REVIEW

Addressing the Soy and Breast Cancer Relationship: Review, Commentary, and Workshop Proceedings

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The impact of soyfood intake on breast cancer risk has been investigated extensively. Much of this focus can be attributed to the soybean being a dietary source that is uniquely rich in isoflavones. The chemical structure of isoflavones is similar to that of estrogen, and isoflavones bind to both estrogen receptors (ERα and ERβ) (although they preferentially bind to and activate ERβ) and exert estrogen-like effects under some experimental conditions. Isoflavones also possess nonhormonal properties that are associated with the inhibition of cancer cell growth. Thus, there are several possible mechanisms by which soy may reduce the risk of breast cancer. However, the role of isoflavones in breast cancer has become controversial because, in contrast to the possible beneficial effects, some data from in vitro and animal studies suggest that isoflavones, especially genistein, the aglycone of the main soybean isoflavone genistin, may stimulate the growth of estrogen-sensitive tumors. Limited human data directly address the tumor-promoting effects of isoflavones and soy. Because the use of soyfoods and isoflavone supplements is increasing, it is important from a public health perspective to understand the impact of these products on breast cancer risk in women at high risk of the disease and on the survival of breast cancer patients. To this end, a workshop was held in November 2005 to review the existing literature and to make research recommendations. This paper summarizes the workshop findings and recommendations. The primary research recommendation is that the impact of isoflavones on breast tissue needs to be evaluated at the cellular level in women at high risk for breast cancer. [J Natl Cancer Inst 2006;98:1275–84]

The possibility that soyfoods reduce breast cancer risk first attracted widespread attention in 1990, when participants at a workshop sponsored by the National Cancer Institute concluded that there were several putative chemopreventive agents in soybeans and recommended funding research in this area *(1)*. Among the various purported soybean chemopreventive agents, isoflavones have received the most attention; approximately 9000 papers have been published on these soybean constituents, about 20% of which involve cancer investigations. Although it is now recognized that the physiologic properties of isoflavones make them potentially applicable as chemopreventive agents for many types of cancer *(2)*, most of the initial focus was on breast cancer *(3)*. This potential role in breast cancer can be attributed to the historically low breast cancer incidence rates in Asia *(4)*, where diets are rich in soyfoods; research demonstrating the potential for isoflavones—which have a similar chemical structure to the hormone estrogen—to exert antiestrogenic effects *(5)*; and early epidemiologic *(6)* and rodent *(7)* studies showing associations between soy intake and reduced risk of breast and mammary cancer, respectively.

Despite the impressive amount of research conducted during the past 15 years, no clear consensus has emerged regarding the breast cancer preventive aspects of isoflavones. Although the limited epidemiologic data modestly support an inverse association between soy intake and breast cancer risk, many of the case–control and prospective studies have important limitations *(8,9)*. Study limitations include the usual issues of sample size, dietary measurement error, and whether a study was specifically and appropriately designed to address the soy–breast cancer hypothesis. Comparison across studies is complicated by the variation in exposure measures used (e.g., intake of soy protein, soyfoods, or isoflavones; urinary or serum isoflavone levels) and the variation in amount and types of soy products consumed *(9)*.

Rodent studies have shown that when isoflavones or soy protein are given before the administration of chemical carcinogens *(10–13)* or the implantation of cancer cells *(14–16)*, mammary tumor development and/or growth is generally inhibited, although there are several exceptions *(17–20)*. Furthermore, as discussed later, the timing of soy or isoflavone exposure relative to the implantation of cancer cells or the administration of carcinogens may be a critical factor in determining whether tumor development and growth is suppressed or enhanced in rodent models *(1)*. In any event, there is little clinical evidence from largely short-term studies that soy or isoflavones favorably affect markers of breast cancer risk, including breast tissue density *(21,22)*, serum estrogen concentrations *(23,24)*, and breast cell proliferation *(25)*. Some studies have found that, with high soy intake, estrogen metabolism is favorably altered *(26)* and menstrual cycle length increased *(23)*; however, the impact of these changes on breast cancer risk is uncertain.

