mammographic and pathologic correlation. Through 2006, there are 1,289 samples with complete data. The subgroups of the study include all breast conservation therapy cases (combined), excision alone, and excision with radiotherapy. The two endpoints of the study are any recurrence and invasive recurrence. Analyses include log rank for individual factors, Cox multivariate for significant factors, and logistic regression.

Because most local recurrence occurred at or near the primary lesion, the most important cause of local recurrence appears to be inadequate excision. Factors found to be significant by multivariate analysis for

any recurrence in the all breast conservation therapy group include nuclear grade, size, margin width, necrosis, age, and treatment. In the excision-only group with invasive recurrence, the significant factors by multivariate analysis included tumor size and margin width. The univariate analysis log-rank test for the all breast conservation therapy group with any recurrence found that nuclear grade, age, tumor size, margin width, necrosis, Van Nuys class, any presence of comedo, predominant presence of comedo, suspicious mammogram, National Surgical Adjuvant Breast and Bowel Project (NSABP) margins, Lagios criteria, and treatment were all significant factors in predicting recurrence. In the same group, Cox multivariate analysis showed that treatment, margin widths, necrosis, nuclear grade, tumor size, and age were significant factors. Cox multivariate analysis indicated a 63 percent reduction in recurrence following radiotherapy. The invasive plus local recurrence rate in the excision-only patients after 12 years of followup was 28 percent, which is similar to the NSABP 12-year followup rate of 32 percent. The invasive recurrence rate after 12 years of followup in these excision-only cases is 12 percent, similar to the NSABP rate of 16 percent.

When the cases of invasive recurrence in excision-only cases are broken down further, it is observed that margins less than 1, age less than 50 years, and a nuclear grade of 3 are factors predicting recurrence. In cases with all three factors present, the recurrence rate is 45 percent; in cases with none of these present, the recurrence rate is 4 percent. The USC/Van Nuys Prognostic Index (USC/VNPI) uses these factors plus tumor size in a scoring system. Cases with USC/VNPI scores of 4, 5, or 6 have a high rate of recurrence-free survival. Those with USC/VNPI scores of 7, 8, or 9 have an intermediate rate of recurrence-free survival. In those patients with scores of 10, 11, or 12, who have a low rate of recurrence-free survival, mastectomy is recommended. In using logistic regression to predict invasive recurrence in all breast conservation therapy cases, the only significant factor is time to recurrence. The same factor was significant in excision-only cases. The conclusion is that it is not possible to predict any recurrence or invasive recurrence with the current data.

### **Impact of Pathology Practice on Outcome in Published Studies of DCIS and Recommendations for a Uniform Pathology Protocol**

Michael Lagios, M.D., The Breast Cancer Consultation Service, Tiburon, CA

The most significant factors for local recurrence of DCIS are nuclear grade, tumor size, margin width, and age (patients less than 40). Problems in establishing prognostic features in DCIS are that the disease is not palpable, invasion must be excluded, total extent must be calculated, and all margins must be examined and margin widths assumed. If only a tissue sampling of DCIS is performed, then invasion cannot be excluded, extent cannot be calculated, and margins only will be sampled.

Previous studies of DCIS have included retrospective (e.g., the NSABP B-17 Phase III Randomized Study) and prospective (e.g., the USC/Van Nuys study) examinations. Limitations of the Bijker, et al. study include nonsequentially processed tissue sampling and the fact that only 22 percent of all reports recorded tumor size. Limitations of the Wong (JS), et al. study include nonsequentially processed tissue sampling, estimated (not measured) tumor size, and inclusion of only sampled margins. Additionally, following clinical evaluation only 76 percent of recurrences were true recurrences; 23 percent were *de novo* events in other quadrants.

The definition and identification of pathologic prognostic factors is highly dependent on methodology. The USC/Van Nuys database is based on resections that are entirely and sequentially embedded with rigorous mammographic-pathologic correlation; however, randomized trials did not demand such methodology and cannot be used retrospectively. As a result, the NSABP B17 study did not find that nuclear grade, tumor size, or margins were statistically significant prognostic indicators. The EORTC 10853 study was able to define nuclear but not other features as statistically significant. Therefore, the prognostic value of specific features only can be assessed within a pathologic protocol that permits

complete analysis: total sequential, correlated tissue processing. The minimal pathologic requirement for the evaluation of DCIS is that the resection must be completely and sequentially examined microscopically. Future DCIS intervention trials should: (1) correlate preoperative imaging, specimen radiography, and postexcision studies; (2) include complete sequential tissue processing of oriented specimens; and (3) calculate tumor size, measure margin widths, exclude microinvasive foci, and classify by grade (nuclear grade and necrosis).

### **Panel Discussion**

*Moderator:* Laurel Habel, M.D., Kaiser Permanente Northern California, Oakland, CA

*Panelists:* Elizabeth Claus, M.D., Ph.D., Yale University School of Medicine, New Haven, CT; Frederic Waldman, M.D., Ph.D., University of California at San Francisco Comprehensive Cancer Center, San Francisco, CA; Roland Holland, M.D., Ph.D., Radboud University Medical Center, Nijmegen, The Netherlands

Dr. Elizabeth Claus stated that the group assembled at this workshop should determine if existing data can be used and how and what needs to be collected. Existing DCIS data include approximately 10,000 population-based cases that primarily are from case-control studies with some from case-only studies. Most of these should have good interview data and paraffin block samples. Some also may have blood, saliva, and other samples, and a small percent may have mammographic data. Approximately 5,000 cases from clinical trials and other cohorts are available, making a total of approximately 15,000 cases available to researchers. At least 12,000 of these are population-based. Given an average risk of 1 to 2 percent per year for a recurrent event and at least 5-year followup, there is a probability of obtaining at least 1,500 outcomes for examination. This workshop should be used to clarify what data exist, if collaboration is feasible, if meta-analysis should be performed on interesting data sets, and exploit the existing mechanisms for collaboration.

Dr. Frederic Waldman commented that DCIS must be seen as multiple diseases. Low-grade and high-grade differences are the most obvious aspect of this paradigm. Extensive DCIS and local DCIS may be different diseases, and preliminary results show that small DCIS shows the most genomic changes. It must be determined what is meant by aggressive DCIS. Overlap of characteristics in different lesions is consistent with what is seen histologically, which in turn is consistent with what is observed genetically, which in turn relates to the biology; all of these factors are intertwined. Regarding the precursor of invasive cancer, it can be agreed that it probably has a precursor and this precursor may look like DCIS. If DCIS and invasive cancer are examined synchronously by genomic alterations, the pairs that are found together look like each other, and the ones that are found in different patients do not. Obviously, they are clonally related if they are found synchronously. DCIS is related to its own recurrences clonally.

Dr. Holland reiterated the salient points presented in each of the talks of this session. He added that in terms of margins, 15 percent of patients with 10 mm negative margins still have recurrent disease. Therefore, it may be necessary to treat patients with high-grade but small DCIS with radiotherapy. Additionally, although the absolute number is small, fully differentiated disease associated with deaths at distant metastases was almost 77 percent. Not only is the central pathology review important, but also a strict protocol for criteria needs to be developed.

### *Discussion*

Dr. Bruno Cutuli commented that the final data are available for the Swedish DCIS trial, which followed 1,000 patients either with or without radiotherapy. After 5 years, there is a 22 percent recurrence rate without radiotherapy and 7 percent with radiotherapy. In the population younger than age 45, there is an increase in the interest of radiotherapy. Radiotherapy rates can be different in young women as compared to control.

Dr. Deborah Winn reiterated that the morning's presentations spoke of how tumor characteristics predict outcome and asked panelists to comment on other factors, such as obesity, and how they affect the etiology of disease. Dr. Claus responded that this was not a topic of this particular session, and she was not sure if any data regarding this would be presented at the workshop. She, however, does have data that indicate that clinical factors do not appear to affect the disease.

Dr. Jack Cuzick commented that he believed that the length of followup needs to be expanded to 20 years. The treatment of invasive cancer indicates that cardiovascular mortality with radiotherapy does not start to occur until 10 years out and overwhelms the gain seen associated with breast cancer mortality. The long-term cardiovascular mortality may be a significant factor in treatment decisions. Long-term followup and not just local recurrence followup is needed. Twenty-year followup data indicate that ER-positive patients have more recurrences than ER-negative patients, which also highlights the need for long-term followup. Dr. Claus stated that the challenge to long-term followup is that the disease changes over time, and what is found at long-term followup may be different than is what is diagnosed today.

A participant expressed concern about ducts that are cut in cross-sections near the margin, in which the margins are called in relation to how close the cross-section is to the edge of the tissue, but it is difficult to determine if the axis is traveling up and toward the margin or down. He asked if others had concerns about specimens being labeled as negative margins that are actually not negative and how the assessment can be improved. Another participant asked how the tissue was being processed. The participant responded that the tissue is processed in a standard method and that he had not been shown a better method to trace the path of a DCIS duct toward the margin, especially if calcifications are present. Dr. Holland responded that in a two-dimensional slide, one can never be absolutely sure if the margin is negative or if there is residue outside. Studies have shown that there will be gaps, even in three-dimensional studies, between two involved segments. The quantity of involvement also determines successful establishment of negative margins.

