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Chemoprevention of Breast Cancer: The Italian Experience

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Abstract The activity of our group is focused on the conduction of chemoprevention clinical trials of breast cancer in at-risk subjects, among which we include women on hormone replacement therapy (HRT). The role of the insulin-like growth factor (IGF) system and of mammographic breast density as surrogate biomarkers for breast cancer prevention is also being investigated. The IGF system is involved in human carcinogenesis of several solid tumors. IGF-I is a potent mitogen for breast cancer cells; elevated circulating IGF-I levels have been associated with a higher risk of premenopausal breast cancer, prostate and colorectal cancer in prospective studies. Both tamoxifen and the synthetic retinoid fenretinide (4-HPR) have been shown to decrease plasma IGF-I levels. A trial of their combination is ongoing in premenopausal women with increased risk for breast cancer. Mammographic breast density has also been associated with an increased risk of breast cancer in several prospective studies. In this article, we discuss the rationale for selection of appropriate cohorts, candidate agents, and putative surrogate biomarkers in our breast cancer prevention trials. Moreover, updated results of the secondary prevention trial of 4-HPR and of the primary prevention trial of tamoxifen are presented. Finally, the rationale for a reduction of tamoxifen dose in future prevention trials is provided. J. Cell. Biochem. Suppl. 34:84–96, 2000.

Key words: chemoprevention; breast cancer; tamoxifen; fenretinide; IGF-I; surrogate biomarkers

The limited impact of the conventional cytotoxic approach to cancer cure and our increased understanding of cancer biology has generated efforts to devise a rational, mechanism-based approach to the inhibition of carcinogenesis [Sporn, 1996]. Among these new strategies, chemoprevention takes advantage of the pharmacological use of natural or synthetic agents to interfere with the carcinogenesis process at an early event stage. The progress made in cardiovascular diseases following a more comprehensive approach to the process of atherosclerosis, including timely treatment of risk disorders associated with end-stage cardiovascular events, has provided an important model for the chemoprevention of carcinogenesis.

APPROPRIATE COHORT SELECTION FOR BREAST CANCER PREVENTION TRIALS

As breast cancer is the most frequent malignancy in women in Western countries, attempts to prevent this disease by natural or synthetic agents have received increased attention. Different target populations for breast cancer chemoprevention may be recognized. A first level may involve primary prevention trials in a wide population of healthy women who have a higher, albeit moderate, risk of low-penetrance genetic factors (e.g., one first-degree relative with breast cancer) or life-style (e.g., delayed pregnancies), or because of exposure to known promoting agents (e.g., hormone replacement therapy (HRT)). Due to limited statistical power,

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however, these studies are extremely costly. A second level involves a limited population at very high risk because of highly penetrating genetic predisposition to cancer (e.g., *BRCA-1* and *BRCA-2* mutation carriers). Trials in this type of population may prove very efficient, but our limited understanding of the physiologic function of these genes has so far prevented the rational choice of effective agents. A third level may involve secondary prevention trials in subjects with premalignant or early malignant lesions (e.g., breast atypical hyperplasia and carcinoma in situ or microinvasive disease) or long-term survivors after adjuvant treatment. Although women with intraepithelial or minimally invasive breast cancer have a good prognosis, they have an elevated relative risk of the development of a second primary breast cancer compared with the general population, particularly before menopause. Data presented in the literature [Hankley et al., 1983] indicate that the incidence of contralateral breast cancer in premenopausal women is approximately 1% per year, that is, approximately 8–12 times higher than that of the general population in the same age range in northern Italy and the United States [Parkin et al., 1992]. Not surprisingly, the average incidence of premenopausal breast cancer in Italy is similar to that of the population in the United States. The rate of ipsilateral breast cancer, defined as outside the original quadrant, is even higher. Unpublished data from our trial of 4-HPR indicate an incidence of two to three times that of contralateral breast cancer. Thus, the overall risk of developing a second primary breast cancer in premenopausal women is 20–30 times higher than that of an age matched population. Therefore women with a diagnosis of noninvasive (ductal carcinoma in situ [DCIS]) or minimally invasive breast cancer are an important target for breast cancer chemoprevention and represent one of the most suitable cohorts for efficacy assessment of candidate agents through feasible and efficient clinical trials.

AGENT SELECTION

Tamoxifen

Tamoxifen is a nonsteroidal triphenylethylene derivative that can be classified as a first-generation selective estrogen receptor (ER) modulator. Tamoxifen is widely used for the palliative endocrine treatment of advanced breast cancer and as adjuvant therapy to control micrometastatic relapse and new primaries in women surgically treated for early breast cancer. It has been investigated in three large cooperative phase III trials for prevention of breast cancer in at-risk women. Preliminary results of two of these studies, the Italian Tamoxifen Prevention Study and the Royal Marsden Tamoxifen Chemoprevention Trial have recently been published [Veronesi et al., 1998; Powles et al., 1998] while the third study, the National Surgical Breast and Bowel Project P-1 Trial (NSABP P-1) has been reported in full [Fisher et al., 1998]. In addition to breast cancer chemoprevention, these trials are investigating other possible benefits of tamoxifen suggested by previous clinical trials: decreased cardiovascular morbidity and mortality, as well as prevention of osteoporosis in postmenopausal women [Nayfield, 1995].

