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The Breast Cancer Course for Researchers: An In-Depth Interdisciplinary Review of the State of the Science

On March 26-27, 2003, The Susan Love MD Breast Cancer Research Foundation, in partnership with the California Breast Cancer Research Program, hosted it's first-ever Breast Cancer Course for Researchers: An Interdisciplinary Review of the State of the Science. More than 100 researchers, including geneticists, basic scientists, surgeons, and epidemiologists, attended the conference, which provided a unique opportunity to learn about and discuss breast cancer science from an array of research perspectives.

"It was becoming increasingly clear to me that many researchers weren't aware of how breast cancer science was approached in fields outside of their own," says Susan Love, MD, the foundation's president. "And if the surgeons don't know what the basic scientists are doing, or the epidemiologists don't know what the radiologists have learned, we're never going to solve the breast cancer puzzle. The idea was to bring people together so that they could learn from each other and better understand how their own work fits into the broader field of breast cancer research."

The California Breast Cancer Research Program was eager to partner with the Foundation in producing this type of conference. The California Breast Cancer Research Program (CBCRP) was eager to partner with the Foundation in producing this type of conference. "This program was important to us because the goals of the Breast Cancer Course for Researchers are the same as those of our program: to eliminate breast cancer by leading innovation in research, communication, and collaboration," says CBCRP Director Marion H.E. Kavanaugh-Lynch, MD, MPH. "I feel confident that the researchers who attended this conference will use the information they gained to improve their own research and to advance what we know about breast cancer."

Prominent breast cancer experts served as Breast Course Faculty and gave state-of-the-art overviews of the epidemiology, basic science, and clinical aspects of breast cancer-to provide the "whole picture" of this disease. The four sections included in the program were (1) Normal Biology of the Breast—What We Know and Don't Know, (2) The Epidemiology of Breast Cancer, (3) Hormones and the Breast, and (4) Clinical Breast Cancer: What We Know and Don't Know. The schedule was structured to encourage attendees to participate, both in a group forum and to interact with each other.

I. The Normal Breast—What We Know and Don't Know

Faculty: Mary Helen Barcellos-Hoff PhD (Lawrence Berkeley National Laboratory)
Roy Jensen MD (Vanderbilt University)

Human Breast Development and Physiology

Roy Jensen, MD, an associate professor of pathology and cell biology at Vanderbilt University School of Medicine in Nashville, Tennessee, kicked off the course by reviewing the development and physiology of the mammary gland. He described how the breast develops in the fetus and how puberty sets the stage for the development of the breast ducts and the creation of the mature breast, which contains 8-15 lobes and terminal duct lobular units and 10-100 acini.

Then, when a woman reaches her mid-20s, her breasts slowly begin to atrophy. At menopause, when her progesterone and estrogen levels decrease, this atrophy accelerates. This results in a loss of the ducts and the terminal duct lobular units and in the replacement of the fibrous stroma with fat (which is why mammography now shows a much clearer image).

Dr. Jensen explained that the final differentiation of the ducts does not occur until pregnancy (which may explain why women who have no children or have them at an older age are at increased risk for breast cancer). During pregnancy the terminal duct lobular units gradually increase and the fat and stroma gradually decrease. By the third trimester, 90 percent of the breast is comprised of glands and the number of acini has increased. These changes enable the breast to produce 1-2 ml of milk per gram of breast tissue per day.

Dr. Jensen also noted that with each pregnancy the gland looks less and less like a nulliparous gland and more and more like a developed gland. (These changes that occur during pregnancy and lactation may explain observed differences in breast cancer risk between countries in which women have many children beginning at a young age and those in which women have fewer children later in life.)

"Of Mice and Women"

Mary Helen Barcellos-Hoff, PhD, group leader for cancer and tissue biology at Lawrence Berkeley Laboratory in Berkeley, California, followed with her presentation "Of Mice and Women." Dr. Barcellos-Hoff explained that mice models are needed in breast cancer research because the time it takes a cancer to develop in mice is dramatically condensed. This allows researchers to use mice to study several processes including the development of cancer from start to finish, interventions that may lead to new diagnostics, and drugs that may lead to patient-specific therapies.

Mice are an especially good tool because of the similarities between the human and mouse mammary gland, the fact that mice have 10 mammaries, and the availability of many mouse strains with different genetic backgrounds, including humanized and knockout mice. Also, in both mice and humans, cancer is spontaneous, ovarian hormone dependent, and its frequency increases with age and some hormone exposure.

Dr. Barcellos-Hoff also noted that by studying pregnant mice researchers have been able to observe how the composition of breast tissue changes rapidly after weaning and then returns almost—but not completely—to its prior state. For example, during pregnancy the ductal tree blossoms and become dense and then, when lactation ends, remains more developed than nulliparous tissue but still resembles its previous state.

There are, of course, important differences between mice and humans. For example, mice are more likely to develop estrogen receptor (ER) negative tumors, whereas the cancers women develop are more likely to be ER positive. Further, as Dr. Love pointed out during the discussion that followed these presentations, human breast ducts, unlike mouse breast ducts, intertwine. Thus, when micro-dissections are conducted on human breasts it is not always possible to determine where one ductal system begins and another ends.

Cell Culture and Biology

Next, Dr. Barcellos-Hoff discussed cell culture and biology, the intracellular and extracellular signaling of epithelial cells, and how to develop and design the mice models and mice studies that are most likely to provide substantive results. This led into Dr. Jensen's discussion on human cell lines and the problems researchers face in using human mammary epithelial cells for research studies. He pointed out that, not only is it difficult to obtain tissues and culture cells, but the cells have a finite proliferative lifespan, not all lineages grow in culture, and serum is required for growth of keratin 19 positive cells.

Unlike the human mammary epithelial (HME) cells, the human breast cancer cells are not only easy to obtain and easy to culture but there are some lines that express functional estrogen. In addition, these cells are well characterized for genetic changes in oncogenes and tumor suppressor genes, and many lines that are tumorigenic in vivo are available.

