Progress in breast cancer chemoprevention

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Abstract

Over the past years there have been significant advances in breast cancer treatment and early detection. For the first time, a decrease in cancer mortality has been observed. Recently, much progress has been made in the understanding of carcinogenesis partly due to available new technologies to detect early molecular changes in the tissue. The knowledge of breast cancer carcinogenesis has provided possible opportunities to prevent breast cancer. Currently, several clinical breast cancer prevention trials are ongoing. This paper reviews issues related to breast cancer chemoprevention including identification of high risk cohorts, endpoint biomarkers, current and new chemopreventive agents as well as models to evaluate these agents.

Introduction

Breast cancer is the most common cancer in women worldwide and continues to be a major health problem (Guillot et al. 1996). Even though the incidence and mortality has decreased recently (Hortobagyi 1998), many women still develop breast cancer and ultimately die from this disease. It is estimated that in the year 2001 192,000 women in the US will be diagnosed with breast cancer, and 40,200 will die of this disease (Greenlee et al. 2001).

Research in breast cancer now extends in many directions, from identification of genes that predispose to breast cancer, to cellular models for preneoplastic disease, investigation of the tumor and its local environment, and finally to the identification of risk factors for the development of breast cancer and possible models of breast cancer prevention including the use of chemopreventive agents. In this review, we aim to discuss risk factors that are associated with breast cancer, identify target populations for breast cancer chemoprevention trials, and summarize the status of current molecular markers and potential agents for the use of breast cancer chemoprevention.

Risk factors

Epidemiological studies have identified several factors that are associated with an increased risk of developing breast cancer. One of the best known risk factors for breast cancer is family history. It is known that 5–10% of breast cancers are due to inherited genetic mutations (Newman et al. 1988, Yang & Lippman 1999). Two of these genes, BRCA-1 and BRCA-2 have recently been cloned and, together, are estimated to be involved in 60%–70% of all hereditary breast cancer (Ford et al. 1998). Women with mutations of these genes have an approximate 50%–80% life time risk of developing breast cancer (Easton et al. 1993, Struwing et al. 1997). Germine mutations in the tumor suppressor gene p53 are also associated with increased breast cancer risk and account for 1% of breast cancers in young women (Malkin et al. 1990). But it needs to be pointed out that most women with a family history do not have genetically inherited disease; it is important to distinguish this group from the genetically inherited group, since the former carries a lower life time risk of developing breast cancer. In fact, a 30-year-old woman with a mother and sister diagnosed with unilateral breast cancer has up to an 18% life time risk of breast cancer; this increases to 25% if they had bilateral breast cancers (Anderson & Badzioch 1985).

Epidemiological data also strongly suggest an association between ovarian hormones and the risk of breast cancer. Indeed, this notion is supported by observations where prolonged estrogen exposure, such as early menarche (MacMahon et al. 1970), late menopause (Trichopoulos et al. 1972), nulliparity, and late age at first pregnancy (MacMahon et al. 1970) were found to be associated with increased risk of breast cancer. Studies reporting on the relationship between breast cancer risk and abortion are controversial (Newcomb et al. 1996, Melbye et al. 1997), as are
the studies on lactation and breast cancer risk (Layde et al. 1989); recent data suggest that prolonged lactation can actually reduce breast cancer risk (Newcomb et al. 1994).

Multiple studies have reported on the exogenous use of hormones and breast cancer risk. One recent meta-analysis revealed that the use of hormonal replacement therapy was associated with a small increased risk (Sillero-Arenas et al. 1992). Another meta-analysis revealed essentially the same results; in this particular report the increased risk was noted in patients who used estrogen for at least 5 years (Steinberg et al. 1991). Studies investigating the relationship between the use of oral contraceptives and breast cancer revealed that oral contraceptives slightly increased the risk of breast cancer, especially if they were used before the first birth (Meirik et al. 1986, UK National Case Control Study Group 1989). Other studies have found no such negative correlation (Malone et al. 1993).

Previous breast biopsy is also associated with an increased risk of breast cancer. Individuals with a histopathological diagnosis of proliferative breast disease have up to twice the normal risk of increased risk of breast cancer; the relative risk increases to 4.5–5 if atypia is present (Page & Dupont 1992). Lobular carcinoma in situ (LCIS) is also known to be a risk factor; specifically, the relative risk of LCIS patients for developing breast cancer has been estimated to be 7–9 times that of the normal population, with an absolute life time risk of 20% (Grooff et al. 1993).

The evaluation of risk factors for breast cancer, mainly by epidemiological studies, has helped to develop models to identify women at increased risk. The most commonly used model is the Gail model, which used data from 4496 matched pairs of cases in the Breast Cancer and Diagnosis and Demonstration Project (Gail et al. 1989). The risk factors for this model included age at menarche, age at first live birth, number of previous breast biopsies, and number of first-degree relatives with breast cancer. This model was validated by two subsequent studies (Bondy et al. 1994, Spiegelman et al. 1994). Unfortunately, the model does not take into account the risk in individuals who have second-degree relatives diagnosed with breast cancer. It also underestimates the risk in individuals with a history of previous LCIS or ductal carcinoma in situ (DCIS) and may overestimate the risk in women with nonproliferative disease at biopsy.

Breast cancer carcinogenesis and chemoprevention

Carcinogenesis is characterized by a long delay between the first exposure to a known or suspected carcinogen and the eventual occurrence of cancer, and by a steep rise in tumor incidence after this latency period. During this period, multi-step processes occur including tumor initiation, tumor promotion, and tumor progression (Pitot 1989, Kellog et al. 1995). This multi-step nature of neoplastic development has been derived from several experiments on skin carcinogenesis (Rous & Kidd 1941, Morttram 1944, Berenblum & Shubik 1947) and from the proposed concept of tumor progression in mouse mammary carcinogenesis (Foulds 1954). In general, newly acquired genetic alterations and molecular damage accompany the progression phase. The accumulation of these genetic alterations then provides the basis for the transition from a pre-malignant to a malignant state (Kramer & Srivastava 1994, Srivastava & Kramer 1994).