An interim analysis of the NSABP P-1 involving 13,388 participants has led to the early closure of the study [Fisher, 1998]. It was shown that 20 mg/day of tamoxifen can approximately halve the incidence of invasive and in situ breast cancer and decrease by approximately 20% the incidence of osteoporotic bone fractures. Compared with the placebo group, however, women aged 50 or older receiving tamoxifen had more than a twofold increased risk of early-stage endometrial cancer and a threefold increased risk of pulmonary embolism [Fisher, 1998]. Altogether, these results underline the importance of strategies aimed at reducing tamoxifen's toxicity while retaining its activity, particularly in postmenopausal women. Notably, the results of the European trials do not appear to confirm the striking benefit observed in the NSABP P1 trial [Veronesi et al., 1998; Powles et al., 1998]. Based on the results of the NSABP trial, however, the Food and Drug Administration has approved the use of tamoxifen to reduce the risk of breast cancer in subjects at increased risk as assessed by the Gail model. This provides the first example of a medication approved and marketed as a cancer preventative agent, a concept which is likely to be expanded in clinical practice in the near future.

The therapeutic efficacy of tamoxifen has largely been proven. In a recent update of the worldwide overview of data on 37,000 women with early breast cancer from 55 randomized trials of adjuvant tamoxifen treatment [Early Breast Cancer Collaborative Group, 1998], this compound, given for 1 year, 2 years, and 5 years

of adjuvant treatment gave a corresponding proportional mortality reduction (mean ±SD) of 12% ± 3, 17% ± 3, and 26 ± 4. In terms of other outcomes among all women studied (i.e., including those with "ER-poor" tumors), the proportional mean ±SD reductions in contralateral breast cancer were 13% ± 13, 26% ± 9, and 47% ± 9 in the trials of 1, 2, or about 5 years of adjuvant tamoxifen. The incidence of endometrial cancer was approximately doubled in trials of 1 or 2 years of tamoxifen and approximately quadrupled in trials of 5 years of tamoxifen (although the number of cases was small and these ratios were not significantly different from each other). An excess of deaths from endometrial cancer was observed in the tamoxifen arm. The absolute decrease in contralateral breast cancer was about twice as large as the absolute increase in the incidence of endometrial cancer. Tamoxifen had no apparent effect on the incidence of colorectal cancer or, after exclusion of deaths from breast or endometrial cancer, on any of the other main categories of cause of death (total nearly 2,000 such deaths; overall relative risk 0.99 ± 0.05 SD). These results show that tamoxifen given for some years can substantially increase the 10-year survival of women with ER-positive tumors and of women whose tumors are of unknown ER status, with the proportional reductions in breast cancer recurrence and in mortality appearing to be largely unaffected by other patient characteristics or treatments.

Moreover, the recent results of the NSABP B-24 Trial [Fisher et al., 1999] have shown that women with ductal carcinoma in situ treated by lumpectomy and radiation therapy had additional benefit from subsequent tamoxifen treatment. In the tamoxifen group fewer breast cancer events at 5 years were reported compared to the placebo group (8.2 vs 13.4%, respectively; $p = 0.0009$). The advantage was mainly due to a decrease in the rate of invasive cancer (especially in the ipsilateral breast), but was noticeable also in the rate of invasive and noninvasive cancers in the contralateral breast.

In general, tamoxifen is well tolerated. Experience from adjuvant therapy clinical trials reflects a 5–10% dropout rate [Nayfield, 1995]. However, the compliance rate among women in the European chemoprevention studies appears lower, approximately 70–75% at 2 years [Veronesi et al., 1998; Powles et al., 1998]. Hot flushes and other vasomotor symptoms are the most commonly reported side effects; approximately 15–20% of women receiving tamoxifen develop hot flushes attributable to the drug. These symptoms appear more commonly among younger women [Powles et al., 1990] despite the elevated levels of estradiol and total estrogens reported among premenopausal patients receiving tamoxifen [Jordan et al., 1991]. The other most frequently reported side effects (15–20%) are vaginal discharge and dryness, urinary disturbances secondary to urogenital atrophy, nausea, gastrointestinal disturbances, rapid pulse, and weight gain. Menstrual irregularities have also been observed. Antithrombin III activity is decreased in postmenopausal patients, and this may in part account for the increased risk of venous thromboembolic events that has been reported in two large adjuvant trials [Fisher et al., 1989; McDonald et al., 1995] and in two prevention trials [Fisher et al., 1998; Veronesi et al., 1998]. Ocular effects (retinal deposits, keratinopathy, cataract) have occurred at high doses; however, some recent reports suggest a lower incidence of such disorders at the chemopreventive trial dose of 20 mg/day [Gorin et al., 1998].

A major concern remains, however: the increased risk of endometrial cancer associated with tamoxifen administration. While an increased risk was initially observed only in Scandinavian trials at daily doses of 40 or 30 mg [Rutqvist et al., 1993], the NSABP B-14 trial in women with stage I breast cancer receiving tamoxifen for 5 years as adjuvant treatment at the daily dose of 20 mg has demonstrated a sevenfold risk of endometrial cancer compared with placebo subjects and a more than twofold risk compared with the population-based rates [Fisher et al., 1994].

More recently, the NSABP P1 trial [Fisher, 1998] has shown an increase of early-stage endometrial cancer in postmenopausal women in the tamoxifen group compared with the placebo group (37 vs 18, respectively). However, all cases except one in the placebo group were at an initial stage and no death from endometrial cancer has been reported in the tamoxifen arm.