Dr. Cutuli commented that modern radiotherapy is completely different than that performed in the past. In DCIS, only the breast without lymph node involvement is treated.

## **INTEGRATING POTENTIAL PROGNOSTIC BIOMARKERS OF SUBSEQUENT DCIS AND INVASIVE BREAST CANCER INTO CLINICAL PRACTICE AND TREATMENT TRIALS FOR WOMEN WITH DCIS**

### **Overview of State-of-the-Field and Challenges in Collaboration**

Laura Esserman, M.D., University of San Francisco, San Francisco, CA

Several cancer reduction strategies target preneoplasia (e.g., cervical intraepithelial neoplasia lesions for cervical cancer and polyps for colon cancer). Is DCIS the preneoplasia target for breast cancer? In 2007, the *New York Times* reported that cancer incidences have fallen 2 years in a row, with the reduction in breast cancer being attributed to reduction of hormone replacement therapy, not diagnosis. The question then is: After 20 years of mammography screening, has the right premalignant lesion been identified? A graph of incidence rates of invasive cancer and DCIS from 1999 to 2003 show that the two have paralleled each other. Of DCIS diagnoses over the years, most have been of the noncomedo type. In the case of prostate cancer, the single best biomarker for prediction of diagnosis is a biopsy. The yearly annual hazard rates of progressing to cancer as a result of DCIS, LCIS, or *BRCA1/2* are similar, but DCIS treatment is compelling because the site of occurrence is known and there is a chance for cure. The risk and timing of progression from all premalignant lesions, however, is not well characterized. It is likely that LCIS is a marker for invasive lobular cancer, whereas atypia may be a marker for invasive ductal cancer. Patients with atypia and a 5-year Gail score of more than 2 percent have a 15 percent chance of developing cancer within 4 years, which breaks down to a 9.7 percent chance of invasive ductal cancer and a 5.3 percent chance of DCIS.

The critical pieces of information that drive a decision to intervene are the likelihood of progression to invasive cancer, the timing of progression to invasive cancer, and the ability to stop the progression. The timing of the progression should determine the focus of the intervention. In shorter progression times, the focus should be treatment; in longer progression times, the focus should be prevention. Intermediate progression times may indicate a combination of both treatment and prevention. The likelihood of progression determines if intervention is desired, and this is patient-preference sensitive. Grade appears to affect the timing of recurrence; if high-grade DCIS progresses to invasive cancer, it will do so within 5 to 7 years. Because no adjuvant treatment has shown survival benefit for DCIS and many if not most of all DCIS patients receiving mastectomy may never have progressed to invasive cancer, another approach should be investigated.

One approach is to determine what markers can be used to distinguish serious DCIS from not serious DCIS. Genomic studies have been useful in determining the relationship between *in situ* and invasive disease and *in situ* lesions show clonal genomic alterations similar to their invasive counterparts; however, alterations differ according to histologic grade and tumor size, and genomics may not necessarily distinguish who is going to progress. Because ER status is predictive in DCIS, it may be a good target for prevention. Tamoxifen continues to reduce the risk of breast cancer recurrence in ER-positive patients even after it has stopped, so this treatment can be considered as one approach to prevention. Atypical hyperplasia also predicts benefit from tamoxifen. Combinations of markers that include ER, COX-2, *p16*, macrophages, and proliferating macrophages also may be another approach to be considered, especially as it has been shown that COX-2 coupled with proliferation identifies women with increased risk of developing a subsequent tumor event. Noncomedo lesions are unlikely to be the precursors of lethal breast cancer, whereas comedo lesions tend to progress in a 5-year timeframe and should be used to explore other prevention options for ER-negative cancers. Age is a marker of more global risk; therefore, increased attention should be paid to prevention.

The paradigm can be changed in the following ways to accelerate progress in DCIS treatment and prevention: (1) understand the biology of the tumors that present; (2) find the right set of markers to rapidly predict response and introduce new agents, which will allow for a more tailored therapy; and (3) use a neoadjuvant approach for more targeted therapy trials to introduce new agents. Several trials are underway to change the paradigm, including the I SPY TRIAL (*Investigation of Serial studies to Predict Your Therapeutic Response with Imaging And moLecular analysis*), which aims to integrate imaging and tissue biomarkers, and the DCIS Neoadjuvant Study, the goal of which is to study and assess the effects of anti-estrogenic therapy on DCIS morphology and biomarker expression. It has been found that magnetic resonance imaging (MRI) is a surrogate biomarker for pathology because it shows good correlation of disease extent with pathology after neoadjuvant treatment with chemotherapy. As MRI also captures the heterogeneity of DCIS, imaging may be linked with pathology to categorize DCIS and predict tumor biology.

The Comparison of Operative to Medical Endocrine Therapy for DCIS (known as COMET) Trial is an opportunity for collaboration that allows a safe way to move forward, including risk reduction and watchful waiting. The DCIS Statin Biomarker Trial and the M.D. Anderson Neoadjuvant Herceptin Therapy Trial also provide opportunities for collaboration. Risk assessment tools, however, will be needed to help patients feel comfortable about participating in these trials. It is necessary for researchers to learn from the prostate cancer community, change perceptions, and work together to investigate data in a different manner. Methodology is needed that will allow new biomeasures to be incorporated into risk models; there is a precedent for this. The key finding that progression to invasive cancer is actually low, at variable rates, over years and not weeks should be leveraged because it is the ideal opportunity in which to study promising biomarkers and agents for breast cancer prevention using novel clinical trial designs.

## **Approaches to Evaluation and Validation of Therapeutically Relevant Biomarkers**

Annette Molinaro, Ph.D., Yale University School of Medicine, New Haven, CT

To avoid toxicity and expense, improved tools for selecting individual patients for treatments and accurate prediction of who will respond and who will not are needed. Although new technologies for genomic profiling have been developed, none have made it into clinical practice because it is difficult to develop biomarker classifiers and sufficiently validate them. Prognostic factors only can be used when therapeutically relevant. It is difficult to substantiate therapeutic values because there is no defined primary endpoint or there are too many primary endpoints. Also, the lack of reproducibility between laboratories, specimens, researchers, and so forth is an issue. The approach to evaluating and validating therapeutic value is to develop a classifier for addressing a specific therapeutic question (i.e., perform a developmental study), verify internal validity, translate the results to a common platform for broad clinical application, and verify the reproducibility and external validity of the classifier. A classifier is a completely defined mathematical function that maps the biomarker values to a set of prognostic categories and typically corresponds to a predicted clinical outcome. A classifier is not a list of biomarkers or genes, because such a list does not allow for prospective clinical validation.

A developmental study is analogous to Phase II of a clinical trial, with the goal of developing a completely specified classifier and corresponding hypotheses; the clinical value of the classifier cannot be evaluated in the same study. The main steps to developing a classifier are to: (1) select a prediction model; (2) split the sample data into training and test sets; (3) perform feature selection; (4) fit the model to the training set; and (5) estimate the prediction accuracy with the test set. Because it is possible to find a perfect classifier even when no signal is present, to avoid overfitting or chance, some form of a training or test set must be used. It is important to remember that there should be no adjustment of the model or fitting on the test set and that feature selection is done within the training set. After statistical significance is assessed and prediction error is estimated, determine if the prediction error confidence interval includes chance. Split sample (in which two-thirds of the sample is placed in the training set and one-third is placed in the test set) and leave-one-out cross-validation (in which the training set is  $n - 1$ , the test set is 1 observation, and the validation is repeated  $n$  times until each observation is in the test set once) can be used for internal validity. Following internal validation, the following questions will be answered: (1) Is the classifier sufficiently accurate? (2) Does it exceed or enhance the prediction accuracy of standard prognostic factors? (3) Is it worthy of further investigation? If the genomic classifier is worthy of further investigation, then its broad clinical application can be examined via external validation. This independent validation of prediction accuracy for the completely specified classifier determines if patients benefit (e.g., better efficacy, reduced incidence of adverse events, etc.) versus not using the classifier.

It is important that the classifier not be validated with the same data with which it was built. Editors, reviewers, and investigators need to verify internal and external validity of all classifiers put forth.

## **MOLECULAR PREDICTORS OF RESPONSIVENESS TO DRUG THERAPY, INCLUDING SUBGROUPS DEFINED BY GENETIC RISK**

### **A Systems Approach To Predicting Drug Response**

Joe W. Gray, Ph.D., Lawrence Berkeley National Laboratory, Berkeley, CA

Breast cancers are heterogeneous, but subsets respond more homogeneously. If these subsets can be identified, the information can be used to guide therapy and marker selection. The researchers' approach takes advantage of the ability to characterize cancer at the DNA level (e.g., genome, copy number) and the RNA level (e.g., gene expression). Breast cancers resolve into several molecularly distinct groups. Simple tumors are ER/PR positive, p53 normal, have a low Ki67 index, and show chromosome 1/16 aberrations, similar to lobular cancers, whereas complex tumors are ER/PR negative, p53 aberrant, possess a high Ki67 index, and show chromosome 8/20 aberrations, similar to *BRCA* cancers. Amplifier

tumors show high-level amplification at chromosome locations 8p, 11q, 17q, and/or 20q. Some subpopulations are associated with poor outcome despite treatment. *HER-2/neu*-positive cancers have been shown to have the worst outcome, with the “triple negative” subset of these cancers having the poorest prognosis. Amplifier and basal tumors also have a poor prognosis. Using these data, the researchers developed a 10-gene amplifier classifier and a 12-gene basal tumor classifier.