Sporn and Newton in 1979 defined chemoprevention as ‘prevention of cancer by the use of pharmacological agents that inhibit or reverse the process of carcinogenesis’. As mentioned earlier, in the process of carcinogenesis, altered states of cell and tissue differentiation are characteristic of premalignant lesions long before they become invasive, which offers a window of time and a target for chemical intervention. In some circumstances, it is possible to reverse the abnormal differentiation with an agent that is essentially non-cytotoxic (Greenwald et al. 1995, Lippman et al. 1995, Hong & Sporn 1997). Even though the precise mechanism of breast cancer carcinogenesis is not known, it is thought that the terminal duct lobular units (TDLUs) are the major stem cell component giving rise to premalignant breast lesions; ductal hyperplasia (DH), atypical ductal hyperplasia (ADH), DCIS and LCIS are thought to be histological manifestations of a continuum process leading from the premalignant to the malignant state (Allred et al. 2001). Genetic alterations and molecular changes occur during this process and for effective breast cancer chemoprevention development, it is extremely important to identify specific molecular abnormalities that can be monitored as biological endpoint biomarkers during specific pharmacological interventions. Potential biological biomarkers which are thought to be involved in breast cancer carcinogenesis are discussed below.

Intermediate and surrogate end points in breast cancer

Intermediate biomarkers of cancer are phenotypic, genotypic, and molecular changes that occur during carcinogenesis. Currently, there are no validated surrogate endpoint biomarkers for breast cancer in the context of chemoprevention trials with invasive cancer as the definitive end points; therefore, the development of intermediate biomarkers as surrogate endpoints for clinical chemoprevention trials for breast cancer is very important. Because of the shorter latency to intermediate biomarker end points and the smaller cohorts required for treatment, planning short term prevention trials and evaluating potential biomarkers in this setting is very critical to the progress of chemoprevention. To be most useful, intermediate biomarkers should be on the causal pathway of breast cancer and reflect biological changes along the carcinogenic process so that they can be used to monitor the
efficacy of a potential breast cancer preventive intervention (Dunn & Ford 2001). It would be helpful to show that these markers can undergo modulation with a chemopreventive intervention and that this modulation will ultimately lead to a decrease in breast cancer. Potential molecular markers for the use of breast cancer chemoprevention are summarized in Table 1.

Table 1 Molecular markers for the use of breast cancer prevention.

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Markers of genetically altered cells

Epigenetic markers

Even though it is known that endogenous and exogenous estrogen promotes the development of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer 1996), it is also known that early initiating events must first take place to sensitize breast epithelial cells to growth factor regulated cancer promotion. Cells in the breast epithelium of high-risk patients may comprise the pool of genetically altered cells that are subject to growth factor-mediated cancer promotion. Epigenetic alterations, such as hypermethylation, could reflect the presence of DNA-damaged cells. Methylation is the main epigenetic modification in humans (Baylin et al. 1998) – it is one of the mechanisms for inactivating tumor suppressor genes (Graf et al. 1995). In particular, hypermethylation of normally unmethylated CpG islands in many tumor suppressor genes correlates with loss of expression (Baylin et al. 1998). Abnormal methylation in breast cancer has been observed in the tumor suppressor genes p16 (Woodcock et al. 1999), BRCA1 (Manicini et al. 1998), estrogen receptor (Ottaviano et al. 1994), progesterone receptor (Lapidus et al. 1996), retinoic acid receptor (RAR) β2 (Sirchia et al. 2000), and E-cadherin (Graf et al. 1995). Methylated markers could potentially serve as biomarkers to identify high-risk women, as well as intermediate endpoint markers in short-term chemoprevention trials (Yang et al. 2001).

Microsatellite instability and loss of heterozygosity

Other markers of genetic alterations include microsatellite instability (MSI) and loss of heterozygosity (LOH), which are thought to contribute to the initiation and progression of human breast cancer (Bieche & Lidereau 1995, Coleman & Tsongalis 1995). MSI has been found in breast carcinomas on 1q, 3p, 6p, 6q, 7p, 11p, 16q, 17p, 17q, 18q, 19q, and Xq (Aldaz et al. 1995, Karnik et al. 1995). The emergence of MSI may involve defects in DNA replication or mismatch repair mechanisms, which are considered to be a driving force in the multistage process of carcinogenesis (Ionov et al. 1993, Boyer et al. 1995, Loeb 1991, 1998). The incidence of MSI in breast cancers ranges from 12% to 100% (Patel et al. 1994, De Marchis et al. 1997, Tomita et al. 1999). It is not yet clear at what stage of breast cancer development a progression of MSI becomes manifest, but MSI has been reported as an early event in tumorigenesis (Yee et al. 1995). LOH has been observed in both in situ and invasive breast carcinomas on multiple chromosomal arms, including 1p, 1q, 3p, 6q, 7q, 11p, 11q, 13q, 16q, 17p, 17q, and 18q (Radford et al. 1995, Tsuda & Hirohashi 1995, Devilee et al. 1997). This type of genetic alteration may indicate deletion of the remaining normal allele of a tumor suppressor gene, which is a generally accepted mechanism of carcinogenesis initially hypothesized by Knudson (1977) in his two-hit theory. Furthermore, in one study 50% of atypical ductal hyperplasia cases showed at least one focus of LOH, suggesting that LOH is an early event in breast carcinogenesis (Lakhani et al. 1995).

Telomerase

Another marker with which to evaluate the presence and modulation of genetically altered cells is telomerase activity. Increased telomerase activity is an early event in the development of breast cancer, which leads to immortalization of genetically altered cells (Herbert et al. 2001a,b). A study evaluating the role of telomerase during multi-stage pathogenesis of breast cancer revealed 14% enzyme activity in benign breast disease, 67% in fibroadenomas, 92% in DCIS, and 94% in invasive breast cancer (Bednarek et al. 1997, Yoshima et al. 1998). Given its presence in early lesion, telomerase could be an endpoint marker and potential target for chemopreventive interventions.