The Synthetic Retinoid 4-HPR

Natural retinoids play a crucial role in cellular proliferation and differentiation, but their poor clinical tolerability has prevented the use of these compounds as cancer preventive agents. Toxic symptoms that may be acceptable in treat-

ing established cancer are not considered acceptable for reducing cancer risk. One of the less toxic vitamin A analogues studied for breast cancer chemoprevention is 4-HPR, a synthetic amide derivative of *all-trans*-retinoic acid [Kelloff et al., 1994a; Costa et al., 1994]. The inhibition of chemically induced mammary carcinoma in rats by 4-HPR was first described by R. Moon and M.B. Sporn [Moon et al., 1979]. On this basis, 4-HPR was proposed for chemoprevention trials in human breast cancer. This compound has been studied extensively and proved less toxic than many other retinoids [Kelloff et al., 1994a; Costa et al., 1994; Naik et al., 1995].

Since early reports showing adverse retinal and dermatologic effects at daily doses of 600–800 mg had succeeded in halting the clinical development of this compound [Kaiser-Kupfer et al., 1986; Kingstone et al., 1986], a phase I/II study led us to identify 200-mg/day administration with a monthly 3-day interruption as the best tolerated schedule to permit partial retinol recovery and storage in the retina [Costa et al., 1989; Rotmensz et al., 1991]. In contrast to retinoic acid, it was demonstrated that (1) 4-HPR blood levels remain constant during administration for as long as 5 years [Formelli et al., 1989, 1993], (2) the drug selectively accumulates in the human breast [Metha et al., 1991], (3) a significant decline of plasma retinol levels is responsible for the increased rod thresholds that occur in a certain proportion of subjects [Decensi et al., 1994]. It was also shown that 4-HPR causes an early drop in plasma retinol concentrations [Formelli et al., 1989] and that the plasma level of N-(4-methoxyphenyl)retinamide (the principal metabolite of 4-HPR) is also associated with the retinol decrease [Torrisi et al., 1994].

Although 4-HPR was synthesized nearly 20 years ago, its mechanism of action has only recently been partially elucidated. This retinoid appears to be the prototype of a new class of selective ligands of the retinoic acid receptors in which the transactivation function can be separated from the transrepression function [Fanjul et al., 1994, 1996]. This selective binding to the nuclear receptors is likely to be the basis for its specific biological activities and its favorable pharmaceutical properties [Fanjul et al., 1996]. Interestingly, 4-HPR is a potent inhibitor of the pivotal AP-1 transcription factor that regulates the *jun-fos* proto-oncogene-mediated cell growth signal [Fanjul et al., 1994]. Since the AP-1 motif is required for IGF-I-mediated estrogen proliferative stimulation [Umayara et al., 1994], this may be an important pathway by which 4-HPR inhibits breast proliferation. Indeed, 4-HPR has been shown to inhibit the estrogen target genes pS2 and PgR in breast cancer cell lines, providing further evidence for an interference with the ER signal transduction pathway [Kazmi et al., 1996]. Moreover, 4-HPR appears to be a potent inhibitor of the IGF system in breast cancer cell lines, and this is an important mechanism of tumor cell growth inhibition by the retinoid [Favoni et al., 1998].

In recent years, 4-HPR has been shown to be active both in vitro and in vivo against mammary, bladder, lung, ovary, cervix, neuroblastoma, leukemia, and prostate preclinical models [Lotan, 1995]. A characteristic feature of 4-HPR is the ability to inhibit cell growth through the induction of apoptosis, rather than differentiation, an effect that is strikingly different from that of the parental compound *all-trans*-retinoic acid and that may occur even in retinoic acid resistant cell lines [Delia et al., 1993; Ponzoni et al., 1995]. On the basis of the selective accumulation of 4-HPR in the human breast [Moon et al., 1979] and the good tolerability in humans [Costa et al., 1989], in 1987 we started a phase III trial aimed at reducing contralateral breast cancer. Preliminary findings of this trial are discussed below.

Rationale for the Combination of Tamoxifen and 4-HPR

The concept of combining multiple agents with different activities to enhance activity and minimize toxicity has been pursued for quite some time in cancer chemoprevention research [Sporn, 1980]. In addition to a mechanistic rationale for this combination, synergistic efficacy has been observed in vivo in animal studies of tamoxifen/tamoxifen citrate in combination with 4-HPR at lower less toxic doses [Ratko et al., 1989]. The dietary combination of 4-HPR with tamoxifen citrate (0.125 mg/kg diet, or ca. 0.011 nmol/kg per body weight per day) showed synergistic activity against methylnitrosurea (MNU)-induced mammary carcinogenesis in older rats [Kelloff et al., 1994a].

Subchronic (90-day) toxicity studies of tamoxifen citrate at 0.4–32 mg/kg body weight per day (Ig), alone and in combination with 4-HPR, in

two species have been performed. In female Beagle dogs, no synergistic toxicity was observed with a capsule formulation of combined agents. Moreover, subtoxic doses of tamoxifen combined with 4-HPR have produced synergistic chemopreventive effects in rat mammary carcinogenesis models [Kelloff et al., 1994b]. A phase I trial of 20 mg tamoxifen per day in combination with increased doses of 4-HPR has recently been performed [Cobleigh et al., 1993]. In consecutive cohorts of three metastatic breast cancer patients each, levels of 4-HPR were 100, 200, 300, and 400 mg/day with a 3-day drug holiday per month. Duration of treatment ranged from 2–14 months, with a total of six patients receiving ≥6 months of therapy. Adverse effects (anemia, altered hepatic enzymes) were attributed to progressive disease, and all combinations were safe and well tolerated. In a recent pilot study in at-risk women, the combination of tamoxifen 20 mg/day and 4-HPR 200 mg/day was also well tolerated [Zujewski et al., 1997].