But researchers who work with human breast cancer cells have other problems they face. For example, Dr. Jensen noted, these cells were typically established and grown years or decades ago using undefined culture condition. In addition, most cell lines were obtained from metastatic breast cancer samples, which means a strong selection bias is present. Further, because these cells have been in culture for years, and since it is known that they evolve and change over time, researchers must be aware that just because one lab reports a finding in some cells does not mean that another lab can repeat the study and obtain the same findings.

Another option for researchers use are the early passage human breast cancer cells. The advantage of these cells, Dr. Jensen said, is that they can be culled from both primary and metastatic sites. The disadvantages are that it is very difficult to propagate the neoplastic cells and that, typically, an overgrowth of normal HME cells and fibroblasts occurs. As a result, even under the best conditions cells can only be cultured about 10% of the time. Another option is the MCF-7 breast cancer cells, which were developed in 1973 from a metastatic pleural fluid aspirate. These cells express functional estrogen receptors, are responsive to estrogen in vitro, and are dependent on estrogen in vivo.

Overall, the greatest problem that researchers confront, Dr. Jensen said, "is that we don't know now how to get cancer cells to grow in vitro. We just don't know how to do it." Not only do the HME cells not respond to luminal cells, which are believed to give rise to most breast cancers, but the cell lines available are the result of selection processes that are not fully understood. Further, all of the cell lines and primary cell cultures that are available have limitations that affect data interpretation.

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Following this, Dr. Barcellos-Hoff presented information on experimental models in vivo. She discussed genetically engineered mice and their hormonal environment and the differences between mouse and human tumors. (For additional information the presenters recommended the National Institute of Health's website http://mammary.nih.gov.)

Tumor Development in Women and Mice

Breast tumors in humans and breast tumors in mice develop in different ways. Most human breast cancer begins as ductal carcinoma in situ (DCIS). DCIS is a precursor for invasive disease, but it does not show any ability to invade the surrounding stroma. More cases of DCIS have been diagnosed due to more extensive use of mammography screening. There are two different forms of DCIS. The large comedo DCIS is relatively rare, but women with this type of DCIS have a 50% chance of their cancer becoming invasive over the next three years. The more common type of DCIS is small, non-comedo DCIS. Women with this type of DCIS only have a 25% chance of their cancer becoming invasive over the next ten years.

Tumors are both graded and staged. Tumors are graded histologically by examining such factors as how differentiated the cells are, whether there are tubules as there would be in a normal breast, and the cells' proliferation rate and S-phase factor. This grade represents an assessment of how biologically aggressive the tumor is believed to be. The tumor's stage represents the clinical and pathological assessment of the natural history of the tumor, its size, degree of lymph-node involvement, and degree and location of metastases. Although both the tumor's stage and grade are used to predict survival, Dr. Jensen noted that, "we don't do a good job of predicting for individual patients. We are good at predicting survival for a large population but we need more data to be more specific for individual women."

The natural history of human breast cancer differs from the natural history of breast cancer in mice. One problem with the mouse models, said Dr. Jensen, "is that many of the genetically engineered tumors lead to lesions that are more like DCIS and not to classic patterns of invasion. In addition, these lesions may not look anything like a human breast cancer."

In conclusion, Dr. Jensen stated that to advance this field of research and to maximize the potential of what mice models can provide to the understanding of human breast cancer we need models that reflect the molecular complexity of breast cancer, research that goes beyond the endpoint of the development of mammary cancer, models that are adequate for experimental therapies, and a better understanding of precursor lesions in rodents.

II. The Epidemiology of Breast Cancer

Faculty: Steven Cummings MD (University of California, San Francisco)
Sue Hankinson ScD (Harvard Medical School)

The second day of the Breast Cancer Course began with lectures on the epidemiology of breast cancer given by Steven Cummings, MD, a professor of medicine and epidemiology and biostatistics and associate chair for clinical research at the University of California,

San Francisco, and Susan Elizabeth Hankinson, ScD, an associate professor of medicine at Brigham and Women's Hospital and Harvard Medical School in Boston.

Epidemiology Nomenclature

Dr. Hankinson began by discussing epidemiological nomenclature and the statistical methods epidemiologists use to determine a disease's frequency of occurrence and a person's risk of developing a specific disease. Relative risk (RR) (which is the same as risk ratio, hazard ratio, odds ratio, and rate ratio) measures the strength of the association between exposure and disease (no association = RR1). Risk difference (or attributable risk) is the risk of disease in the exposed population due to the exposure by subtracting out the risk of disease in the non-exposed. Relative risk is a better indicator of the strength of the association, but risk difference is important because it shows the public health impact by delineating the excess number of cases occurring due to a specific exposure.

Dr. Hankinson then described the different types of epidemiological studies. Clinical trials are studies in which women are randomly assigned to treatment. Observational studies are typically either cohort studies, in which healthy women are followed over time to assess their health status and exposure before disease occurs, or case-control studies, which compare cases (those with the disease) to controls (those who don't have the disease) after disease has occurred.

Similar epidemiological studies sometimes provide differing results. This may happen because the exposure/disease relationship varies between study populations. It is also possible that researchers' findings differ because they are measuring different things or a different range of exposure. For example, two studies measuring physical activity could be measuring different types of women or different types or degrees of activity (recent activity verses lifetime activity), which could affect disease risk. Also, some studies may have a confounding or selection bias that is affecting the result. Or, differences may just be due to chance. "This is why replication of study results is very important," said Dr. Hankinson, "and why you need to consider the overall body of evidence, not just the most recently published study. No study stands alone."

Descriptive Epidemiology of Breast Cancer

Dr. Hankinson then went on to explain the descriptive epidemiology of breast cancer. These statistics allow researchers to better understand rates of disease in specific groups, to gain information about incidence and mortality over time, and to look at international variations in breast cancer rates. (In this instance the numbers are "age-adjusted" to remove variations in age between countries.)

Looking at age-specific rates, one can see that the biggest increase in breast cancer rate occurs around menopause. And by looking more closely at various types of tumors, one can see that the proportion of ER-negative cancers diagnosed is larger in young women than in older women. It can also be seen that the rate of ER-negative tumors is staying flat while the rate of ER-positive tumors is increasing. In discussing this point, Dr. Hankinson made reference to a recent article in the *Journal of the American Medical Association*, "Trends in Incidence Rates of Invasive Lobular and Ductal Breast Carcinoma" (March 19,

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2003) that found that while the rates of ductal carcinoma have stayed constant, the rates of lobular cancer have gone up.