STK15/BTAK

Finally, the centrosomal serine/threonine kinase 15/breast tumor amplified kinase (STK15/BTAK), which plays a role in chromosomal instability, could also serve as a marker for altered genetic status (Miyoshi et al. 2001). Indeed amplification of the STK15/BTAK gene and overexpression of STK15/BTAK mRNA was shown in human breast cancers (Sen et al. 1997, Zhou et al. 1998, Tanaka et al. 1999).
indicating that it might be involved in breast cancer carcinogenesis.

**Markers of proliferation and apoptosis**

Imbalances in proliferation and apoptosis, by favoring the promotion of genetically altered cells in the breast tissue, most probably play a role in breast cancer promotion. In fact, apoptosis occurs regularly during normal growth and development of the mammary gland. One of the most dramatic examples of apoptosis is evident during the remodeling of the breast that accompanies post-lactational involution. Transgenic mouse models have demonstrated that overexpression of some growth factors, such as epidermal growth factor (EGF), can block this remodeling and serve as survival factors for the mammary epithelium (Rosfjord & Dickson 1999). Overexpression of epidermal growth factor receptor (EGFR) is considered an important autocrine stimulatory pathway for breast carcinoma cell growth, and its expression is associated with an enhanced metastatic potential in model systems (Fox et al. 1994, Schroeder et al. 1997). Interestingly, recent data has shown that abnormal ploidy and EGFR expression is present in benign breast epithelium in women who later develop breast cancer (Fabian et al. 1997).

p53 serves a multi-functional role as a transcriptional regulator; it mediates G1-S growth arrest and plays a critical role in maintaining DNA integrity by facilitating apoptosis of DNA-damaged cells. Mutations of p53 are present in up to 50% of invasive breast cancers, and its loss of function is associated with a high proliferation index and poor clinical outcome (Allred et al. 1993, 1994). In most instances, p53 abnormalities become appreciable just before the transition from preinvasive to invasive disease (Davidoff et al. 1991, Thor et al. 1992); in fact, in a case control study in more than 4800 women, the presence of p53 mutations in benign epithelium was found to be an early marker for later breast cancer development (Rohan et al. 1998).

Another proliferative factor is Her-2/neu, which is over-expressed in about 30% of breast cancers (Coussens et al. 1985, Chrysogelos & Dickson 1994). The activation of the Her-2/neu oncogene triggers a cascade of growth signals that results in gene activation (Sundaresan et al. 1998). Its over-expression is associated with loss of estrogen receptor, aggressive clinical behavior, increased metastatic potential, and altered sensitivity to hormonal and chemotherapeutic agents (Pegram et al. 1997). Recently, it was shown that Her-2 exerts its proliferative effect also by increasing cyclooxygenase-2 (Cox-2) expression; in that study it was shown that Cox-2 inhibition blocked the proliferative effect of Her-2 (Vadlamudi et al. 1999).

Another survival and proliferation factor is COX-2, which is now believed to play an important role in breast carcinogenesis. COX-2 expression was shown in up to 88% of invasive breast cancers (Parrett et al. 1997, Hwang et al. 1998, Masferrer et al. 2000, Arun et al. 2001), and it was also found to be increased in DCIS lesions (Soslow et al. 2000). COX-2, via increasing prostaglandin levels, can stimulate the transformation and growth of mammary epithelial cells (Bandyopadhyay et al. 1987, Subbaramaiah et al. 1996). In one study, mammary gland involution was delayed in COX-2 overexpressing transgenic mice, with a decrease in the apoptotic index of mammary epithelial cells (Liu et al. 2001). Furthermore, COX-2 inhibitors may be specific inhibitors of normal epithelial cell proliferation and growth of malignant cells (Erickson et al. 1999, Ding et al. 2000, Joki et al. 2000, Marrogi et al. 2000). Therefore, COX-2 could be a potential marker with which to follow chemopreventive interventions, and might also serve as a target itself for selective COX-2 inhibitors. In continuation, there is recent data suggesting an interaction between COX-2 and the aromatase pathway in the breast epithelium. Indeed, aromatase was recently shown to be activated by COX (Zhao et al. 1996, Zhou et al. 2001), leading to estrogen-induced proliferation. Aromatase expression is highest in or near breast tumor sites (Bulun et al. 1993), suggesting that it is involved in autocrine and paracrine mechanisms of initiation and progression of breast cancer.

Insulin-like growth factor (IGF)-I and IGF-II are potent epithelial mitogens that stimulate growth of human breast cancer cells and prevent apoptosis (Pollak et al. 1988, Van den Ber & Yee 1996, Stoll 1997, Valentinis et al. 1999). IGFs are also involved in the later stages of progression and invasion by enhancing epithelial cell migration (Mira et al. 1999). These effects are mediated through the IGF-I receptor (IGF-IR), which is overexpressed in most breast cancer cell lines and in malignant breast tissue (Clarke et al. 1997). In addition, circulating IGF-I levels were found to be increased in patients with breast cancer compared with healthy women, and, more importantly, increased levels of IGF-I were shown to be associated with subsequent development of premenopausal breast cancer (Hankinson et al. 1998). Further studies showed serum IGF-I modulation with tamoxifen (Decensi et al. 1999), suggesting it’s potential as an intermediate endpoint marker for chemoprevention trials.

Another proliferation marker, Ki-67, was found to be increased in breast tissue with ductal hyperplasia, which was adjacent to tissue with invasive breast cancer (Mommers et al. 1998). If ductal hyperplasia is an early event in breast carcinogenesis, Ki-67 could serve as an early proliferation marker for monitoring chemopreventive drug effects.

