Breast Cancer Chemoprevention: Studies With 4-HPR Alone and in Combination With Tamoxifen Using Circulating Growth Factors as Potential Surrogate Endpoints

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Abstract Fenretinide (4-HPR), a synthetic derivative of retinoic acid, has proven effective at inhibiting *in vitro* breast cancer cell growth and preventing the progression of chemically induced mammary carcinoma in rodents. Our group has made a particular effort with regard to this molecule in clinical studies aimed at evaluating its pharmacology, toxicity, and efficacy in breast cancer prevention. We have demonstrated that 4-HPR blood levels remain constant during administration for as long as 5 years, that the drug accumulates in the human breast, and that it induces a significant decline of plasma insulin-like growth factor-I (IGF-I) levels. To date, 2,972 Stage I breast cancer patients have been randomized to evaluate the efficacy of a 5-year administration of 4-HPR to prevent new contralateral primary breast cancers. Compliance to protocol and treatment is high and tolerability of the drug is good; only 51 women out of 1,397 (3.6%) had to interrupt drug intake due to toxicity. The only potential limitation to the extensive use of 4-HPR is diminished dark adaptation, which occurs in about one-fourth of the patients and is dependent on the decline of plasma retinol below the threshold level of 100 ng/ml. Plasma levels of (4-methoxyphenyl)retinamide (4-MPR), the principal metabolite of 4-HPR, which are higher in elderly women with a high percentage of adipose tissue, are the major determinants of the retinol decrease. However, about 50% of the patients with altered dark-adaptometry are asymptomatic and the alterations are promptly reversible upon drug discontinuation. Since the combination of 4-HPR with the antiestrogen tamoxifen has shown a synergistic activity in the prevention of breast cancer in preclinical models, it is currently an important avenue of investigation in an attempt to reduce human breast cancer incidence and mortality. Moreover, a dose reduction of one or both agents in an effort to minimize toxicity while maintaining activity, would represent a major improvement in cancer chemoprevention. For these reasons, a randomized study of different dose combinations in Stage I–II breast cancer patients using a number of circulating growth factors as surrogate endpoints has been initiated. The main endpoint is the reduction of plasma IGF-I levels.   © 1993 Wiley-Liss, Inc.

Key words: Breast cancer, chemoprevention, EGF, 4-HPR, growth factors, IGF-I, retinoid, retinol, tamoxifen, TGF-β

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STUDIES WITH 4-HPR ALONE

Fenretinide [N-(4-hydroxyphenyl)retinamide (4-HPR)], a synthetic derivative of retinoic acid, has proven effective at inhibiting *in vitro* breast cancer cell growth [1] and preventing the pro-

gression of chemically induced mammary carcinoma in rodents [2]. Recently, our group has made a particular effort in conducting clinical studies aimed at evaluating 4-HPR's pharmacology, toxicity, and chemopreventive efficacy in breast cancer.

Pharmacology and Endocrinology

Most studies on the pharmacokinetics of 4-HPR and its effect on plasma retinol levels have been conducted in breast cancer patients who participated in our Phase I trial, and who continued to be treated and followed for 5 years [3,4]. The plasma concentrations of 4-HPR, its main metabolite N-(4-methoxyphenyl)retinamide (4-MPR), and retinol were measured by HPLC [3] after different doses and at different times during and after treatment.

Plasma retinol level reduction. During the Phase I study, it was shown that 4-HPR causes an early reduction of plasma retinol concentrations in humans [3]. Twenty-four hours after a single dose of 200 mg, the concentrations of retinol and of its specific transport protein, retinol binding protein (RBP), are reduced an average of 38% and 26%, respectively, in treated patients. The reduction of plasma retinol concentration is proportional to the dose. In patients whose blood was collected 12 hours after the last dose of either 100, 200, or 300 mg 4-HPR/day given for 5 months, the concentrations of the drug and its metabolite increased linearly with the dose; in contrast, the plasma retinol showed a dose-related linear decrease. The reduction of plasma retinol levels is associated with the interaction of 4-HPR with RBP and interference in the RBP-transthyretin (TTR) complex formation [5]. Since the reduction of plasma retinol was dose-dependent and associated with diminished dark adaptation [6–8], it was decided to periodically interrupt drug treatment to increase plasma retinol concentrations, thus allowing storage of retinol in the retina. A 3-day treatment interruption at the end of each month was prescribed for patients in the ongoing trial.