RATIONALE FOR BIOMARKERS SELECTION IN BREAST CANCER PREVENTION TRIALS

High costs are inherent to prevention trials using clinical endpoints, where a huge number of subjects and years of follow-up evaluation are necessary. Even in a high-risk population in which the rate of second primary breast cancers is approximately 3% per year (i.e., premenopausal women with a personal history of breast cancer), to detect a 15% reduction in the hazard of breast cancer in a 2-year trial of of 4-HPR with a 3-year follow-up would require in excess of 10,000 patients. New large trials may not be justified, and preliminary information can be gathered only from a smaller study of surrogate endpoints. If this is successful, then there is a stronger case for justifying a larger trial. Also, the risk of unexpected detrimental effects has recently been highlighted [α-Tocopherol, β-Carotene Cancer Prevention Study Group, 1994], and great emphasis has been put on the search for intermediate, surrogate endpoints.

Surrogate endpoints are biological markers or events that may be assessed or observed before the clinical appearance of the disease and that bear some relationship to the development of that disease. They are intermediate in the sense that they occur sometime between a given intervention that affected the disease process and the time of the clinical diagnosis of the disease. The use of surrogate endpoint biomarkers (SEBs) in pivotal cancer chemoprevention trials may lead to a rational choice of agents that are likely to affect cancer incidence in subsequent phase III trials.

The importance of developing non invasive methods to assess SEBs has recently been emphasized [Kelloff et al., 1997]. In particular, the higher feasibility and acceptability of serum/plasma-based SEBs over tissue-based SEBs are likely to increase the efficiency and ethicality of clinical prevention trials. Furthermore, recruitment rate is likely to be higher when noninvasive techniques are employed.

Circulating Plasma IGF-I

The IGF system plays a pivotal permissive role in cell proliferation of both epithelial and mesenchymal tissues in at least three different ways: (1) it is highly mitogenic, (2) it protects normal and tumor cells from apoptosis, (3) it is required in several types of cells for the establishment and maintenance of the transformed phenotype and for tumorigenesis [LeRoith et al., 1992; Baserga 1995; LeRoith et al., 1995]. There is also growing experimental, epidemiological, and clinical evidence that the IGF system is important in breast carcinogenesis [Bruning et al., 1992; Stoll, 1993; Kazer, 1995]. A summary of the rationale for the use of circulating IGF-I as a SEB for breast cancer is provided in Table I. In vitro, IGF-I is one of the most potent mitogens for breast cancer cell lines,

TABLE I. Rationale for the Use of Circulating IGF-I as a Surrogate Biomarker for Breast Cancer

1. Mitogenic, antiapoptotic, tumorigenic in experimental systems [Baserga, 1995]
2. Stimulates normal mammary epithelial proliferation in primates [Ng et al., 1997]
3. Mediates estrogen effect in breast cancer cells [van der Burg et al., 1990]
4. Activates ER pathway in the absence of E2 [Kato et al., 1995a]
5. Has prognostic effect in breast cancer tissue [Foekens et al., 1989; Bonneterre et al., 1990]
6. Modulated by tamoxifen [Wakeling et al., 1989] and 4-HPR in vitro [Favoni et al., 1998] and in vivo [Torrisi et al., 1993]
7. Reflects 4-HPR preventive activity [Torrisi et al., 1998]
8. Predicts premenopausal breast cancer risk [Hankinson et al., 1998]

where it mediates the estrogen action [van der Burg et al., 1990]. Importantly, the interaction between IGF-I and ER is mutual, since IGF-I has been shown to function as a potent stimulatory factor of the estrogen signaling pathway in the absence of estrogen [Kato S et al., 1995]. Several studies have recently demonstrated that the antiproliferative effect exerted by retinoids on breast cancer cell lines is mediated by the inhibition of the IGF-stimulated growth [Fontana et al., 1991; Adamo et al., 1992]. In humans, 4-HPR was shown to modulate plasma IGF-I levels [Torrisi et al., 1993]. As IGF-I can stimulate normal epithelial breast proliferation and promote breast cancer cell growth both in vitro and in vivo, and because higher circulating IGF-I levels have been found in breast cancer women compared with healthy controls, the change in IGF-I levels may be considered as a potential surrogate endpoint of breast cancer inhibition [Torrisi et al., 1998]. Indeed, recent results from the Nurses' Health Prospective Study [Hankinson et al., 1998] indicate that premenopausal women with higher IGF-I levels have an increased risk of developing breast cancer compared with women with lower levels (Table II). Moreover, our observation that the modulation of IGF-I by 4-HPR reflects its clinical effect on second primary breast cancers supports the role of this biomarker as a suitable surrogate endpoint in chemoprevention clinical trials of breast cancer [Decensi et al., 1997].

