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Oestrogens, Oestrogen Receptors and Breast Cancer

D.J. Bentrem[a], P. Gaiha[b], V.C. Jordan[b,c,*]

[a]*Department of Surgery, Northwestern University Medical School, Chicago, IL 60611, USA*
[b]*Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL 60611, USA*
[c]*Robert H. Lune Comprehensive Cancer Center, Northwestern University Medical School, Chicago, IL 60611, USA*

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Abstract

Tamoxifen has been the endocrine treatment of choice for all stages of oestrogen receptor positive breast cancer for 20 years and the first chemical therapeutic to be tested to reduce the incidence of breast cancer in high-risk women. It is now clear that the oestrogen receptor is proving to be an invaluable target for the treatment and chemoprevention of breast cancer. The success of tamoxifen clinically can be quantitated: 400 000 women are alive today because of the application of 5 years of adjuvant tamoxifen therapy in node-positive and node-negative breast cancer. This advance has resulted in vigorous efforts to reduce side-effects and to improve objective response rates by the rapid application of laboratory principles. Tamoxifen is known to have a mixture of oestrogen-like and anti-oestrogen actions so it is reasoned that completely anti-oestrogenic agents would enhance treatment response rates while lowering the incidence of oestrogen-like side-effects such as endometrial cancer and blood clots. A new pure anti-oestrogen, fulvestrant, that destroys the oestrogen receptor, is available after drug resistance to tamoxifen develops. The group of drugs known as aromatase inhibitors block the production of oestrogens from androstenedione and testosterone in the body fat of postmenopausal women. New agents such as anastrozole, exemestane, and letrozole have shown promise as new treatment modalities for advanced breast cancer. Most importantly, the successful testing of anastrozole as an adjuvant treatment for breast cancer has enhanced enthusiasm for the evaluation of aromatase inhibitors and selective oestrogen receptor modulators (SERMs) to be tested as chemopreventives. SERMs express anti-oestrogenic actions in the breast but oestrogen-like actions in bone and lower circulating cholesterol. This insight not only allowed the safe application of tamoxifen to well high-risk women to test the worth of an 'anti-oestrogen' to prevent breast cancer, but also caused a paradigm shift in the strategy of chemoprevention. The question was posed that if, tamoxifen prevents breast cancer but an added benefit is the maintenance of bone density, why not develop a drug to prevent osteoporosis or atherosclerosis that prevents breast cancer in the general population as a beneficial side-effect? Raloxifene is the result of this new strategy to seek multifunctional medicines for women's health. Raloxifene is currently available for the treatment and prevention of osteoporosis but is being tested in high-risk postmenopausal women for the prevention of breast cancer against tamoxifen in the study of tamoxifen and raloxifene (STAR trial) and for the prevention of coronary heart disease (CHD) in a placebo-controlled trial in women at high risk for CHD called raloxifene use for the heart (RUTH).

Keywords: Breast cancer; Anti-oestrogens; Tamoxifen; Raloxifene; Anastrozole; Drug resistance

1. Introduction

The breast is the leading site for cancer incidence and the second most common site for cancer death among US women [1]. As a result, treatment and chemoprevention have been the focus of laboratory work and clinical trials for the past 30 years. However, as the pace to introduce new treatment modalities has quickened, it is perhaps valuable to reflect upon the origins of the most successful strategy that has propelled research from the era of nonspecific chemotherapy to the present era of targeted treatments. At the beginning of the 20th century, Professor Paul Ehrlich was the first to reason that a parasite or cancer could be targeted selectively by a chemical therapy to cure disease without harming the host [2]. Even though a cure for cancer remained elusive, he demonstrated a logical approach to a complex problem: Identify a selective target, test a drug in the laboratory and then conduct clinical studies.

In 1936, Professor Antoine Lacassagne suggested that, if breast cancer was caused by a special hereditary

* Corresponding author. +1-312-908-4162.

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sensitivity to oestrogen, then the disease could be prevented by developing a therapeutic antagonist to "prevent the congestion of oestrone" in the breast [3]. However, there were no therapeutic antagonists of oestrogen at that time, nor was there a target for drug design. Furthermore, there was little interest in treating breast cancer with new hormonal drugs, and most of the endocrinology research focused on understanding reproduction. The first non-steroidal anti-oestrogen, MER25, was described by Lerner and co-workers in 1958 [4] and identified as a possible contraceptive. This drug failed in clinical trial because the large doses caused neurotoxicity [5], so drug discovery then switched to triphenylethylene-based compounds that resulted first in clomiphene and then tamoxifen [6,7].