Monitoring of 4-HPR, 4-MPR, and plasma retinol concentrations during a 5-year chronic treatment regimen. As is generally the case with chemopreventive agents, the ongoing prevention studies require 4-HPR administration for long periods of time, i.e., 1 to 5 years. Daily chronic administration of 200 mg 4-HPR results in an average plasma concentration of 350 ng/ml (i.e., approximately 1 µM) 14 hours after drug intake, which remains constant throughout the treatment period [4]. Concentrations of 4-MPR similar to those of the parent drug increase slightly but significantly during the first 35 months of treatment; however, after 5 years, they are similar to those found at 5 months. Saturable accumulation of this metabolite, which is more polar than the parent drug, might explain this behavior. Retinol concentrations are reduced from 493 ng/ml to approximately 170 ng/ml (i.e., by 65%), and this reduction is constant during the 5-year treatment period [4].

Kinetics of elimination after 5 years of chronic treatment. After the 5-year treatment period, 4-HPR was cleared from the plasma in an average $t_{1/2}\beta$ of 27 hours, evaluated through blood collected from 14 patients between 12 and 86 hours after the last dose [4]. The rate of 4-MPR elimination was slower than that of the parent drug, with an average $t_{1/2}\beta$ of 54 hours. Long-term elimination of 4-HPR and 4-MPR, as well as retinol recovery following drug discontinuation after the 5-year continuous treatment, have been investigated over a 12-month period. At 6 and 12 months after drug interruption, plasma 4-HPR concentrations were at the limits of detection (0.01 µM), whereas the concentrations of 4-MPR were approximately 5 times higher. Baseline retinol concentrations (500 ng/ml) recovered after 1 month [4].

Distribution of 4-HPR in the human breast. Evaluation of 4-HPR concentrations in breast biopsies of a small sample of patients confirms accumulation in the breast as already demonstrated in rodents [2], and as previously reported in other breast cancer patients [9]. The tissue concentrations of 4-HPR in all but one sample were 1.4–8.2-fold higher than those in plasma [4]. 4-MPR, which is more lipophilic than 4-HPR, accumulated in the breast to an even greater extent; this was particularly evident after long-term treatment. This may be relevant for the chemopreventive effect of 4-HPR since 4-MPR has the same potency as 4-HPR in in vitro differentiation assays [10]. The highest concentrations

of both 4-HPR and 4-MPR were found in fat. Evidence that 4-HPR accumulates not only in fat but also in the epithelial cells of the breast is supported by the fact that the concentrations of 4-HPR and 4-MPR in nipple discharge (which is secreted by the breast gland) are 10 and 30 times higher, respectively, than those found in plasma [4].

Fenretinide lowers plasma insulin-like growth factor-I (IGF-I) levels in breast cancer patients.

Although the mechanisms by which retinoids exert their effects on proliferation and differentiation are poorly understood, interaction with several growth factors is plausible. For this reason, we studied the effect of 4-HPR on IGF-I in a cohort of 60 consecutive breast cancer patients participating in the Phase III trial [11]. Thirty-two women assigned to receive 4-HPR (200 mg p.o. daily) and 28 randomized controls entered the study. Age, menstrual status, time since surgery, and body mass index (BMI) [weight (kg) divided by squared height (m^2)] were not significantly different between the two groups. No endocrine or metabolic alterations were present at randomization or during follow-up. IGF-I levels were determined by radioimmunoassay after acid ethanol extraction, using commercially available kits (blinded as to allocated arm) at randomization and during follow-up at a mean interval of 10.8 ± 0.3 months.

At baseline, there was no difference in IGF-I levels between the two groups (mean ± SE: 4-HPR, 152.9 ± 9.4 ng/ml; control, 159.2 ± 7 ng/ml; p = 0.59). After a mean time of 10.8 ± 0.3 months, IGF-I levels declined significantly ($15.3 \pm 5\%$) in treated patients as compared with baseline (134.6 ± 8.1, p = 0.003), while no change was shown in controls (163.3 ± 7.4, p = 0.5). Post-treatment values were also significantly lower when compared to follow-up values in controls (p = 0.011).