Both antiestrogens and retinoids can regulate IGF-I synthesis in humans [Pollak et al., 1990; Torrisi et al., 1993]. As they act through nuclear receptors belonging to the same steroid/thyroid/retinoid superfamily, they likely interfere with IGF-I through common pathways. For instance, estrogens have been shown to regulate IGF-I gene expression through the transcription factor AP-1 [Umayara et al., 1994], while retinoids can negatively regulate AP-1-responsive genes [Schüle et al., 1991]. Moreover, the hormonal regulation of IGF-I synthesis appears to be complex with estrogens acting as dose-dependent stimulatory or inhibitory agents [Pollak et al., 1990]. Specifically, physiological concentrations such as those achieved through transdermal HRT tend to increase IGF-I levels, while pharmacological doses such as those achieved in the liver circulation after oral administration induce a decline of IGF-I levels.

TABLE II. Relative Risk of Breast Cancer by Plasma IGF-I Levels

Cases/control: 397 breast cancers/620 age-matched controls	
Time from blood collection to diagnosis	8 months (1–57)
All, top versus bottom quintile of IGF-I	RR = 0.99 (0.65–1.50)
Postmenopausal, top versus bottom quintile	RR = 0.85 (0.53–1.39)
Premenopausal, top versus bottom quintile	RR = 2.88[a] (1.21–6.85)
Premenopausal <50 years, top versus bottom tertile	RR = 7.28* (2.40–22.0)

Based on Hankinson et al. [1998].
[a]Adjusted for IGFBP-3.

Mammographic Density

There is also a consistent line of evidence that a higher mammographic density is associated with an increased risk of breast cancer (≥50% ≈ RR = 4–5 compared with women with lucent mammograms). There is a high degree of consistency among eight epidemiological studies that the higher category of breast density (>75%) has an approximately fivefold increased risk of breast cancer compared with the lower category. This conclusion is based primarily on three prospective nested case-control studies [Boyd et al., 1995a; Byrne et al., 1995; Kato I et al., 1995] (Table III).Thus, in contrast to the conflicting results provided by the qualitative classification of Wolfe, a quantitative assessment (by visual inspection) of the percentage of breast density appears to be uniformly associated with an increased risk of breast cancer. Moreover, at variance with most other risk determinants, this factor may potentially be modified by some forms of intervention [Ursin et al., 1996; Boyd et al., 1997], providing the endpoint for new preventive interventions.

Dense breasts at mammography are associated with a higher incidence of early preneoplastic lesions (e.g., atypical hyperplasia and DCIS) and are characterized by a predominant stromal component, supporting the contention that stromal-epithelial interactions play a significant role in breast carcinogenesis [Boyd et al., 1992]. Boyd and colleagues have shown that the use of mammographic density ≥50% as an entry criterion for a chemoprevention trial can select a population with an incidence of breast

TABLE III. Effect of Percentage Mammographic Density on Breast Cancer Risk in Prospective Studies

Reference	No. of cases	No. of controls	Density (%)	Adjusted odds ratio (OR)	95% CI
Saftlas et al. [1991]	67	58	45–65	3.8	2.1–3.6
	45	33	>65	4.3	2.1–8.8
Boyd et al. [1995a]	66	31	>75	6.1	2.8–13
Byrne et al. [1995]	194	136	>75	4.4	3.1–6.1
	576	554	50–74	2.8	2.1–3.6
Kato I. et al. [1995]	37	99 (pre)	>66	3.6	1.7–7.9
	48	81 (post)	>66	2.1	1.1–3.8

cancer which is 4–5 times higher than the age standardized incidence rate in that age range [Boyd et al., 1995b]. While visual (manual) quantification of density can readily be applied as an entry criterion for defining at-risk subjects, quantitative measurement of density by instrumental or computerized methods can more reliably be applied to assess variations during intervention [Boyd et al., 1995a; Byrne et al., 1995].

Results from a randomized trial of low dietary fat intervention have shown that density can be modulated, particularly in the perimenopausal group [Boyd et al., 1997]. Tamoxifen has also been shown to be associated with a reduction of mammographic density in a recent pilot study [Ursin et al., 1996]. By contrast, HRT can increase mammographic density by 17–73% according to different methods and studies [Laya et al., 1995, 1996; Stomper et al., 1990]. Thus, there is evidence for a hormonal regulation of mammographic density as well as for a modulation by active agents that can modulate breast cancer risk, indicating that this factor may be another suitable surrogate endpoint for breast cancer in chemoprevention trials.

ONGOING BREAST CANCER CHEMOPREVENTION TRIALS

Phase III Multicenter Trial of 4-HPR for the Prevention of Contralateral Breast Cancer

A large multicenter phase III trial of the synthetic retinoid 4-HPR to prevent contralateral breast cancer was initiated in 1987 and accrued until 1993. A detailed description of the study design and recruitment has recently been published [De Palo et al., 1997]. Briefly, 2,972 women with a history of stage I breast cancer were randomized to 4-HPR or no intervention for 5 years. The primary endpoint of the study was the occurrence of contralateral breast cancer as the first malignant event. The eligible women were diagnosed with stage I invasive breast cancer or DCIS within the previous 10 years and have undergone definitive surgery without adjuvant hormone or chemotherapy. The women were randomized to 200 mg/day of 4-HPR versus no intervention. Although the limitation of toxicity evaluation without a placebo group was realized, the use of placebo was not considered advantageous becaue of the long duration of intervention, the large size of the placebo capsule, and the likelihood that nonspecific effects of an intervention are generally early events. The women are followed with a complete medical examination and blood tests every 6 months, a mammogram and chest radiography every year, and a bone scan every 18 months. The results are expected to mature at the end of 1999, when most women will have completed their 7-year follow-up management period.

