ELSEVIER

European Journal of Cancer 41 (2005) 1980–1989

European Journal of Cancer

www.ejconline.com

SERMs in chemoprevention of breast cancer

Milena Gasco [a], Alessandra Argusti [a], Bernardo Bonanni [b], Andrea Decensi [a,b,*]

[a] *Division of Medical and Preventive Oncology, E.O. Ospedali Galliera, 16128 Genoa, Italy*
[b] *Division of Chemoprevention, European Institute of Oncology, 20141 Milan, Italy*

Received 22 April 2005; accepted 22 April 2005
Available online 16 June 2005

Abstract

Selective estrogen receptor modulators (SERMs) play a key role in breast cancer chemoprevention. Tamoxifen has been shown to reduce breast cancer incidence by 30–40% in at-risk subjects in large phase III trials. However, toxicity may be a limiting factor. Thus, different strategies are being pursued to improve the risk: benefit ratio of using these compounds in chemoprevention. Firstly, the second generation SERM raloxifene is currently undergoing evaluation in comparison with tamoxifen in a large phase III trial. Also, lower doses of tamoxifen are being assessed in phase II–III trials. In addition, the combination of hormone replacement therapy (HRT) or aromatase inhibitors and tamoxifen at low doses may reduce the risks while retaining the benefits of either agents. Finally, new agents that interfere with the onset of ER-negative breast cancer are being sought for combination chemoprevention since almost a third of breast cancers will not be sensitive to hormonal modulation.

Keywords: Tamoxifen; Chemoprevention; Breast neoplasms; Clinical trial

1. Introduction

The rationale behind cancer chemoprevention is based on the hypothesis that the multi-step process of carcinogenesis can be modulated, arrested or reversed by natural or synthetic agents. Chemoprevention can be divided into primary (to prevent the onset of disease in healthy individuals at risk); secondary (to treat a population with a premalignant condition in order to arrest the development of cancer) or tertiary (to protect subjects cured of an initial cancer against second primary tumours).

SERMs play a key role in breast cancer chemoprevention. These agents antagonise estrogens in some tissues and mimic their action in others. The mechanism for tissue selectivity appears to be related to differences in their molecular and three-dimensional structures, which affect the transcriptional activity of the activated estrogen receptor. For example, tamoxifen and toremifene act as estrogen antagonists in breast tissue and as estrogen agonists in the endometrium. Conversely, raloxifene behaves as an estrogen antagonist in both the breast and the endometrium.

Studies of tamoxifen have shown that chemoprevention can successfully cover all three settings of prevention: (a) primary chemoprevention, as shown in the NSABP P-1 trial in healthy women at increased risk according to the Gail's model [1]; (b) secondary chemoprevention, as described in the NSABP B-24 trial, in which patients with ductal carcinoma *in situ* (DCIS) benefited from tamoxifen for prevention of ipsilateral and contralateral breast cancer [2]; and (c) tertiary chemoprevention, as demonstrated in the EBCTCG meta-analysis, wherein tamoxifen was associated with prevention of contralateral breast cancer in definitively treated breast cancer patients [3]. Based on the results

* Corresponding author. Tel.: +39 010 563 4501; fax: +39 010 5748 1090.
E-mail address: andrea.decensi@galliera.it (A. Decensi).

0959-8049/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2005.04.017

of the NSABP P-1 study, the Food and Drugs Administration has approved the use of tamoxifen as a chemopreventive agent in women with individual risk $\geqslant$1.66% in 5 years according to the Gail's model, which includes age, age at menarche, age at first pregnancy, first degree family history of breast cancer and number of biopsies for benign disease, with or without atypical hyperplasia (http://bcra.nci.nih.gov/brc/).

However, tamoxifen causes undesirable side effects, including an increased risk of endometrial cancer and venous thromboembolic events (VTE). Other SERMs are therefore entering the field of clinical cancer prevention and a large number of agents are likely to be tested in the next few years. In this review, we shall summarise the results of the major chemoprevention trials for breast cancer published so far and discuss some of the current issues in the field of clinical breast cancer prevention, with special emphasis to the search of strategies to optimise tamoxifen use.

2. Important issues in chemoprevention trials

The high costs of large chemoprevention studies have prompted the search for intermediate, surrogate endpoints of intervention efficacy. These are defined as biological markers or events that may be assessed or observed before the clinical appearance of the disease and are associated with the development of that disease. Reduction in cancer incidence among populations receiving a chemopreventive intervention may require years to evaluate. Therefore, monitoring intermediate markers that correlate with a reduction in cancer incidence would allow the prompt evaluation of potentially active chemopreventive agents. Moreover, the identification of risk biomarkers would provide insight into critical pathways associated with carcinogenesis and additionally characterise the activity of investigational agents. Analysis of molecular pathways, levels of circulating proteins or expression of histological markers can be used as intermediate surrogate endpoints, thus reducing time, sample size and costs of large trials which utilise conventional clinical endpoints. A typical example of a validated surrogate endpoint in clinical trials is cholesterol in cardiovascular medicine. Although there are increasing data on the validity of this strategy [4–6], no definitive surrogate endpoint biomarker has been established so far in the field of breast cancer chemoprevention. Several studies [7–13] and a recent meta-analysis [14] have shown that high circulating levels of insulin-like growth factor-1 (IGF-I) and low levels of insulin-like growth factor binding protein-3 (IGFBP-3) are associated with an increased breast cancer risk in premenopausal women. These findings support their putative role as breast cancer risk biomarkers. Genetic polymorphisms in the IGFBP-3 gene have recently been shown to be associated with the level of blood IGFBP-3 protein and an increased breast cancer risk [15]. An association between a polymorphism in the estrogen receptor α gene and postmenopausal ductal breast cancer risk has also been suggested, particularly in women with high body mass index and therefore higher levels of circulating endogenous estrogens [16]. Premalignant lesions, such as hyperplasia and atypical hyperplasia, are also a potential source of intermediate markers. Ductal lavage is a promising and non-invasive method to obtain breast epithelial cells from the ductal system for cytopathologic analysis to provide individualised risk assessment [17]. Several studies are ongoing, which evaluate endpoint biomarkers in ductal lavage fluid, such as atypia and Ki-67 expression, in order to identify factors associated with breast cancer initiation and progression.