In normal dividing cells, the G1 to S transition is regulated by cyclin E (Dou et al. 1993, Keyomarsi et al. 1995). In several studies, cyclin E expression was increased and associated with death and/or relapse from breast cancer (Keyomarsi et al. 1994, Nielsen et al. 1996, Porter et al. 1997). In a very recent study, it was found that cyclin E proved to be the most powerful independent predictor for survival in stage I-III breast cancer (K Keyomarsi, personal
communication). Cyclin E could serve as a marker to identify high-risk patients and to monitor chemopreventive interventions.

The expression of the bcl-2 gene in human breast cancer ranges from 32% to 85.9% (Bhargava et al. 1994, Hellemans et al. 1995). The presence of bcl-2 protein immunostaining has been shown to be associated with a low apoptotic index in malignant mammary epithelium. As a result, tissue kinetics could shift towards the preservation of genetically aberrant cells, thereby facilitating tumor progression (Chan et al. 1993). Bcl-2 was shown to inhibit wild-type p53-induced apoptosis (Zhang et al. 1997). Further studies have suggested that an imbalance in the bax and bcl-2 ratio might be responsible for the dysregulation of apoptosis and proliferation (Bargou et al. 1995, Binder et al. 1996). A recent study showed increased bcl-2 expression in patients who received preoperative chemotherapy and still had residual disease (Ellis et al. 1998); in the same way, bcl-2 could serve as a marker to evaluate chemopreventive drug interventions as well.

Angiogenetic markers – vascular endothelial growth factor (VEGF)

Angiogenesis, the formation of new blood vessels from the existing vascular network, is essential for continued tumor development, growth, and metastasis (Folkman 1992). Studies have suggested that angiogenesis is an early event in breast carcinogenesis; indeed it was shown that VEGF (vascular permeability factor), one of the most potent of angiogenic factors, is expressed in nearly all DCIS lesions (Guidi et al. 1997). VEGF production is increased by mutant p53 (Kieser et al. 1994) and COX-2 (Oshima et al. 1996, Tsujii et al. 1998) and has potential not only as a marker but also as a target for inhibition and prevention.

Chemopreventive agents

Since the target population for primary breast cancer prevention is high-risk but otherwise healthy individuals, much emphasis needs to be placed on the development of active but nontoxic chemopreventive agents. Currently used and potential agents are discussed below and summarized in Table 2.

Hormonal agents

Selective estrogen receptor modulators (SERMs)
The term selective estrogen receptor modulator (SERM) was recently used to describe compounds that interact with the estrogen receptor but have tissue-specific activities (Mitlak & Cohen 1997). A variety of compounds classified as antiestrogens are known to have both estrogen-agonistic and estrogen-antagonistic properties (Baker & Jaffe 1996).

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<th>Table 2</th>
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Tamoxifen, one of the SERMs, is a triphenylethylenederivative with both estrogenic and anti-estrogenic activities (Furr & Jordan 1984). Tamoxifen’s antiestrogenic activity is attributed generally to its ability to bind to and translocate estrogen receptor (ER) to the nucleus, where it inhibits estrogen-mediated events leading to cell growth (Guillot et al. 1996). There is evidence that tamoxifen interferes with the initiation and promotion of tumors in experimental systems and inhibits the growth of malignant cells by a variety of mechanisms, including inhibition of protein kinase C (Issandou et al. 1990), up-regulation of c-myc expression (Kang et al. 1996), modulation of the expression of growth factors such as EGF and IGF-I, inhibition of enzymes involved in the synthesis of estrogens and signal transduction (Cuzcick 1996), and induction of oxidative stress with inducing activation of the nuclear transcription factor kappa B (NF-kB) (Jordan 1974, Jordan & Allen 1980, Jordan et al. 1980, 1990, Fertini et al. 1999). Specifically, tamoxifen blocks estrogen-induced mammary tumor growth (Jordan & Allen 1980, Jordan 1983) and prevents the development of mammary carcinoma in rats (Jordan 1974, 1976).

Clinical evidence for tamoxifen as a chemopreventive agent is derived from several clinical trials that have demonstrated the efficacy of tamoxifen in the adjuvant therapy of breast cancer (Fomander et al. 1989, Nayfield et al. 1991; Trials: Breast Cancer Trials Committee 1987, Nolvadex Adjuvant Trial Organisation 1988, CRC Adjuvant Breast Trial Working Party 1988). In one of the key studies, the National Surgical Adjuvant Breast and Bowel Project (NSABP) randomized lymph node-negative, estrogen receptor-positive patients post operatively to tamoxifen or placebo (Fisher et al. 1996). A significant advantage in disease-free survival, distant disease-free survival, and survival was seen for patients randomized to receive tamoxifen. In addition, there was a statistically significant reduction in the incidence of second primary tumors in the contralateral breast. Recently, the Early Breast Cancer Trialists’ Cooperative Group (1998; EBCCTCG) reported a meta-analysis overview on over 36 000 early breast cancer patients; tamoxifen reduced the incidence of contralateral breast cancers by 26% at 2 years.
in a subset of more than 7000 women who took tamoxifen for approximately 5 years. Based on these data, the NSABP launched a randomized, double-blind, placebo controlled trial of tamoxifen in high-risk women and showed a reduction of 49% in the incidence of invasive breast cancer (Fisher et al. 1998). This study is discussed in detail below. Finally, another set of data supporting tamoxifen’s chemopreventive effect derives from the NSABP B-24 study which showed a 43% reduction in the incidence of invasive breast cancer in patients with DCIS (Fisher et al. 1999).