The adverse events in the experimental arm that most commonly led to discontinuation of 4-HPR were alterations in dark adaptation with an abnormal ERG (n = 24), significant dermatological disorders (n = 19), and severely altered liver function tests (n = 9). However, the toxicity in the control group included an alteration in liver function tests (n = 20), abnormal lipid profiles (n = 4), and dermatological disorders (n = 2). Adverse events included all nonneoplastic adverse events for which treatment was discontinued, related, or unrelated to 4-HPR. Serious adverse events seen were ocular disorders such as retinal dialysis, vitreous detachment, and retinopathy as the most common alterations. Overall, there were 29 patients with ocular events in the 4-HPR group versus 12 in the control group. However, because of the high rate of retinal function tests in the 4-HPR group and the lack of placebo, an

increased detection bias is likely. Other adverse events included disturbances of the liver and gastrointestinal system (12 4-HPR vs 5 control), the cardiovascular system (18 vs 9, including 7 vs 4 ischemic heart diseases and 8 vs 3 arrhythmias), the neural system (9 vs 7), the renal system (3 vs 0), and other (12 vs 12).

A total of 250 subjects (8.5 % of the evaluable subjects) dropped out during the study, with a refusal rate of twofold in the 4-HPR arm. The predominant reasons ascertained for refusal in the 4-HPR arm were subjective intolerance (n = 40), fear of side effects (n = 35), other psychological reasons (n = 20), and external advice (n = 8). Refusal in the control group was due primarily to unwillingness to conform to the follow-up procedures (n = 63). Compliance to treatment as assessed by capsule count appears to be high, as approximately 90% of the population taking 4-HPR had a drug intake >80% as assessed by capsule count. These figures are reasonably good for such a prolonged study and illustrate the good level of adherence one may expect from a secondary prevention trial in high-risk subjects.

An exploratory analysis of the study was performed after a median of 75 months or two-thirds of the person-years of total follow-up times. The analysis shows a reduction in contralateral breast cancer in the premenopausal women who have received 4-HPR: the risk of contralateral breast cancer was reduced by approximately 40% (of borderline statistical significance). By contrast, a nonsignificant trend to an increase in contralateral tumors was observed in postmenopausal women [Decensi et al., 1997]. Interestingly, a similar pattern was observed on the change in circulating IGF-I in a subset of 78 consecutive women from the phase III trial [Torrisi et al., 1998]. Specifically, whereas IGF-I levels in untreated controls decline with age, a reverse effect is observed with 4-HPR treatment. The joint effect of age and 4-HPR treatment and on plasma IGF-I is reported in Figure 1.

Although qualitative interactions (differences in kind) are known to be uncommon in clinical trials [Yusuf et al., 1991], the influence of the dramatic hormonal changes that occur at menopause, the complex incidence pattern of breast cancer over age (the Clemmensen's hook at menopause), and the different therapeutic approach for pre- and postmenopausal patients

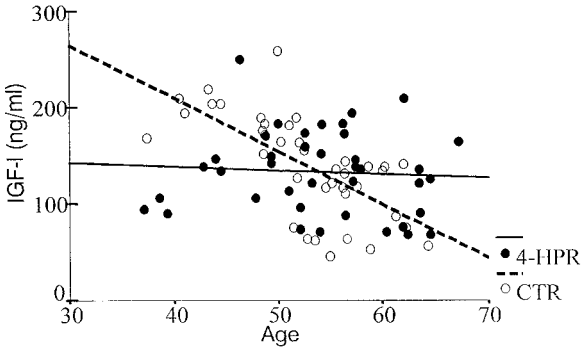

Fig. 1. Case of qualitative interaction between 4-HPR treatment and age on the change in plasma IGF-I. In untreated controls, IGF-I levels decline with age; a reverse effect is observed during 4-HPR treatment.

suggest two different disease entities [de Waard et al., 1979]. Another example of qualitative interaction with menopausal status is the effect of body weight on the risk of breast cancer. Lean premenopausal women are at higher risk of breast cancer than are overweight women, in direct contrast to the effect seen in postmenopausal women [London et al., 1989]. Thus, the complex results of our trial appear to be biologically plausible, even though they derive from post hoc observations and are therefore hypothesis generating.

Phase III Multicenter Trial of Tamoxifen in Healthy Women

A second large multicenter breast cancer chemoprevention trial was initiated in October 1992. The study, designed as a double-blind placebo-controlled trial, evaluates the effect of tamoxifen, given at daily dose of 20 mg p.o. for 5 years, on the prevention of breast cancer in healthy women. Eligible subjects are well women aged 35–70 years, who had undergone prior hysterectomy for nonmalignant conditions. A detailed description of the trial has recently been published [Veronesi et al., 1998]. The primary endpoint of this study is the incidence of breast cancer. Secondary endpoints are the incidence of cardiovascular events and bone osteoporotic fractures as well as the toxicity of treatment. Recruitment was stopped December 31, 1997 with 5,408 women randomized. As of this date, 1,404 (26%) subjects dropped out of the study. The main reasons for dropout are shown in Figure 2. Several factors external to the study contributed to the high rate of withdrawal, including bad publicity in the media