Multiple regression analysis, using Δ IGF-I as the dependent variable and age, treatment, and their interaction as covariates, showed that treatment was the only determinant of IGF-I decrease (Table I). In addition, the interaction between treatment and age was significant, indicating a different, age-dependent behavior of Δ IGF-I in the two groups. Specifically, the decrease induced by 4-HPR administration was much more pronounced in patients less than 50 years of age (mean reduction $26.7 \pm 11.6\%$), suggesting a potential interaction with sex hormones, while an age-related decline was seen in controls (Fig. 1).

Tolerability

One of the main reasons oncologists are attracted to 4-HPR is its tolerability compared to other retinoids. As shown by a randomized Phase I study of different doses of 4-HPR [12], no acute or severe toxicity is observed with this retinoid. The same occurs with long-term, daily oral administration of 200 mg 4-HPR, as shown by the evaluation of 53 patients treated for 42 months [13]. Dermatologic and metabolic alterations are uncommon, and no liver function abnormalities are observed [14]. The only potential limitation to extensive use of 4-HPR is the occurrence of diminished dark adaptation. Estimation of this secondary effect by the Goldmann-Weekers dark-adaptometry test, which can

TABLE I. Multiple Regression of the Difference (Δ) in IGF-I Levels Between Follow-up and Baseline

VARIABLE	β	SE (β)	t-TEST	p
CONSTANT	42.78	49.30	0.87	0.389
Treatment	−167.09	65.02	−2.57	0.013
Age	−0.76	0.97	−0.15	0.432
Age * Treatment	2.73	1.24	2.19	0.032

Overall F test = 4.98; p = 0.004; r^2 = 0.22
β = regression coefficient, SE (β) = standard error of β, CI = confidence interval of β, Test = Student's t-test with 56 degrees of freedom
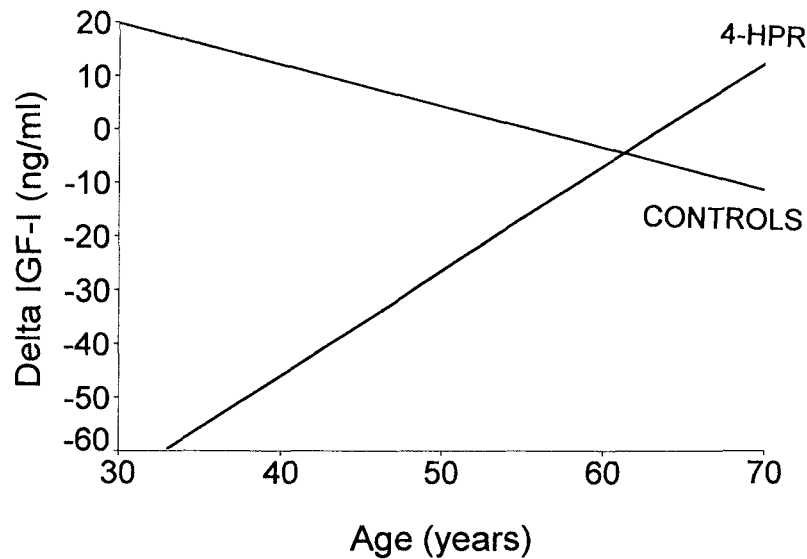
Fig. 1. Age-dependent behavior of Δ IGF-I. Data are expressed as expected values. (Reprinted with permission of the publisher, American Association for Cancer Research, Inc., from an original article in *Cancer Research.*)

very sensitively detect subclinical vitamin A deficiency, has shown that there is a 23% incidence of mild and a 26% incidence of moderate alteration of dark-adaptometry associated with the drug-induced decline of plasma retinol below the threshold levels of 160 and 100 ng/ml, respectively, in women treated with 200 mg 4-HPR daily. However, about 50% of the treated patients with altered dark-adaptometry were asymptomatic, and the alterations of dark-adaptometry are promptly reversed upon drug interruption [6]. Interestingly, the decrease of plasma retinol was inversely related to treatment duration (Table II). Plasma 4-MPR, which is higher in elderly women with a higher percentage of adipose tissue (Table III), is the major determinant of decreased retinol (Table II). Moreover, the significant interaction of age with other covariates (BMI, plasma 4-HPR, age) is indicative of a selective biological effect exerted by age on both plasma retinol and 4-MPR levels (Tables II and III). For instance, retinol levels decreased with increasing age in women with higher plasma concentrations of 4-HPR, while an opposite trend was shown in women with lower plasma concentrations of 4-HPR (Table II). Toxicity of the ocular surface, frequently observed on administration of natural and synthetic retinoids [15], is negligible in 4-HPR-treated patients [6].