Considerable enthusiasm remains for the possibility that soyfood intake contributes to the low breast cancer rate in Asia but increasingly it appears that childhood and/or adolescence is the critical period of exposure. This hypothesis, which is supported by both epidemiologic *(27–29)* and animal *(30,31)* data, is

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consistent with the mounting evidence that early life events greatly impact breast cancer risk *(32)*. However, it should be noted that although there is some evidence in rodents that in utero isoflavone exposure affects adult gene expression *(33,34)*, there is no evidence that such exposure reduces mammary cancer risk *(30,33–36)*. For example, in utero genistein exposure shifted the coat color of heterozygous viable yellow agouti offspring toward pseudoagouti *(33)* and the feeding of genistein and daidzein to Wistar-Kyoto rat dams during gestation caused the cardiac myocytes of their adult offspring to be shorter than in counterparts originating from mothers fed with a phytoestrogen-free casein-based diet *(34)*.

In addition to the potential protective effects, some data suggest that isoflavones could promote breast cancer. In vitro, genistein stimulates the growth of estrogen-sensitive mammary cancer cells *(37)*, and in ovariectomized athymic mice, dietary genistein *(37)* and genistin *(38)* stimulate the growth of existing estrogen-sensitive mammary tumors. Consequently, in recent years, the estrogen-like effects of isoflavones have raised concerns that soyfoods are contraindicated for women at high risk of breast cancer and breast cancer patients with estrogen-sensitive tumors—approximately two-thirds of women with postmenopausal breast cancer are in this category *(39)*. Numerous review articles and commentaries have been published on this topic *(40–44)*.

Establishing the impact of soy intake on women at high risk for breast cancer and breast cancer patients is clearly an important public health goal. Many women at high risk underestimate their chance of getting breast cancer, and many at low risk overestimate it *(45)*. Thus, it is possible that many women will either unnecessarily avoid soy or consume it when perhaps they should not. Moreover, many breast cancer patients consume soy and often list "anticancer effects" as a reason for doing so *(46)*. Soyfoods, because of their purported health benefits, have become increasingly popular among non-Asians although partly because much of the soy protein added to traditional Western foods has a reduced isoflavone content, daily per capita isoflavone intake is quite low in the United States *(47–49)* and in Europe *(50,51)*—typically less than 3 mg. It is however, much higher among health-conscious individuals and vegetarians *(52,53)*.

To gain an understanding of the current state of knowledge regarding the safety of soy for breast cancer patients and for women at high risk for breast cancer and to identify research initiatives with the potential to resolve this controversy, a workshop was held on November 3, 2005. This meeting was organized by one of the authors (M. Messina) and was funded by the United Soybean Board. With one exception (W. McCaskill-Stevens), each of the 13 workshop participants received an honorarium for their participation, and all were given an opportunity to review the manuscript.

This review has three parts. First, a background section on isoflavones is provided. Next, the workshop proceedings are presented. Finally, an outline of the conclusions and the recommendations of the workshop are presented.

Background

Isoflavones are a subclass of flavonoids that have a limited distribution in nature; among commonly consumed foods, they are found in dietary-relevant amounts only in the soybean *(54)*. The three soybean isoflavone aglycones—genistein, daidzein, and glycitein—are present in raw soybeans and in nonfermented soyfoods almost entirely as β-glycosides (genistin, daidzin, and glycitin), to which either an acetyl or malonyl group is attached *(55)* The biologically active form of isoflavones is the aglycone, but during digestion the glycoside is efficiently hydrolyzed such that the form in which isoflavones are ingested does not appear to markedly impact overall absorption and the resulting biologic effects *(56)*. Genistein–genistin, daidzein–daidzin, and glycitein–glycitin make up approximately 50%, 40%, and 10%, respectively, of the total isoflavone content of the soybean. Each gram of soy protein in soybeans and in traditional Asian soyfoods contains approximately 3.5 mg of isoflavones (isoflavone weight throughout this paper refers to the aglycone weight) *(55)*. However, processing reduces the isoflavone content of some soy protein products by as much as 80% *(55)*. The daily isoflavone intake from traditional soyfoods of older Japanese adults ranges from 25 to 50 mg *(57–60)*.