This presentation generated a great deal of discussion about different types of cancers and whether all tumors start ER-positive but them some become ER-negative. This had been a popular theory but the fact that more ER-negative tumors are being seen in young women has called this belief into question. It is possible that there two completely different paths and that some women's tumors start ER-negative while others start ER-positive. It was noted that ER-positive cells regulate ER-negative cells in mice. Additionally, Dr. Dan Hayes noted that since not all cancers are totally ER-positive or totally ER-negative that it might be better to describe these tumors as either being "hormone-dependent" or "not hormone-dependent" instead of describing them as ER-positive or ER-negative.

During the discussion a question was raised as to why observational studies are even done, since the best data comes from controlled trials. In response, Dr. Hankinson explained that while the controlled trials did provide the best information, there were clear limits as to when they can be used. "There are many things that we will never be able to have trials for," she explained, "and there are some things that we can't test that way. When this occurs, we need to make decisions with the best information that we have. But we have to be careful."

For example, the results of the Women's Health Initiative, a controlled trial, contradicted the observational data that had previously been available on women and HRT use. "But that doesn't mean we should disregard the other findings," said Dr. Hankinson. "Instead of thinking this has totally changed the answer about heart disease and hormone replacement therapy (HRT); for example, we need to think even more about why there are differences. Were the observational studies truly wrong or are there other factors that we aren't thinking about? This is why consensus panels are so important. We need to think, does this flip our understanding or just add another piece to the big picture."

Breast Cancer Risk Factors

Family History

Next, Dr. Steven Cummings explained the risk factors for breast cancer. Although family history is known to be a risk factor, the actual excess risk women face because they have a family history isn't known. It does appear that family history is a stronger risk factor for younger women than it is for older women. Also, family history is a strong risk factor for ER-negative but not ER-positive breast cancer. "This means that when we think of family history as a risk factor, we need to think about estrogen-receptor negative disease," said Dr. Cummings. This is important because family history is one of the risk factors that is used to enroll women in the prevention trials--but these trials use tamoxifen, which is only effective in women with ER-positive disease.

Parity

Parity appears to decrease a woman's risk for breast cancer by 7% per birth (without nursing), and the risk is decreased further for women who had their first child at a young age. Each year of breast-feeding also lowers a woman's risk of breast cancer. There is no data as to whether this reduction is true for both ER-positive and ER-negative disease or if the reduction in risk is additive.

BMI

The relationship between Body Mass Index (BMI) and breast cancer risk appears to be different for pre- and postmenopausal women. For those who are premenopausal, said Dr. Cummings, the thinner a woman is the greater her risk. But in postmenopausal women, the larger a woman is the greater her risk. "Why does that flip at menopause?" said Dr. Cummings. "No one is entirely sure. It's probably about estrogen. But it remains unexplained." He also noted that when you look at the other factor in BMI—height—there does appear to be a relationship between height and breast cancer, with taller women having a greater risk than short women.

In young women, risk is increased for those who have a family history and a lower BMI. In a study of 400 twins in which one woman had developed breast cancer, researchers found that earlier breast development was the strongest risk factor. Also, the twin who developed breast cancer was more likely to weigh less, to be taller, and to be childless.

Other relationships between BMI and breast cancer risk have been observed. Recent data from the Women's Health Initiative, for example, indicate that BMI and breast cancer risk doesn't stay strong in women in their 70s. Further, change in weight appears to be a stronger predictor of risk than baseline weight (if looking at weight gain after age 18). "Why would change in weight be such an important factor," said Dr. Cummings. "Higher caloric intake? Hormonal change? We don't know."

Alcohol

Alcohol use had been found to increase breast cancer risk and, Dr. Cummings said, this might be because alcohol induces enzymes that activate pro-carcinogens, increase estradiol levels, and may inhibit DNA repair. Whether alcohol only increases the risk for ER-positive breast cancers, though, isn't known.

Tobacco

Although smoking was thought to be a risk factor for breast cancer, a recent meta-analysis found the relative risk to be only 1.1—essentially no association. "It's possible," said Dr. Cummings, "that it might be stronger for ER-negative disease than it is for ER-positive disease." It's also possible that "because smokers tend to weigh 5-10 pounds less that they have lower estradiol and that this lower estradiol induced by smoking counters the affect of the carcinogen. But we would need much more analysis to determine that smoking is safe for the breast."

Diet

The relationship between diet and breast cancer has been explored in a number of studies, but meta-analyses have failed to find a relationship between fruit and vegetable intake and breast cancer. It's also currently not known whether eating meat increases breast cancer risk or if it is the type of meat that is eaten that is most significant. For example, some studies have found that those women who rarely eat meat or who only eat rare meat have a lower breast cancer risk than do those women who eat well-done meat.

Radiation

Exposure to radiation is known to increase risk and this risk emerges 10-25 years after exposure. However, noted Dr. Cummings, in those women who have received radiation

there is no significant increase in the risk of presenting with disease that has metastasized, and their cancers are said to look the same as other breast cancers.

Breast Density

Next, John Shepherd, PhD, a physicist and an assistant adjunct professor in the department of radiology at the University of California San Francisco, discussed breast density and the fact that it increases breast cancer risk. Dr. Shepherd explained that there is a decrease in breast density as a woman ages and that this relationship mirrors the decrease in bone density that is also seen as women age. Researchers at UCSF have found that breast density appears to have the same relationship to both ER-negative and ER-positive tumors, Dr. Shepherd said. They have also found breast density to be inversely related to BMI: lean women with low BMIs have denser breast tissue, whereas women with the highest BMIs have more fatty tissue in the breast.

Limitations.

Dr. Cummings then explained the limitations of risk factor studies. Most of these studies have looked at all breast cancer, he said "but what are now needed are studies that pay more attention to phenotypes and to ER status. Also, most of the research has been focused on reproductive hormonal factors. We need new ideas." These ideas are likely to come from advances in molecular epidemiology. Looking to the future, he said, "the most important and fruitful areas are likely to be those that address why parity and breast feeding are protective and how weight change affects risk, because this could offer clues to reducing risk."