The researchers identified molecular-subset-specific therapies by correlating cellular responses of cancer cell lines with their molecular characteristics. Genome copy number, expression, proteins and phosphoproteins, DNA sequence information, and promoter methylation were analyzed to determine which molecular characteristics are associated with drug response; the drug chosen in the initial study was lapatinib, a dual-function kinase inhibitor designed to inhibit *HER-2/neu* and epidermal growth factor receptor (*EGFR*). Correlative analyses revealed the presence of subsets that respond to lapatinib, with a continuum of response levels. Although there is no association between *EGFR* levels and drug response, cells that have amplified *HER-2/neu* have the strongest response to lapatinib. From these results, a quantitative lapatinib response indicator was developed that works relatively well. Specific signaling pathways are associated with lapatinib response, so tests can be developed utilizing genes found in the pathway that make the most biological sense. The next step is to determine if other drugs that are more effective in resistant populations can be selected and in so doing generate a pharmacopeia of specific drugs and markers that can be chosen on an individual basis for the best clinical response. The study is now testing all drugs approved by the U.S. Food and Drug Administration (FDA) for a cancer indication.

Another goal of the study is to develop therapeutic agents that are targeted to specific molecular abnormalities. As discussed earlier, high-level amplification is associated with poor outcome in some patients despite treatment. As a result of amplification, 66 genes are overexpressed (deregulated) and associated with a poor prognosis; *HER-2/neu* is a prototypic example of this phenomenon. Researchers are investigating the correlation of the gene expression of these genes with drug sensitivity using more than 60,000 compounds in 60 cell lines. Similar explorations of developing new therapeutics also are being carried out in the Bay Area Breast Cancer Translational Research Program, a Specialized Program Of Research Excellence (SPORE). The overall idea is to bring together markers and therapeutics to simultaneously carry them forward clinically.

### **New Markers and Drug Therapies Being Examined in DCIS Trials**

Saraswati Sukumar, Ph.D., Johns Hopkins University School of Medicine, Baltimore, MD

Early detection assays must be minimally invasive, possess diagnostic value, be observer independent, specific (i.e., low number of false positives), and low cost. Current diagnostic techniques need a critical mass for detection and provide low specificity. Computer-aided detection of DCIS is a refinement that led to 91 percent detection of lesions in one sample. Direct cell analysis, the cytologic analysis of cells obtained from the breast ducts, is labor intensive, often acellular, does not sample the entire breast, and has low sensitivity and high specificity. Random fine needle aspiration has yet to be tested in tumor-bearing breast. In terms of cell-free DNA in plasma or serum, microsatellite DNA are too infrequent, but genes silenced by promoter methylation are promising. Serial analysis of gene expression (commonly referred to as SAGE) identifies multiple changes leading to invasive breast cancer, and it is possible that underexpressed genes are silenced by methylation.

Any panel of markers developed will need to be able to detect 100 percent of invasive carcinomas and 95 percent of DCIS cases. The panel should utilize markers that are frequently methylated in breast cancer but that have no or low methylation in white blood cells and normal breast tissue. The panel must have high sensitivity and specificity. Methylation-specific polymerase chain reaction (MSP) currently is being used to detect cancer cells. It is a qualitative test that has a sensitivity of 1:1,000; however, it requires an abundance of DNA template, and sometimes difficulties arise in interpreting genes with normal low

levels of methylation. Quantitative multiplex (QM)-MSP answers these challenges by providing a quantitative, absolute method of detection with high sensitivity that allows for the detection of methylated DNA in the presence of a large excess of unmethylated DNA, provides simultaneous assessment of multigene promoter hypermethylation status from limited amounts of DNA, and simultaneously detects up to four genes in the same well using multiple fluorogenic probes.

QM-MSP was used to determine if ductal lavage cytology reveals the presence of breast tumors and if it can enhance the detection of cancer in cells retrieved from ductal lavage. The study found that a panel of nine methylation markers can be evaluated successfully by QM-MSP in more than 90 percent of cases of ductal lavage from a single slide. The study also found that in high-risk women, cumulative methylation is low, whereas in ducts of women with cancer, cumulative methylation is significantly higher. Additionally, when cytology is positive (marked atypia or malignant) there is 100 percent correlation with positive QM-MSP. In ductal lavage cells, when comparing QM-MSP to cytology, QM-MSP is more sensitive, cytology is more specific, and both are equally accurate. Although QM-MSP can provide a valuable adjunct to cytology in the clinic, ductal lavage is not an effective way to sample the breast for early detection because not all ducts are lavaged. Therefore, sampling method still remains a challenge for breast cancer detection. Random periareolar fine needle aspiration (RPFNA) and serum are being investigated as alternatives to ductal lavage. Using QM-MSP, methylated genes in the serum of women with metastatic breast cancer can be detected in all samples, and multiple genes in RPFNA samples can be detected consistent with other methods of detection.

Treatment of DCIS needs to include a prevention arm. The laboratory studied intraductal instillation of anticancer agents (DOXIL<sup>®</sup>) for treatment and prevention and found that, in *HER-2/neu* transgenic mice, in the treatment and prevention settings, DOXIL<sup>®</sup> administered intraductally achieves longer tumor-free survival and lower tumor incidence compared to intravenously administered drug. The next step is to determine if the intraductal approach has translational potential and in what platforms can it be tested. A Phase I clinical trial is underway to test the feasibility and safety of intraductal administration and determine potential side effects, including pain, inflammation, or changes in the structure of the duct network. Thus far, the study has shown that the method can identify the correct cancer-containing duct in each case, as proven by subsequent mammogram. Another clinical question is if chemotherapy should be administered to DCIS patients prior to or instead of lumpectomy. Whether targeted DOXIL<sup>®</sup> therapy administered intraductally combined with chemotherapy can be used for the systemic management of invasive breast cancer needs to be investigated as well.

### **Group Discussion**

*Moderator:* Wortia McCaskill-Stevens, M.D., M.S., Division of Cancer Prevention, National Cancer Institute, Bethesda, MD

*Panelists:* Laura Esserman, M.D., University of San Francisco, San Francisco, CA; Annette Molinaro, Ph.D., Yale University School of Medicine, New Haven, CT; Joe W. Gray, Ph.D., Lawrence Berkeley National Laboratory, Berkeley, CA; Saraswati Sukumar, Ph.D., Johns Hopkins University School of Medicine, Baltimore, MD

Dr. Schnitt commented that a lot of emphasis in identifying markers has been in the epithelial cells, but the role of myoepithelial cells and myoepithelial-stromal interaction in DCIS also is important. Because the epithelial cells may not be the whole or right answer, he asked the group to comment on potential targets in myoepithelia or stromal cells. Dr. Sukumar responded that there will not be drastic changes in stromal fibroblasts, but the myoepithelial cell does deserve a lot of attention. Studies have shown that the myoepithelial cells are the most important cell type to hold the ductal structure together. Markers in myoepithelial cells may be the most significant, as new research will show. Other studies have shown that stromal cell markers are not significant. There is no doubt that there is interplay and cross-talk between myoepithelial and stromal cells, but the correct marker has not been identified yet.

Dr. Gray commented that whether one focuses on a tumor intrinsic property depends on what stage of the disease is being treated. Once local containment is lost, then focus will be on the tumor properties. If attempting to contain the disease, as in DCIS, then it is appropriate to use a variety of strategies that speak to the molecular cross-talk. Dr. Tlsty asked if recently published data, which showed that a fully tumorigenic cell modified by outside signals reverts to a normal phenotype with no changes in genotype, contradicts Dr. Gray's comment. Dr. Gray responded that once the cells escape the local environment, containment possibility is lost. The data are in the local context and do not speak to invasive disease.

Dr. Esserman commented that another interesting cell type is the macrophage. Proliferating macrophages are a risk factor for resistance to therapy and death. This was seen only in high-grade DCIS in the large lesions, so it may be a marker of a different haplotype, pathway, or disease. There may be a hypoxic signature in the genotype of these tumors.

Dr. Steven Shak commented that systematic, logical, stepwise development of a diagnostic test or assay is important and cannot be overemphasized. When moving to a validation study to determine if the assay is appropriate for clinical use, regulatory factors should be incorporated that allow for standardization. He mentioned the SEER study showed that a recent decrease in the incidence of invasive breast cancer, possibly as a result of the decrease in hormone replacement therapy, and asked Dr. Esserman if the incidence of DCIS was examined. Dr. Esserman replied that it was investigated. One possible explanation for the recent decrease is the elimination of the DCIS, but this is doubtful. There has not been a similar drop in DCIS. Either hormone replacement therapy has no impact on DCIS or there is a reservoir of disease that does not progress.

Dr. Gray asked Dr. Shak about his comment regarding standardization of assays. His approach has been to use Clinical Laboratory Improvement Amendments (CLIA)-approved assays, which are not FDA-approved. To follow that pathway, it almost is necessary to work closely with the private sector and ensure that the financial support necessary to get to the FDA is in place. He asked for Dr. Shak's comments on this. Dr. Shak replied that collaboration should be encouraged, especially between academia, industry, regulatory agencies, and advocates. There are two pathways one can follow, the CLIA pathway and the FDA pathway, and there is a role for each. If CLIA could be enhanced so that patient safety is ensured and the FDA makes some changes in its method, there is real potential, and everyone needs to be involved in the discussion on how to improve these processes.