In response to the consumption of dietary amounts of isoflavones provided by soyfoods or extracts, postprandial isoflavone levels can reach the low micromolar range; however, at least 95% of the isoflavones in serum are conjugated and thought to be largely biologically inactive *(56)*. Although isoflavones are extensively conjugated in both rats and humans, a higher percentage of both genistein and daidzein appear in the free or aglycone form in rats *(61)*. Isoflavones have short half-lives (approximately 8 hours), and nearly all are excreted within 24 hours after ingestion *(56)*. There is considerable interindividual variation in gut bacterial metabolism of genistein and daidzein *(62–64)*, which leads to markedly different serum and urinary concentrations of the isoflavones and their metabolites in different individuals *(62–64)*. This variation, coupled with differences in biologic activity among the isoflavonoids, has been offered as a possible explanation for the often inconsistent results from clinical trials *(63)*. The varied chemical composition of the many soy products used in these trials further complicates interpretation of the literature *(65)*.

Isoflavones bind to both estrogen receptors (ERα and ERβ) and exert some estrogen-like effects in vitro *(66,67)*. However, in clinical studies, estrogen-like effects are often not observed *(68–70)*. This discrepancy is not surprising because ER binding alone is a poor predictor of in vivo activity *(71)*. ER-binding ligands often have very different and sometimes opposite physiologic effects, depending upon the manner in which the ligand–receptor complex interacts with coactivators and corepressors within the cell *(72–74)*. Isoflavones have traditionally been considered weakly estrogenic, having 10^{-5}–10^{-2} less activity per mole than 17β-estradiol *(75–77)*. However, in some in vitro systems, genistein and daidzein and their metabolites exert effects even greater than those of estradiol *(78,79)*. Isoflavones are not unique in this regard; this phenomenon has been demonstrated for a number of other compounds, including resveratrol *(79,80)*. It is difficult to accurately estimate the relative estrogenic activity of ER-binding ligands because it depends on many factors, including the dose and the type of tissue used in the study.

Although isoflavones can bind to both ERα and ERβ, they preferentially bind to and activate ERβ *(81–83)*; for this reason, they are sometimes classified as selective estrogen receptor modulators (SERMs) *(69,84,85)*. The selectivity of isoflavones may depend in part on the relative tissue distribution of the two ERs. However, isoflavones also possess a variety of nonhormonal properties; thus, classifying isoflavones as phyto-SERMs does

not capture all of their properties *(2,86,87)*. The preferential binding of isoflavones to ERβ may have implications related to breast cancer risk; some data suggest that, when activated by certain ligands, ERβ inhibits mammary cancer cell growth as well as the stimulatory effects of ERα *(88)*. But the precise role of ERβ activation in breast cancer is unclear *(89)*.

In vitro, the isoflavone genistein inhibits the growth of most types of cancer cells, including both hormone-dependent and -independent breast cancer cells, through a variety of mechanisms *(2,90,91)*. However, its effect on the growth of ER-positive (+) cells is biphasic *(37,92–94)*. Genistein inhibits the growth of MCF-7 cells at higher (>10 μM) concentrations, whereas it stimulates growth at relatively low and physiologically relevant concentrations (<1 μM). Current thinking is that growth stimulation and inhibition by isoflavonoids occur through estrogen-dependent *(95)* and -independent mechanisms, respectively *(96)*. Genistein's estrogen-independent mechanisms include modulating genes that are related to the control of cell cycle and apoptosis, inhibiting the activation of nuclear factor-κB and Akt signaling pathways and inhibiting the activity of several enzymes and growth factors that control growth and differentiation *(97–100)*. Furthermore, isoflavones have antioxidant activity *(101)* and may stimulate the immune system *(102–104)* and inhibit angiogenesis *(105)*.

The clinical relevance of the in vitro data is a matter of considerable debate. A potentially important consideration is the extent to which the addition of physiologic levels of estrogen to culture medium affects the cancer cell growth-stimulatory effects of genistein. Some studies show that, in a high-estrogenic environment, genistein does not stimulate growth and can inhibit it *(106)*, whereas others show a modest increase in growth with genistein *(14,107–109)*. The hormonal milieu may also be an important factor determining the in vivo effects of isoflavones.