An alternative biomarker approach to monitoring the efficacy of chemoprevention may be detection of genomic DNA shed from malignant breast cancer cells into peripheral blood. Genetic and especially epigenetic changes occurring in cancer cells, such as aberrant methylation within CpG sequences of specific genes, can readily be detected in circulating DNA [18]. The principle of using circulating nucleic acids as a biomarker to detect tumours cells has been convincingly established in several major solid tumour types including oesophageal [19] and prostate carcinomas [20]. Recent evidence suggests that methylated sequences from the RASSF1A and HIC-1 genes could be detected in DNA eluted from the surface of peripheral blood cells of breast cancer patients even when methylated DNA was not detected in plasma from the same patients [21]. Other alternative candidate genes which may merit analysis include 14-3-3σ in which methylation is a frequent early event in breast cancer [22]. Although additional work will be required to verify sensitivity, specificity and ability to detect preinvasive disease, the simplicity and non-invasiveness of this approach make it an attractive option to consider in future strategies for assessing outcome in cohorts of patients receiving chemopreventive agents.

Another crucial aspect is the definition of the appropriate target population(s) by identification of criteria for selection of high-risk subjects [23]. Some examples of these criteria are summarised in Table 1.

3. Tamoxifen studies

3.1. The NSABP P-1 study

This study [1] recruited 13,388 women with a breast cancer risk $\geqslant$1.66% in 5 years according to the Gail's model, which is equivalent to an average 60 year old woman's risk or to the risk of a woman with history of lobular carcinoma *in situ*. Women were randomised to

Table 1
Risk factors for breast cancer other than the Gail's model

• Intraepithelial neoplasia	ADH,[a] LCIS, DCIS, or atypical cells in FNAs or ductal washing
• Prior breast cancer	
• High hormone exposure	For example, high IGF-I or low IGFBP-3 in premenopausal women, high estradiol levels in postmenopausal women, HRT use, high mammographic density
• Gene mutation carriers	For example, BRCA1 and BRCA2
• Single nucleotide polymorphisms	For example, TGF β1

[a] Atypical ductal hyperplasia.

receive either tamoxifen 20 mg/day or placebo. This trial gave such positive results that an interim analysis led to early closure. It was shown that tamoxifen reduces the risk of invasive and non-invasive breast cancer by 49% and 50%, respectively (two-sided $P < 0.00001$ and $P < 0.002$) and decreases the occurrence of estrogen receptor (ER)-positive tumours by 69%, with no effect on estrogen receptor (ER)-negative tumours. The protective effect of tamoxifen was seen in women of all age groups. Risk was also reduced in women with history of lobular carcinoma *in situ* by 56% and by 86% in those with atypical hyperplasia. All women in any category of predicted 5-year risk had a risk reduction with tamoxifen. Interestingly, tamoxifen produced an overall 20% reduction in the incidence of osteoporotic bone fractures. However, women aged 50 or older had a four-fold increased risk of early stage endometrial cancer, a three-fold increased risk of pulmonary embolism and a significant excess of cataract [1].

Based on the trial results, the Food and Drug Administration approved the use of chemopreventive tamoxifen in high-risk individuals. This study provides the first example of a medication approved and marketed as a cancer preventive agent. However, the striking results of the NSABP P-1 trial were only partially confirmed in European trials [24–27].

3.2. The Royal Marsden trial

In this pioneering study [24], 2494 healthy women aged between 30 and 70, at increased risk of breast cancer because of family history, were accrued and randomised in a double blind fashion to receive either tamoxifen 20 mg/day or placebo for up to 8 years. The initial analysis after a median follow-up of 70 months, when the study had adequate power to detect a 50% reduction of breast cancer in the tamoxifen arm, reported the same overall frequency of breast cancer in both arms ($P = 0.8$). Interestingly, women who were already on HRT, mostly by oral route, at the time of recruitment showed an increased risk of breast cancer compared to non-HRT-users, whereas those who started HRT while on trial had a significantly reduced risk. There were four cases of endometrial cancer in the tamoxifen arm *vs* one in the placebo arm and seven cases *vs* four of VTE and pulmonary embolism. In an updated analysis after a median follow-up of 123 months, there was still no significant reduction of breast cancer on tamoxifen (69 on tamoxifen *vs* 82 on placebo), despite a trend for risk reduction in women on HRT who took tamoxifen (hazard ratio, HR = 21%; $P = 0.12$) [25].

3.3. The Italian study

This study recruited 5408 healthy women aged 35–70 years who had had prior hysterectomy for non-malignant conditions [26]. At a median follow-up of 81.2 months, there was a non-significant trend to fewer cases on tamoxifen (34 *vs* 45 events). Tamoxifen significantly reduced the incidence of breast cancer in the high-risk group (3 *vs* 15 events, $P = 0.003$), defined on the basis of baseline as well as reproductive and hormonal characteristics, whereas no such effect was seen in the low-risk group ($P = 0.89$). No difference was observed in the subset of women who had never taken HRT. Conversely, women who had taken HRT at some point before or during the study ($n = 1584$) had fewer breast cancers in the tamoxifen arm (6 on tamoxifen *vs* 17 on placebo, HR = 0.35, 95% confidence interval, CI, 0.14–0.89). There were 28 VTE on placebo and 44 on tamoxifen (HR = 1.63, 95% CI, 1.02–2.63), 80% of which were superficial phlebitis, accounting for all the excess due to tamoxifen within 18 months from randomisation. In multivariate regression analysis, age $\geqslant$60 years, height $\geqslant$165 cm and diastolic blood pressure $\geqslant$90 mmHg had independent detrimental effect on VTE risk during tamoxifen, whereas transdermal estrogen therapy during tamoxifen was not associated with any excess of VTE (HR = 0.64, 95% CI = 0.23–1.82) [27].

3.4. The IBIS trial

In the IBIS trial [28], 7152 women aged 35–70 at increased risk for breast cancer were recruited for a double blind, placebo controlled, tamoxifen study. Nearly all participants (97%) had a family history of breast cancer. At a median follow up of 50 months, tamoxifen treatment resulted in a 33% reduction of breast cancer incidence compared to placebo ($P = 0.01$). A non-significant two-fold relative increase (11 *vs* 5) of endometrial cancer was observed ($P = 0.20$) in the tamoxifen arm. Thromboembolic events were significantly increased in

the tamoxifen arm ($P = 0.001$). Major VTE increased significantly on tamoxifen within three months of major surgery, immobilisation or fracture. No differences in bone fractures and cataract were observed.