In the discussion on breast density that followed it was noted that some postmenopausal women have high breast density and that there is dramatic variance in breast density at a given age. Additionally, while the epithelial cells are the same in low and high dense breast tissue the stroma increases in high density tissue; this can be seen surgically.

This led into a discussion of the best way for epidemiologists and molecular biologists to work together. For example, epidemiologists have blood and cell samples that were collected years ago that could be tested by molecular biologists who now have more advanced molecular techniques. Working together, it may be possible to determine specific molecular pathways that could lead to testable hypotheses.

"My frustration as an epidemiologist," said Dr. Cummings, "is that I don't know what's coming or how to store samples or what to get so that it can be used in five or ten years. So this cross talk about methodology is very important. What we are going to need are molecular biologists and epidemiologists collaborating and forming large groups because the large studies we need can't be done by just one lab or one group."

To take advantage of future developments researchers will need to develop patient consent forms that are good over time and that take into account that it is not clear how samples may be used in the future to test hypotheses. Epidemiologists also said they needed to get more information from molecular biologists about when new tests will be available so that they don't use up their samples on testing that is really not good enough. In response, it was suggested that perhaps the National Cancer Institute could develop a program that brings expert epidemiologists and microbiologists together to discuss these issues and concerns.

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Endogenous Sex Hormones and Breast Cancer

Dr. Hankinson then discussed endogenous sex hormones and breast cancer and described the epidemiological data that supports a relationship between hormones and breast cancer. This data includes age-incidence curves as well as studies that indicate relationships between breast cancer and reproductive risk factors, obesity, circulating endogenous hormones, and exogenous hormones, such as the birth control pill and hormone replacement therapy.

Dr. Hankinson explained that because endogenous hormones are typically analyzed after menopause there is little data on circulating levels of estrogens and risk of breast cancer in premenopausal women. "There is a real paucity of data from prospective studies," she said, "so we can't say anything about this at this time. Hopefully in two to five years we will have more information."

In the 1990s, case-control and cohort studies of postmenopausal women that looked at exogenous hormone use found an increased relative risk for current hormone users and recent hormone users. No discernable association was seen for women who had used hormones in the past; their risk was the same as that of women who had never used hormones.

The Women's Health Initiative, the randomized controlled trial of HRT use, challenged the findings of these case-control and cohort studies with the finding that HRT appeared to increase breast cancer risk. A recent sub-analysis of the WHI data, Dr. Hankinson said, "suggests that those women who had used HRT prior to the start of the trial had an increase in risk, which fits with the observational studies that indicated there seemed to be a duration effect from HRT use."

Studies designed to look at circulating estrogens and the risk of breast cancer in postmenopausal women who did not use HRT have looked at different types of estrogens and androgens. These studies found that relative risk was associated with higher levels of estradiol and testosterone. And while it appeared that both were contributing independently, said Dr. Hankinson, more research needs to be done to see if this is the case.

Estrogen Levels and Raloxifene

After the raloxifene trial found a decrease in breast cancer risk in women taking raloxifene, baseline estradiol levels collected prior to randomization were analyzed to see if raloxifene was of greater benefit to a certain group of women. This analysis indicated that the greatest reduction in risk was seen in those women who started the trial with the highest estrogen levels. In fact, there was no reduction in risk observed for those who started the trial with the lowest estrogen levels. This finding will be looked for in the NSABP-P1 prevention trial, as it has important implications for determining which women at high risk for breast cancer should consider hormonal prevention options.

Prolactin

Dr. Hankinson and others have also been looking at whether plasma prolactin levels are an independent factor in breast cancer risk. She said that in her own study it did seem to

be a factor, but she emphasized that this was only one study and that it remains to be seen whether other researchers will reproduce this result.

IGF

The relationship between height and breast cancer risk and birth weight and breast cancer risk has directed attention to the question of whether Insulin-like Growth Factor-1 (IGF-1) may contribute to breast cancer risk. To date, studies have found no relationship in postmenopausal women and IGF levels. However, an inverse relationship was seen in premenopausal women. This is something researchers are continuing to try to sort out, said Dr. Hankinson, along with whether IGF and mammographic density interact with one another as well.

One of the problems in studying endogenous hormones in premenopausal women, Dr. Hankinson stated, is that the tissue samples that are available typically don't include information as to what time during the menstrual cycle they were collected—which may influence the findings. Further, there is a lot of variability in the techniques that are currently used to measure estradiol.

In the discussion that followed the question was raised as to why there wasn't more research looking at the effect of progesterone on breast cancer risk. Dr. Hankinson explained that this was because most of the research is on postmenopausal women, and their progesterone levels are very low and hard to measure. She mentioned that she would be presenting data at the upcoming American Association of Cancer Research conference from a study that did not find an association between circulating progesterone and breast cancer risk. And she noted that because there are so few studies in premenopausal women it would be hard to even begin to assess the role progesterone might play in this group.

It was also noted that concentrations of these hormones are higher in the breast than in the blood, and that the levels seen in the breast are not necessarily correlated with those seen in the blood. This may indicate that blood levels of hormones may not be the most relevant measure.

III. Hormones and the Breast

Faculty: Steffi Oesterreich PhD (Baylor College of Medicine)
Richard Santen MD (University of Virginia)
Douglas Yee MD (University of Minnesota)

History of Early Hormonal Breast Cancer Therapy

Richard Santen, MD, a professor of medicine at the University of Virginia School of Medicine, in Charlottesville, began the session on endocrinology by describing how hormonal manipulation has been used as breast cancer treatment. The first clinical observations of hormones and breast cancer were published in the 1920s, when the idea first began to surface that some women might have tumors that are hormone dependent. This observation led to the first use of hormonal therapies, such as oophorectomy (which was actually first done in 1898), adrenalectomy, and hypophysectomy. Later, oncologists

began to use high-dose estrogens in the form of DES and then high doses of androgens as breast cancer treatment.