Raloxifene, another SERM, is a nonsteroidal benzothiophene compound, chemically distinct from tamoxifen and estradiol, that binds to estrogen receptors to competitively block estrogen-induced DNA transcription in the breast and endometrium (Brzozowski et al. 1997, Grese et al. 1997). Raloxifene has a high binding affinity to estrogen receptor (Black et al. 1983). In animal models, raloxifene has estrogen agonistic activity on bone and circulating lipoproteins, but estrogen antagonistic activity on mammary tissue and the uterus (Baker et al. 1998). Raloxifene is a potent inhibitor of breast cancer cell growth in culture (Poulin et al. 1989, Anzano et al. 1996). The preclinical rationale for its use as a chemopreventive agent comes from the in vivo data, which show that raloxifene inhibits the growth of dimethylbenzanthracene-induced rat mammary carcinoma (Clemens et al. 1983). More importantly for the proposed evaluation as a preventive agent, raloxifene reduces the incidence of N-nitrosomethylurea-induced tumors (Gottardis & Jordan 1987, Anzano et al. 1996) if given after the carcinogen but before the appearance of palpable tumors. The clinical evidence for a preventive effect of raloxifene comes from 2 clinical studies: The Multiple Outcomes of Raloxifene Evaluation (MORE) trial randomized 7704 postmenopausal women (mean age 66.5 years) who had osteoporosis (hip or spine bone density at least 2.5 S.D. below normal mean or had vertebrate fractures) and no history of breast or endometrial cancer, to placebo or to 60 or 120 mg raloxifene daily. The risk of breast cancer was reduced by 76% at 3 years, with a total of 40 cases of breast cancer confirmed (Cummings et al. 1999). The second database pooled all placebo-controlled raloxifene trials and included 10 553 women monitored for, on average, 3 years. In this group, a 54% reduction in the incidence of breast cancer in the raloxifene-treated patients was observed (Jordan et al. 1998). In terms of toxicity, raloxifene seems to have a favorable profile; it has less estrogenic activity in the uterus than tamoxifen, and it only increases the growth of human endometrial carcinomas under laboratory conditions by about 50% of that noted with tamoxifen (Gottardis et al. 1990). Indeed, this preclinical finding was confirmed in the MORE trial (Cummings et al. 1999) which demonstrated no increased endometrial cancer occurrence in the raloxifene group compared with the placebo group. In fact, there is evidence suggesting an antiproliferative effect of raloxifene in the endometrium in postmenopausal women (Huster et al. 1996). Given these preclinical and clinical results and raloxifene’s favorable toxicity profile, raloxifene is now being tested against tamoxifen as a chemopreventive agent for breast cancer in high risk postmenopausal women in a large-scale national trial (STAR trial) (discussed below).

Other newer generation SERMs, such as toremifene, droloxifene, idoxifene, LY 353,381.HCL, EM 652 and faslodex (ICI 182,780) are developed or currently being developed for the treatment of breast cancer and are potential agents for chemoprevention (O’Regan & Jordan 2001). Other promising agents include selective ERα and ERβ modulators, which will have tissue-specific activity and a favorable toxicity profile (Jordan & Morrow 1999).

Aromatase inhibitors

The conversion of androgens to estrogens, the final step in estrogen synthesis, can be blocked by aromatase inhibitors. Aromatase activity, by increasing local estrogen synthesis, may play an early role in breast cancer carcinogenesis (Bulun et al. 1993); in fact, in vivo models have shown that aromatase expression in breast tissue can induce the development of premalignant lesions (Tekmal et al. 1996). Several aromatase inhibitors, including anastrazole, letrozole, vorozole, and exemestane have been shown to be effective in the treatment of metastatic breast cancer with a favorable toxicity profile (Goss et al. 1999, Buzdar 2000, Kaufmann et al. 2000, Nabholz et al. 2000, Mouridsen et al. 2001). These agents are currently in clinical trials as adjuvant hormonal agents and are certainly candidate chemopreventive agents (Kelloff et al. 1998, Spicer & Pike 2000, Goss & Strasser 2001, Ingle 2001). In preclinical models, aromatase inhibitors, including anastrazole, letrozole, vorozole, and exemestane inhibited the appearance or caused regression of DMBA- or methyltrinitrosourea (MNU)-induced mammary carcinomas in rats (Schieweck et al. 1988, Bhatarah et al. 1990, De Coster et al. 1992, Schieweck et al. 1993, Gunson et al. 1995, Lubet et al. 1998). Currently, short term chemoprevention trials of aromatase inhibitors in postmenopausal women are planned. The only concern with these agents are the effects of estrogen depletion in target organs, such as the cardiovascular system and bones. This, perhaps, could be overcome by considering lower doses of highly potent agents, such as letrozole, which might inhibit local estrogen synthesis in the breast without decreasing ovarian estrogen production (Santen et al. 1999).

Ovarian ablation

For many years ovarian ablation, induced either by surgical oophorectomy, radiation, gonadotropin releasing hormone (GnRH) agonists, or chemotherapy, has been used for the treatment of breast cancer. The EBCTCG meta-analysis overview reported a significant improvement in disease-free and overall survival for women who underwent ovarian ablation as adjuvant therapy (Cochrane Database 2000). Several studies also reported on the preventive effect of
oophorectomy. Overall, oophorectomy reduced the risk of breast cancer by 20% to 50% in premenopausal women, even when hormonal replacement therapy was used (Brinton et al. 1988, Meijer & van Lindert 1992, Parazzini et al. 1997, Schairer et al. 1997). Surgery after the age of 50 did not seem to have a preventive effect (Schairer et al. 1997). Finally, the preventive effect of bilateral prophylactic oophorectomy in genetically high-risk women was addressed in a recent trial, which revealed a 50% to 70% breast cancer risk reduction in women with BRCA1 mutations who underwent bilateral prophylactic oophorectomy (Rebeck et al. 1999).

Another way to achieve ovarian ablation is to use GnRH agonists. These agents were shown to be effective in the metastatic and adjuvant treatment of breast cancer (Kaufmann et al. 1989, Saphner et al. 1993, Hoffken & Kath 2000). GnRH agonists are currently being investigated in the chemoprevention of breast cancers (Manni 1999). Recent data, showing a decrease in contralateral breast cancers by 40% when GnRH agonists are used in the adjuvant setting, supports this approach (Baum 1999). Current studies are focusing on ovarian suppression with GnRH agonists plus adding back low doses of estrogen and progesterone, which would be insufficient to induce breast cancer, but would be enough to have a protective effect on the cardiovascular system and preserve bone density (Spicer & Pike 2000).