A participant commented about the drop in invasive cancer seen following the decreased use of PREMPRO™, which was not seen in Canada, that showed a decrease in both ER-positive and -negative cancers. At the same time this occurred, there was a problem with insurance in the United States that may have had some affect on this phenomenon. Dr. Esserman responded that it was interesting because the drop was seen only in premenopausal women versus postmenopausal women and in primarily ER-positive cancers. Dr. Kerlikowske added that cancers are detected more when more screening mammography is done, and data indicate that the slight decrease in screening mammography does not account for the larger drop in breast cancer incidence. Also, the screening population only has a small portion of women affected by hormone replacement therapy. The SEER Registry, however, has not seen a significant decrease in invasive cancer or DCIS, so she is not convinced that hormone replacement therapy is the cause. Dr. Esserman asked if Dr. Kerlikowske had the national screening rates. Dr. Kerlikowske responded that she was speaking to the Breast Cancer Surveillance Consortium rates. Dr. Ballard-Barbash added that estimated national screening rates are not available except by self-report and National Health Interview Survey data, which show about a 3 percent drop in screening. A participant added that the Northern California Kaiser population was examined and showed a concomitant decrease in incidence that paralleled hormone replacement therapy use. This population is a screening population, so there was not a decrease in screening. There is a possibility that the decrease in incidence

is not an either/or situation, and both or neither screening or hormone replacement therapy use may be responsible.

Dr. Henry Kuerer commented about Dr. Esserman's and Dr. Shelley Hwang's DCIS neoadjuvant herceptin trial, stating that the high-grade, comedo DCIS lesions most likely to recur also are most likely to respond to herceptin. Prevention to invasive cancer is the goal, because there is not much risk if DCIS is contained. He asked for comments about patients in which invasive cancer may be missed in this study as well as on the progress of the NSABP trial. Dr. Esserman commented that herceptin is an accepted treatment for invasive cancer, and therefore it is an acceptable treatment for DCIS. A participant added that the NSABP DCIS herceptin-radiation trial protocol has been written, and the trial should be active in a few months. Dr. Hwang explained that the concern of missing patients with invasive cancer was considered, and patients with palpable disease are not included in the trial, nor are any cases that are remotely suspicious for microinvasion or invasive cancer. All patients receive screening MRI to determine eligibility. Despite all of the precautions, two study patients had invasive cancer at the time of surgical excision, so it is important to recognize that when active surveillance/watchful waiting studies are performed, there is this possibility. This study was designed to remove all residual disease surgically and then treat at 3-months postoperative. All patients in the study were ER-positive.

Dr. McCaskill-Stevens asked Dr. Gray about target genes with therapy and predictions. Dr. Gray responded that there is no quantitative, direct method of prediction, but there may be the ability to state which subset of tumors are likely to respond. A small set of candidate markers that can be identified to evaluate clinically risk indicators is needed. Dr. Esserman added that one of the next steps of the I SPY TRIAL was to head in this direction.

Dr. Weaver commented that when looking for aggressive genotypes with what has been learned about genetic profiles, generally there is a fair amount of tissue with which to work. He asked how this can be applied to using the material available for premalignant lesions, which usually is core biopsy material. As most of the tissue has been consumed in the diagnostic process, he asked how the remainder can be used to move forward therapeutically to predict which patients will progress. Dr. Gray responded that in the I SPY TRIAL a few core biopsies are taken prior to the initiation of the neoadjuvant therapy, and most molecular biologists at the workshop have learned how to perform CGH, expression profiling, sequencing, and tissue protein lysate arrays out of one core. Additionally, as technologies advance, less nucleic acid is needed for the assays.

### **Imaging: State of the Science and New Directions**

Laura Liberman, M.D., Memorial Sloan-Kettering Cancer Center, New York, NY

Although mammography has been the gold standard for screening, it does have limitations for use in DCIS cases. Breast MRI is an alternative to mammography that can find cancer even following a negative mammogram. In six studies of 1,044 women, of the cancers found by MRI, 30 percent were DCIS. In three studies, the sensitivities of mammography, ultrasound, and MRI were compared. MRI was much more sensitive in cases of invasive cancer than both mammography and ultrasound (90-100% vs. 28% and 41%, respectively). The same held true for DCIS cases (83% vs. 33% and 0%, respectively). Taken together, the results indicate that MRI holds a particular advantage for the diagnosis of DCIS. On MRI, approximately 70 percent of DCIS cases present as nonmass cancer and 30 percent as a mass. The morphology of the enhancement is clumped or irregular, and the distribution is ductal or segmental. In terms of kinetics, on an MRI DCIS shows low initial rise with a long time to peak, and in addition to washout also may have plateau or persistent enhancement. If the morphology is suspicious, a biopsy should be performed no matter what the kinetics show. In one study of 101 cases, MRI found 39 cases of breast cancer that mammography did not. Of these cases, 77 percent were classified as high grade, 56 percent were ER and PR negative, and 51 percent were *HER-2/neu* negative.

Studies are underway to determine how often MRI identified additional sites of cancer in the contralateral and ipsilateral breast in women with biopsy-proven cancer, as well as what percentage of additional sites are DCIS versus invasive and what the yield of MRI is if the index cancer is DCIS. Of the cancers found in the contralateral breast by MRI in the study, one-half were DCIS and one-half were invasive. The cancer rate was the highest in women with a strong family history (first degree relative with the disease) and those whose index cancer was invasive lobular carcinoma. In the study of the ipsilateral breast, MRI found additional sites of cancer in 27 percent of the samples, with most located in the same quadrant as the index cancer. Again, additional sites of cancer were more frequent in women with a strong family history and those with invasive lobular carcinoma. Data suggest that these additional sites may be clinically important.

MRI imaging was used in the assessment of residual breast cancer following biopsy. Results showed that MRI identified 86 percent of residual disease (sensitivity) and had 69 percent specificity. Positive and negative predictive values were 79 and 78 percent, respectively. If margins are positive, however, a negative MRI does not spare re-excision. In examining MRI in recurrent disease following breast conserving therapy, scars and post-radiotherapy changes did not interfere with MRI interpretation. Breast MRI-guided biopsy is a promising method of detection, but needs and challenges still remain. MRI-guided vacuum-assisted biopsy has the advantages of being less invasive, leaving no scar or deformity, and is less costly. MRI can identify otherwise occult DCIS, but it is unknown if detecting these cancers improves survival. Other areas to be investigated include determining which patients are most likely to benefit from MRI and how interpretation and biopsy can be optimized.

### *Discussion*

A participant asked what the parameters would be of a prevention trial for invasive cancer and DCIS. Dr. Liberman responded that optimization of the menstrual cycle, so that testing occurred in weeks 2 or 3, would be a start.

Dr. Kerlikowske asked who the women were who received MRI and what were the indications. Dr. Liberman replied that the criteria were specific and dependent on the study in question. Increased risk factor did increase MRI findings. The Sloan-Kettering criteria included genetic predisposition, familial history of breast cancer, and a personal history of breast cancer. Recurrent breast cancer cases were studied to assess the extent of the disease. Dr. Esserman added that a high risk justified the tests. The criteria for ordering an MRI in the I SPY TRIAL were dense breast and a BI-RAD score of 3 or 4.

A participant commented that the data for screening women who are carriers provide the rationale. The bulk of the data that exists is in invasive disease. The 10-year local fail rate is 5 percent or less. MRI provides the capability to find disease and also may increase the mastectomy rate. Cancer therapeutics must be judged by patient-centered outcomes. Serial sub-gross sectioning finds additional cancer, and if it is found, it must be treated. Treatment must do right by the patient.

Dr. Liberman commented that a randomized clinical trial with death as the endpoint will never be approved.

A participant asked if the researchers investigated whether recurrences were separate primary cancers or an extension of the first cancer. Dr. Liberman responded that the cancers appeared to be separate on imaging, but cancers that appear separate on imaging can be pathologically contiguous.

Dr. Esserman commented that it was remarkable how different the patterns of disease are, and this is an opportunity to learn. It is possible that those patients with unicentric disease may have a different process and those with multicentric disease may be the ones who should receive radiotherapy, but it should not be used to upgrade mastectomy.

Dr. Holland commented that a study from 1984, in which the index tumors were 5 cm or less, and the additional foci studies cannot be compared to current studies. In terms of multicentricity, he does not approve of the other quadrant definition. The definition should be at least a 3 cm distance between the two lesions. The Sloan-Kettering data indicate that multicentric disease is present in only 5 to 6 percent of patients.

A participant asked if there was something about the MRI technology more likely to detect high-grade lesions, as the lesions identified are more likely to be high grade. Dr. Liberman replied that there was nothing to support this in her data, but a German group is performing detection on the basis of vascularity.