Simultaneously, the nature of the target began to evolve: In 1962, Jensen and Jacobson [8] demonstrated that [^{3}H]oestradiol bound to oestrogen target tissue including uterus, vagina and pituitary gland in the female rat. Further work confirmed the oestrogen receptor's (ER) role as a nuclear protein and transcripton factor [9–11]. More importantly for the application of basic knowledge to cancer therapy, more than 60% of breast cancers were found to contain measurable levels of ER. In general, patients who are ER-positive have a longer disease-free interval and longer survival times than women with receptor-negative disease [12,13]. Furthermore, the responsiveness of breast cancer to endocrine ablation and/or anti-oestrogen therapy is now proven to correlate with the ER target [7,14–16]. Thus, a research focus by the academic community working with industry, beginning in the early 1970s, facilitated the successful development of tamoxifen as a breast cancer treatment [7,17,18]. Subsequent research has built on the knowledge of tamoxifen's actions and the enhancement of the effectiveness of agents that use the ER as a target.

2. Selective modulation

The clinical pharmacology of non-steroidal anti-oestrogens is complex and cannot be described as simply a blockade of oestrogen action and is now referred to as selective oestrogen receptor modulation (SERM) to describe the site-specific effects [19]. Tamoxifen and the other triphenylethylene antioestrogens bind with high affinity to tissues throughout the body. The high level of protein binding results in drug accumulation and slow excretion [20]. Tamoxifen functions through the ER [21,22] but the biological effects of oestrogen may be mediated by two receptors, oestrogen receptor-α (ERα) and oestrogen receptor-β (ERβ) (Fig. 1). The cloning of ERα in 1986 [10,23] focused research efforts on the existence of only one ER protein in all target tissues. Ten years later, ERβ was identified in the rat prostate [24] and in humans [25]. The two ERs share a conserved structure with six functional domains, A–F. ERβ is homologous to ERα at the ligand binding domain (58%) and DNA binding domain (95%). The remaining domains are not well conserved [26]. The discovery of ERβ has advanced our understanding of oestrogen signalling and may explain responses to oestrogen in tissues in which ERα is undetectable [27]. Furthermore, the existence of ERα and β subtypes provides a possible explanation for the tissue selectivity of SERMs although knowledge of SERM action in breast cancer and the development of drug resistance in the form of 'tamoxifen stimulated growth' is almost certainly targeted to ERα exclusively (see Fig. 2).

The agonist/antagonist activity of the ER–tamoxifen complex is determined by cell context. Tamoxifen functions as an antagonist in most ER-positive breast tumours, but displays a paradoxical agonist activity in bone density [28–30] and serum lipid levels, and partial agonist activity in the uterus [19,31] (Fig. 3). Tamoxifen exhibits oestrogen-like effects in postmenopausal women, including a decrease in luteinising and follicle-stimulating hormone [32]. Tamoxifen has been shown to increase bone density in the lumbar spine, radius and femur by 1–2% [30,33]. Several studies have also shown that tamoxifen also has oestrogen-like effects on serum lipid profiles [34–36]. Low-density lipoprotein cholesterol is reduced by approximately 15%, and high-density lipoprotein cholesterol is maintained. There is no prospective clinical evidence that tamoxifen will reduce the risk of coronary heart disease, but it is important to state that with the finding that hormone-replacement therapy (HRT) *does not* significantly reduce the risk for CHD [37] but, in fact, on balance is detrimental to women's health; there are opportunities in medicinal chemistry to develop new selective medicines.

It is well known that tamoxifen produces a partial agonist action in the human uterus [31,38]. The most significant finding is an increase in the stromal component, rather than endometrial hyperplasia [39,40]. Tamoxifen does not increase the risk of endometrial cancer in premenopausal women, but does increase the risk by 3- to 4-fold in postmenopausal women [41]. Importantly, the stage and grade of endometrial cancers observed in women taking tamoxifen are the same as the general population [42].

3. Drug resistance to tamoxifen

Although tamoxifen is classified as an anti-oestrogen for the use as a breast cancer treatment, the action of the drug is subverted by the molecular configuration of the cell that can convert tamoxifen from an antioestrogen to an oestrogen. Thus, tamoxifen-stimulated (resistant) breast cancer growth can be demonstrated both in