Oncologists found that regardless of which hormonal therapy was used about 30% of the women would respond to treatment. But, explained Dr. Santen, because there was no way to know who would respond--everyone was treated the same way. This changed once researchers developed a method to differentiate between ER-negative and ER-positive tumors. These tests, though, are not 100% accurate. Currently about 80% of tumors are correctly classified while 5% are incorrectly classified as ER-negative and 15% are misclassified as ER-positive.

Currently, the hormonal treatments for premenopausal women include GNRH agonists and oophorectomy. For postmenopausal women the treatments include the anti-estrogen tamoxifen and the aromatase inhibitors, which work by blocking the synthesis of estrogen. These treatments are used both in the adjuvant and metastatic setting and can be used in the metastatic setting until the cancer becomes hormone resistant.

Assessing Estrogen

Not only does estrogen fuel breast cancer but the breast is an endocrine gland that makes estrogen. This is significant, because it means that the breast doesn't just get estrogen from the blood. This understanding of the breast, Dr. Santen said, is supported by the fact that a highly specific antibody for aromatase is on breast cancer cells.

Techniques that can calculate and differentiate between the amounts of estrogen made in the breast and that made elsewhere in the body have found that levels of estrogen made in the breast vary from woman to woman. It has also been shown that there are differences among women between estrogen uptake and synthesis. And it is because estrogen gets into breast tissue that the levels of estrogen in the breast are the same in both premenopausal and postmenopausal women.

Researchers have also found that estrogen blocks apoptosis, which means that one of the ways that a tumor will grow in response to estrogen is by blocking cell death. This, in turn, is why using an aromatase inhibitor to block estrogen synthesis can stimulate apoptosis and slow tumor growth.

Estrogen and Metastasis

But why is it that women whose tumors initially have been stopped by estrogen then go on to metastasize? Dr. Santen described a model he developed to try to account for this finding. This model proposes that the breast cells change in their new environment and that the cells that have been deprived of estradiol for a long time paradoxically then begin to have a marked increase in apoptosis. "This suggests that breast cancer cells can adapt and may have stimulation of apoptosis under the right circumstances," Dr. Santen said, "and that we need to reconsider the use of high-dose estrogen for the treatment of breast cancer."

The commonly accepted hypothesis as to how estrogen causes breast cancer is that estradiol induces carcinogenesis by stimulating cellular proliferation genes. After enough

cell divisions an error can occur, which leads to the development of a mutation. And when the mutation is not repaired, breast cancer will occur.

A second more controversial hypothesis, Dr. Santen said, is a genotoxic hypothesis, that suggests that estradiol can be metabolized to 4-hydroxy estrogen, and that it is this compound that leads to depurination of DNA. This naked spot on the DNA is then repaired with error-prone DNA.

The fact that quinone conjugates are found in high concentrations in women with breast cancer and in the cancer itself, said Dr. Santen, indicates that it is possible that the development of breast cancer may involve both cellular proliferation genes and DNA mutations from these metabolites. It is important to think about these dual pathways when discussing cancer treatment because an anti-estrogen like tamoxifen blocks estrogen whereas the aromatase inhibitors will also block the amount of phenotoxic metabolites that develop.

Estrogen—A Direct Mutagen?

Whether estrogen is a direct mutagen remains very controversial. The in vivo evidence that supports this genotoxic hypothesis, Dr. Santen explained, comes from studies that have used an estrogen receptor knockout transgenic mouse model. These mice are chosen because they have no functioning ER alpha or ER beta, which means they have no ER receptor. Further, these mice have very high LH and estrogen levels but no estrogen receptors and they don't develop breast cancer. To get them to develop cancer they must be crossbred with the Wnt-1 transgenic mice (which can develop breast cancer).

When an oophorectomy (removal of the ovaries) is performed on these mice it reduces the incidence of breast cancer. In the absence of estrogen receptors, this can be attributed to a metabolite phenomena. Studies conducted on these mice have found that after 12 months 50% of the mice will develop breast cancer. But if the ovaries are taken out, only about 20% will develop breast cancer, Dr. Santen said, "which indicates that estrogen is doing something to influence breast cancer that has nothing to do with the receptors." This finding has implications for breast cancer prevention research "because unlike tamoxifen, which is now used for breast cancer prevention, the aromatase inhibitors block both sides of the pathway."

The best data available on aromatase inhibitors for breast cancer treatment comes from the Anastrazole and Tamoxifen Alone or in Combination (ATAC) trial. Early results from this trial indicate that anastrazole (an aromatase inhibitor) may be a better a drug than tamoxifen (an estrogen blocker), especially in its ability to prevent contralateral breast cancer.

In the ensuing discussion it was noted that the ATAC trial has only followed women for four years and that there is concern about the higher risk of fractures seen in women on anastrazole. (Fractures are more likely because the aromatase inhibitors lower plasma estrogen, which leads to a reduction in bone density.) In addition, because this is a whole new area of physiology, it's not clear if anastrazole and the low levels of estrogen it leads to are going to affect the nervous system or increase a woman's risk for Alzheimer's disease or heart disease.

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Dr. Cummings also pointed out that how tamoxifen is preventing cancer isn't clear. It's not known if tamoxifen is preventing breast cancer by actually stopping breast cancer from developing, if it is curing cancers that were already there through apoptosis, or if it is stopping cancers from growing any further.

Molecular Action of the Estrogen Receptor

Next, Steffi Oesterreich, PhD, an assistant professor at Baylor College of Medicine, in Houston, explored the molecular action of the estrogen receptor and the role of co-factors, such as coactivators and corepressors, and non-genomic factors in this process. She discussed the research that is using fluorescent recovery after photo bleaching (FRAP) and ChiP Essay Chromatin Immunoprecipitation to look at the dynamics of the estrogen receptor. My goal, Dr. Oesterreich said, "is to convince you that estrogen regulation is very complex and that it is a big challenge to develop simple models. We have to always remember to interpret results within the context of multifaceted estrogen receptor action. At this point we really don't yet know what certain things mean. But we do know that we need to look at more than estrogen and that we need to look at many genes at a time."