Efficacy: The Phase III Study

On the basis of experimental data showing the peculiar accumulation of 4-HPR in the rodent mammary gland [2] and its tolerability in humans [12,13], a large randomized chemoprevention trial was started in 1987 with the assumption that if 4-HPR succeeds in preventing second primaries in breast cancer patients, it may possibly be useful for a wider group of high-risk subjects, such as members of families with a high incidence of breast cancer [16]. Study participants are breast cancer patients between the ages of 33 and 68. In order to be eligible, patients must have had an operated breast cancer (T1–2), without axillary lymph node involvement, and without evidence of local recurrence and/or distant metastases. Patients were randomized to receive 200 mg 4-HPR daily for 5 years (with a 3-day drug holiday at the end of each month) versus no treatment.

To date, 2,972 patients had been randomized (1,496 in the 4-HPR group and 1,476 in the control group). Protocol and treatment compliance is high (drug compliance >90% in 81% of women and >80% in another 10% of women). Tolerability of the drug is good; only 51 of 1,397 women (3.6%) interrupted drug intake due to toxicity.

TABLE II. Multiple Regression of Plasma Retinol

VARIABLE	β	SE (β)	t-TEST	p
CONSTANT	–791.60	321.51	2.46	0.020
Plasma 4-HPR	1.84	0.75	2.47	0.022
Plasma 4-MPR	–0.28	0.10	2.96	0.008
Age	14.90	5.50	2.71	0.013
BMI	4.23	4.72	0.89	0.380
Length of treatment (yrs)	3.28	1.21	2.71	0.013
4-HPR * Age	0.03	0.01	2.58	0.017

Overall F test = 3.24, p = 0.02; r^2 = 0.48

TABLE III. Multiple Regression of Plasma 4-MPR

VARIABLE	β	SE (β)	t-TEST	p
CONSTANT	6904.22	1754.41	3.94	<0.001
Plasma 4-HPR	6.52	2.04	3.20	0.004
Age	–211.92	52.77	4.02	<0.001
BMI	–181.14	68.26	2.65	0.015
Age * Age	1.59	0.52	3.09	0.006
BMI * Age	3.31	1.18	2.80	0.010
4-HPR * Age	–0.11	0.04	3.12	0.005

Overall F test = 4.64, p = 0.004; r^2 = 0.57

ACTIVITY OF 4-HPR PLUS TAMOXIFEN USING CIRCULATING GROWTH FACTORS AS POTENTIAL SURROGATE ENDPOINTS

Rationale for Combining the Two Agents

Tamoxifen (TAM), a triphenylethylene anti-estrogen, is the standard adjuvant treatment for postmenopausal estrogen receptor positive (ER+) breast cancer patients. A significant benefit in survival has also been seen in premenopausal ER+ patients; mortality reduction rates of 11% and 17% have been observed with estrogen receptor-negative (ER–) tumors and node-negative (N–) tumors, respectively [17]. The 39% decrease in contralateral breast cancer demonstrated in a meta-analysis [17] of all TAM adjuvant studies has given the most compelling evidence for the use of TAM as a preventive agent in women at risk for breast cancer. Moreover, a reduction in myocardial infarction mortality [18,19] and blood cholesterol [20], and a benefit against osteoporosis [4], have also been associated with the administration of this antiestrogen. For these reasons, several large controlled trials evaluating the efficacy of TAM in the chemoprevention of breast cancer have been implemented, including one by our group [22].

Beyond the classical mechanisms of competitive binding to the ER, several other mechanisms have been proposed to explain the activity of TAM [23], including growth hormone (GH)-dependent and -independent decrease of tissue

and circulating IGF-I levels [24,25], and an increase in peritumoral expression of transforming growth factor-β (TGF-β) in humans [26].

Although the mechanisms by which retinoids affect proliferation and differentiation are poorly understood, interaction with several growth factors is plausible. Retinoic acid has indeed been shown to increase TGF-β expression in several organs in the mouse [27]. We have recently demonstrated that administration of 4-HPR significantly lowers plasma IGF-I levels in women with early breast cancer [11].