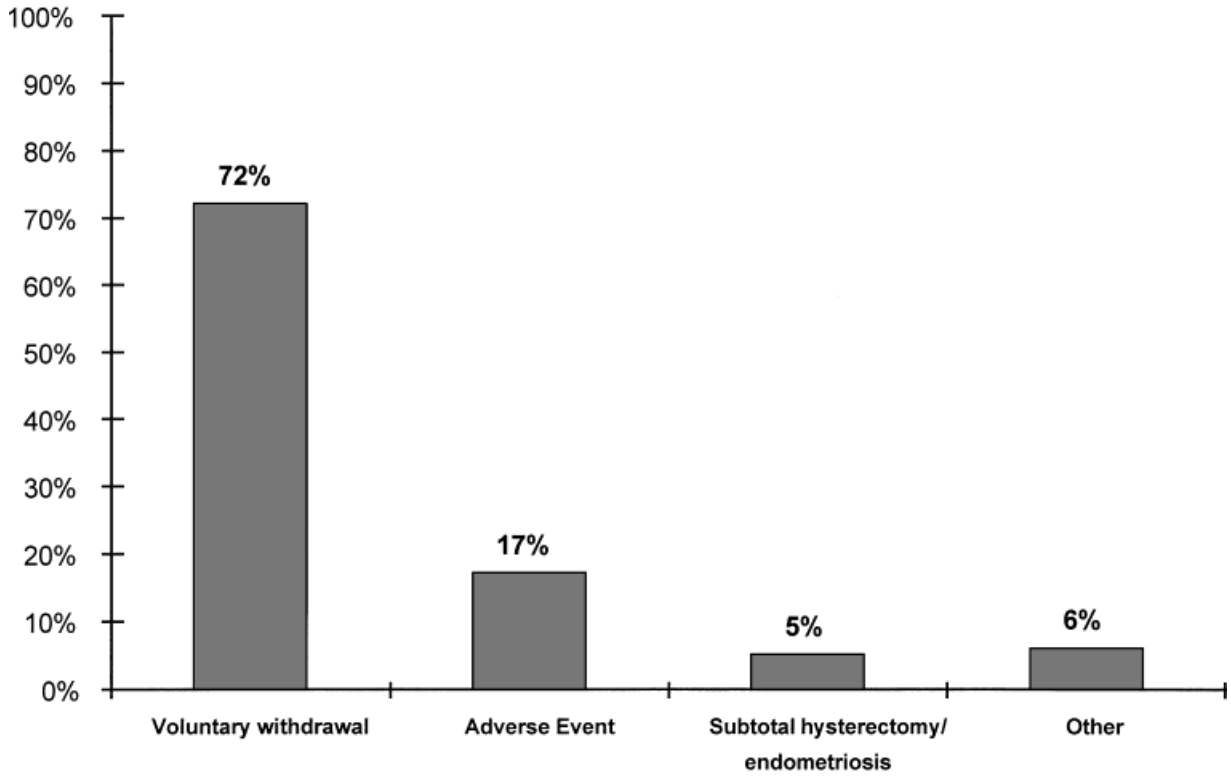

Fig. 2. Main reasons for dropout rate in the Italian Tamoxifen Trial.

after the inclusion of tamoxifen in the list of class A carcinogens by the International Agency on Cancer Research (IARC) in 1996. Because of the low rate of recruitment, the Data Monitoring Committee advised to stop recruitment before the planned date.

Preliminary results after a median of 46 months show no difference in breast cancer incidence between arms [Veronesi et al., 1998]. Of the 41 cases of breast cancer that have occurred so far, 22 cases were in the placebo group and 19 cases in the tamoxifen-treated group. Among women on intervention from more than 1 year, there was a trend to a beneficial effect of tamoxifen (11 in the tamoxifen arm versus 19 in the placebo arm, $P = 0.16$). A borderline significant reduction of breast cancer was observed among women who were HRT users and received tamoxifen. Compared with 8 cases of breast cancer occurred among the 390 HRT users who were on placebo, there was 1 case of breast cancer among the 362 HRT users who were receiving tamoxifen (RR = 0.13, 95% confidence interval [CI] = 0.02–1.02). There was an increased risk of venous vascular events (38 women on tamoxifen versus 18 women on placebo, $P = 0.0053$), consisting mainly of superficial phlebitis, and 15 cases versus 2 cases of severe hypertriglyceridemia in the tamoxifen and placebo arms, respectively, $P = 0.0013$).

As the combination of tamoxifen and transdermal HRT might reduce risks and side effects of either agent, we tested their combined effect on several cardiovascular risk factors, including blood cholesterol levels, within the trial [Decensi et al., 1998a]. Compared with small changes in the placebo group, tamoxifen was associated with changes in total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol of −9%, −14%, and −0.8%, respectively, which were similar in continuous HRT users and never-HRT users. By contrast, the decrease induced by tamoxifen of total and LDL cholesterol was blunted by two-thirds in women who started HRT while on tamoxifen. Thus, the beneficial effects of tamoxifen on cardiovascular risk factors are unchanged in current HRT users, while they may be attenuated in women who start transdermal HRT while on tamoxifen.