Growth Factors

Next, Doug Yee, MD, leader of the Breast Cancer Research Program at the University of Minnesota Cancer Center, in Minneapolis, discussed growth factors and their relevance to cancer. Each cell is constantly monitoring its environment because it is waiting to be told what to do: to survive, to proliferate, or to metastasize. Normal cells require signals from the extracellular space to proliferate, to die, to differentiate, or to move—all of which are things that cancer cells also do. When cancer begins to occur, cells use the same signals from the extracellular space to go from a normal epithelial cell to a transformed epithelial cell as well as to maintain the malignant phenotype.

Her2/neu is a viral oncogene related to the EGFR family, Dr. Yee explained. EGFR family members bind to multiple ligands and are transmembrane tyrosine kinases. The monoclonal antibody Herceptin is used specifically to treat tumors that are HER2/neu positive, but it is limited by the fact that multiple molecules are activated downstream of HER2.

Insulin-like Growth Factors (IGFs) can also play a role in cancer because they affect proliferation, survival, and metastasis. This has been demonstrated in the research that has shown that IGF-1 liver-specific knockout animals are less likely to develop colon cancer. Because IGFs are already bound to a partner they can't be activated merely by overexpression, they require ligands. Dr. Yee said that researchers believe that once the IGFs are activated then adhesion mortality and proliferation occurs. It is also possible that IGF receptors and steroid hormone receptors interact, and that growth factor signaling might not only enhance estrogen receptor functioning but influence how the tumor responds to treatment with selective estrogen receptor modulators (SERMS).

The literature addressing whether tumors that are Her2/neu positive are tamoxifen-resistant is controversial, said Dr. Yee. Some studies have found that women who have HER2/neu negative tumors do better on tamoxifen than do those who were HER2/neu

positive, which suggests that there are signaling pathways activated by Her2/neu that mediate tamoxifen resistance. Other studies, however, have not found this to be the case.

Changing the Hormonal Milieu

In the discussion that followed, Dan Hayes MD mentioned that some women who are first treated with tamoxifen and then later treated with other available drugs may respond to tamoxifen when it is introduced again. "This means," said Dr. Hayes, "that they are only resistant for awhile, and it's possible that other hormone therapies might work again as well." For this reason, he said, "we need to think of hormone dependence and independence and hormone resistance as separate categories."

This led Dr. Love to note, "just the fact of changing the hormonal milieu may be more important than anything else. In the old days all we had was DES and when women began to show resistance just stopping the drug would generate another good response due to DES withdrawal."

Dr. Santen noted that if the ovaries are removed from a premenopausal woman and she has a relapse 18 months later, you can lower her estrogen with an aromatase inhibitor and she will respond again. "So there is clearly hypersensitivity," he said, "and this is the focus of our lab. What I believe is happening is that when we deprive the cell of estrogen we turn on growth factor pathways. And if we understood that process we could better understand the sequence and whether the cells that upregulate pathways are the same cells that are adapting to therapy and then becoming sensitive to apoptosis. If we knew this we could then give high-dose estrogen to knock out the cells we don't want. Or, for example, by cycling between oophorectomy and high-dose estrogen, we could use the fact that the cells adapt to the oophorectomy to set them up to be killed by high-dose estrogen."

Dr. Susan Love added to this by noting that "Dr. Richard Love reported that in his research on women in Vietnam he found that when women had an oophorectomy and a mastectomy, the women who had their surgeries in the second half of their menstrual cycle did 30% better then did those women who had their surgeries during the first part of their menstrual cycle." This was true, Dr. Love said, whether the women's tumors were ER-positive or ER-negative, which means "it's not just long-term use of hormones but the hormonal milieu at the particular moment that is critical."

IV. Clinical Breast Cancer: What We Know and Don't Know

Faculty: Daniel F. Hayes MD (University of Michigan)
Funmi Olopade MD (University of Chicago)

Risk and Prevention

Olufunmilayo Olopade, MD, a professor of hematology and oncology in the department of medicine and the director of clinical cancer genetics at the University of Chicago, began the discussion on clinical breast cancer with an overview of what we know and don't know about risk and prevention. Most breast and ovarian cancer is sporadic. Family clusters only account for 15-20% of breast cancers, and hereditary factors only account for 5-10% of both breast and ovarian cancer cases. However, even within the sporadic group, it is

possible that there are genes that are involved in estrogen metabolism and growth pathways that might preferentially affect different individuals and influence breast cancer risk.

BRCA1 and BRCA2

The discovery of BRCA1 and BRCA2 has been significant in assessing risk. But it's not without its own complications, said Dr. Olopade, especially when genetic testing is brought into the clinic. Sometimes geneticists must work with women who learn that they carry one of the BRCA genes but who do not have a strong family history of the disease. There are also families in which those without the BRCA gene have developed breast cancer and those who do have the gene have not, complicating how these women assess their risk.

BRCA does not account for all hereditary cancer. About 20-40% of hereditary cancer can be attributed to BRCA1 while about 10-30% of hereditary cancers can be attributed to BRCA2. Thus, undiscovered genes account for 30-70% of all hereditary cancers. "The reason we haven't found BRCA3 yet," said Dr. Olopade, "is that we don't have the tools to look at inherited susceptibility."

BRCA1 tumors have a distinct phenotype. They have a high proliferation fraction, tend to be ER-negative and HER2 negative, and they frequently have TP53 mutations and chromosome 17 aberrations. In contrast, BRCA2 tumors are indistinct from sporadic tumors. They are more heterogeneous, usually ER-positive, and typically affect women at an older age. For these reasons, hormone manipulation is more likely to prevent BRCA2 tumors than BRCA1 tumors. Also, risk for women with BRCA2 mutations does not taper off in postmenopausal women as it does for women with BRCA1. In contrast, non-BRCA1 or 2 familial tumors tend to be low grade and are more likely to be invasive lobular cancers.

Risk Estimation Models

Doctors and geneticists rely on a number of risk estimation methods to help women assess their probability of developing breast cancer and determine whether they want to consider genetic testing or chemoprevention. Risk-estimation methods include the Claus Model, the Gail Model, and the Parmigiani and Berry Model. The models used to assess the probability that a woman carries a mutation include the Couch model, the Myriad model, BRCAPRO, and the Lakhani model.