The combined administration of TAM and 4-HPR has proven additive or synergistic in both the growth inhibition of MCF-7 cells [28] and the prevention of N-methyl-N-nitrosourea (MNU)-induced mammary carcinoma in the rat [29]. The concept of different agents separately inhibiting tumor occurrence and/or progression of hormone-dependent and -independent breast cancers is very intriguing. Hypothetically, TAM would prevent ER+ tumors, and retinoids ER– tumors. At present, this is purely theoretical but worth further investigation, especially since the two agents have been shown to act by the same molecular mechanism [30]. Moreover, estrogen and retinoid receptors are part of the same superfamily of nuclear receptors, and reciprocal interaction at the molecular level has been suggested.

Toxicity is a crucial aspect, particularly in chemoprevention studies which often require prolonged administration to healthy people. Although these two agents have been shown to be relatively non-toxic, TAM has been associated with an increased risk of endometrial cancer, possibly in a dose-dependent manner, with the risk increasing at 40 mg daily [23,31]. It is also carcinogenic in the rat liver in a dose-dependent manner [32], although no substantial increase in human hepatic cancers has been reported [31]. At a daily dose of 200 mg, 4-HPR appears to be less toxic than other natural and synthetic retinoids; however, about one-fourth of the patients experience diminished dark adaptation [6]. It is noteworthy that TAM administration has been sporadically associated with retinal deterioration [33]. Although uncommon, dermatologic toxicity due to 4-HPR may be encountered. A dose reduction of one or both agents which maintains activity would therefore represent a major improvement in cancer chemoprevention.

Role of Growth Factors in Breast Cancer

In recent years, there has been ample evidence of the crucial role of peptide GFs in the development of breast cancer [34–36]. Synthesis of some GFs may be under hormonal control and may thus represent one mechanism through which hormones (including retinoids, vitamin D, etc.) regulate sensitive tissues [37]. Several GFs play a critical role in differentiation and proliferation of normal and transformed breast epithelium through a complex pattern of stimulatory [epidermal growth factor (EGF)/TGF-α, IGFs] and inhibitory (TGF-β) actions. The role of GFs is not confined to the epithelium since stromal cells are involved in the secretion of and response to some GFs. The latter may thus exert an autocrine and/or paracrine regulatory action on breast cancer growth. However, some GFs are found in large amounts in circulation, thus suggesting an even more complex network of interactions between sites of production and target tissues. GFs have been involved not only in the promotion of breast cancer but also in later stages of the neoplastic process, such as the progression to hormone independence and resistance, or the increase of invasive and metastatic potential [38].

Study Design

The proposed pilot study is preliminary to a larger clinical trial which will evaluate the chemopreventive activity of the combination of TAM and 4-HPR in breast cancer. The present study will use the modulation of circulating growth factors, some with mitogenic and some with antiproliferative effects on the breast, as intermediate endpoints in a population at risk, i.e., women already surgically treated for Stage I–II breast cancer. Moreover, this pilot study will assess the least toxic chemopreventive combination regimen. Finally, as the relationship between circulating GFs, their modulation by agents, and the prognosis of breast cancer has never been elucidated, our study will evaluate these interactions within the setting of a randomized clinical trial.

The design of the study is a five-arm randomized trial comparing different dose combinations of TAM plus 4-HPR versus TAM alone in Stage I–II breast cancer candidates to adjuvant TAM administration. The main endpoint is de-

creased plasma IGF-I at 12 months. Other major endpoints are the modifications in plasma levels of IGFBP-3, serum TGF-β1, urinary EGF, and plasma retinol at 12 months. A sixth, non-randomized cohort of 80 Japanese patients treated with TAM (20 mg daily) alone will be studied for the same endpoints in order t_ evaluate any race-related biological differences in the modulation of the biomarkers.