## **FRIDAY, February 2, 2007**

### **Synopsis and Facilitated Discussion of Key Issues From Day 1**

Rachel Ballard-Barbash, M.D., M.P.H., Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD; Leslie Bernstein, Ph.D., University of Southern California, Keck School of Medicine, Los Angeles, CA; Tracy Lively, Ph.D., Cancer Diagnosis Program, National Cancer Institute, Bethesda, MD

Dr. Leslie Bernstein commented that the previous day's presentations and discussions had illustrated four categories of problems and challenges: making the diagnosis, decisions that affect incidence, treatment decisions, and prevention. Researchers are not always in agreement on solutions in these areas. In terms of making the diagnosis, how DCIS is classified at diagnosis affects how it is going to be treated. Also, moving toward a point of knowing what molecular characteristics allow the watchful waiting of breast tumors in certain subgroups of patients is highly desirable. In examining the incidence of DCIS along racial and ethnic lines, because rates are constant between all races in DCIS but not invasive cancer, an important question is if DCIS differs from invasive cancer. Use of technology also affects incidence. When determining treatment decisions, the endpoint must be established whether it is survival, recurrence, or something else. As the survival rate currently is approximately 98 percent, it is difficult to increase it more than that. Many of the presentations had implications for prevention. The means for early detection are being developed, and this will be a major step toward prevention. Slowing down the progression of cancer may be the best opportunity for prevention.

Dr. Tracy Lively commented that she was struck by the optimism of the previous day's presentations, which indicated that within 5 years researchers expected significant differences in diagnosis and treatment as a result of the advancements in technology. Another interesting point was that researchers tend to agree that followup of at least 10 years is necessary with DCIS. The path to developing new tools has been laid out. The first step is to outline exactly what the clinical needs are. The previous day's speakers, whether they were coming from the perspective of biology, epidemiology, or genomics, each focused their talks on decisions with which doctors and patients struggle. A big challenge is how to prioritize markers. It is necessary to focus on the real clinical questions and design a feasible study to answer those questions. If researchers produce new ideas, new collaborations, and new methods and work together to solve the challenges facing DCIS, then the NCI will do its best to help researchers make it happen.

Dr. Ballard-Barbash charged the group with determining what areas are needed to move forward to advance the research and frame the key questions that need to be answered. The products of the breakout sessions will be used by the NCI and other organizations to move forward.

A participant commented that one theme that has emerged from this workshop is how much is being done for women with DCIS despite how little risk they have, that is, the theme of overtreatment. She suggested a paper or series by the assembled experts be published to help the clinical community understand the nuances of the various research results that are being published that affect treatment

decisions and skew these decisions toward overtreatment. A publication providing the real numbers of the impact of radiation and tamoxifen will be helpful. Dr. Ballard-Barbash added that this would be a good idea to move forward, perhaps even including the relevance of new methodologies. Dr. Tlsty stated that this workshop should be captured on paper.

### **Meeting the Research Challenges: Update on Current Population-Level DCIS Research**

Laurel Habel, M.D., Kaiser Permanente Northern California, Oakland, CA

Population-level studies can help meet the research challenge by providing: secular trends in incidence, treatment, and mortality; characteristics of DCIS tumors (e.g., *HER-2/neu* status) in the general population; the etiology of DCIS and different DCIS subgroups; the prognostic value of patient and clinical factors, pathologic features, and standard tumor markers; a prognostic index; and populations for the discovery and validation of new markers. There have been many descriptive as well as outcome/prognosis population studies, but not many quality-of-life or risk factor studies. Overall, there are relatively few DCIS studies, probably because up until the mid-1980s, it was rarely diagnosed and even now is diagnosed in relatively small numbers. Additionally, breast cancer researchers are interested primarily in risk factors and outcomes for invasive cancer. Other reasons include the low risk of breast cancer mortality, the fact that there are few new treatments to evaluate, and there is minimal tissue for marker studies. The recent descriptive studies of DCIS incidence and treatment provide information on patterns and trends and allow the study of unselected populations but very few collect pathology materials or information on recurrence. The quality-of-life studies focus on recent diagnoses, so information is not available at this point on long-term outcome. Only some of the current risk factor studies have tissue blocks, and the ones that do only possess a small portion of the overall sample. Outcome studies on treatment and clinical factors focus on larger studies, and only some have pathology materials. Outcome studies on risk factors encompass a relatively small number of cases and an even smaller number of recurrence incidents. Phase III random clinical trials are the best source of pathology materials. Current studies are examining a variety of questions related to risk of disease, treatment, and outcomes, and the source populations and designs vary. Some studies are retrospective, whereas others are prospective, and there are a relatively small number of studies with both clinical data and biospecimens.

A current DCIS biomarker study aims to examine the risk of recurrence and patient and clinical factors, histopathologic features of the index DCIS, and tumor markers (immunohistochemistry and gene expression). The second goal of the study is to develop a prognostic index that uses a combination of factors. The study cohort is comprised of 3,100 eligible DCIS patients out of a potential of 3,700 and examines recurrences ( $n = 340$ ) versus controls (nonrecurrences). Histopathologic features and DCIS classification systems, immunohistochemistry, and gene expression profiles will be investigated. Only those treated with breast conserving therapy were selected for the cohort, which is mostly white with a well-rounded mixture of age groups. Preliminary findings of the patient and clinical factors indicate that the risk of recurrence is higher in younger women, those with symptomatic DCIS, and black women. Risk is slightly higher in premenopausal women and those with a history of benign breast disease, but recurrence is not associated with BMI or family history. Results of the mammographic findings indicate that scattered calcifications approximately double the risk of a recurrence, and risk is elevated among patients with highly dense breasts, but the increase largely may be for contralateral cancer. The pathology findings indicate that the risk of recurrence is increased for comedo necrosis, larger tumors, and status of margins but not nuclear grade. Additionally, the risk is higher for tumors with poor cell polarity or stromal inflammation and among patients with ER- and PR-negative tumors, possibly p53-positive tumors. Women with ER-/PR-/HER2+ tumors also appeared to be at increased risk of recurrence. The overall findings indicate that no single factor is strongly prognostic, but a few are modestly or weakly prognostic.

Next steps include gene expression studies, the development of a prognostic index that combines clinical and pathological features and markers, validation of the index in other populations, and collaborations with other breast SPOREs. It also is important to remember that retrospective tumor retrieval rates vary substantially, retrieval rates of slides and blocks are lower on patients who have a recurrence, central review is important because not all DCIS is really DCIS, and expert breast pathologists are busy and the reviews of hundreds of patients and thousands of slides take time. Additionally, most DCIS tumors are small and many patients have only one block with tumor; therefore, exhaustion of tumor material is a concern. Marker studies must be prioritized and possible bias as a result of over-representation of larger tumors must be considered. Finally, a big study is never big enough.

To move forward to meet the research challenge of DCIS, researchers must: incorporate biomarkers into new studies; combine populations to increase sample sizes; establish a set of key variables and definitions to collect on study populations; develop a prognostic index that combines clinical factors and markers into a single score; follow earlier cohorts for long-term outcomes; conduct prospective studies of new therapies; validate promising markers and risk scores in multiple independent populations; and bring together population scientists, clinical scientists, and basic scientists.

### *Discussion*

Dr. Katz commented that in terms of the point that stated a study can never be too big, actually a big study can be too big, and it definitely can be sufficiently large enough to demonstrate that there is not much clinical utility to the conceptual approach to evaluating a predictive model with predetermined variables. His study concluded a low, null finding in a low-risk population. Many patients prefer mastectomy because of the perceived risk. Studies show that when a surgeon is asked to make a choice regarding mastectomy for the low-risk group, the percentage that recommend mastectomy is near zero. When patients are asked to make the same decision, approximately 50 percent opt for mastectomy. The question is how to decrease the risk further in the low-risk group.

Dr. Kerlikowske advised that adjusting for breast density and BMI may be significant. Dr. Habel responded that breast density was adjusted for, and BMI still was not significant. It is possible that the study size was too small, because it was expected that BMI would be a significant risk factor.

Dr. Kerlikowske commented that in terms of looking for predictors with a subsequent event being invasive cancer versus a DCIS event, the markers can be very different. She suggested that the data be broken out and presented both ways. Dr. Habel replied that the data are being analyzed in a variety of ways, but so far there has been no strong prognostic factor. She agrees that DCIS and invasive cancer may be two different entities.

A participant asked if risk of recurrence in the context of some cells being missed on excision (i.e., positive margins) was considered. Dr. Habel responded that she did not have time to present everything that her laboratory is investigating, but that has been examined. Nothing significant is seen with cancerization of the lobules, and the status of margins is not telling the whole story. Stronger prognostic factors in addition to margins are desired.

Dr. Kuerer asked what the median followup was for patients in the study. Dr. Habel responded that the median followup at this point was 5 years, with the longest followup being 12 years. Dr. Kuerer replied that the median followup at M.D. Anderson Cancer Center was 18 years. At 18 years, a significant finding is seen in terms of nuclear grade as a risk factor for recurrence. As was pointed out in the previous day's discussions, long follow-up times are needed to obtain a more accurate picture of this disease. Dr. Habel responded that the laboratory plans to investigate if risk factors change over time.

Dr. Eileen Rakovitch commented that she believes that the incidence of invasive cancer following a DCIS event is prevalent enough that studies are justified. The Sunnybrook Health Sciences Centre in Toronto has initiated a large cohort study of more than 8,000 patients of which 5,700 had breast conserving surgery. The goal of the study is to determine who proceeds to invasive cancer. In looking at a Sunnybrook cohort of 130 women treated by lumpectomy alone compared to those who received radiotherapy, findings indicated that *HER-2/neu* predicted for the development of invasive recurrence. A larger cohort, however, is needed to validate this finding. This will allow the determination of those patients that may need mastectomy instead of radiotherapy because they are at a higher risk.