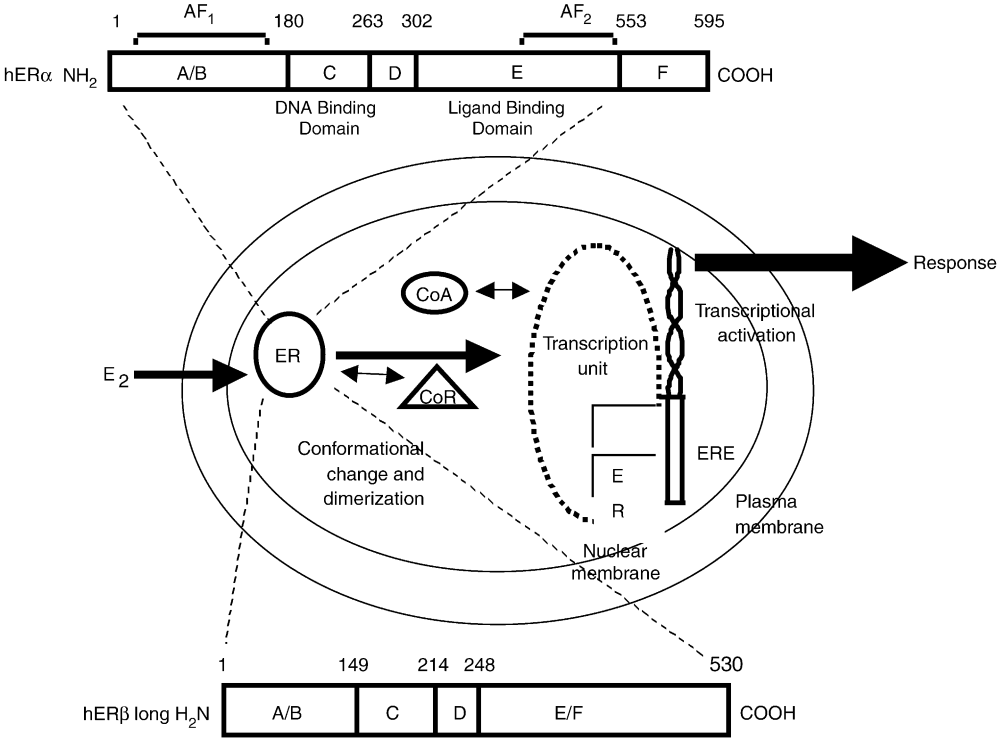

Fig. 1. The oestrogen receptor (ER) signal transduction pathway. Gene transcription is initiated with either a selective ER modulator (SERM) or oestrogen binding to the ER (α or β) which induces a conformational change in the receptor. After interaction with various corepressors (CoR) or coactivators (CoA), the transcription unit binds either to an oestrogen-response element (ERE) in the promotor region of an oestrogen-responsive gene or through a protein/protein interaction (fos and jun) at the AP-1 site. Gene transcription can produce either stimulatory or inhibitory signals depending on the target site.

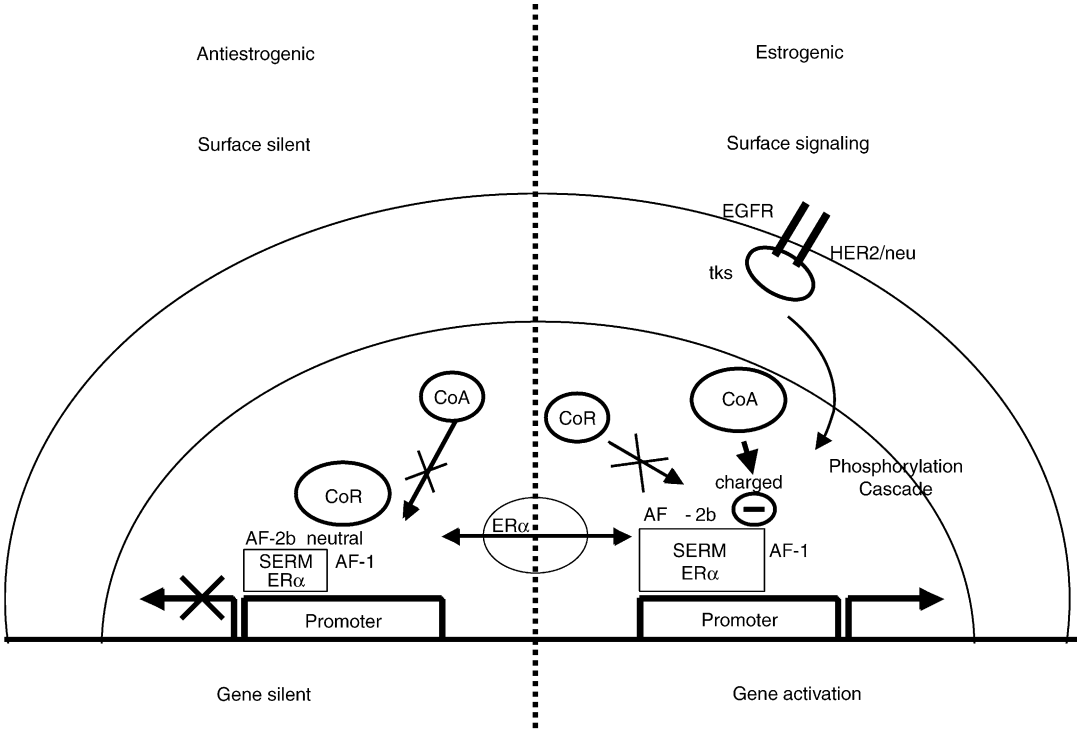

Fig. 2. Integrated mechanism for the target site-specific action of selective ER modulators (SERMs) in breast or uterine cancer. The two extremes of antioestrogenic or full oestrogenic actions are shown. Oestrogen-like actions could occur in cells expressing an excess of coactivators (CoAs) and/or a decrease in corepressors (CoRs). The charged surface of a tamoxifen oestrogen receptor (ER) complex at AF2b prevents CoR binding. The oestrogenic action would be amplified by surface signaling with dimers of epidermal growth factor receptor (EGFR) and HER2/neu activating tyrosine kinases (tks). The phosphorylation cascade can activate AF-1 on ERa directly or activate the excess of CoAs in a high ER environment. Reduced levels of ER prevent the signal transduction pathway and promote antioestrogenic actions in a surface silent cell.