**Retinoids**

Retinoids represent a promising group of agents for the prevention of breast cancer. Fenretinide, a synthetic amide of retinoic acid, induces apoptosis through mechanisms partly involving the retinoid receptors (Torrisi et al. 2001) and has been extensively studied (Brown & Lippman 2000). In *in vivo* models, fenretinide prevented the development of N-nitroso-N-methylurea-induced breast cancer in rats (Moon et al. 1979). Recently, fenretinide was also shown to decrease plasma IGF-I levels in early breast cancer patients, suggesting its potentially chemopreventive effect (Torrisi et al. 1998). Indeed, in a recent large phase III trial, fenretinide use was associated with a reduction of second breast malignancies in premenopausal women with a history of previous breast cancer (Veronesi et al. 1999).

The rexinoid LGD 1069 was shown to be effective against established carcinogen-induced rat mammary tumors (Gottardis et al. 1996); in another study, LGD 1069 prevented the development of mammary tumors in the N-nitroso-N-methylurea-induced rat mammary carcinoma model (Bischoff et al. 1998) and could be considered for chemoprevention.

**Non-steroidal anti-inflammatory agents – COX-2 inhibitors**

Non-steroidal anti-inflammatory drugs (NSAIDs), especially COX-2 inhibitors, could represent a mechanism-based chemopreventive approach for carcinogenesis. Epidemiological studies investigating the relationship between NSAID use and breast cancer have reported conflicting results; some of the studies have failed to show a significant relationship (Paganini-Hill et al. 1989, Egan et al. 1996), whereas other studies did show a relationship between NSAID use and reduction in breast cancer by 30–40% (Schreinemachers & Everson 1994, Harris et al. 1996, Sharpe et al. 2000). The exact reasons for the conflict in data are not known, but it is possible that it is partly due to the fact that only a subset of breast cancers, such as ER-negative or Her-2/neu-positive cancers express COX-2. Early studies that showed elevated levels of prostaglandin (PG) in breast tumors were the first to suggest the importance of COX expression (Tan et al. 1974); the levels were particularly increased in patients with metastatic disease (Bennett et al. 1977). Later, COX-2 expression was shown in breast cancer cell lines; COX-2 mRNA was especially increased in the ER-negative and highly aggressive MDA MB-231 human breast cancer cell lines (Liu & Rose 1996). Recently, several studies have reported increased COX-2 expression in human breast tumors (Parrett et al. 1997, Hwang et al. 1998, Masferrer et al. 2000, Soslow et al. 2000, Arun et al. 2001). Data possibly linking COX-2 to breast carcinogenesis come from a recent study demonstrating that overexpression of COX-2 from the mouse mammary tumor virus promoter was sufficient to cause breast tumor formation in more than 85% of multiparous mice (Liu et al. 2001). Furthermore, it has been shown that the selective COX-2 inhibitor, celecoxib, has a growth inhibitory effect on breast cancer cell lines and induces regression of DMBA-induced mammary tumors in rats (Alishafie et al. 2000). Finally, the potential for COX-2 inhibitors as chemopreventive agents in breast cancer was demonstrated in several *in vivo* mouse models in which COX-2 inhibitors reduce the development of carcinogen-induced mammary tumors (Harris et al. 2000, Nakatsugi et al. 2000, Lu et al. 2001). Currently, several phase I and phase II chemoprevention trials are planned to evaluate the COX-2 inhibitors in the context of breast cancer chemoprevention.

**Other potential agents**

Several other agents are considered for the chemoprevention of breast cancer, including the monoterpens limonene and perillyl alcohol. The proposed anti-tumor and preventive mechanism includes induction of apoptosis, cell cycle arrest, and the inhibition of posttranslational modification of proteins that are involved in signal transduction and in increasing the expression of IGF-IIR (Gould 1995), a putative breast tumor suppressor gene (Oates et al. 1998). Perillyl alcohol is currently under clinical phase I testing (Ripple et al. 1998). The isoflavone, genistein, which is found in high concentrations in soybeans and soy products, inhibits estradiol synthesis by inhibiting aromatase, inhibits DNA topoisomerase...
and the expression of DNA transcription factors c-fos and c-jun and is currently under clinical trial (Wang & Chen 1994, Barnes 1997).

Signal transduction modulators such as EGFR inhibitors and farnesyltransferase inhibitors (FTIs) are also proposed as potential chemopreventive agents for breast cancer (Kelloff et al. 1996, 1997, Lantry et al. 2000).

Finally, demethylating agents are also promising agents. DNA methylation plays an important role in gene expression in breast cancers. Since epigenetic changes are potentially reversible, demethylating agents, such as 5-aza-C, can be exploited as potential chemopreventive agents (Yang et al. 2001). They are especially promising for the prevention of ER-negative breast cancers, given that demethylation of the ER gene induces re-expression of the ER (Ferguson et al. 1995).

**Phase II chemoprevention trials**

The ultimate clinical goal of chemoprevention studies is to reduce cancer incidence, and the gold standard of chemoprevention trials is a large, long-term, randomized study in which cancer incidence is the end point. Since these are long term, costly studies and require a large patient population, it is very important first to perform short term clinical phase II studies with the agent under consideration (one with a strong mechanistic or experimental basis for inhibition of carcinogenesis) in an appropriate cohort of patients using intermediate endpoint markers that would be validated in preparation for future large, phase III chemoprevention trials (Dunn et al. 1998).

**Target populations and sample acquisition models for phase II trials**

As discussed earlier, individuals with abnormal breast histology, including ADH, LCIS, DCIS, or with BRCA 1 or BRCA 2 mutations, because of their increased risk of breast cancer, are candidates for chemopreventive interventions. A newer model would also include patients with prior breast cancer history, since their risk of developing a second primary in the contralateral breast is two or more times higher than women with no history of breast cancer (Chen et al. 1999).