An interim analysis of the UK pilot prevention trial has also been published [Powles et al., 1998]. In this study, 2,494 healthy women, aged 30–70, at increased risk of breast cancer because of family history were accrued between 1986 and 1996. They were double-blind random-

ized to receive tamoxifen 20 mg/day of placebo for ≤8 years. The primary endpoint was the occurrence of breast cancer. After a median follow-up of 70 months, the results demonstrate the same overall frequency of breast cancer in both arms (tamoxifen 34, placebo 36, RR = 1.06 [95% CI 0.7–1.7], $P = 0.8$). Interestingly, women who were already receiving HRT (mostly by the oral route) when they entered the trial showed an increased risk of breast cancer as compared with nonusers, while the subjects who started HRT while on trial had a significantly reduced risk.

Comparison of the preliminary results among the three studies [Veronesi et al., 1998; Powles et al., 1998; Smigel, 1998] might suggest that the efficacy of tamoxifen varies depending on the type of population: high in moderate-risk subjects (U.S. trial), moderate in low-risk subjects (Italian trial), and none in women at high risk (UK trial). Given the complexity of the issue, however, further results are clearly demanded.

As appropriately discussed in a recent editorial [Pritchard, 1998], after the European trials have so far failed to confirm the results of the U.S. study, doubts may rise on the advisability of a rush to prescribe tamoxifen widely for prevention. There is still a need for a longer follow-up evaluation of current studies to better realize the balance between risks and benefits in different populations and to confirm the U.S. data. Moreover, no reliable data on mortality are provided by all the trials and these will be needed too to assess the ultimate preventive efficacy of this drug.

Phase II Trial of Tamoxifen at Low Doses

Although tamoxifen is the most widely described anticancer drug and is currently being evaluated as a multidisease preventative, its minimal active dose is unknown. This is quite surprising, given the observation from in vitro experiments [Sutherland et al., 1987] of a plateau of tumor growth inhibition above the concentration level that saturates the ER and the lack of a dose-response effect between 20 mg and higher daily doses observed in the worldwide overview of the tamoxifen trials as an adjuvant treatment [Early Breast Cancer Trialists' Collaborative Group, 1998]. In addition, the dose-response relationship on liver cancer induction observed in the rat model [Li et al., 1997] and the potential dose-relationship on the risk of endometrial cancer which is inferred from clinical trials [Rutqvist et al., 1995] provide substantial background to study lower doses of tamoxifen as a plausible attempt to minimize toxicity while retaining activity. Finally, the half-life of tamoxifen (on the order of 1 week) suggests that a once-a-day regimen may lead to an unnecessary drug accumulation in target issues.

All these considerations prompted us to study the biological activity of tamoxifen at reduced doses in a recent trial in healthy women, where we showed that 10 mg/day and 10 mg every other day were comparable to the conventional dose of 20 mg/day of tamoxifen in modulating a broad spectrum of intermediate biomarkers of several diseases, including cardiovascular disease, breast cancer, and osteoporosis [Decensi et al., 1998b].

In two sequential randomized trials, a total of 127 healthy hysterectomized women aged 35–70 years were allocated to one of the following four arms: placebo (n = 31) or tamoxifen at 20 mg/day (n = 30), in the first trial; and tamoxifen 10 mg/day (n = 34) or tamoxifen 10 mg per alternate day (n = 32), in the second trial. Comparison was made between baseline and 2-month measurements of various parameters: total cholesterol (which was the primary endpoint), LDL, and HDL cholesterol, triglycerides, lipoprotein(a), blood cell count, fibrinogen, antithrombin III, osteocalcin and, in a subgroup of 103 women, IGF-I. After adjustment for the baseline values, the changes in total cholesterol and IGF-I were of the same magnitude in all three tamoxifen arms; a similar pattern was observed for most of the other parameters. No change occurred in the placebo

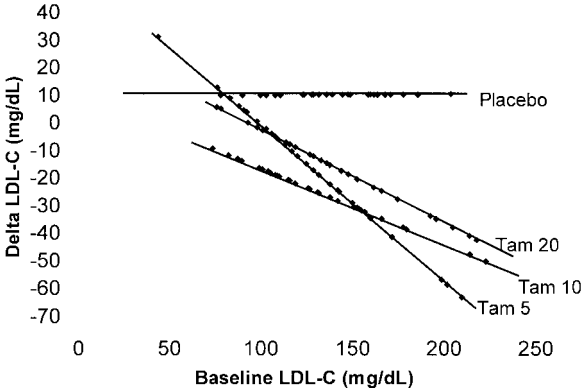

Fig. 3. Effect of 2 months of tamoxifen at different doses on the change in LDL cholesterol according to baseline LDL cholesterol values. The slope of the relationship between the change in LDL cholesterol and the baseline values in all tamoxifen groups is different from that in the placebo group ($P = 0.08$, F-test).

arm except for fibrinogen levels. The behavior of LDL-cholesterol in the four treatment arms is illustrated in Figure 3.

These results indicate that a 75% reduction in the conventional dose of tamoxifen does not affect the activity of the drug on a large number of biomarkers, including several surrogate markers of cardiovascular disease and circulating IGF-I, a likely surrogate marker for breast cancer. Future trials are clearly warranted to assess the efficacy and the safety of tamoxifen at low doses. Finally, comparison of low-dose tamoxifen with novel selective ER modulators with potentially improved safety profiles [Delmas et al., 1997] could provide important clues for the choice of safe and effective preventive approaches for a wide range of estrogen-related diseases.

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