The in vitro growth-stimulatory effects of genistein were not fully appreciated (perhaps because they conflicted with the prevailing hypothesis) until dietary genistin was shown to stimulate the growth of existing estrogen-sensitive tumors in athymic ovariectomized mice *(37)*. Even before this finding was published however, observations in humans suggested that soyfoods had the potential to exert estrogen-like effects on breast tissue. In a pilot study, premenopausal but not postmenopausal women who consumed isoflavone-rich isolated soy protein (which is ≥90% protein) had a two- to sixfold increase in nipple aspirate fluid volume compared with those who did not *(110)*. Of greater concern was that epithelial hyperplasia was detected in seven of 24 postmenopausal women when they consumed soy. However, one important limitation of this study was the lack of a control group. Research published 3 years later by a different group showed that the consumption of textured vegetable (i.e., soy) protein for 2 weeks resulted in increased pS2 levels in breast biopsies taken from premenopausal women *(111)*. The pS2 protein is expressed in response to estrogen *(112)*; its activation in breast tissue in response to textured vegetable protein suggests that constituents of the intervention product are eliciting an ER–mediated response *(113)*. However, because of the short duration of this study and because breast cell proliferation was not increased, in contrast to the findings from a subset of this cohort that were published 1 year earlier *(114)*, the authors of this study concluded that the long-term implications of these findings were unclear *(111)*. Nevertheless, these latter two studies *(110,111)* suggested that soy has the potential to increase breast cancer risk and provided at least some of the impetus for further work in this area, the results of which form part of the discussion of the workshop proceedings.

Workshop Proceedings

Epidemiologic Studies

Not surprisingly, concern over the effects of isoflavones on breast cancer risk is based in part on the role of estrogen in the etiology of the disease and on data suggesting that conventional hormone therapy increases risk of the disease *(115)*. These data were briefly reviewed by W. McCaskill-Stevens (National Cancer Institute, Bethesda, MD), who noted that, in the Women's Health Initiative (WHI), risk of breast cancer was increased by 26% in response to the combination of estrogen plus progestin *(116)*, whereas it was decreased by 23% in response to estrogen alone *(117)*. In the Million Women Study, both treatments increased risk, but the risk associated with the use of combination hormones (odds ratio [OR] = 2.00, 95% confidence interval [CI] = 1.88 to 2.12) was higher than that of estrogen alone (OR = 1.30, 95% CI = 1.21 to 1.41) ($P<.001$) *(118)*. The differing effects of estrogen and estrogen plus progestin on breast cancer risk, as highlighted by these studies and for which there are considerable supporting data *(119,120)*, are noteworthy because isoflavones do not demonstrate progestin activity in vitro *(121)*.

Both the WHI and the Million Women Study addressed the risk for generally healthy postmenopausal women to develop breast cancer, not on the impact of hormone therapy on the prognosis of breast cancer patients. In a comprehensive review, Creasman *(122)* recently concluded that there is relatively little evidence that conventional hormone therapy is contraindicated for breast cancer patients, although this issue remains highly controversial *(123)*. In agreement with this conclusion, Durna et al. *(124)* found in a small study involving premenopausal breast cancer patients that hormone therapy use after diagnosis of breast cancer was not associated with increased breast cancer recurrence or mortality. However, there may be little chance of obtaining substantially more insight on this topic because the oncology community in general advises their ER+ breast cancer patients not to use postmenopausal hormones.

The impact of soy intake on recurrence or survival of breast cancer patients can be evaluated in an epidemiologic setting. In this regard, Anna Wu (University of Southern California, Los Angeles, CA) presented the experimental design for her ongoing investigation of the effects of lifestyle factors on breast cancer prognosis among Asian-Americans. A total of 1378 case patients are in the study, including 489 Chinese, 383 Japanese, and 506 Filipino women, all of whom reside in the Los Angeles area. Data are being collected about initial treatment and tumor characteristics—tumor stage and size, lymph node status, extent of disease, histology, differentiation, grade, laterality, and estrogen–progesterone receptor status—as provided by the Los Angeles County Cancer Surveillance Program (member of the Surveillance End Results Program). Telephone interviews are being conducted 5 years after initial diagnosis of breast cancer to determine lifestyle characteristics, including body weight, physical activity, herbal and vitamin supplement use, and dietary pattern (main food groups include soy, tea, fruits and vegetables, red meat, white meat, fish, and alcohol). Postdiagnostic follow-up data also being collected include the number and type of breast surgeries and use of tamoxifen, raloxifene, aromatase inhibitors, herceptin, chemotherapy,

radiation, and other conventional treatments, as well as the use of alternative or complementary therapy. Data collection will be completed in 2008.