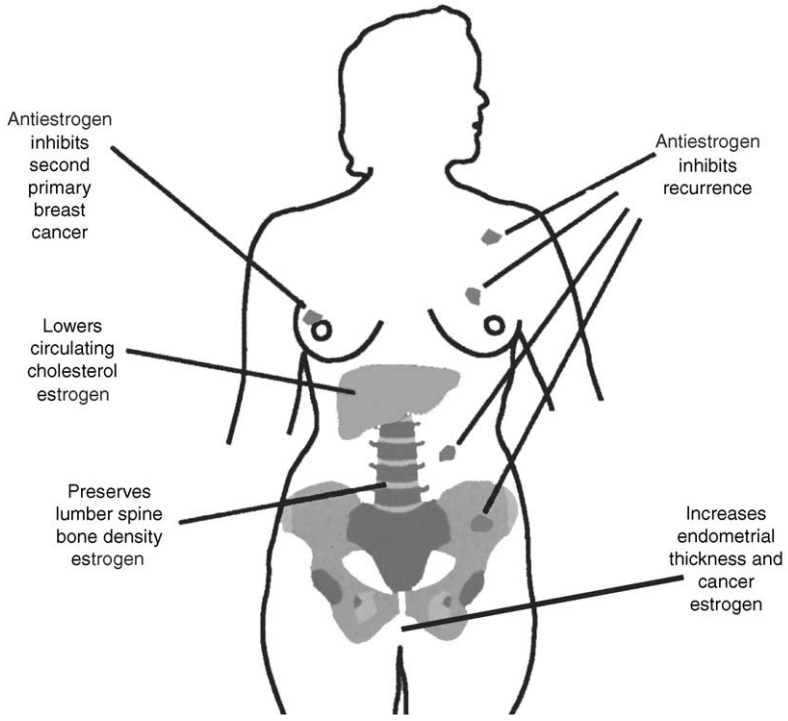

Fig. 3. Site-selective effects of adjuvant tamoxifen therapy.

the laboratory [43] and clinically in advanced breast cancer [44].

A molecular model of tamoxifen and SERM action during the development of drug resistance is now emerging. Indeed, the extensive literature on the molecular pharmacology of tamoxifen can be used to evaluate the critical associations noted in clinical studies. Knowledge of the triumvirate of ER, cell-surface signalling and coactivators has been used previously to understand the actions of SERMs (Fig. 2). Shang and Brown [45] have recently shown that the concentration of an ER coactivator (SRC-1) can enhance some ER-mediated gene activation in endometrial cancer cells where tamoxifen has oestrogen-like effects. Levels of SRC-1 were transiently increased or decreased in cells to modulate gene activation by tamoxifen. However, Brown's group has also shown that cell-surface signalling can enhance the breast cancer-related coactivator, SRC-3 phosphorylation and ER activation [46]. If the triumvirate of ER level, HER2/neu expression and coactivator SRC-3 is critical for cancer-cell survival during resistance to tamoxifen, there needs to be an innate support mechanism so that the signal transduction pathways are coordinated to work in harmony. Arguably, the most important component of tamoxifen-stimulated growth is the ER. Anti-oestrogen binding to the ER increases the transcription of the HER2/neu gene by releasing ER coactivators from the oestradiol ER complex. These coactivators then activate the HER2/neu promoter [47]. This cancer-cell survival system facilitates cell-surface signalling and, ultimately, subverts tamoxifen's action through the phosphorylation of coactivators [46] and ER (Fig. 2). Importantly, tamoxifen ER complexes have a built-in mechanism to enhance their own long-term survival so that the complex can become promiscuous at gene targets within the cell. The oestradiol–ER complex is a powerful intracellular messenger that needs to be ubiquitinised and destroyed rapidly by the proteosome. However, the tamoxifen–ER complex tends to accumulate in the cell because of poor ubiquitinisation [48]. The destruction process is controlled through a region around D538 that is exposed to and apparently impaired on the surface of the tamoxifen–ER complex [49].

Apparently, the coactivator SRC-3 is a rate-limiting factor for oestrogen-dependent growth of MCF-7 breast cancer cells in culture [50]. Nevertheless, oestrogen causes a reduction in SRC-3 mRNA but anti-oestrogens cause an increase in SRC-3 mRNA [51] through an increase in transforming growth factor α (TGFα), so a complete self-regulated system is in place for breast cancers to acquire resistance eventually. The hypothesis has recently been supported using retrospective clinical samples. Patients with ER-positive breast cancers, high HER2/neu and elevated SRC-3 were noted to fail adjuvant tamoxifen rapidly [52].

4. Alternative treatments for oestrogen blockade: pure anti-oestrogens and aromatase inhibitors

The question must be asked whether the physician can subvert the power of the triumvirate by the judicious

use of new approaches to endocrine therapy. There are two approaches; either the ER target can be blocked and prematurely destroyed by a pure anti-oestrogen [53], or the synthesis of oestrogen can be prevented with an aromatase inhibitor so no oestradiol ER complex will form.