DNA sequencing is used to detect coding-region mutations in the BRCA1 and BRCA2 genes. Others tests that can be performed to look for these mutations include ASO and protein truncation assays and heteroduplex analysis. But because BRCA1 and 2 are large genes, said Dr. Olopade, even though a number of tests are available "it is hard to do good analyses and each of these tests may miss large deletions or noncoding."

Women with BRCA mutations are at higher risk for developing ovarian cancer, but they are not more likely to develop the disease at a younger age than other women are. This is why women with BRCA mutations are typically asked to consider a prophylactic oophorectomy when they are in their 40s.

Not every woman with an altered BRCA gene will go on to develop cancer. Currently researchers are looking at what factors affect penetrance, exploring what other modifier genes may be affecting how BRCA acts, and studying whether risk is affected by estrogen levels along with BRCA. Whether there is a transient increase in risk during pregnancy is especially significant for women with BRCA1 or 2. Typically, having a child at an early age is protective, said Dr. Olopade, "but women with BRCA1 get early onset disease, so we need to know how to counsel them about having children because we know that reproductive factors do affect risk in these individuals."

Surveillance options for women who know they are BRCA1 or BRCA2 carriers include monthly breast self-exams, early clinical surveillance, annual or semi-annual clinical breast exam, annual mammography, investigational MRI, ultrasound, and ductal lavage.

To reduce risk, women can try chemoprevention, surgical prevention (prophylactic mastectomy or oophorectomy), or lifestyle modifications. Prophylactic mastectomy has been proven to be effective in reducing breast cancer risk, and prophylactic removal of the ovaries and fallopian tubes has been shown to be effective in reducing breast cancer risk by 50% and ovarian cancer risk by 96%.

What We Know and Don't Know

Dr. Dan Hayes presented the last lecture of the conference on "Clinical Breast Cancer...What We Know and Don't Know." Dr. Hayes noted that the "recent decrease in breast cancer mortality is a sign that we are making a difference." Statistics show that in 1985 a steep drop in cancer deaths occurred due to widespread use of adjuvant tamoxifen, and, said Dr. Hayes, "I think the curves will continue to drop because I think screening does improve mortality. So we are making progress—but not enough. Each year, 40,000 women will die of breast cancer and that is not acceptable and that is why we are here."

There is not any one single identifiable lifestyle change that can reduce breast cancer risk. But researchers are studying possible options. The NSABP P-1 study, the tamoxifen prevention trial, found a 50% reduction in primary breast cancers, specifically hormone-dependent cancers. However, the risk of developing ER-negative cancer was the same whether women took tamoxifen or not. And, as Dr. Hayes noted, even though the P-1 trial has shown a reduction in the development of cancer, it has not yet been shown "that we will reduce mortality with tamoxifen because we are reducing the cancers we are most likely to cure anyway."

Not all high-risk women should take tamoxifen. Side effects such as hot flashes, vaginal discharge/atrophy, and weight gain as well as toxicities such as deep venous thrombosis, pulmonary embolism, stroke, and endometrial cancer must be taken into account. "In the adjuvant setting most of this is irrelevant," said Dr. Hayes. "But in the chemoprevention setting only the individual woman can decide if this is worth it."

The NSABP-P-2 trial, also known as the STAR trial, is comparing tamoxifen to raloxifene in the prevention setting. The problem with both of these drugs, though, is that they only prevent about 50% of cancers and they have side effects. Other new agents that are now being considered for prevention include other selective estrogen receptor modulators

(SERMS), aromatase inhibitors, and selective estrogen receptor downregulators (SERDS), like fulvestrant.

Concerns have been expressed about the use of aromatase inhibitors for prevention. Dr. Hayes pointed out that these drugs only prevent about 50% of breast cancers and can lead to bothersome side effects or unacceptable toxicities, such as osteoporosis. And even though osteoporosis can be treated with another drug, "then you are chasing one drug with another," said Dr. Hayes, "and if we are not improving mortality then you have to begin to wonder if it's worthwhile."

Prevention research takes a long time to show results. But if it was possible to find surrogate markers of activity, Dr. Hayes explained, then shorter-term trials could be conducted to test some of these new hypothesis and new drugs.

Screening

Dr. Hayes also discussed the debate over breast cancer screening. He noted that there have been nine prospective randomized trials looking at whether screening reduces mortality. "People ask how can you question screening," he said, "but if we are not improving mortality then why would we do screening. The fact is that we over treat most women in order to help the group that benefits from treatment. So if we start from the fact that we are over treating a lot of women, and then are finding conditions that are actually non-morbid and over treating them, we have to ask what we are doing. But when we put the nine trials together and don't throw any out, then we see a small, maybe 25-30% proportional reduction in those women who were screened. And because of that, I think it's worth it."

Dr. Hayes said he believes there is no reason to start mammography screening at age 40 because the odds for women having breast cancer at that age are so low. "But maybe 50 is waiting too long," he said, "and the recommendation should be to start at 45." In addition to not knowing when to start screening, it's also not known how often women should be screened, or when screening should stop.

Mammography is the current screening tool, but better modalities are needed. MRI can be used in high-risk women, but PET scans are too expensive and impractical. Digital mammography is no different than regular mammography; it is just another way to store an image. Ductal lavage can also help with risk-assessment, but it is currently not a screening tool and it is only recommended for high-risk women.

The discussion that followed explored risk reduction and the pros and cons of oophorectomy, mastectomy, and aromatase inhibitor use.

Dr. Santen raised a question regarding the increased diagnosis of DCIS: Given that more DCIS is being found in women in their 40s, he said, would that not mean that fewer women would go on to develop invasive disease in their 50s? In response, Dr. Love explained, the problem right now is that "we don't know how to tell good verses bad in terms of DCIS. And since most DCIS is not going to do anything then we are over treating 70% of women. But women who are diagnosed with DCIS are told that their cancer was picked up early and they have extensive treatment."