Relevant to this study are recently published findings from a prospective epidemiologic study conducted in Shanghai involving 1459 Chinese breast cancer patients *(125)*. During the approximately 5-year follow-up period, 240 deaths occurred, but there was no association between the intake of soy protein or isoflavones before diagnosis of breast cancer and disease-free survival. The relationship between soy protein intake and breast cancer survival did not differ according to estrogen–progesterone receptor status, tumor stage, age at diagnosis, body mass index, waist-to-hip ratio, or menopausal status. Also, the results were not affected when the analysis was restricted to only women with ER+ tumors (63% of the total). One limitation of this study is that soy intake was determined only at baseline; however, when the analysis was restricted to only women who reported "no dietary change" during the follow-up period, the results were similar to findings involving all women.

Animal Studies

It was research demonstrating that dietary genistein *(37)* and genistin *(38)* stimulate the growth of estrogen-sensitive mammary tumors in rodents that first raised concern that isoflavones might be contraindicated for breast cancer patients. This research was conducted by William Helferich and colleagues at the University of Illinois, who used a model in which athymic BALB/c (nude) ovariectomized mice are subcutaneously injected with MCF-7 cells and implanted with estrogen pellets to stimulate estrogen-dependent tumor growth. Once tumors have reached a cross-sectional area of approximately 40 mm^2, the estrogen pellets are removed from all groups except the positive control. In mice that are fed the standard AIN-93G diet, tumors regress completely; however, diets containing either isoflavone-rich isolated soy protein *(126)* or isoflavone extracts *(127)* stimulate tumor growth in the mice. Furthermore, in this model, dietary genistein negates the ability of tamoxifen to inhibit tumor growth *(128)*. In a recent publication from this group *(129)*, dietary genistein stimulated estrogen-dependent tumor growth in athymic BALB/c ovariectomized mice implanted with silastic implants containing low levels of estradiol that produced plasma estradiol concentrations similar to those found in postmenopausal women. These data indicate that genistein can act as an ER agonist and can stimulate estrogen-dependent tumor growth in vivo.

A potentially crucial observation from Helferich and colleagues is that mice exposed to more processed soy products had faster tumor growth than mice exposed to less processed soy products even if the amount of genistein in both products was the same *(127)*. A diet containing soy flour, which is minimally processed, did not promote tumor growth, although tumors did not regress to the extent that they did with the control diet lacking soy. The mechanism behind this processing effect is unclear, although two explanations have been proposed: one is that processing causes greater increases in serum levels of free genistein *(130)* and the other is that compounds removed during processing inhibit the tumor-stimulatory effects of isoflavones and/or directly inhibit mammary tumor growth *(131)*. In contrast to genistein, daidzein only modestly stimulated the growth of MCF-7 cells in this mouse model. Moreover, tumor growth was not at all stimulated by equol, a bacterially derived metabolite of daidzein that stimulates MCF-7 cell proliferation in vitro *(132)*.