Dr. Kimberly Van Zee asked what the local recurrence rate was. Dr. Habel responded that at 5 years followup, it differed for patients treated with radiotherapy versus excision alone. Rates were similar to that of the NSABP B17 study, approximately 10 percent in those treated with radiotherapy.

A participant asked if there was a difference in the invasive recurrence rate between irradiated and nonirradiated groups. Dr. Habel replied that her findings indicate that there is a lower percentage of invasive recurrent cancer in the irradiated group.

A participant commented that it might be advantageous to examine samples from the 2 to 3 percent of DCIS patients who had death as the outcome to determine what differences can be found in their tissue. Another participant added that it would be interesting to combine and evaluate all of the serious recurrences from all studies.

A participant commented about the extent that genetic determinants affect recurrence in tamoxifen-treated patients. In a cohort of 895 patients, those with low risk recurrence scores (50% of the cohort), the risk of local recurrence was 4 percent. In those with high recurrence scores (25% of the cohort), the risk of local recurrence was 16 percent, or four times that of the low-risk group. This was true in the mastectomy and lumpectomy cohort. Biology definitely is an important factor as well as treatment.

A participant commented that how relevant some of these studies are to clinical practice is a legitimate question. In clinical practice, many DCIS patients are excluded from breast conservation therapy because of their many positive margins. Excluding these patients leaves a group with relatively limited disease who have negative margins. Determining the recurrence rate in the group with positive margins is relevant. The relative importance of extended disease, adequacy of excision, and biological factors is the question. How much risk is mechanical (i.e., accuracy of excision) versus biological? If better methods of removing all involved tissues/ducts are developed, it will be possible to assess how much biology matters after all cancer has been removed. In trying to determine the best margins for excision, it may be beneficial to examine the markers in the normal breast tissue surrounding the tumor instead of those markers in the DCIS itself. Markers in the normal tissue surrounding the DCIS may be the best predictors of complete margins.

Dr. Hwang asked if there is a difference between groups that already have been analyzed versus those that have not been analyzed yet that might change the findings after their analysis. Dr. Habel responded that the pathology review has been blinded, with cases mixed with noncases, so there should not be a major difference. The exception is that the immunohistochemistry may be biased, because the cases with the smallest tissue amount have been saved until last. As these probably represent those cases with smaller tumors, the analysis could change.

Dr. Waldman asked if biology may be able to predict if it is the same tumor returning or a new neoplasia in the ipsilateral breast. Dr. Habel responded that 20 to 30 percent of the recurrences are expected to be a new neoplasia in the ipsilateral breast.

A participant commented that in terms of the mechanical versus biology issue, good data exist that indicate that margins are a significant factor. Biology aside, generally if the margins are good, recovery can be expected. Margins need to be predicted better. Another participant agreed that margins are important. Although those patients that receive mastectomy probably have the worst biology, they have zero recurrence. Perhaps this information can somehow be extrapolated to a smaller procedure. Another participant commented that margins are important, but researchers should not be restricted to thinking of margins as ink and ducts near ink.

Dr. Porter commented that excluding mastectomy patients from studies makes it difficult to determine what the risk factors are for DCIS. Dr. Habel responded that these patients are included in the risk factor studies. It is the prognostic studies in which they generally are not included.

## **MEETING THE RESEARCH CHALLENGES: STRATEGIES FOR COLLABORATIVE RESEARCH AND SHARING RESOURCES**

### **Panel: Strategies for Addressing Common Concerns in Collaborative Research and How To Maximize Existing Resources To Advance Research**

Panelists: Graham Colditz, M.D., Dr.P.H., Washington University School of Medicine, St. Louis, MO; Larissa Nekhlyudov, M.D., M.P.H., Harvard Medical School, Boston, MA; James Dignam, Ph.D., University of Chicago, Chicago, IL

Dr. Colditz stated that there are two concerns regarding collaboration: (1) access to resources and the priorities of the collector of the data versus the collaborator; and (2) the progression of academic careers, particularly that of junior faculty, following a major contribution to team science. Data resources include questionnaires, blood-based samples, and tissue samples. Questionnaire data are combined easily. DNA samples from the Breast and Prostate Cancer and Hormone-Related Gene Variants Cohort Consortium Study, the Cancer Genetic Markers of Susceptibility Study, and InterLymph Consortium can be shared; however, blood is less readily shared as a result of its nonrenewable nature. For nonrenewable resources, priority should go to the collector, as he developed the hypothesis for the collection and then collected, catalogued, and prepared the samples. In terms of large-scale biomedical efforts, the Institute of Medicine has discussed the issue and recommends that big science address its own challenges and difficulties. Team science can be small scale (e.g., R01 grants, program projects) or large-scale (e.g., SPORES, transdisciplinary Centers, consortiums). Tenure and promotion generally require a set criteria of activities that may be compromised by involvement in a large-scale collaboration; tenure committees need to reward instead of penalize investigators involved in collaborative efforts. Consortia-type activities already are a common feature of the epidemiology programs at the NCI, but training programs do not offer any recommendations on how to prepare for participation in these large-scale efforts. Additionally, promotion criteria do not yet reflect such activities. Junior investigators can be supported for promotion by educating academic leaders that major contributions through team science can be accomplished and working to revise promotion rules to accommodate team science. Also, the peer-review process for consortia must be improved, and journals should allow long author lists. Funding of the basic components that feed into the consortia activities must be maintained, and best practices need to be identified.

Dr. Nekhlyudov described the Cancer Research Network (CRN), an NCI-funded consortium comprised of 11 Health Maintenance Organizations (HMO)—soon to be expanded to 13—with access to more than 10 million patients. Advantages of collaborative efforts include large sample sizes, a wide range of expertise, geographic diversity, enhanced productivity, and the potential for mentorship for junior investigators. Disadvantages include the cost, the Institutional Review Board (IRB) process, Health Insurance Portability and Accountability Act regulations, differences in protocol, and the potential for junior investigators to be lost. There are solutions to overcome these difficulties. Indirects from other

sites are no longer a part of the prime site budget, which should reduce cost. Implementing a standardized language across all sites (e.g., the CRN's Virtual Data Warehouse, in which the claims-based data terminology is being created to be standard across all 13 sites), assigning multiple principal investigators, standardizing IRB processes, and providing training for consortium leaders and junior personnel are other possible solutions.

Dr. Dignam commented that sharing has been mandated among some cooperative groups. This mandated sharing creates a tremendous resource, but the groups mining the data have invested much effort and time collecting and managing the data sets. Sharing the data may allow outsiders utilize the data for their own analysis, which the group managing the data wishes to do but does not have time to do. On the whole, though, it has been a positive experience. Data collection methods need to be improved so that data retrieval can be more meaningful. When a canonical form set is created, individual collaborators then all want it amended to include their data needs and desired variables. Clinical trials generally are focused on the mission but recently have been broadened to include quality-of-life issues. Additionally, expertise in clinical trials is being expanded to include behavioral science experts. This expansion brings a whole new set of opportunities for collaboration.

### *Discussion*

Dr. Ann Geiger commented that CRN is required by DCCPS to conduct formal evaluations every year, and the opportunity to use the skills that determine if the collaboration is going well should not be overlooked. She asked the panel to describe what skill set leaders of large collaborations need to possess to be successful. Dr. Colditz replied that two helpful skills are the ability to devise an overall plan early in the process and the ability to effectively run meetings. Attending management training classes or reading management/leadership books also may be helpful. Dr. Nekhlyudov added that a leader must be very clear about the direction the collaboration should take and needs to communicate this vision. The leader must take charge but also be willing to listen to others' opinions and be respectful of their time.

Dr. Solin commented that a common concern is the increased regulatory paperwork burden, which can be exhausting. He suggested that a national, standardized IRB be implemented so that project investigators do not have to deal with multiple IRBs when attempting to collaborate. Dr. Colditz responded that a local HMO is concerned about the burden within their research system and would want control over their own institutional review and not trust it to another institution's IRB. Dr. Solin clarified that the standardized IRB would be nationally mandated and possibly managed via the NCI. Dr. Nekhlyudov commented that an HMO's primary concern is not research, and it may be difficult to get them to comply with a national IRB. Dr. Esserman asked if the organization was present to conduct clinical medicine in such a way that a molecular component could be added. A national mandate might be a good place to begin. Researchers must get organized in the collection and storage of specimens and data, and baseline, common standards must be in place.

Dr. Ballard-Barbash informed participants that the Department of Health and Human Services (HHS) asked for suggestion about how to improve health care delivery so that health care and research both can move forward. The NCI offered its input, and the HHS has stated that this issue is a priority. Researchers should offer input as well. If the initiative moves forward, funding will come from HHS.