A question was asked about whether there was research underway exploring why pregnancy was protective, since that might provide more information as to how breast cancer works. Dr. Hayes responded by explaining that there has been discussion about developing preventive methods that would mimic early first pregnancy or late menarche. The problem, though, is that it is not known what it would mean to interfere with young women's hormonal systems early on to try to prevent breast cancer.

A question also was raised about the higher mortality rate from breast cancer in African American women. Dr. Olopade responded by pointing out that because African-American women are more likely to develop early-onset breast cancer (between the ages of 30-45); their age-incidence curve is not the same as that of white women. Also, the Gail Model, which is widely used for risk assessment, does not fit African-American women. "I am astonished at how many African-American women do not meet the eligibility criteria to go on to a prevention study because what is in the model doesn't completely apply to their situation," said Dr. Olopade. To address this problem, researchers are working on modifying the Gail Model to better fit African-American women; some data on this was presented at last year's American Society for Clinical Oncology conference.

Treatment

Next, Dr. Hayes discussed primary therapy for breast cancer treatment. NSABP studies on DCIS found that whether treatment was wide excision alone, wide excision and radiation, wide excision, radiation, and tamoxifen, or wide excision and tamoxifen, the survival rate was 99% at 10 years. Right now, though, it is not known who only needs wide excision, which is why these other treatments are recommended.

Reviewing the history of mastectomy and breast preservation, Dr. Hayes noted that in the 1890s Dr. Halstead began doing what became known as the Halstead Radical Mastectomy. Dr. Halstead believed that the more tissue that was excised the better the chance of a cure. Thus, his mastectomy included removal of the entire chest wall and breast along with 50-100 lymph nodes. The procedure was very disfiguring and resulted in large numbers of women developing lymphedema.

Mastectomy remained the primary treatment until large studies comparing mastectomy with breast conserving therapy plus radiation found equal survival for both groups. This NSABP study has now followed women for more than 20 years, and the survival data hasn't changed.

Today, whether a woman has a mastectomy or breast conserving therapy is typically determined by how large her tumor is, if her cancer is multifocal, if she has a contraindication to radiation, or if she prefers one surgery to the other. (Despite the data some women still feel better about having a mastectomy.) Still, said Dr. Hayes, mastectomy is done too often. Breast conserving therapy should be done 75% of the time, but the rate is much lower, especially in the United States.

Currently the standard of care for radiation therapy is to irradiate the whole breast and then give an added boost to the area where the tumor was removed. Researchers are now looking at whether radiation could be done in three to five days at a very high dosage

instead of spreading the dosage out over six weeks. Researchers are also now studying intraoperative irradiation, which could provide an important treatment option.

Prognosis

Next, Dr Hayes discussed prognosis and prediction. A tumor marker is only clinically useful if it is reliably prognostic or predictive. A prognostic tumor marker provides information about whether (not how) a patient should be treated; prognostic factors can be strong, modest, or weak. Currently clinical stage, lymph node status, and tumor size are the primary prognostic factors used to determine whether a woman should have chemotherapy. Grade, profile, and lymph node invasion are also taken into account; ER and HER2 status are not. In some instances, the added benefit of chemotherapy may be very small, maybe 1-2%. Even so, there are patients who will still want to have chemotherapy to receive this benefit.

Whether a woman has bone mets is much more significant than whether she has lymph mets. Thus, Dr. Hayes said that he believes the "wave of the future" will be looking at bone tests to determine the status of the bone marrow cells. "If a woman is IHC positive in the bone marrow, she will have worse survival then if she is IHC negative," he explained. "And if she is node negative and IHC negative, her cure rate will be high."

Other prognostic markers currently being studied include UPA/PA1-1, cyclin E levels, and gene expression patterns. Currently these markers are not routinely used because their prognostic ability has not been validated.

Predictive Factors

Predictive factors determine how a woman should be treated. To date, only three factors been validated: estrogen receptor status, which is an excellent predictive factor for benefit from tamoxifen; HER2 status, which is a predictive factor for treatment with Herceptin; and 2-4Her2, which is a predictive factor for a good response to doxorubicin. Before any new factors are used they will have to be highly validated. "This is risky business," said Dr. Hayes. "There is the danger of having patients told the wrong thing and making the wrong decisions."

Adjuvant chemotherapy came into use in the 1960s. Chemotherapy is used because of the studies that have found it to proportionally reduce the risk of recurrence or death. For example, ovarian ablation offers a 16% proportional reduction in recurrence or death, tamoxifen offers a 25% proportional reduction, and chemotherapy offers a 25% proportional reduction. Although chemotherapy has been widely touted, said Dr. Hayes, "it is not the best. Hormone therapy is actually probably more effective than chemotherapy in ER-positive women."

For women with metastatic disease, no cure is possible. Chemotherapy and hormonal therapy are used in this instance to keep a woman feeling as good as she can for as long as she can. This means balancing the benefit of a drug verses its toxicity.

In the discussion that followed, Dr. Hayes was asked what he thought the major unmet needs were in breast cancer treatment. "The most unmet medical need," he said, "is a cure

for metastatic breast cancer. We also need markers in the newly diagnosed setting that will allow us to understand who needs to be treated and how they should be treated. In addition, we need to be better able to categorize women who are at risk. Right now most women diagnosed with breast cancer have no risk factors, which means for 85-90% of women we have no idea why they developed the disease, and we need to figure out what is going on with these women."

A question was raised about a recent study that found that CMF appeared to be equivalent to hormonal therapy. "Many have felt for years that the effect of chemotherapy is that it stops the ovaries," explained Dr. Hayes, "and it does appear that ovarian ablation and CMF-type chemo are about the same in effectiveness. But in most women the combination of chemotherapy and endocrine therapy is better than either alone. Although in older women this is not necessarily true. Chemotherapy is less and less effective as one gets older, but hormone therapy is not."

The need to put breast cancer risk in context with the risks women face from other disease was also discussed. The point was made that although many women are terrified of breast cancer, a woman newly diagnosed with invasive disease actually has an 80-85% chance of being cured. Thus, more work needs to be done to get the general public and oncologists to recognize that the prognosis is actually good.

Dr. Susan Love then thanked everyone for attending the conference and formally brought the Breast Cancer Course for researchers to a close.