In a similar model to that used by Helferich's group *(126,127)*, Lilian Thompson (University of Toronto) also observed tumor-stimulatory effects of isoflavone-rich isolated soy protein although they were not as pronounced *(133)*. According to Thompson, tumors initially regressed with dietary soy protein to the same extent as with the control diet, which did not contain soy, but after 10–12 weeks soy protein stimulated tumor regrowth. In contrast, flaxseed, a rich source of lignans, which like isoflavones are also diphenolic compounds classified as phytoestrogens, did not alter tumor growth from that in response to the control diet alone, i.e., tumor regression occurred to a similar extent. Furthermore, the addition of flaxseed to the isoflavone-rich isolated soy protein–containing diet caused tumor regression that was similar to the regression that occurred in response to the control diet. Thus, flaxseed inhibited the tumor-stimulatory effects of soy protein. In comparison to isoflavone-rich isolated soy protein alone, the addition of flaxseed caused a decrease in tumor cell proliferation and an increase in tumor cell apoptosis. Similar effects were noted when genistein and the enterolignans enterolactone and enterodiol were injected (10 mg/kg body weight), both alone and in a combination of all three *(134)*. Tumors regressed initially in response to genistein alone and then stopped regressing after prolonged exposure, whereas the tumors continued to regress in response to the enterolignans and in response to the combination of enterolignans and genistein. As was observed for the combination of flaxseed and isoflavone-rich isolated soy protein, the addition of enterolignans markedly inhibited genistein-induced tumor cell proliferation compared with genistein alone. Finally, only the enterolignans increased apoptosis; there was no effect of the combination of genistein and the enterolignans on the percentage of cells that underwent apoptosis. Neither flaxseed nor the enterolignans inhibited the skeletal benefits of isoflavone-rich isolated soy protein or genistein, respectively *(135)*.

There is however considerable debate about the merits of using animal models of breast cancer to predict effects in humans. A specific criticism *(136)* of the athymic ovariectomized mouse model as used by the research groups of Helferich *(126,127)* and Thompson *(133)* is that, unlike pre- and postmenopausal women, these mice do not produce sufficient endogenous estrogen to promote or to even maintain tumors. Thus, this model is biased toward finding that even weakly estrogenic compounds stimulate the growth of existing estrogen-sensitive mammary tumors. However, mammary tumor stimulation has been noted in other rodent models in response to both dietary *(18,20,129)* and subcutaneously injected *(137)* genistein, including those in which estrogen levels are more reflective of the hormonal milieu of postmenopausal women *(129)*.

Another criticism of the animal studies is the use of high oral doses of isoflavones. Studies using multiple treatment doses suggest that isoflavones at 200–500 ppm in the diet yield serum concentrations in rodents that are within the range observed in humans who consume soyfoods or use soy isoflavone supplements, whereas doses of approximately 1000 ppm result in excessive isoflavone concentrations *(132,138,139)*. In addition, serum isoflavonoid molar ratios differ between rodents and humans because the rodent gut bacteria effectively convert daidzein to the metabolite equol, whereas only 30%–50% of humans carry bacteria with this metabolic capacity *(61,63)*. Furthermore, even in humans who are classified as equol producers, genistein is the

predominant isoflavone in the serum in response to the ingestion of soy or mixed isoflavones, whereas equol predominates in most other species, including both rodents and monkeys *(61)*.

Angela Brodie (University of Maryland) described the different rodent models that are available for studying mammary cancer. These include aromatase-overexpressing transgenic mice, the BRCA1 conditional mutant mouse model, and the human ER+ MCF-7 aromatase (MCF-7C_a) cell xenograft model *(140,141)*. In the first two models estrogen levels are regulated by the host. In the third model, however, estrogen-dependent tumor growth is regulated by the tumors, which produce their own estrogen. Thus, this third model may better reflect the hormonal environment of women than the first two. Furthermore, in the ER+ MCF-7C_a model, mice are also often injected with androstendione to provide greater substrate for estrogen production, resulting in rapidly growing tumors that are sensitive to both antiestrogens and aromatase inhibitors. This model offers an opportunity to study the effect of soy and isoflavones on the growth of estrogen-sensitive mammary tumors.

Clinical Studies

As discussed previously, the hormonal milieu may affect the biologic activity of isoflavones. Therefore, it is important to have a clear understanding of the hormonal environment of both pre- and postmenopausal women in general and of normal and cancerous breast tissue in particular. Jürgen Geisler (Haukeland University Hospital, Norway) noted that in postmenopausal women, plasma concentrations of estrone, estradiol, and estrone sulfate are 60–80 pmol/L, 10–20 pmol/L, and 400–500 pmol/L, respectively *(142)*. Breast tissue estrogen levels are largely determined by uptake from serum, by local production in tumor cells or in surrounding tissues, and by the metabolism of estrogens in breast and peripheral tissues. Free estrogens are taken up by breast tissue against a concentration gradient—estradiol, estrone, and estrone sulfate concentrations are 10–20 times, 2–10 times, and 10–20 times higher, respectively, in breast cancer tissue than in plasma *(142)*. Furthermore, plasma estrogen levels do not predict tissue estrogen levels in postmenopausal breast cancer patients *(142)*. Thus, it appears that, despite having lower serum estrogen levels, postmenopausal women have breast tissue estrogen concentrations that are similar to those of premenopausal women.