4. Overview of prevention trials with SERMs

A recent meta-analysis has been conducted [29], which included the 28,406 healthy subjects participating in all four major primary prevention trials of tamoxifen, the 7705 osteoporotic women enrolled in the multiple outcome raloxifene endpoint (MORE) study and the 14,170 patients participating in adjuvant trials where the effect on contralateral breast cancer was assessed. The tamoxifen prevention trials showed a 38% (95% CI 28–46) reduction of breast cancer incidence. ER-positive cancers were decreased by 48% (36–58; $P < 0.0001$), whereas no effect was seen on ER-negative breast cancer (HR ratio 1.22 [0.89–1.67]). Age had no apparent effect on tamoxifen efficacy. Rates of endometrial cancer were increased in all tamoxifen prevention trials (relative risk, RR 2.4 [1.5–4.0]) and in the adjuvant trials (RR 3.4 [1.8–6.4]), whereas no increase has been seen so far with raloxifene. Venous thromboembolic events were increased in all tamoxifen studies (RR 1.9 [1.4–2.6] in the prevention trials; $P < 0.0001$) and with raloxifene (RR 3.1, CI 1.5–6.2). Overall, there was a non-significant 9% reduction of breast cancer mortality with tamoxifen as a primary preventive agent.

Clear evidence is now available that tamoxifen can reduce the risk of ER-positive breast cancer. New approaches are needed to prevent ER-negative breast cancer and to reduce tamoxifen-related adverse events. Such approaches include tamoxifen at lower doses and the use of aspirin to reduce the risk of VTE. In addition, newer agents such as raloxifene and the aromatase inhibitors await evaluation.

5. Raloxifene

This agent is another SERM with estrogen antagonist action in both breast and uterus [30] and agonist action in bone. It maintains bone density and lowers LDL cholesterol in postmenopausal women [31]. It is potentially less hazardous than tamoxifen, as it has not been associated with increased risk of endometrial cancer. A recent report from the CORE study (follow-up to the MORE trial) showed that raloxifene for 8 years reduced the incidence of newly diagnosed breast cancer by 66%, with a marked effect on ER-positive tumours (risk reduced by 76%) and no effect on ER-negative tumours and non-invasive cancers [32,33]. There is no reported increase of endometrial cancer so far, whereas the effects on VTE look similar to those of tamoxifen [29].

Raloxifene is being evaluated in comparison with tamoxifen in the STAR study, a large primary prevention trial of tamoxifen 20 mg/day *vs* raloxifene 60 mg/day for 5 years. The study population includes postmenopausal women at high risk based on the Gail's model and women with previous lobular carcinoma *in situ*. A total of 19,747 subjects have been recruited in order to assess the advantages of raloxifene over tamoxifen. The accrual has been completed in November 2004. The main outcome measure is breast cancer incidence and the results are expected in 2006.

6. Tamoxifen at lower doses

The use of tamoxifen as a chemopreventive agent may be limited by the risk of endometrial cancer and VTE. A simple and economic approach to retain tamoxifen efficacy while reducing the risks may be a dose reduction. The rationale for this approach is summarised in Table 2 and supported by several observations. In a study conducted by us, standard dose tamoxifen (20 mg/day) and two different lower doses (10 mg/day and 10 mg on alternate days) were administered for two months to a cohort of 127 healthy women [34] and changes in serum biomarkers regulated by the estrogen receptor were evaluated, including lipid profile, blood cell count, fibrinogen, antithrombin III, osteocalcin and IGF-I. No evidence for a concentration-response relationship was observed for most of the biomarkers. The concept of a dose reduction was further supported by the observation that tamoxifen has very high tissue distribution, ranging from 5 to 60 times its blood concentrations [31,35], and a prolonged half-life (9 and 13 days for tamoxifen and metabolite *X*, respectively) [31,36]. Also, the breast tissue level attainable with 10 mg per alternate days still exceeds the *in vitro* growth inhibitory concentration of tamoxifen in breast cancer cell lines. Interestingly, a recent cross-sectional study conducted in older, nursing home residents in New York State long-term facilities has shown a significant reduction of bone fracture rate among breast cancer women taking tamoxifen 10 mg/day [37]. The concept of a dose reduction has further been assessed in a preoperative trial [38] in which 120 women with breast cancer were treated with either 20 mg or 5 mg or 1 mg/day of tamoxifen for 4 weeks before surgery. The effects of different doses of tamoxifen on breast cancer proliferation were studied using Ki-67 expression as the main surrogate endpoint marker. The change in Ki-67 expression induced by lower doses of tamoxifen was comparable to that achieved with the standard dose, implying that tamoxifen at low doses retains antiproliferative activity. Several blood biomarkers of tamoxifen

Table 2
Tamoxifen: rationale for a dose reduction

- Binding to ER follows a saturation kinetics
- Twenty milligram per day is as effective as 30–40 mg/day in the global meta-analysis
- The endometrial effect is dose dependent
- Animal data show complete inhibition of tumour formation at a dose equivalent to 1 mg/day in humans
- Preoperative clinical trials show similar antiproliferative effects of 1 mg and 5 mg/day compared to 20 mg/day

estrogenicity associated with the risk of breast cancer, cardiovascular disease and bone fracture showed dose–response relationship, suggesting that low doses of tamoxifen may be associated with reduced, favourable and unfavourable, estrogenic effects of tamoxifen. Taken together, these findings provide a strong rationale for the formal assessment of low dose tamoxifen in a preventive context.

7. Hormonal replacement therapy and tamoxifen

Prolonged use of HRT may increase breast cancer risk due to increased expression of estrogen receptors in the breast tissue, leading to enhanced sensitivity to the mitogenic effect of estrogens [39]. The addition of a SERM may reduce this growth promoting effect. Notably, in a subgroup analysis of the Italian Study, there was a significant difference in the incidence of breast cancer between tamoxifen and placebo among women who had used HRT at some point during the trial ($P = 0.022$), whereas no difference was recorded among women who had never used HRT [26]. On the other hand, the use of tamoxifen is associated with increased risk of endometrial cancer and the use of progestins in the HRT regimen might reduce this tamoxifen-related side effect. A number of studies indicate that the combination of HRT and a SERM such as tamoxifen may reduce the risks (breast cancer, endometrial cancer and VTE), while retaining the benefits, of either agent alone [26,28]. These findings provide strong justification for studying the effect of the combination of HRT and tamoxifen in chemoprevention.

The HOT study (HRT opposed by tamoxifen), a phase III trial addressing this issue, has recently been launched in Italy [40]. As of March 31, 2005, approximately 1650 healthy postmenopausal women on HRT have been randomised to receive tamoxifen 5 mg/die or placebo for 5 years. The study is powered to detect a 40–50% reduction in the incidence of invasive breast cancer and DCIS in the tamoxifen arm.