### **DCIS and the Development of Diversity During Breast Cancer Evolution**

Craig Allred, M.D., Washington University School of Medicine, St. Louis, MO

Several models of the evolution of DCIS have been put forth; one compelling model is the Wellings-Jensen model, developed more than 30 years ago and based on the evidence of histological continuity. In this model, the malignant stem cell compartment resides somewhere in the normal terminal duct lobular unit. Some of these cells proliferate, undergo hyperplasia, and develop into lesions called hyperplastic

enlarged lobular units (HELUs). Some subsets of HELUs develop into ADH, some of which progress further to DCIS, and finally some develop into an invasive phenotype. The continuum is defined by artificial stages, but there are biological characteristics distinguishing one stage from another. The transition from the normal compartment to HELUs is growth. The transition from HELUs to ADH includes changes in epithelial adhesion and polarity. The transition from ADH to DCIS includes histologic, biologic, and genetic diversity that is not seen in previous precursors. Other evidence to support this model includes the fact that these lesions are increasingly more common in cancerous breasts, there are escalating risk factors for developing invasive breast cancer, and the lesions share genetic alterations with invasive breast cancer and homology with genetically engineered mouse models.

Generally, DCIS is considered to be of two types, noncomedo (low grade) with good histological and pathological features and comedo (high grade) with bad histological and pathological features. ADH is accepted as a nonobligate precursor of noncomedo DCIS because they look similar; ADH is not accepted as a precursor to high-grade lesions because of their differences, so a co-precursor is speculated to exist. This is, however, a false assumption. DCIS is a very complex, broad histological and biological continuum. Along this continuum from well to poorly differentiated, there is a correlation between pathology and biomarker profiles. This pattern is replicated in invasive cancer, so this diversity arises first in DCIS and is later propagated independent of invasion to invasive cancer. Gene expression microarray data show the same pattern of subtypes. The real question is what the source of this diversity is. The hypothesis is that a subset of ADH progress to low-grade DCIS, some of which then progress to higher grade DCIS by the stochastic accumulation of additional genetic and epigenetic alterations that influence cell morphology and function.

Evidence to support the progression of low-grade to high-grade DCIS is that: ADH is a risk factor for invasive breast cancer and DCIS; there is histobiological continuity between cases and histobiological continuity/diversity within cases; diversity increases with time; and the lesions possess the same chromosomal defects (e.g., mutated p53, which is highly correlated with any kind of diversity) that increase over time. Other events also can contribute in nongenomic means, including apocrine metaplasia (APOM). APOM is the transformation of breast epithelium to cells that are histologically and biologically similar to those in apocrine sweat glands. APOM is associated with a complete loss of ER expression and is common in all types of benign breast epithelium. Using an apocrine histological scoring system, more than 42 percent of DCIS samples had a score of at least 1, and 15 percent showed significant apocrine histology ( $\geq 2$ ). Those with high scores were correlated with loss of ER. Additionally, the apocrine gene signature associated with 30 percent of the ER-negative invasive tumors and looked like ER-positive tumors. The progression from DCIS to invasive breast cancer is a major step. Seminal studies have shown that there are very few differences in gene alteration between noninvasive and invasive tumor cells in the breast epithelium; however, some studies have shown many differences in gene expression between stromal cells in noncancerous and cancerous breasts. The stromal cells may be fundamentally involved in progression, whereas the genotypic alterations reside in the tumor epithelium.

In summary, substantial histological and biological diversity originates in DCIS. The diversity in these lesions is primarily stochastically or randomly acquired and highly correlated with genetic instability. The tumor microenvironment must be taken into account in the progression to invasive disease.

### *Discussion*

Dr. Harry de Koning asked if Dr. Allred considered the issue of regression. Dr. Allred responded that there was some evidence of regression in cervical cancer literature. He is not aware of similar breast cancer studies, but some investigators have speculated that some of the histological appearances of some

DCIS, especially high-grade DCIS, show a space with a scar instead of necrosis in the middle. In these cases, regression is assumed. Although it may be possible for regression to occur, it is not common.

A participant commented that DCIS is heterogeneous within and between tumors and tools must be carefully selected or the heterogeneity may be missed. In terms of *HER-2/neu*, fluorescent *in situ* hybridization (FISH) allows the analysis of cell-to-cell heterogeneity. Although, in most cases, tumors are homogeneous. Dr. Allred agreed that this was the case when examining *HER-2/neu*, but ER, PR, and p53 are not overlapping defects. Only 1/10,000 of the tumor is examined in FISH. Clones compete for the dominance of the tumor.

A participant asked how the gene signature of apocrine change, which is a differentiation pattern, may be used to understand the proliferation of ER-negative cells. Dr. Allred replied that this is a good idea, but he has not thought in terms of proliferation because he is interested in ER status. Apocrine metaplasia is not associated with genetic alterations, and a good candidate may be downregulation of methylation. He is attempting to determine if there is an upstream signature to explain why ER is being turned off, but proliferation could be another endpoint.

Dr. Esserman asked if Dr. Allred thought that it was important for investigators to begin recording the percentage of diversity so that this information is available for future collaboration. Dr. Allred agreed that this was a good idea, because diversity is definitely important.

### **Breast Cancer Advocates**

Bambi Schwartz and Helen-Louise Ittner, University of California at San Francisco, San Francisco, CA

Ms. Ittner thanked the group for including her in the meeting. She also thanked investigators for their research efforts. Her mother was diagnosed with breast cancer, and her mother's treatment in the 1950s was very different than her own treatment in the 1990s because of the advances made by breast cancer researchers. She urged investigators to collaborate, because all of the positive gains made by collaboration eventually trickle down and affect everyone living with breast cancer.

Ms. Schwartz thanked the SPORE programs but mentioned that she was disappointed that the patient hardly was mentioned in any of the talks. She also is upset by the resistance to collaboration. From the patient's point of view, the goal of researchers is to help patients not to receive promotion and tenure. Patients do not care about politics. One topic not discussed was the dialogue between patient and doctor. When diagnosed with DCIS, patients have a fear of dying of the disease just as they would with a diagnosis of invasive breast cancer. Community doctors look to the researchers to provide answers, but it appears as though the researchers do not care about what the community doctors have to say. Investigators should listen to community doctors.

### ***Reports From Breakout Sessions***

#### **Breakout Session: What Must Be Done To Facilitate Moving Promising Findings From Small Validation Studies To Evaluation in Large Population Studies and Clinical Trials?**

Ann M. Geiger, Ph.D., M.P.H., Wake Forest University School of Medicine, Winston-Salem, NC

The key problems to this issue are that no single study is large enough and there is limited tissue and patient information. The major challenge is the decision of when to move from a small to a large trial. Determination of who decides and how the decision is made is critical, as well as what type and how much evidence needs to be collected to inform the decision. The group recommends that a committee to build on meeting discussions and to lay the foundation for the future be formed. Such a committee will: weigh the merits of study registry, consortium, or similar activities to catalog, maintain, and disseminate an updated description of existing resources and then develop a plan; resolve tension between voluntary

versus mandated participation in shared resources; and promote sharing of experiences (i.e., successful methods for data pooling and tissue banking). The second recommendation involves a change of culture. Clinicians, basic scientists, and population scientists need to work together before biomarkers are ready to move from small to large studies because it is important to develop the science together. Because of the differences between academic and community clinicians, both groups must be involved. Models of funding-induced change include SPORes, the Early Detection Research Network, and translational networks. Articles about which biomarkers to move from small to large studies are a possible resource.

### *Discussion*

Dr. Tlsty commented that technically any tissue-based evidence belongs to the women from which it was collected. Dr. Geiger responded that levels of consent differ, and studies must take these varying levels of consent into consideration.

### **Breakout Session: What Are the Possible Collaborative Approaches for Testing Markers Identified in Basic or Cellular Research in Small-Scale Studies Prior to Testing on a Cohort Sample?**

Isis Mikhail, M.D., Dr.P.H., M.P.H., Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD

The chosen endpoint should be the development of invasive cancer, and because of the limited data, retrospective studies examining this endpoint are not recommended. It is important to remember, however, that prospective studies need a large sample size to be reliable. Four phases of such studies could be used in collaborations: (1) discovery; (2) small study prediction of prognosis, (3) initial outcomes, and (4) large-scale cohort study for validation. The first two stages could be performed independently, but the third and fourth phases would require collaboration. Statisticians could collaborate and create a table with the numbers required for statistical significance that could be shared among collaborators so that the necessary sample size for significance is widely known. The Urinary Bladder Cancer Consortium is a good role model of collaboration. The group recommends that pathologists be given standard training to assure intraobserver consistency. Standardized pathological observations will facilitate future collaborations. Additionally, good communication is necessary for successful collaboration. The best course is to continue an open dialogue with other DCIS researchers.

### *Discussion*

Dr. Tlsty asked how Dr. Kerlikowske obtained pathology samples from hospitals. Dr. Kerlikowske responded that she keeps in contact with 63 hospitals and is persistent in asking for samples before they are thrown out. She offers her assistance in making the donation of the samples as easy as possible for the hospital, including picking them up herself. Sometimes contacting many people in the hospital until one finally says yes is necessary. At this point, she has so many contacts that hospitals now contact her before samples are thrown away. This is a time- and labor-intensive process. The cost of each block ranges from free to \$300, depending on the source hospital. A participant suggested that hospitals be reminded that ultimately the research using the samples is for the patients, and the hospital does not want to be associated with any behavior that negatively impacts patients and public perception. Another participant commented that some areas in which a lot of studies are taking place may be willing to share samples, but there is intense competition from various studies to obtain these samples. Additionally, hospitals in such areas are inundated with requests and simply may not be able to keep up with these requests. In such areas, hospitals and study administration have an agreement that only one person from each study is designated to contact hospitals with tissue sample requests. Also, SEER has spent money on discard repositories in Los Angeles, Hawaii, and Iowa.