Clearly there is a need to determine the effect of soy consumption on markers of breast cancer risk in high-risk women and breast cancer patients. Unfortunately, few if any noninvasive or minimally invasive assays for markers of breast cancer risk have been identified. One that has been used extensively is breast tissue density. Dr Norman Boyd (Ontario Cancer Institute, Toronto, ON) noted that differences in the parenchymal pattern of the breast on mammography reflect differences in the amounts of stromal, epithelial, and fat tissue present in the breast *(143)*. Stroma and epithelium are radiologically dense, whereas fat is lucent. Women who have extensive areas of mammographically dense breast tissue have a 4–6 times higher risk for breast cancer than women with little or no density *(144)*. Furthermore, menopausal hormone (combined estrogen plus progestin) interventions, which are known to increase breast cancer risk, also increase breast tissue density *(145)*. Nonetheless, the effects of hormone therapy on breast cancer risk do not appear to be mediated by effects on breast tissue density *(146)*. These data suggest that if an intervention alters breast density, it does not necessarily follow that the intervention will alter breast cancer risk; conversely, interventions may alter risk of breast cancer without changing density.

Several investigators have examined the impact of either soyfoods or isoflavones on breast tissue density in intervention *(21, 22,147–149)* and epidemiologic *(150–154)* studies. Gertraud Maskarinec (Cancer Research Center of Hawaii, Honolulu, HI) discussed three intervention studies: a 1-year study that examined the impact of isoflavone supplements derived from red clover on mammographic density in postmenopausal women *(21)*, a 1-year study in which premenopausal women were given 100 mg/d soybean isoflavones in supplement form *(147,148)*, and a 2-year study in which premenopausal women consumed two servings of soyfoods per day that provided approximately 50 mg of isoflavones *(22,149)*. Maskarinec concluded that these studies showed that there is no effect of 1–2 years of soy or isoflavone consumption on breast density in premenopausal women. No published studies have examined the impact of soy isoflavones on density in postmenopausal women; however, red clover isoflavones, which lead to blood isoflavone concentrations similar to those achieved with the ingestion of soyfoods *(155)*, had no effect *(21)*. Thus, these studies show evidence of neither harm nor benefit on breast cancer density, in contrast to the effects of hormone therapy, which increases breast tissue density *(156)*.

Jeffrey A. Tice (University of California San Francisco, San Francisco, CA) also discussed the effects of soy on breast tissue density. Tice and his colleagues carried out a double-blinded study in which 47 postmenopausal women at high risk (defined by Gail risk $\geq 1.67\%$ and mammographic breast density $\geq 50\%$) for breast cancer were randomly assigned to either a daily dose of 25 g casein or 25 g isoflavone-rich isolated soy protein that provided approximately 50 mg of isoflavones. At 6 months, there were no differences between the groups in the change in breast density timed to the menstrual cycle; there were also no differences in circulating levels of insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), or the IGF-1 : IGFBP-3 ratio. Thus, these results are consistent with the lack of effect of isoflavones on breast density in the studies reviewed by Maskarinec.

In the not too distant future, more information about the impact of soy on breast tissue density will be available. Lee-Jane Lu (The University of Texas Medical Branch, Galveston, TX) presented the experimental designs for her two ongoing double-blinded, randomized, placebo-controlled, parallel group studies. Both trials are 2 years in duration and involve healthy premenopausal women aged 30–42 years not using contraceptive medications. In one study, women will consume daily either 40 g soy protein without isoflavones or 40 g casein, and in the other, women will take a placebo or 130 mg/d isoflavones as a supplement. Serum hormones, bone density, and breast density will be measured at baseline and yearly during the intervention period. There will be approximately 100 women per group in each study. Thus, this research, both because of the duration and size, may provide the most definitive data to date on the effect of both soy protein and isoflavones on breast tissue density in premenopausal women.