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REFERENCES

1. Marth C, Bock G, Daxenbichler G. (1985) Effect of 4-hydroxyphenylretinamide and retinoic acid on proliferation and cell cycle of cultured human breast cancer cells. J Natl Cancer Inst 75:871–875.
2. Moon RC, Thompson HJ, Becci PL, Grubbs CJ, Gander RJ, Newton DL, Smith JM, Phillips SL, Henderson WR, Mullen LT, Brown CC, Sporn MB. (1979) N-(4-hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer. Cancer Res 39:1339–1346.
3. Formelli F, Carsana R, Costa A, Buranelli F, Campa T, Dossena G, Magni A, Pizzichetta M. (1989) Plasma retinol level reduction by the synthetic retinoid fenretinide: A one year follow-up study of breast cancer patients. Cancer Res 49:6149–6152.
4. Formelli F, Clerici M, Campa T, Di Mauro MG, Magni A, Mascotti G, Maglia D, De Palo G, Costa A, Veronesi U. (1993) Five-year administration of fenretinide: Pharmacokinetics and effects on plasma retinol concentrations. J Clin Oncol 11:2036–2042.
5. Berni R, Formelli F. (1992) In vitro interaction of Fenretinide with plasma retinol-binding protein and its functional consequences. FEBS Lett 308:43–45.
6. Decensi A, Torrisi R, Polizzi A, Gesi R, Brezzo V, Rolando M, Rondanina G, Orengo MA, Formelli F, Costa A. (1994) Effect of the synthetic retinoid fenretinide on dark-adaptation and the ocular surface. J Natl Cancer Inst 86 (in press).
7. Kaiser-Kupfer MI, Peck GL, Caruso RC. (1986) Abnormal retinal function associated with Fenretinide, a synthetic retinoid. Arch Ophthalmol 104:69–70.
8. Kingstone TP, Lowe NJ, Winston J, Heckenlively J. (1986) Visual and cutaneous toxicity which occurs during N-(4-hydroxyphenyl)retinamide therapy for psoriasis. Clin Exp Dermatol II:624–627.
9. Mehta RG, Moon RC, Hawthorne M, Formelli F, Costa A. (1991) Distribution of Fenretinide in the mammary gland of breast cancer patients. Eur J Cancer 27:138–141.
10. Swanson BN, Newton DL, Roller PP, Sporn MB. (1981) Biotransformation and biological activity of

N-(4-hydroxyphenyl)retinamide derivatives in rodents. J Pharmacol Exp Ther 219:632–637.
11. Torrisi R, Pensa F, Orengo MA, Catsafados E, Ponzani P, Boccardo F, Costa A, Decensi A. (1993) The synthetic retinoid fenretinide lowers plasma IGF-I levels in breast cancer patients. Cancer Res 53:4769–4771.
12. Costa A, Malone W, Perloff M, Buranelli F, Campa T, Dossena G, Magni A, Pizzichetta M, Andreoli C, Del Vecchio M, Formelli F, Barbieri A. (1989) Tolerability of the synthetic retinoid fenretinide (4-HPR). Eur J Cancer Clin Oncol 25:805–808.
13. Rotmensz N, De Palo G, Formelli F, Costa A, Marubini E, Campa T, Crippa A, Danesini GM, Delle Grottaglie M, Di Mauro MG, Filiberti A, Gallazzi M, Guzzon A, Magni A, Malone W, Mariani L, Palvarini M, Perloff M, Pizzichetta M, Veronesi U. (1991) Long term tolerability of fenretinide (4-HPR) in breast cancer patients. Eur J Cancer 27:1127–1131.
14. Pizzichetta M, Rossi R, Costa A, Guindani A, De Palo G. (1992) Lipoproteins in Fenretinide (4-HPR)-treated patients. Diabetes Nutr Metab 5:71–72.
15. Gross EG, Helfgott MA. (1992) Retinoids and the eye. Dermatol Clin 10:521–531.
16. Vasen HFA, Beex LVAM, Cleton FJ, Collette HJA, van Dongen JA, van Leeuwen FE, Crommelin MA, Meera Khan P. (1993) Clinical heterogeneity of hereditary breast cancer and its impact on screening protocols: The Dutch experience on 24 families under surveillance. Eur J Cancer 29A:1111–1114.
17. Early Breast Cancer Trialists' Collaborative Group. (1992) Systemic treatment of early breast cancer by hormonal, cytotoxic, or immunotherapy. Lancet 339: 1–15, 71–85.
18. McDonald CC, Stewart HJ. (1991) Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial. BMJ 303:435–437.
19. Rutqvist LE, Mattsson A, for the Stockholm Breast Cancer Study Group. (1993) Cardiac and thromboembolic morbidity among postmenopausal women with early-stage breast cancer in a randomized trial of adjuvant tamoxifen. J Natl Cancer Inst 85:1398–1406.
20. Love RR, Newcomb PA, Wiebe DA, Surawicz TS, Jordan VC, Carbone PP, DeMets DL. (1990) Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. J Natl Cancer Inst 82:1327–1332.
21. Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL. (1992) Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 326:852–856.
22. Costa A. (1993) Breast cancer chemoprevention. Eur J Cancer 29A:589–592.
23. Cobleigh MA, Dawlatshahi K, Deutsch TA, Mehta RG, Moon RC, Minn F, Benson AB III, Rademaker AW, Ashenhurst JB, Wade JL III, Walter J. (1993) Phase I/II trial of tamoxifen with or without fenretinide, an analog of vitamin A, in women with metastatic breast cancer. J Clin Oncol 11:474–477.