Preliminary results of a pilot 4-arm randomised study of 210 postmenopausal HRT users, receiving either tamoxifen at 1 mg/day, 5 mg/day and 10 mg/week or placebo, have recently been presented [41]. The primary endpoint was the modification of serum IGF-I levels. Secondary endpoints included changes of endometrial thickness and modifications in the levels of several circulating biomarkers. In a preliminary analysis, all doses of tamoxifen significantly decreased plasma IGF-I levels and ultrasensitive C-Reactive Protein (CRP). Endometrial thickness was non-significantly increased by any of the tamoxifen dose levels. Final results are awaited shortly.

8. Tamoxifen *vs* aromatase inhibitors

The new third generation aromatase inhibitors have shown efficacy in advanced breast cancer and have a favourable toxicity profile. Each of these compounds leads to a nearly total blockade of peripheral conversion of androgens to estrogens, by inhibiting the aromatase enzyme complex, and a marked decline in circulating estrogen levels. They offer another approach to local control, prevention of recurrence and prevention of primary breast cancers, which may be superior and/or complimentary to the use of SERMs.

A very large trial (ATAC) [42] has evaluated the role of anastrozole both alone and in combination with tamoxifen compared with tamoxifen in the adjuvant setting for early breast cancer. A total of 9366 patients were enrolled and more than 1000 recurrences or deaths were recorded. After a median follow-up of 33 months, the 3-year disease-free survival (DFS) of the hormone receptor-positive patients was significantly increased in the anastrozole arm (89.4% *vs* 87.4% on tamoxifen, $P = 0.013$). Incidence of contralateral breast cancer was also significantly lower with anastrozole than with tamoxifen (odds ratio OR = 0.42 [0.22–0.79], $P = 0.007$). Results with the combination were not significantly different from those with tamoxifen only. The side effect profile of anastrozole was favourable, with fewer endometrial cancers ($P = 0.02$), vaginal bleeding and discharge ($P < 0.0001$), cerebrovascular events ($P = 0.0006$), venous thromboembolic events ($P = 0.0006$) and hot flushes ($P > 0.001$) compared to tamoxifen. However, there were significant increases in musculo-skeletal disorders and bone fractures ($P < 0.0001$) in the anastrozole arm. At a median follow-up of 68 months, the advantage of anastrozole was confirmed for both DFS ($P = 0.01$) and contralateral breast cancer (35 *vs* 59, HR = 0.52, 95% CI, 0.38–0.88, $P = 0.01$) [43]. Whereas the overall safety profile of anastrozole remained consistent with the original report, there was a slight trend to a higher risk of ische-

mic cardiovascular disease on anastrozole compared to tamoxifen (127 *vs* 104, OR = 1.23, 95% CI, 0.95–1.60).

The IES trial evaluated the role of exemestane in the adjuvant setting [44]. After 2–3 years of tamoxifen therapy, 4742 patients were randomised between switching to exemestane or continuing tamoxifen for the remainder of the 5 years of treatment. After a median follow up of 30.6 months, DFS was 91.5% in the exemestane group *vs* 86.8% in the tamoxifen group ($P = 0.001$). In addition, exemestane significantly reduced the risk of contralateral breast cancer (HR = 0.44, 95% CI, 0.20–0.98, $P = 0.04$). Thromboembolic events were more common with tamoxifen ($P = 0.007$), as were gynecologic symptoms, vaginal bleeding and muscle cramps. Exemestane was associated with a higher incidence of arthralgia and diarrhea. Fractures were also more frequent in the exemestane group, although the difference did not reach statistical significance.

The MA-17 trial evaluated the role of letrozole for 5 years *vs* placebo in postmenopausal women who had completed 5 years of tamoxifen therapy [45]. A total of 5187 women were recruited. The trial was terminated after a median follow-up of 2.4 years due to a significant improvement in DFS in the letrozole group (estimated four-year DFS 93% *vs* 87% with placebo, $P \leqslant 0.001$). Similarly, letrozole reduced the risk of new contralateral breast cancer (HR = 0.57, 95% CI, 0.43–0.75, $P = 0.00008$). Hot flushes, arthritis, arthralgia and myalgia were more frequent in the letrozole group ($P < 0.05$), whereas vaginal bleeding was more common with placebo ($P = 0.01$). There was no statistically significant difference in the rate of cardiovascular events and new bone fractures. In an updated analysis, the study reported not only an overall benefit in DFS, but also a survival advantage in women with node-positive disease [46]. This is the only trial to date to demonstrate a survival advantage for an aromatase inhibitor in the adjuvant setting.

A forth adjuvant trial, the BIG 1-98 study, has recently been presented at the St. Gallen conference [47]. More than 8000 postmenopausal women with hormone receptor-positive early breast cancer were randomised to the following arms: tamoxifen for 5 years, letrozole for 5 years, tamoxifen for 2 years followed by letrozole for 3 years, and letrozole for 2 years followed by tamoxifen for 3 years. At a median follow-up of 26 months, letrozole was associated with a significant 19% reduction in the risk of relapse ($P = 0.003$) and a noticeable 27% decrease in the risk of distant metastases compared with tamoxifen ($P = 0.0012$). Letrozole significantly reduced vaginal bleeding, hot flushes, and endometrial cancer compared to tamoxifen. Hypercholesterolemia, grade 3–5 stroke and other cardiovascular events were more common on letrozole. As expected with estrogen deprivation therapy, the number of new bone fractures was 5.8% on letrozole and 4.1% on tamoxifen. Grade 3–5 thromboembolic events were more common with tamoxifen. In patients who remained free of cancer recurrence, more deaths due to stroke (7 *vs* 1) or cardiac events (26 *vs* 13) were reported in the letrozole compared to the tamoxifen arm.

Given the encouraging results on contralateral breast cancer risk, aromatase inhibitors hold promise as chemopreventive agents in postmenopausal women and are being evaluated in such setting. The IBIS-II trial will randomise 6000 postmenopausal women aged 40–70, at increased risk for breast cancer, to receive either anastrozole or placebo. Increased risk is determined from family history, previous benign disease with evidence of proliferation, mammographic dysplasia, and nulliparity. A parallel trial of 4000 women with DCIS will also be conducted, except that randomisation will be between tamoxifen and anastrozole. Three NCIC-CTG trials (MAP.1, MAP.2 and MAP.3) are comparing letrozole to placebo, exemestane to placebo, and exemestane with or without celecoxib to placebo in postmenopausal women at increased risk of breast cancer. These studies will provide essential information on the long-term safety of a total estrogen withdrawal in healthy women on several target systems, including bone, cardiovascular and brain.