Direct histologic analysis of breast tissue—short of monitoring for tumor development—provides the optimal approach for determining cancer risk. Melanie Palomares (City of Hope Comprehensive Cancer Center, Duarte, CA), presented the results of her pilot randomized controlled trial in which postmenopausal

breast cancer survivors were given either a placebo or an isoflavone supplement (100 mg/d) for 1 year. To qualify, women had to have a history of unilateral stage I–II infiltrating ductal or infiltrating lobular carcinoma or ductal carcinoma in situ and not to have used estrogen-modulating therapy, including SERMs, aromatase inhibitors, hormone therapy, or hormonally active herbal supplements within 3 months of enrollment. Also, women were excluded if their baseline diet included more than three servings of soyfoods per day (average 10 mg/d of isoflavones).

Normal breast tissue from the contralateral breast was sampled using ultrasound-guided core biopsy at baseline and at 6 and 12 months. At none of the time points were there statistically significant differences in cell proliferation (Ki67 index), histology (hyperplasia with or without atypia), or ER expression between the two groups. However, because of the small sample size (n = 23) of this study the findings should be interpreted cautiously. Interestingly, the baseline Ki67 indices were higher and the incidence of hyperplasia in these women was greater than what has been observed for healthy individuals in other studies, supporting the observation that breast cancer patients are at an increased risk of developing contralateral breast cancer *(157)*.

Finally, Carol Fabian (University of Kansas Medical School, Kansas City, KS) reviewed her research demonstrating the use of random periareolar fine-needle aspiration (RPFNA) for obtaining breast tissue to study the effects of different interventions on breast cancer risk *(158)*. The advantages of this approach include the capacity to assess precancerous changes at the tissue level, the availability of tissue for other response biomarkers (e.g., Ki67) and those predictive of response (e.g., ER expression), and minimal discomfort on the part of the subject. Disadvantages include interpretation and sampling variance; approximately 25% of a placebo-treated group will show improvement (40% will show overall categoric change) when a high-risk cohort member exhibiting hyperplasia +/− atypia is treated for 6 months. In one 6-month study using RPFNA among women on a stable dose of hormone therapy, letrozole reduced cell proliferation (Ki67 index) by two-thirds but did not affect breast tissue density, thus emphasizing the importance of analyzing tissue to assess risk. Dr Fabian presented the experimental design for an ongoing study in which RPFNA will be used to investigate the effects of the plant lignan secoisolariciresinol diglycoside on breast cancer risk in premenopausal women at high risk for breast cancer.

It is clear from the above discussion that biomarkers of breast cancer risk are limited and often their association with causality is not well understood. The clinical studies presented as part of this workshop suggest that biomarkers measured in the target tissue (e.g., breast tissue hormone concentrations or epithelial cell proliferation), rather than surrogate measures (e.g., breast density or serum hormone concentrations), may be more appropriate for evaluating the impact of an intervention on risk of breast cancer. The limitations of these existing biomarkers highlight the critical need to develop biomarkers definitively linked to breast cancer as an outcome.

Workshop Conclusions and Research Recommendations

Neither the existing animal nor human data allow definitive conclusions to be drawn about the effect of soyfoods or isoflavones on breast cancer risk in high-risk women and on the survival of breast cancer patients. There is an important public health imperative to determine the safety of soyfoods in both groups of women. Definitively establishing that soyfoods do not adversely affect the survival of breast cancer patients may not be possible. To do so will likely require conducting a long-term intervention trial in which tumor recurrence or survival are endpoints. However, conducting such studies may be prohibitively expensive and raise ethical concerns. Assessing the potential impact of soyfoods on breast cancer risk in high-risk women is possible by examining cancer risk markers (e.g., cell proliferation, apoptosis) using breast tissue samples obtained via RPFNA or ultrasound-guided biopsies. Such research is urgently needed and should be designed to determine both safety and efficacy. Careful consideration should be given to the types of soy products used for such interventions; emphasis should be placed on using products that allow findings to be extrapolated to as broad a range of soy products as possible.

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Notes

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