24. Pollak M, Costantino J, Polychronakos C, Blauer SA, Guyda H, Redmond C, Fisher B, Margolese R. (1990) Effect of tamoxifen on serum insulin-like growth factor I levels in Stage I breast cancer patients. J Natl Cancer Inst 82:1693–1697.

25. Huynk HT, Tetenes E, Wallace L, Pollak M. (1993) *In vivo* inhibition of insulin-like growth factor I gene expression by tamoxifen. Cancer Res 53:1727–1730.

26. Butta A, MacLennan K, Flanders KC, Sacks NPM, Smith I, McKinna A, Dowsett M, Wakefield LM, Sporn MB, Baum M, Colletta AA. (1992) Induction of transforming growth factor $\beta 1$ in human breast cancer *in vivo* following tamoxifen treatment. Cancer Res 52:4261–4264.

27. Glick AB, McCune BK, Abdulkarem N, Flanders KC, Lumadue JA, Smith JM, Sporn MB. (1991) Complex regulation of TGFβ expression by retinoic acid in the vitamin A-deficient rat. Development 111:1081–1086.

28. Fontana JA. (1987) Interaction of retinoids and tamoxifen on the inhibition of mammary carcinoma cell proliferation. Exp Cell Biol 55:136–144.

29. Ratko TA, Detrisac CJ, Dinger MN, Thomas CF, Kelloff GJ, Moon RC. (1989) Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. Cancer Res 497:4472–4476.

30. Sporn MB, Roberts AB, Glick AB, Luckert PH, Pollard M. (1992) Interaction of retinoids and transforming growth factor-β in the chemoprevention of cancer. In Sporn MB (ed): "Control of Growth Factors and Prevention of Cancer," ESO Monograph. Berlin: Springer-Verlag, pp 37–49.

31. Fornander T, Rutqvist LE, Cedermark B, Glas U, Mattsson A, Silfverswärd C, Skoog L, Somell A, Theve T, Wilking N, Askergren J, Hjalmar ML. (1989) Adjuvant tamoxifen in early breast cancer: Occurrence of new primary cancers. Lancet 1:117–120.

32. Williams GM, Iatropoulos MJ, Djordjevic MV, Kaltenberg OP. (1993) The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. Carcinogenesis 14:315–317.

33. Pavlidis NA, Petris C, Briassoulis E, Klouvas G, Psilas C, Rempapis J, Petroutsos G. (1992) Clear evidence that long-term, low dose tamoxifen treatment can induce ocular toxicity. Cancer 69:2961–2964.

34. Lippman ME, Dickson RB, Bates S, Knabbe C, Huff K, Swain S, McManaway M, Bronzert D, Kasid A, Gelmann EP. (1986) Autocrine and paracrine growth regulation of human breast cancer. Breast Cancer Res Treat 7:59–70.

35. Osborne CK, Arteaga CL. (1990) Autocrine and paracrine growth regulation of breast cancer: Clinical implications. Breast Cancer Res Treat 15:3–11.

36. Aaronson SA. (1991) Growth factors and cancer. Science 254:1146–1153.

37. Roberts AB, Sporn MB. (1992) Mechanistic interrelationships between two superfamilies: The steroid/retinoid receptors and transforming growth factor-β. Cancer Surv 14:205–220.

38. Brunner N. (1990) Human breast cancer growth and progression: Role of secreted polypeptide growth factors. Int J Cancer 5(C):62–66.