'Pure' anti-oestrogens are, by definition, anti-oestrogenic in all target tissues [53] because there is destruction of the ER in target sites [48,54,55]. The pure anti-oestrogens are effective in laboratory models as second-line treatment for tamoxifen-stimulated breast [56,57] and endometrial cancer [58,59]. Clinically, it must be administered by intramuscular injection because of low oral potency. Following successful phase II studies [60], randomised trials were initiated comparing fulvestrant (Faslodex®) with another hormonal therapy, the aromatase inhibitor anastrozole, in-patients with tamoxifen-refractory advanced breast cancer. Fulvestrant and anastrozole are equivalent treatments [61,62]. Further large randomised clinical trials are underway and will address remaining questions regarding effects of long-term therapy on bone mineral density and serum lipid profiles.

After menopause, circulating oestrogen levels drop to about 20% of those of premenopausal women and, in the absence of cyclic ovarian function, remain at steady-state concentrations. In postmenopausal women, oestrogens (oestrone and oestradiol) are produced mainly through aromatisation of androgen precursors (androstenedione and testosterone) [63], particularly in breast tissue where oestrogen concentrations are higher than plasma concentrations. Immunohistochemical analysis and *in situ* hybridisation techniques have shown that the aromatase and precursor mRNA are expressed in the epithelial cells of the terminal ductal lobular units and in the surrounding stromal cells of normal breast tissue [64]. Oral aromatase inhibitors have been shown to reduce aromatase activity and oestradiol concentrations within tumoral tissue of patients with primary breast cancer [65].

Third-generation aromatase inhibitors are divided into steroidal suicide inhibitors, type I (exemestane), which bind to the aromatase enzyme and destroy function through covalent interaction and nonsteroidal oral inhibitors, and type II (anastrozole and letrozole), which are competitive inhibitors of aromatase action. Whereas tamoxifen, ovarian ablation, and second-generation aromatase inhibitors reduce oestrogen effects to some degree, only third-generation aromatase inhibitors reduce circulating oestrogens to nearly undetectable levels without an effect on the formation of adrenal corticosteroids or aldosterone.

All of the third-generation oral aromatase inhibitors have now been tested in phase III trials as second-line treatment of postmenopausal hormone-dependent breast cancer [66]. They have shown clear superiority compared with the conventional therapies and are therefore considered (at least) established second-line hormonal agents. Exemestane is an orally active steroidal aromatase inhibitor that has demonstrated efficacy in the treatment of postmenopausal patients with advanced breast cancer [67,68]. This compound exhibits a good tolerability and safety profile, which may result from its highly selective mechanism of action. Exemestane binds irreversibly to the aromatase enzyme causing inactivation of the enzyme. Exemestane is a potent inhibitor of aromatisation-reducing oestrogen synthesis *in vivo* by greater than 97% [69]. The most frequently reported drug-related adverse events are hot flushes, nausea and fatigue, which are consistent with the oestrogen-suppressive effects of the drug [70]. Discontinuation due to adverse events is rare. Exemestane is a safe and well-tolerated alternative for the treatment of postmenopausal patients with advanced breast cancer.

Anastrozole is the first third-generation aromatase inhibitor for the treatment of advanced breast cancer. It inhibits the aromatase enzyme by competitively binding to the haeme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of oestrogen biosynthesis. Two studies with the selective aromatase inhibitor anastrozole versus tamoxifen for advanced breast cancer were recently published [71,72]. In both studies, response rates were similar. In the North American study [71], time to progression (TTP) was in favour of anastrozole (median TTP of 11.1 months versus 5.6 months for anastrozole and tamoxifen). In the larger, mainly European study, 668 patients were randomised to receive either anastrozole 1 mg once daily or tamoxifen 20 mg once daily, and TTP was similar for both treatments supporting the use of anastrozole as an alternative treatment to tamoxifen in postmenopausal women with advanced breast cancer [72]. Side-effects included nausea ~15%, hot flashes ~12%, arthralgias in 10–13% [73] and vaginal dryness ~2%.

Letrozole, another potent selective aromatase inhibitor, has shown efficacy as a second-line therapy of advanced breast cancer in postmenopausal women after tamoxifen has failed [74,75]. In a recent trial as first-line therapy for postmenopausal women with advanced breast cancer, letrozole was as effective as tamoxifen with a longer time to progression (41 weeks versus 26 weeks) [76]. A small pilot study has also looked at letrozole in the neoadjuvant setting. In a series of 24 patients with large operable breast cancer, letrozole was effective in reducing tumour volume, allowing all patients to have breast-conserving surgery [77].