Currently, several models are being used to test drug effects on surrogate endpoint biomarkers (Fabian et al. 1998). In the short term DCIS model, women with DCIS are randomized to placebo or drug in the interval between biopsy and definitive surgery. In the core biopsy hyperplasia model, women undergo a core biopsy of a palpable or mammographically abnormal area, and are then randomized to receive drug or placebo, and then undergo a second core needle biopsy. Two other models include the use of four quadrant or random periareolar fine needle aspirations (FNA). Indeed, earlier studies reported on the association between abnormal cytology obtained by FNA and breast cancer risk; breast cancer incidence was 18% among women who had evidence of proliferative cytology diagnosed in samples obtained by FNA (Wrensch et al. 1993). In a more recent study using the random periareolar FNA model, hyperplasia with atypia was associated with a short-term increased risk of developing breast cancer in high risk women at a rate of 3% per year (Fabian et al. 2000b).

Finally, the most exciting and promising approach to define intermediate endpoints is the less invasive method of ductal lavage. In a very recent study, ductal lavage was compared with nipple aspiration in high-risk women. Study results revealed a higher yield of epithelial cells with ductal lavage compared with nipple aspiration; only 27% of nipple aspirate fluid samples were adequate for diagnosis whereas 78% of ductal lavage samples were adequate (Dooley et al. 2001). Given these encouraging results, ductal lavage will certainly be the method of choice for future prevention studies where the modulation of cytological as well as other molecular markers, such as methylation, proliferation and apoptosis with a chemopreventive intervention, can be safely monitored.

Currently, two prospective, placebo controlled studies are testing biomarker evaluation with a potential chemopreventive agent. In one study, women with hyperplasia or atypical hyperplasia, diagnosed by examination of FNA samples, were randomized to difluoromethylornitine (DFMO) or placebo for 6 months, after which they underwent second FNAs to assess biomarker modulation (Fabian et al. 2000a). The second study, using the short-term presurgical model, randomized patients with DCIS with or without early invasive cancer to tamoxifen and N-[4-hydroxyphenyl]retinamide versus placebo for 12–28 days to evaluate the modulation of several markers including ER and Ki-67 (Singletary et al. 2000). Both studies proved the feasibility of biomarker modulation studies; several other studies, evaluating different agents, are currently underway.

**Clinical phase III chemoprevention trials**

**NSABP-P1**

Between 1992 and 1997, the NSABP conducted a large-scale, double-blind, phase III breast cancer chemoprevention trial (BCPT-P1) using tamoxifen versus placebo in 13 388 women at high risk for breast cancer (Fisher et al. 1998). Eligible women had to be 60 years or older, or between the ages of 35 and 59 and have a diagnosis of LCIS or a projected 5-year risk of developing breast cancer greater than 1.66%, according to the modified Gail model (Gail et al. 1989). This study showed a highly statistically significant
reduction in breast cancer incidence, which led the independent BCPT Endpoint Review, Safety Monitoring and Advisory Committee to recommended unblinding 1 year earlier than planned. After a median follow-up of 54 months, a 49% reduction in the incidence of invasive breast cancer ($P<0.00001$), and a 50% reduction of noninvasive cancer ($P<0.0001$) occurred among those receiving tamoxifen. A subset analysis also revealed that women with LCIS had a 56% reduction and women with atypical hyperplasia had an 86% reduction in the occurrence of breast cancer. The decreased risk occurred in women of all ages and in all risk groups. Additionally, the occurrence of ER-positive breast cancer was reduced by 69%; tamoxifen did not reduce the occurrence of ER-negative breast cancers.

In terms of secondary endpoints, tamoxifen reduced the incidence of osteoporotic fractures of the hip, spine and radius, which approached but did not reach statistical significance (Fisher et al. 1998). Also, no difference was seen in the incidence of myocardial infarction, angina, coronary artery bypass, or angioplasty (Fisher et al. 1998).

Side effects of tamoxifen included increased risk of endometrial cancer. The relative risk in the tamoxifen group was 2.5; this increased to 4.01 in women aged 50 or older. All diagnosed endometrial cancers in the tamoxifen group were stage I and no one in this group died of endometrial cancer (Fisher et al. 1998). Furthermore, there was no increased risk of any other malignancies associated with the use of tamoxifen.

Deep vein thrombosis was seen more in the tamoxifen group, again with increased risk in women aged 50 or older (Fisher et al. 1998). The relative risk of deep vein thrombosis in the older group was 1.71. Pulmonary emboli were also seen in the older women with a relative risk of 3. An increased risk of stroke was also seen in women taking tamoxifen, but this did not reach statistical significance. A marginally statistically significant increase in cataract formation and risk of requiring cataract surgery was observed in the tamoxifen group (Fisher et al. 1998).

Quality of life issues were also analyzed in this trial (Fisher et al. 1998). There was no difference in the depression scores between the two groups (Day et al. 1999). A subsequent study, which recruited 488 women from the Royal Marsden Tamoxifen and the International Breast Cancer Intervention Study, also did not find psychosocial- and sexual function-related side effects in the tamoxifen groups (Fallowfield et al. 2001).

The NSABP P-1 study reported increased hot flushes in women taking tamoxifen ~ 81% versus 69% in women taking placebo (Fisher et al. 1998). Vaginal discharge was experienced by 29% of women in the tamoxifen group and 13% in the placebo group. There were no differences observed in irregular menses, weight gain, skin changes, fluid retention and nausea (Fisher et al. 1998).

The issue of whether tamoxifen treated early microscopic disease or indeed prevented it has been an ongoing debate (Wickerham & Tan-Chiu 2001). That the two groups showed early divergence within the first year supports the notion that tamoxifen perhaps treated microscopic disease which was present at study entry. But tamoxifen might exert its preventive effects at very early stages of carcinogenesis and furthermore, at 7 years of follow-up, the use of tamoxifen still remained beneficial, suggesting an ongoing preventive effect. The other support for the preventive effect of tamoxifen is that the occurrence of preinvasive cancers such as DCIS and LCIS was also significantly lowered by tamoxifen.