9. Combination of SERMs and aromatase inhibitors

In the ATAC study, the combination of anastrozole and tamoxifen did not result in any advantage compared to tamoxifen only, possibly due to an adverse pharmacokinetics interaction between the two drugs, which reduces by 30% the bioavailability of anastrozole. Moreover, the estrogenic effect of tamoxifen may prevail in an estrogen deprived environment. However, the combination of low dose tamoxifen with an aromatase inhibitor might provide an advantage by counteracting the risk of bone fractures and ischemic cardiovascular events. The same combination used in the ATAC study, but with a much lower dose of tamoxifen (10 mg/week), is being evaluated in 75 postmenopausal DCIS or lobular carcinoma *in situ* (LCIS) patients randomised to receive anastrozole 1 mg/day or tamoxifen 10 mg/week or their combination for 12 months [48]. Primary endpoint is the plasma concentration of the two drugs. Secondary endpoints include the effects of each treatment on biomarkers of breast cancer, bone turnover and cardiovascular risk. Results are awaited shortly.

10. Combination of SERMs and retinoids

Retinoids play a crucial role in cellular proliferation and differentiation, but their poor clinical tolerability has prevented their use as cancer preventive agents, leading to the synthesis and evaluation of derivatives.

One such compound is (*N*-4-hydroxyphenyl) retinamide (4-HPR) or fenretinide, a synthetic amide derivative of *all-trans* retinoic acid [49], which is one of the less toxic vitamin A analogues studied in breast cancer chemoprevention. 4-HPR induces the generation of reactive oxygen species and promotes apoptosis through both retinoid receptor-dependent and -independent mechanisms [50–53]. It also lowers circulating IGF-I levels, which have been associated with increased risk of premenopausal breast cancer, prostate cancer and colorectal cancer [54–58]. Fenretinide has shown *in vitro* and *in vivo* activity against mammary, bladder, lung, ovary, cervix, neuroblastoma, leukeamia and prostate preclinical models [59]. On the basis of the selective accumulation of fenretinide in the human breast [60] and the good tolerability in humans [49], a phase III trial was started in 1987 to evaluate the role of fenretinide in breast cancer chemoprevention. 2972 women with a history of stage I breast cancer were randomised to fenretinide 200 mg/day or no intervention for 5 years. Primary endpoint was the occurrence of contralateral breast cancer. After a median follow-up of 8 years, the incidence of contralateral breast cancer was comparable in the two arms [61]. However, a beneficial trend (35% reduction) was observed in the subgroup of premenopausal women. Interestingly, modulation of plasma IGF-I levels by fenretinide followed a similar pattern, with IGF-I levels showing a reduction in premenopausal women only.

The potential synergism between fenretinide and tamoxifen was assessed in a 2×2 factorial design biomarker study. A total of 235 premenopausal women at high risk of breast cancer were randomised to either tamoxifen 5 mg/day, or fenretinide 100 mg twice daily, or both agents or placebo for 2 years [62]. Primary endpoints were the changes in IGF-I levels and mammographic percent density. Additional endpoints included changes in endometrial thickness and proliferation, presence of ovarian cysts and atypia in fine needle aspirates (FNA). Recruitment was stopped based on the lack of a synergistic interaction on the primary endpoints. After a median follow-up of 40 months, 35 subjects dropped the study for refusal ($n = 19$) or adverse events ($n = 16$). So far, no difference in the rate of primary or recurrent breast cancer has been observed among arms. Of the two serious adverse events, one stage I endometrial cancer occurred in the fenretinide arm and one optic nerve ischemia in the tamoxifen arm. There was no increased endometrial thickness and no difference in endometrial polyps among the four arms. Mature results are awaited.

11. Chemoprevention strategies in women at genetic risk

Women carrying a germline mutation of either BRCA1 or BRCA2 have a markedly increased lifetime risk of developing breast and/or ovarian cancer, typically at early ages [63–66]. Oophorectomy performed early in reproductive life is effective in reducing breast cancer risk in BRCA1/2 carriers [67], suggesting a potential role for preventive hormone therapy. To date however, no chemoprevention trial has specifically been designed for BRCA1/2 carriers and this area of research remains controversial. BRCA1/2 associated tumours present with distinctive features: BRCA1 tumours are mostly ER- and progesterone receptor (PgR)-negative, whereas BRCA2 tumours are more commonly ER/PgR-positive [68–73]. The lack of ER/PgR positivity in BRCA1 tumours suggests that treatment with tamoxifen, or other hormone therapy, may be less effective in this patient population [69,70].

A favourable trend towards a reduction of contralateral breast cancer in BRCA1 mutation carriers receiving adjuvant tamoxifen was demonstrated in a case-control study [74]. A subsequent analysis was performed by Foulkes *et al*. [75] to assess the impact of BRCA1 mutation status on the response to adjuvant tamoxifen in a retrospective cohort of 292 Ashkenazi Jewish women with first primary invasive breast cancer genotyped for BRCA1 and BRCA2 mutations. It was concluded that tamoxifen might be effective for BRCA1 breast cancer irrespective of the ER status of the tumour, implying that the ER-negative status that occurs in BRCA1 cancers may not have the same effect on response to tamoxifen as it does in the general population. Conversely, recent data from the NSABP-P1 trial indicate that tamoxifen may reduce breast cancer risk in BRCA2 but not in BRCA1 carriers, although the numbers were too small to draw definitive conclusions [76].

Recent reports have shown that the wild-type BRCA1 protein may repress ER function, either directly or indirectly [77]. Therefore, tumours that lack functional BRCA1 protein may be particularly responsive to hormone manipulation. In addition, tamoxifen can induce cell death via oxidative stress in ER-negative cancer cell lines [78], and this effect may be more prominent in BRCA1-null cells, which have profound defects in DNA repair. Thus, tumours that lack both ER and BRCA1 may respond differently to tamoxifen than those that lack only ER. Aromatase inhibitors may be an alternative prevention strategy in BRCA1/2 carriers and are being evaluated in such setting.