## **Breakout Session: What Information on the Natural History of DCIS Is Needed To Enhance Prediction?**

Kathy Cronin, Ph.D., M.P.H., National Cancer Institute, Bethesda, MD

Because intervention occurs in DCIS, investigating the natural history of DCIS, which is not allowed to progress, may not be a relevant question. One recommendation of this group is to combine data from existing studies to develop prediction models, which will allow identification of the lowest risk group. Independent validation will be needed. The development of a list of important markers also is important, and funding and resources for collecting blocks for a biomarker study is necessary. The group also recommended that a trial design that provides information about the natural history of DCIS be encouraged. In this scenario, prediction models would be used to identify low-risk women that are candidates for watchful waiting and/or prevention. This model must be safe, ethical, and accept some risk. Trials that provide information of when and how to intervene along the natural history also should be encouraged, and a cohort that will provide natural history information in the future should be identified. The development of a standardized educational tool to communicate risk to patients may be the key to recruiting women for these trials; this would provide information for physicians and their patients that would help communicate the absolute risk. Additionally, there is a need for studies of quality-of-life issues associated with long-term and aggressive surveillance where patients are observed over a long-term period. The development of a consensus statement from the meeting that would summarize state-of-the-science of DCIS and define the absolute risk of recurrence and survival is highly desirable. This would provide background information for clinicians to use, updated from the consensus report of 10 years ago. This also may provide standardization of margins. Another discussion occurred regarding taking the “carcinoma” out of DCIS, which might change the perceptions of risks involved with the diagnosis of DCIS and distinguish it from invasive cancer.

### *Discussion*

A participant asked if watchful waiting meant stopping any intervention following a needle biopsy in low-grade DCIS. Dr. Cronin responded that there was discussion of that and also a discussion of prevention using tamoxifen. The participant clarified that watchful waiting meant no further surgery and stated that this scenario probably would not happen because patients will not agree with no further surgical intervention for diagnosis even if a drug is available. They might, however, agree to needle biopsy followed by excision to confirm the DCIS diagnosis and then stop intervention at that point. Dr. Hwang agreed that this is a limitation of potential watchful waiting trials; however, there is no way to move forward in understanding DCIS and the natural history of DCIS unless something is known about what happens in women in the absence of active treatment. Retrospective studies indicate that women with unrecognized microinvasive carcinoma that remain untreated do not do any worse than those that are treated. Part of the education and communication project would be to educate patients that there will be a subset of women that will have unrecognized microinvasive disease. The participant commented that the simple endpoint of invasive cancer is restrictive because patients care about other endpoints, including local recurrence.

Dr. Nekhlyudov asked the breast cancer advocates if any effort was being devoted to making women aware of the population-based research. Ms. Ittner responded that most women do not pay much attention to breast cancer topics until they are diagnosed. Her recommendation to newly diagnosed breast cancer patients is to take a tape-recorder with them to capture all of the doctor’s words and to donate their tissue to breast cancer research. Ms. Schwartz added that no education is available regarding research efforts or the connotations of research. Most breast cancer patients are treated at community hospitals versus large university medical centers, where most of the research occurs. Many patients are not aware of research opportunities because many of the community doctors are not interested in being involved in research studies.

Dr. McCaskill-Stevens commented that the NCI has a premalignancy breast cancer committee that reviews proposals regarding funding for breast cancer projects. The committee invited a group from Indiana University to share their experience collecting more than 2,000 normal breast tissue specimens from women, including 14 percent minority specimens. Dr. Ballard-Barbash added that the group developed this as an open resource for collaborative research. Dr. McCaskill-Stevens also described a network of community physicians called the Community Clinical Oncology Program (CCOP) that is comprised of 62 networks across the country, 13 of which are minority-based. CCOP was developed in the mid-1980s to bring academic research into the community and may be another resource for tissues.

A participant stated that pathologists are instructed not to take fresh tissue from DCIS patients and freeze it because every piece needs to be examined to rule out invasion. This underscores the need to have a balance between banking the tissue repository of samples for future research versus ensuring that a diagnosis of invasion is not missed. He has been met with resistance when requesting clinicians to extract a few extra core samples for freezing and banking. The question that then evolves is if researchers can find all of the answers they need with paraffin-embedded tissues so that fresh tissue collection and banking is not needed. He asked what the clinicians' reaction was to taking extra core samples and banking those for the future. Dr. Allred responded that there are ways around this that are labor intensive. Mirror-image frozen sections is one alternative. The participant responded that this is not feasible in core biopsy samples because this results in wasted tissue. Dr. Winchester responded that clinicians do like to know what they are dealing with, and because microinvasion can be subtle, every bit of the tissue should be investigated. Dr. Hwang commented that there is a similar protocol in patients with microcalcifications, but it is difficult to inform patients fully when doing that type of banking. It is difficult to explain to a naïve patient about the implications of banking a core sample. Although it would be highly desirable to have frozen DCIS samples, the technology to perform immunohistochemistry on paraffin-embedded samples is improving at a faster rate than the ability to inform patients of the implication of tissue banking.

Dr. Tlsty asked what happens to the tissue when the pathologist has finished the examination. A participant responded that it remains embedded in paraffin and saved, but it is not fresh. Dr. Habel added that she has tried to persuade clinicians in her organization to adopt these practices, but they will not change clinical practices for research purposes unless it can be proved that it is directly beneficial to the patient.

### **Conclusions and Next Steps**

Rachel Ballard-Barbash, M.D., M.P.H., Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD; Leslie Bernstein, Ph.D., University of Southern California, Keck School of Medicine, Los Angeles, CA; Tracy Lively, Ph.D., Cancer Diagnosis Program, National Cancer Institute, Bethesda, MD

Dr. Lively was impressed that Breakout Groups 1 and 2 both focused on the same question, that is, how to start from a smaller to a larger study or trial. Although these questions are not unique to the DCIS community, the study of DCIS is limited by the availability of tissue. There was an affirmation in both groups that the open dialogue of this workshop is a step in the right direction. Group 3 had less confidence in the ability of a committee to come up with a consensus plan to move the research forward. Open discussion that helps researchers understand who has what resources (e.g., data, tissues, patients, skills) at any given time is beneficial so that researchers can focus on the main research question and facilitate collaboration. She thanked Dr. Ballard-Barbash for her efforts in starting this open discussion by planning this workshop.

Dr. Bernstein also expressed her gratitude to Dr. Ballard-Barbash. She commented that she will investigate how many DCIS samples are available in a certain repository that could be used by

researchers, especially in marker studies. The California Teachers Study will publish a paper this month that reveals that increased physical activity decreases the risk of *in situ* breast cancer. The issue of the patient always must be in the forefront of everyone's minds. She asked if there had been a movement to develop intraepithelial neoplasia classifications similar to those in cervical and prostate cancer. A participant responded that the World Health Organization Working Group on Tumors of the Breast met in 1992 and decided that it was not prudent to move forward in that direction before there were data on which to base a revised classification rather than just changing the name for the sake of changing the name. The goal is to develop a molecular-based classification system as has been done with lymphomas. This raises the question about where ADH fits into the scheme. Dr. Fattaneh Tavassoli at Yale University has proposed that ADH and low-grade DCIS be combined into a single group called ductal intraepithelial neoplasia grade 1, but clinicians currently treat ADH and low-grade DCIS differently. Dr. Bernstein emphasized that cases need to be classified into those truly at low risk and those at high risk.

Dr. Ballard-Barbash reported that many participants asked if this type of a forum could occur on a periodic basis. It also may be worth the effort to have a larger group of researchers convene to debate the issues periodically. One goal of the workshop was to bring various groups together and stimulate discussion and collaboration. One theme that emerged was the concept of standardization of characterization and how that characterization is reported. Creativity often is best fostered in a small, nonregimented group, but resources happen when there is more organization. The NCI understands the challenges and frustrations involved with collaboration. Successful collaborations get more knowledge out more quickly and expand ideas. This is not a one-time effort, but an initial effort to increase the focus on DCIS and how DCIS research can be fostered.

Dr. Weaver commented that there were efforts toward standardization, including standard elements being recorded on pathology reports according to the CAP checklist. Dr. Kerlikowske asked if CAP checklist elements were being incorporated into SEER. Dr. Weaver responded that they did not go into SEER, but it is an attempt to get better volume and margin estimates. Dr. Ballard-Barbash stated that a future goal is to have electronic medical records with more systematic reporting of this type of information so the information could be translated to cancer registries. If these items are standardized, then they can be disseminated more widely.

Dr. Ballard-Barbash explained that the conference planning committee did discuss broadening the workshop to address issues such as quality of life and quality of care. It certainly is relevant to talk about issues other than those that were addressed at this workshop, and participants' suggestions for topics are welcomed. Additionally, another goal should be to think in terms of prevention within the context of early-stage cancer, as it should be a promising area, as evidenced by the success the cardiovascular field has had.

Dr. Ballard-Barbash thanked the participants for their contributions and adjourned the meeting at 2:14 p.m.