Other questions remaining unanswered relate to the optimal duration of tamoxifen treatment. Adjuvant studies evaluating the incidence of contralateral breast cancer revealed benefit if tamoxifen is used for up to 5 years (Early Breast Cancer Trials' Collaborative Group 1998); furthermore the NSABP B-14 study revealed that 5 and 10 years of tamoxifen were equivalent in preventing contralateral breast cancers (Fisher et al. 1996, Lippman & Brown 1999). Currently, 5 years of tamoxifen is recommended for the prevention of breast cancer.

So far, a survival benefit with the use of tamoxifen in the BSABP-P1 group has not been observed, mainly because the study was unblinded. In fact, survival data may never become available.

The impact of tamoxifen on women with high genetic risk, such as BRCA 1 or BRCA 2 mutation carriers is currently being evaluated. In this effort, BRCA 1 and BRCA 2 gene sequencing was performed on all breast cancer cases and a subset of non-breast cancer cases in women who participated in the NSABP-P1 trial (results presented at the American Society of Clinical Oncology Meeting, May 2001). Preliminary results showed that tamoxifen did not reduce the occurrence of breast cancer among women with a BRCA 1 mutation. Given that BRCA1 mutation-related breast cancers are likely to be ER negative (Robson et al. 1998), this finding is consistent with the lack of tamoxifen’s beneficial effect on ER-negative breast cancers. On the other hand, a reduction in breast cancer with tamoxifen was observed among women with BRCA 2 mutations; again this is consistent with tamoxifen’s beneficial effect on ER-positive breast cancers.

Nevertheless, in 1998 tamoxifen was the first drug to be approved for the reduction of breast cancer in high-risk individuals (Chlebowski et al. 1999). But before making the decision to use tamoxifen to reduce the risk of breast cancer, potential benefits need to be weighed against potential side effects, taking the risk of breast cancer, age, race, and comorbid conditions into consideration. A recently developed methodology revealed that tamoxifen was mostly beneficial for younger women with an elevated risk of breast cancer and that women above the age of 50 should receive tamoxifen if their short-term risk is 1% per year for women with a uterus.
and 0.5% per year for women without a uterus (Gail et al. 1999).

**European tamoxifen chemoprevention trials**

The Italian prevention trial enrolled 5408 patients to receive either tamoxifen or placebo (Veronesi et al. 1998). Eligibility included a prior hysterectomy, but increased risk of breast cancer was not required. Unfortunately, 26% of study participants had discontinued therapy, therefore accrual was terminated earlier than planned. At a median follow-up of 46 months, the study did not show any difference in the incidence of breast cancer in either group.

In the second trial conducted at the Royal Marsden Hospital (Powles et al. 1998), 2494 healthy women with a family history of breast cancer were randomized to receive either tamoxifen or placebo. In this trial more than 40% of the participants received concomitant hormonal replacement therapy. After a median follow-up of 70 months, no reduction in the incidence of breast cancer was seen with tamoxifen.

The differences in the results of these 3 studies (Fisher et al. 1998, Powles et al. 1998, Veronesi et al. 1998) can be attributed to the difference in the study population (Pritchard 1998). The Royal Marsden study included more younger women and women with a family history of breast cancer; the Italian study, on the other hand, enrolled women who had undergone a hysterectomy and therefore would be at lower risk for breast cancer. Basically, the difference in the study population and the limited statistical power of the European trials prevents objective comparison among these trials.

**Ongoing phase III trials**

As discussed earlier, the NSABP is conducting a second chemoprevention trial (NSABP-P2/STAR) in postmenopausal women with increased risk. Participants are randomized to either 5 years of tamoxifen at a dose of 20 mg a day or raloxifene at 60 mg a day. Stratification factors include age, relative risk, race, and history of LCIS.

Another ongoing tamoxifen chemoprevention trial is the International Breast Cancer Intervention Trial (IBIS), which is currently being conducted in Europe and Australia. The accrual target is about 7000 women, and more than 6000 have been recruited so far.

**Conclusion**

Tamoxifen is the only chemopreventive agent approved for the prevention of breast cancer in high-risk women as defined by the Gail model (>1.66) and in postmenopausal women aged 60 and older. The risk–benefit ratio in elderly women remains to be carefully assessed. Data regarding the benefit of tamoxifen in genetically high-risk women such as BRCA 1 and BRCA 2 mutation carriers is forthcoming. Raloxifene is another potential SERM for breast cancer chemoprevention and is currently being studied in the STAR trial against tamoxifen.

Since the benefit of tamoxifen in preventing breast cancer was only seen in ER-positive breast cancers, there is an absolute need for a chemopreventive agent for ER-negative breast cancers. COX-2 inhibitors seem to be suitable candidates (Howe et al. 2001). Several phase II chemoprevention and biomarker modulation studies with COX-2 inhibitors are planned and some are already underway. Other promising agents for the prevention of ER-negative breast cancers include polyamine biosynthesis inhibitors (DFMO) (Meyskens & Gerner 1999), vitamin D analogs, retinoids, cyclin dependent kinase inhibitors (Brown & Lippman 2000), telomerase inhibitors (Herbert et al. 2001a), isoflavonoids (Barnes 1997), demethylating agents (Yang et al. 2001), and molecular chemopreventive approaches including targeted gene therapy for BRCA 1 mutation carriers (Fan et al. 1999).

Conducting large-scale phase III chemoprevention studies with cancer incidence as the endpoint requires a large population, is extremely costly, and takes a long time for accrual and follow-up. It is therefore crucial to proceed with short-term clinical phase II chemoprevention trials that test potential agent efficacy via evaluating the modulation of intermediate surrogate endpoints in an appropriate cohort of individuals. The widely accepted model is to obtain pre- and post chemopreventive intervention tissue from high-risk individuals and to assess the biomodulation of specific markers as well as the intervention efficacy. Ductal lavage seems to be a very promising and rather noninvasive method of collecting epithelial cells from high-risk women for analysis of morphological and molecular markers.

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