A lower risk of thromboembolic complications or vaginal bleeding associated with anastrozole or letrozole is seen when compared with tamoxifen [71,72,76]. Given this aspect of the improved safety profile, the US Food and Drug Administration has approved both drugs for use as initial therapy for metastatic disease in postmenopausal

women. However, effects on bone density and serum lipids remain in question with long-term therapy. Preliminary data from an EORTC study which randomised 122 postmenopausal patients with metastatic breast cancer to tamoxifen or exemestane found little negative impact of the oral aromatase inhibitor on serum lipids [78]. The role of aromatase inhibitors in premenopausal breast cancer in combination with chemotherapy and gonadotropin-releasing hormone are areas of future exploration.

5. Adjuvant anastrozole

The recent publication of the ATAC (Arimidex versus tamoxifen and the combination) trial demonstrates that an aromatase inhibitor, as a single adjuvant agent, is superior to tamoxifen or the combination in ER-positive patients. The ATAC trail clearly demonstrates an increase in DFS for anastrozole (1 mg) over tamoxifen (20 mg) during the early analysis at 2.5–3 years of treatment [79]. Most importantly, there are reductions in blood clots, vaginal discharge and endometrial cancer in the anastrozole alone arm compared with either the tamoxifen alone or the combination. Even though there is a small, but significant, number of recurrences in the anastrozole arm compared with tamoxifen, it will be important to establish which patients fail tamoxifen—in other words, which patients will benefit from anastrozole. One line of reasoning focuses upon the triumvirate of cell-surface signalling, coactivators and the ER. A neoadjuvant study demonstrates that patients with high cell-surface signalling (HER2/neu/EGFR) are less likely to respond to tamoxifen [80]. Additionally, the recent report by Osborne [52] indicates that 10% of patients who are HER2/neu-positive with high levels of SRC-3 do not respond to tamoxifen. It is possible that these are the patients who respond to anastrozole by impairing signal transduction because the ER is unoccupied during therapy with an aromatase inhibitor.

6. Prevention trials

Oestrogenic hormones are considered to be the promoter of initiated breast cells and to act over many years to increase the tumour-cell population. Since the promotional stage is characterised by reversibility, there has been great interest in preventing the consequences of prolonged, unopposed oestrogenic stimulation of the breast. Laboratory studies laid the foundation for chemoprevention: tamoxifen prevented carcinogen-induced mammary cancer in rats [81,82]. Subsequently, the decrease in contralateral breast cancers during tamoxifen administration for adjuvant therapy [16], and a favourable toxicity profile in clinical practice, suggested the drug could be evaluated in women at risk for breast cancer.

Four studies have addressed the prevention question, the Royal Marsden Pilot Study [83], the Italian Tamoxifen Prevention Study [84–86], the National Surgical Adjuvant Breast and Bowel Project (NSABP) [41] and the IBIS study [87]. The Marsden study, which enrolled 2494 high-risk women, was designed as a pilot toxicology study to serve as a basis for a nationwide clinical trial in Britain that originally planned to recruit 20 000 high-risk women. This did not occur, but the plan evolved into the IBIS study (see later). The Marsden Study evaluated the problems of accrual, acute symptomatic toxicity, compliance and safety for the use of tamoxifen in a larger trial; however, the Marsden study was also analysed for breast-cancer incidence. No difference in the incidence of breast cancer was observed between groups, with 34 carcinomas in the tamoxifen group and 36 in the placebo group. There is no satisfactory explanation why the tamoxifen arm failed to show a decrease in breast-cancer incidence. The authors suggest that perhaps there was a high population of BRCA-1 and -2 carriers that possibly are hormone unresponsive. There is limited data on the responsiveness to tamoxifen treatment of genetic breast cancer [88]. In a case–control study, Narod and colleagues [89] found tamoxifen protected against contralateral breast cancer for carriers of BRCA1 mutations (Odds Ratio (OR) 0.38, 95% Confidence Interval (CI) 0.19–0.74) and for those with BRCA2 mutations (OR 0.63, CI 0.20–1.50). In women who used tamoxifen for 2–4 years, the risk of contralateral breast cancer was reduced by 75%.

The Marsden study has provided invaluable information about the effects of tamoxifen in healthy women, but we believe the study was underpowered and not designed to answer the question of whether tamoxifen prevents breast cancer.

The Italian study evaluated 5408 low-risk women beginning in October 1992 until December 1997 [84]. Originally, the study intended to enrol 20 000 volunteers without risk factors, but ended prematurely due to poor recruitment and compliance. Prior to enrolment, women were required to have had a hysterectomy to obviate concerns about a possible increased risk of endometrial carcinoma. There was no requirement that participants be at risk for breast cancer and, in fact, 47% had a reduced risk of breast cancer, having had premenopausal oophorectomy with hysterectomy. The incidence of breast cancer did not differ between groups, with 34 cases in the tamoxifen group and 45 in the placebo group with a median follow-up of 76 months [86]. Tumour characteristics, including size, grade, lymph node status and receptor status, did not differ between groups. The results of this study can be explained by the low-risk study population, the high dropout rate, the

young age, and the small number of participants who completed 5 years of tamoxifen treatment. However, high-risk groups for ER-positive breast cancer could be predicted and tamoxifen was found to be extremely effective as a chemopreventive [86]. The conclusion that can be drawn from this study is that the benefit of tamoxifen is likely to be small in women with an average or decreased risk of breast cancer. In contrast, women who are carefully preselected to be at elevated risk can anticipate effective chemoprevention. One important finding is that, in the population of women who took oestrogen-replacement therapy, tamoxifen prevented the development of breast cancer. Studies are planned in Italy to address this interesting finding and evaluate the value of an HRT/SERM combination.