12. Prevention of ER-negative breast cancer

SERMs can reduce the incidence of ER-positive breast cancer in at-risk women, while the incidence of ER-negative cancers does not seem to be affected by these compounds. Similar results are likely with aromatase inhibitors. Approximately 20–30% of all invasive

breast cancers are ER-negative and some ER-positive precancerous lesions might be resistant to tamoxifen intervention. Furthermore, women with a family history of breast and ovarian cancer have a higher risk of developing ER-negative breast cancer compared with the general population. Therefore, strategies to prevent ER-negative tumours are actively being sought and women with BRCA1 mutations, ER-negative DCIS or prior ER-negative breast cancer are potential candidates for phase I and phase II chemoprevention trials with novel agents targeting alternative, important molecular pathways (Fig. 1). A number of trials with new potential chemopreventive agents are currently being planned in women at increased risk for ER-negative breast cancer. These include tyrosine kinase inhibitors, cyclin-dependent kinase inhibitors, ligands for peroxisome proliferator-activated receptor γ (PPAR ligands, glitazones), RXR selective ligands (rexinoids), COX-2 selective inhibitors, demethylating agents, histone deacetilase inhibitors and Vitamin D3 derivatives.

ErbB-2 and EGFR are overexpressed in at least 30% of breast cancers and overexpression has been correlated with poor outcome. EGFR tyrosine kinase inhibitors have shown protective activity against the development of breast cancer in several studies, including inhibition of proliferation in DCIS [79]. However, the safety of these agents is still under investigation, the most common toxicities thus far reported being diarrhoea, skin rash and interstitial pneumonia. Another potential molecular target for ER-negative breast cancer prevention is COX-2 over expression, which has been correlated with breast carcinogenesis. One of the possible mechanisms of action of COX-2 is induction of aromatase through prostaglandin E2, leading to increased levels of estrogens. The chemopreventive activity of COX-2 inhibitors can thus be due to gene-specific activity, antiangiogenetic and pro-apoptotic properties, and tissue specific inhibition of estrogen synthesis [80].

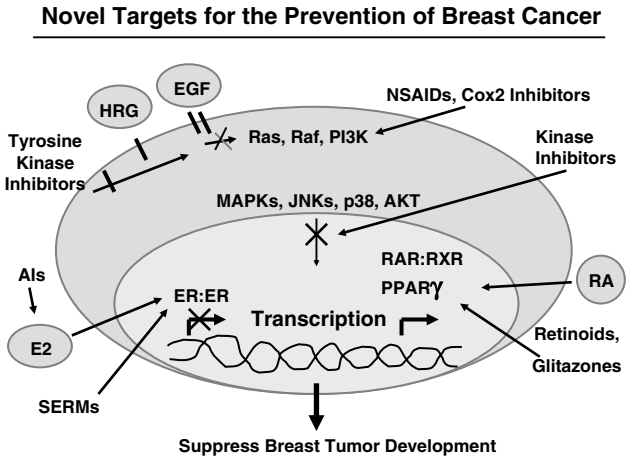

Fig. 1. Selected molecular targets for breast cancer chemoprevention.

13. Conclusions

Cancer prevention is an important public health issue and chemoprevention of breast cancer is a very promising area that has already achieved significant objectives in recent years. Despite the encouraging results of the clinical trials described in this review, several important questions await resolution, including the appropriate duration and dose of tamoxifen, concomitant use of aspirin, development of new SERMs, assessment of long-term efficacy and safety of aromatase inhibitors, a better definition of subjects at increased risk for ER-positive breast cancer, the exclusion of subjects at risk for adverse events and the discovery of agents with activity in ER-negative cancers.

Another issue of primary importance is the identification of risk biomarkers and molecular targets and the introduction of these into chemoprevention trials. Modern molecular technology such as microarrays and proteomics will clearly facilitate identification of such biomarkers and expedite definition of appropriate target populations for intervention. This approach will allow the research in cancer prevention to expand in a cost-effective way in the near future. It is our hope that these efforts will improve our ability to prevent breast cancer and, ultimately, our strategies to reduce breast cancer mortality.

References

1. Fisher B, Costantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998, **90**, 1371–1388.
2. Fisher B, Dignam J, Wolmark N, *et al*. Tamoxifen in treatment of intraductal breast cancer: National Surgical Adjuvant Breast and Bowel Project B-24 randomised controlled trial. *Lancet* 1999, **353**, 1993–2000.
3. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998, **351**, 1451–1467.
4. Lippman SM, Lee JJ, Sabichi AL. Cancer chemoprevention: progress and promise. *J Natl Cancer Inst* 1998, **90**, 1514–1528.
5. O'Shaughnessy JA, Kelloff GJ, Gordon GB, *et al*. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. *Clin Cancer Res* 2002, **8**, 314–346.
6. Kelloff GJ, Boone CW, Crowell JA, *et al*. Risk biomarkers and current strategies for cancer chemoprevention. *J Cell Biochem* 1996, **25**(Suppl.), 1–28.
7. Hankinson SE, Willett WC, Colditz GA, *et al*. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. *Lancet* 1998, **351**, 1393–1396.
8. Yu H, Jin F, Shu XO, *et al*. Insulin-like growth factors and breast cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev* 2002, **11**, 705–712.