The NSABP Protocol P-1 was the first completed prospective, randomised clinical trial to support the hypothesis that tamoxifen can prevent breast cancer in a high-risk population. This clinical trial opened in May 1992 and closed 5 years later after accruing 13 388 high-risk women, a group sufficient to answer the prevention question. Those eligible included women over the age of 60 years, or women over 35 years whose 5-year risk of developing breast cancer, as predicted by the Gail model [90], was equal to that of a 60-year-old woman. Tamoxifen administration reduced the risk of invasive and non-invasive breast cancers by approximately 50% in all age groups [41]. A subset analysis of women at risk with the diagnosis of lobular carcinoma *in situ* demonstrated a 56% reduction of invasive cancers. The most dramatic reduction, 86%, was seen in women at risk due to atypical hyperplasia. As expected, tamoxifen affected only the incidence of ER-positive tumours, which were overall reduced by 69% per year. The incidence of ER-negative tumours in the tamoxifen group did not differ significantly from that of the placebo group. Tamoxifen reduced the incidence of invasive cancers of all sizes, but the greatest difference was seen in tumours $\leqslant$2.0 cm in size. Tamoxifen reduced the incidence of both node-positive and node-negative breast cancer. The beneficial effects were observed for each year of follow-up, with 33% reduction after 1 year, and 69% reduction after 5 years. The absolute number of breast-cancers in the control group also provided a means to validate the Gail model [91] which, despite some limitations, is able to provide useful information on breast-cancer risk for women who plan to participate in annual mammographic screening programs.

The most recent trial to report results with the use of tamoxifen as a chemopreventive is the IBIS trial [87]. A total of 7152 high-risk women aged between 35 and 70 years were randomised to 5 years of tamoxifen (20 mg) or placebo. There was a 32% risk reduction for breast cancer, but the authors cautioned about the use of prophylactic tamoxifen in women at high risk of thromboembolic disease, especially after surgery [87].

These data have driven the evolution of ideas from the treatment of breast cancer toward preventive therapeutics. Although tamoxifen substantially reduces the risk of breast cancer among women at higher than average risk for the disease, it has not been proved that the risk benefits of tamoxifen would benefit the average risk population of women. Nevertheless, current strategies are addressing the concern.

7. Raloxifene as a chemopreventive

Raloxifene, a second-generation SERM, has a high binding affinity for the ER [92,93], and exhibits potent anti-oestrogenic activity. There has been limited clinical experience with raloxifene in the treatment of breast cancer; however, the rationale for the use of raloxifene as a treatment of osteoporosis with the side-effect of breast-cancer prevention was first described in 1990 [94].

Raloxifene reduces the incidence of *N*-nitroso-methylurea-induced tumours if given after the carcinogen [95,96], but before the appearance of palpable tumours. However, as anticipated with a short biological half-life secondary to rapid phase II metabolism and poor bioavailability [97], raloxifene is not superior to tamoxifen at equivalent doses [95].

Raloxifene maintains bone density in ovariectomised rats [29,98–103]. In studies of postmenopausal women, raloxifene acts as a partial oestrogen agonist in bone, increasing bone density by 2.4% in the lumbar spine and the total hip [104].

Raloxifene produces a significant decrease in low-density lipoprotein cholesterol, with maintenance of triglyceride and high-density lipoprotein levels [104–106]. Laboratory data in the rabbit strongly support the value of raloxifene to prevent atherosclerosis [107]. However, data in primates fed high-cholesterol diets did not show any benefit [108]. A prospective, randomised clinical trial is in place to address the question of whether raloxifene has merit for risk reduction of coronary heart disease in high-risk postmenopausal women. The study, Raloxifene Use for the Heart (RUTH), has randomized 10 000 high-risk women to placebo or 60 mg of raloxifene daily for 5 years. Results should be available by 2005 [109]. Venous thromboembolism is a serious adverse effect associated with raloxifene (3.32 events per 1000 woman-years) [110]. The magnitude of the RR is similar to that observed with both hormone replacement therapy [111] and tamoxifen [41].

A current evaluation in women screened to ensure the absence of pre-existing endometrial abnormalities shows that raloxifene, unlike oestrogen, does not increase endometrial thickness [112]. There is no clinical evidence to date to suggest that raloxifene increases the risk of endometrial cancer.