9. Toniolo P, Bruning PF, Akhmedkhanov A, *et al*. Serum insulin-like growth factor-I and breast cancer. *Int J Cancer* 2000, **88**, 828–832.
10. Li BD, Khosravi MJ, Berkel HJ, *et al*. Free insulin-like growth factor-I and breast cancer risk. *Int J Cancer* 2001, **91**, 736–739.
11. Del Giudice ME, Fantus IJ, Ezzat S, *et al*. Insulin and related factors in premenopausal breast cancer risk. *Breast Cancer Res Treat* 1998, **47**, 111–120.
12. Krajcik RA, Borofsky ND, Massardo S, *et al*. Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2002, **11**, 1566–1573.
13. Johansson H, Baglietto L, Guerrieri-Gonzaga A, *et al*. Factors associated with circulating levels of insulin-like growth factor-I and insulin-like growth factor binding protein-3 in 740 women at risk for breast cancer. *Breast Cancer Res Treat* 2004, **88**, 63–73.
14. Shi R, Yu H, McLarty J, *et al*. IGF-I and breast cancer: a meta-analysis. *Int J Cancer* 2004, **111**, 418–423.
15. Ren Z, Cai Q, Shu X, *et al*. Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP-3 protein levels among Chinese women. *Cancer Epidemiol Biomarkers Prev* 2004, **13**, 1290–1295.
16. Wedrén S, Lovmar L, Humphreys K, *et al*. Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. *Breast Cancer Res* 2004, **6**, R437–R449.
17. Arun B. Ductal lavage and risk assessment of breast cancer. *Oncologist* 2004, **9**, 599–605.
18. Herman JG. Circulating methylated DNA. *Ann NY Acad Sci* 2004, **1022**, 33–39.
19. Kawakami K, Brabender J, Lord RV, *et al*. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. *J Natl Cancer Inst* 2000, **92**, 1805–1811.
20. Jeronimo C, Usadel H, Henrique R, *et al*. Quantitative GSTP1 hypermethylation in bodily fluids of patients with prostate cancer. *Urology* 2002, **60**, 1131–1135.
21. Rykova EY, Laktionov PP, Skvortsova TE, *et al*. Extracellular DNA in breast cancer: cell surface-bound, tumour-derived extracellular DNA in blood of patients with breast cancer and non-malignant tumours. *Ann NY Acad Sci* 2004, **1022**, 217–220.
22. Ferguson AT, Evron E, Umbricht CB, *et al*. High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. *Proc Natl Acad Sci USA* 2000, **97**, 6049–6054.
23. Armstrong K, Eisen A, Weber B. Assessing the risk of breast cancer. *N Engl J Med* 2000, **342**, 564–571.
24. Powles T, Eeles R, Ashley S, *et al*. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet* 1998, **352**, 98–101.
25. Powles TJ, Eeles R, Salmon A, *et al*. Update of the Royal Marsden Hospital tamoxifen breast cancer chemoprevention trial. *Proc Am Soc Clin Oncol* 2003, **22**, 94 [Abstract 375].
26. Veronesi U, Maisonneuve P, Rotmensz N, *et al*. Italian randomised trial among women with hysterectomy: tamoxifen and hormone-dependent breast cancer in high-risk women. *J Natl Cancer Inst* 2003, **95**, 160–165.
27. Decensi A, Maisonneuve P, Rotmensz N, *et al*. Effect of tamoxifen on venous thromboembolic events in a breast cancer prevention trial. *Circulation* 2005, **111**, 650–656.
28. Cuzick J, Forbes J, Edwards R, *et al*. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomised prevention trial. *Lancet* 2002, **360**, 817–824.
29. Cuzick J, Powles T, Veronesi U, *et al*. Overview of the main outcomes in breast-cancer prevention trials. *Lancet* 2003, **361**, 296–300.
30. Cummings SR, Eckert S, Krueger KA, *et al*. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomised trial – Multiple Outcomes of Raloxifene Evaluation. *JAMA* 1999, **281**, 2189–2197.
31. Johnston CCJ, Bjarnason NH, Cohen FJ, *et al*. Long-term effects of raloxifene on bone mineral density, bone turnover, and serum lipid levels in early postmenopausal women: three-year data from 2 double-blind, randomised, placebo-controlled trials. *Arch Intern Med* 2000, **160**, 3444–3450.
32. Cauley JA, Norton L, Lippman ME, *et al*. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. *Breast Cancer Res Treat* 2001, **65**, 125–134.
33. Martino S, Cauley JA, Barrett-Connor E, *et al*. Incidence of invasive breast cancer following 8 years of raloxifene therapy in postmenopausal women with osteoporosis: results from the Continuing Outcomes Relevant to Evista (CORE) Trial. *Proc Am Soc Clin Oncol* 2004, **23**, 97 [Abstract 1000].
34. Decensi A, Bonanni B, Guerrieri-Gonzaga A, *et al*. Biologic activity of tamoxifen at low doses in healthy women. *J Natl Cancer Inst* 1998, **90**, 1461–1467.
35. Johnston SR, Haynes BP, Sacks NP, *et al*. Effect of oestrogen receptor status and time on the intra-tumoural accumulation of tamoxifen and *N*-desmethyltamoxifen following short-term therapy in human primary breast cancer. *Breast Cancer Res Treat* 1993, **28**, 241–250.
36. Guerrieri-Gonzaga A, Baglietto L, Johansson H, *et al*. Correlation between tamoxifen elimination and biomarker recovery in a primary prevention trial. *Cancer Epidemiol Biomarkers Prev* 2001, **10**, 967–970.
37. Breuer B, Wallenstein S, Anderson R. Effect of tamoxifen on bone fractures in older nursing home residents. *J Am Geriatr Soc* 1998, **46**, 968–972.
38. Decensi A, Robertson C, Viale G, *et al*. A randomised trial of low-dose tamoxifen on breast cancer proliferation and blood estrogenic biomarkers. *J Natl Cancer Inst* 2003, **95**, 779–790.
39. Khan SA, Rogers MA, Khurana KK, *et al*. Estrogen receptor expression in benign breast epithelium and breast cancer risk. *J Natl Cancer Inst* 1998, **90**, 37–42.
40. Guerrieri-Gonzaga A, Galli A, Rotmensz N, *et al*. The Italian breast cancer prevention trial with tamoxifen: findings and new perspectives. *Ann NY Acad Sci* 2001, **949**, 113–122.
41. Bonanni B, Serrano D, Cazzaniga M, *et al*. A biomarker trial of low-dose tamoxifen in HRT users. *Proc Frontiers in Cancer Prev Res*, 69 [Abstract A2].
42. Baum M, Budzar AU, Cuzick J, *et al*. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet* 2002, **359**, 2131–2139.
43. ATAC Trialists' Group. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 2005, **365**, 60–62.
44. Coombes RC, Hall E, Gibson LJ, *et al*. A randomised trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *New Engl J Med* 2004, **350**, 1081–1092.
45. Goss PE, Ingle JN, Martino S, *et al*. A randomised trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *New Engl J Med* 2003, **349**, 1793–1802.
46. Goss PE, Ingle JN, Martino S, *et al*. Updated analysis of the NCIC CTG MA 17 randomised placebo (P) controlled trial of letrozole (L) after five years of tamoxifen in postmenopausal women with early stage breast cancer. *Proc Am Soc Clin Oncol* 2004, **23**, 87 [Abstract 84].
47. The Big 1-98 Collaborative Group. Letrozole versus Tamoxifen as adjuvant endocrine therapy for postmenopausal women with receptor-positive breast cancer. BIG 1-98: a prospective random-

ized double blind phase III study. In: *Primary therapy of early breast cancer conference*, St. Gallen, Switzerland; 2005 [Abstract S4].
48. Guerrieri-Gonzaga A, Decensi A, Serrano D, *et al*. Anastrozole and low dose tamoxifen in patients with breast carcinoma *in situ*: a pharmacokinetics and biomarker study. *Proc Frontiers in Cancer Prev Res*, 69–70 [Abstract A4].
49. Costa A, Formelli F, Chiesa F, *et al*. Prospects of chemoprevention of human cancers with the synthetic retinoid fenretinide. *Cancer Res* 1994, **54**, 2032s–2037s.
50. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. *Oncogene* 2003, **22**, 7305–7315.
51. Maurer BJ, Metelitsa LS, Seeger RC, *et al*. Increase of ceramide and induction of mixed apoptosis/necrosis by *N*-(4-hydroxyphenyl)-retinamide in neuroblastoma cell lines. *J Natl Cancer Inst* 1999, **91**, 1138–1146.
52. Reynolds CP, Maurer BJ, Kolesnick RN. Ceramide synthesis and metabolism as a target for cancer therapy. *Cancer Lett* 2004, **206**, 169–180.
53. Lovat PE, Di Sano F, Corazzari M, *et al*. Gangliosides link the acidic sphingomyelinase-mediated induction of ceramide to 12-lipoxygenase-dependent apoptosis of neuroblastoma in response to fenretinide. *J Natl Cancer Inst* 2004, **96**, 1288–1299.
54. Chan JM, Stampfer MJ, Giovannucci E, *et al*. Plasma insulin-like growth factor-1 and prostate cancer risk: a prospective study. *Science* 1998, **279**, 563–566.
55. Ma J, Pollack MN, Giovannucci E, *et al*. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-1 and IGF-binding protein-3. *J Natl Cancer Inst* 1999, **91**, 620–625.
56. Hankinson SE, Willet WC, Colditz GA, *et al*. Circulating concentrations of insulin-like growth factor-1 and risk of breast cancer. *Lancet* 1998, **351**, 1393–1396.
57. Giovannucci E, Pollack MN, Platz EA, *et al*. A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. *Cancer Epidemiol Biomarkers Prev* 2000, **9**, 345–349.
58. Decensi A, Veronesi U, Miceli R, *et al*. Relationships between plasma insulin-like growth factor-1 and insulin-like growth factor binding prote-3 and second breast cancer risk in a prevention trial of fenretinide. *Clin Cancer Res* 2003, **9**, 4722–4729.
59. Lotan R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy. *J Natl Cancer Inst* 1995, **87**, 1655–1657.
60. Mehta RG, Moon RC, Hawthorne M, *et al*. Distribution of fenretinide in the mammary gland of breast cancer patients. *Eur J Cancer* 1991, **27**, 138–141.
61. Veronesi U, De Palo G, Marubini E, *et al*. Randomised trial of fenretinide to prevent second breast malignancy in women with early breast cancer. *J Natl Cancer Inst* 1999, **91**, 1847–1856.
62. Decensi A, Bonanni B, Guerrieri-Gonzaga A, *et al*. A randomised 2×2 biomarker trial of low-dose tamoxifen and fenretinide in premenopausal women at high-risk for breast cancer. *Proc Am Soc Clin Oncol* 2004, **23**, 97 [Abstract 1001].
63. Miki Y, Swensen J, Shattuck-Eidens D, *et al*. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994, **266**, 66–71.
64. Wooster R, Stratton MR. Breast cancer susceptibility: a complex disease unravels. *Trends Genet* 1995, **11**, 3–5.
65. Ford D, Easton DF, Bishop DT, *et al*. Risks of cancer in BRCA1-mutation carriers – Breast Cancer Linkage Consortium. *Lancet* 1994, **343**, 692–695.
66. Easton DF, Steele L, Fields P, *et al*. Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12-13. *Am J Hum Genet* 1997, **61**, 120–128.
67. Rebbeck TR, Levin AM, Eisen A, *et al*. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. *J Natl Cancer Inst* 1999, **91**, 1475–1479.
68. Verhoog LC, Brekelmans CT, Seynaeve C, *et al*. Survival and tumour characteristics of breast-cancer patients with germline mutations of BRCA1. *Lancet* 1998, **351**, 316–321.
69. Johannsson OT, Idvall I, Anderson C, *et al*. Tumour biological features of BRCA1-induced breast and ovarian cancer. *Eur J Cancer* 1997, **33**, 362–371.
70. Karp SE, Tonin PN, Begin LR, *et al*. Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. *Cancer* 1997, **80**, 435–441.
71. Loman N, Johannsson O, Bendahl PO, *et al*. Steroid receptors in hereditary breast carcinomas associated with BRCA1 or BRCA2 mutations or unknown susceptibility genes. *Cancer* 1998, **83**, 310–319.
72. Phillips KA, Andrulis IL, Goodwin PJ. Breast carcinomas arising in carriers of mutations in BRCA1 of BRCA2: are they prognostically different? *J Clin Oncol* 1999, **17**, 3653–3663.
73. Wagner TM, Moslinger RA, Muhr D, *et al*. BRCA1-related breast cancer in Austrian breast and ovarian cancer families: specificBRCA1 mutations and pathological characteristics. *Int J Cancer* 1998, **77**, 354–360.
74. Narod SA, Brunet JS, Ghadirian P, *et al*. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Hereditary Breast Cancer Clinical Study Group. *Lancet* 2000, **356**, 1876–1881.
75. Foulkes WD, Goffin J, Brunet JS, *et al*. Tamoxifen may be an effective adjuvant treatment for BRCA1-related breast cancer irrespective of estrogen receptor status. *J Natl Cancer Inst* 2002, **94**, 1504–1506.
76. King MC, Wieand S, Hale K, *et al*. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: National Surgical Adjuvant Breast and Bowel Project (NSABP-P1) Breast Cancer Prevention Trial. *JAMA* 2001, **286**, 2251–2256.
77. Fan S, Ma YX, Wang C, *et al*. P300 modulates the BRCA1 inhibition of estrogen receptor activity. *Cancer Res* 2002, **62**, 141–151.
78. Ferlini C, Scambia G, Marone M, *et al*. Tamoxifen induces oxidative stress and apoptosis in oestrogen receptor-negative human cancer cell lines. *Br J Cancer* 1999, **79**, 257–263.
79. Chan KC, Knox WF, Gee JM, *et al*. Effect of epidermal growth factor receptor tyrosine kinase inhibition on epithelial proliferation in normal and premalignant breast. *Cancer Res* 2002, **62**, 122–128.
80. Davies G, Martin LA, Sacks N, *et al*. Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. *Ann Oncol* 2002, **13**, 669–678.