8. MORE trial

Based on the hypothesis that raloxifene could reduce the incidence of breast cancer as a beneficial side-effect of the prevention of osteoporosis [94], placebo-controlled trials with raloxifene were started in the early 1990s. The Multiple Outcomes of Raloxifene Evaluation (MORE) has randomised 7704 postmenopausal women with osteoporosis and no history of breast or endometrial cancer to placebo, 60 or 120 mg raloxifene daily. Raloxifene reduces the incidence of vertebral fractures by 30% in postmenopausal women with osteoporosis taking 60 mg daily [113], but the risk of non-vertebral fractures did not differ significantly. Analysis of results at 3 years [114] and 4 years [110], confirmed a sustained 72% risk reduction of invasive breast cancer. As noted in the tamoxifen study, raloxifene reduces the incidence of ER-positive breast cancer and has no effect on the incidence of ER-negative breast cancer. This study gave further evidence that increased lifetime oestrogen exposure increased breast-cancer risk. Raloxifene therapy reduced breast-cancer risk regardless of lifetime oestrogen exposure, but the reduction was greater in those with higher lifetime exposure to oestrogen [115]. The longer-term effects of raloxifene 60 mg/day on reducing the incidence of breast cancer in postmenopausal women will be evaluated in the Continuing Outcomes Relevent to Evista® (CORE) trial, which will follow the incidence of breast cancer in a subset of the MORE cohort for an additional 4 years.

9. STAR trial

The widespread acceptance of the worth of raloxifene for breast cancer prevention depends on the results of the Study of Tamoxifen And Raloxifene (STAR) trial that will be completed between 2005 and 2007 (Fig. 4). The primary aim of the STAR trial is to determine whether long-term raloxifene therapy is as effective as tamoxifen in prevention of invasive breast cancer in postmenopausal women identified as high risk. A secondary goal is to compare the net effect of raloxifene and tamoxifen with respect to cardiovascular disease, fracture incidence and general toxicities. It is clear that the activation or suppression of various target sites around a woman's body is similar for tamoxifen and raloxifene, but an evaluation of the overall comparative benefits of the agents will provide an important new clinical database for raloxifene in postmenopausal women. The goal is to recruit 22 000 high-risk postmenopausal women. As of early 2003, more than 15 000 volunteers had been randomised.

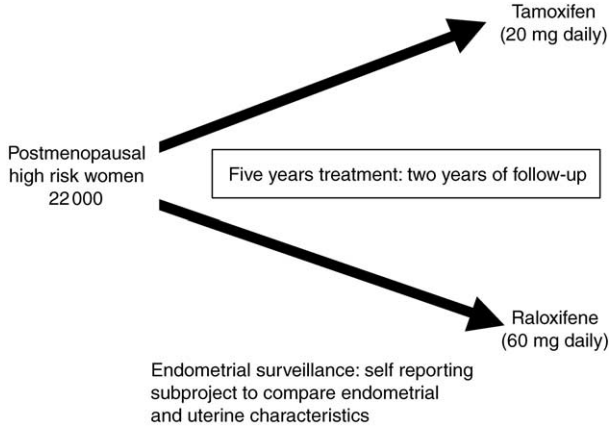

Fig. 4. The study of Tamoxifen And Raloxifene (STAR) trial design—22 000 women are being randomized to either 5-year treatment with tamoxifen or raloxifene to determine whether long-term raloxifene therapy is as effective for the prevention of invasive breast cancer in postmenopausal women identified as high risk.

10. No oestrogen at all for prevention

One of the early positive results in the ATAC trail is the finding that anastrozole is superior to tamoxifen in reducing the risk of contralateral breast cancer [79]. Plans are in place to exploit these early observations (IBIS 2) with the aim of testing whether anastrozole will reduce the incidence of breast cancer in normal-risk postmenopausal women. The goal is to improve on the safety profile of tamoxifen but with further reductions in the incidence of breast cancer. Clearly, these are important studies but the long-term benefits will need to be balanced against the reductions in bone density, osteoporotic fractures, CHD and Alzheimer's disease.

11. Closing the circle

What is to be done, in the future, when all of the endocrine therapies have been used sequentially in the ER-positive patient and there are no further responses? What are the consequences of prolonged oestrogen deprivation on the evolution of tumour biology? Remarkably, a recent clinical report suggests that oestrogen therapy can produce significant responses in patients with antihormone resistant disease [116]. Indeed, this clinical situation has been modelled in the laboratory and it appears that drug resistance to tamoxifen therapy evolves first through a phase (phase 1) where either tamoxifen or oestrogen can induce breast-tumour growth (this explains the efficacy of aromatase inhibitors after tamoxifen failure) [43] but then in phase II of tamoxifen drug resistance, after 5 years of treatment, oestrogen is a tumoricidal agent, even at physiologic levels [117]. Similarly, breast-cancer cells that are oestrogen-deprived for prolonged periods grow spontaneously, but low concentrations of oestrogen now induce apoptosis [118]. Clearly, there are clues to

the evolving tumour biology of breast cancer that should be incorporated into clinical trials but, before this occurs, the mechanism of action of oestrogen to induce apoptosis needs to be thoroughly investigated. Since tamoxifen again becomes an anti-oestrogen following the tumoricidal actions of oestrogen in tamoxifen-resistant breast cancer [117], these data suggest that hormonal scheduling may be able to maintain patients indefinitely before chemotherapy is considered.

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