From the Divisions of Cancer Prevention and Genetics, Breast Surgery, Breast Diagnostics, Gynaecologic Surgery, and Pathology, European Institute of Oncology; University of Milan School of Medicine; Chemoprevention Unit, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, Milan; Medical Oncology, E.O. Ospedali Galliera, Genoa; Medical Oncology, Ospedale S. Bortolo, Vicenza, Italy; Statistics and Modeling Science, Strathclyde University, Glasgow, Scotland, United Kingdom; Hormone Laboratory, Haukeland University Hospital; Section for Endocrinology, Institute of Medicine, University of Bergen, Bergen, Norway; and the Division of Cancer Prevention, National Cancer Institute, Bethesda, MD.

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Corresponding author: Andrea Decensi, MD, Division of Medical Oncology, E.O. Ospedali Galliera, Mura delle Cappuccine 14, 16128 Genoa, Italy; e-mail: andrea.decensi@galliera.it.

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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Randomized Double-Blind 2 × 2 Trial of Low-Dose Tamoxifen and Fenretinide for Breast Cancer Prevention in High-Risk Premenopausal Women

Andrea Decensi, Chris Robertson, Aliana Guerrieri-Gonzaga, Davide Serrano, Massimiliano Cazzaniga, Serena Mora, Marcella Gulisano, Harriet Johansson, Viviana Galimberti, Enrico Cassano, Simona M. Moroni, Franca Formelli, Ernst A. Lien, Giuseppe Pelosi, Karen A. Johnson, and Bernardo Bonanni

ABSTRACT

Purpose

Tamoxifen and fenretinide are active in reducing premenopausal breast cancer risk and work synergistically in preclinical models. The authors assessed their combination in a two-by-two biomarker trial.

Patients and Methods

A total of 235 premenopausal women with pT1mic/pT1a breast cancer ($n = 21$), or intraepithelial neoplasia (IEN, $n = 160$), or 5-year Gail risk $\geq 1.3\%$ ($n = 54$) were randomly allocated to either tamoxifen 5 mg/d, fenretinide 200 mg/d, their combination, or placebo. We report data for plasma insulin-like growth factor I (IGF-I), mammographic density, uterine effects, and breast neoplastic events after 5.5 years.

Results

During the 2-year intervention, tamoxifen significantly lowered IGF-I and mammographic density by 12% and 20%, respectively, fenretinide by 4% and 10% (not significantly), their combination by 20% and 22%, with no evidence for a synergistic interaction. Tamoxifen increased endometrial thickness principally in women becoming postmenopausal, whereas fenretinide decreased endometrial thickness significantly. The annual rate of breast neoplasms ($n = 48$) was $3.5\% \pm 1.0\%$, $2.1\% \pm 0.8\%$, $4.7\% \pm 1.3\%$, and $5.2\% \pm 1.3\%$ in the tamoxifen, fenretinide, combination, and placebo arms, respectively, with hazard ratios (HRs) of 0.70 (95% CI, 0.32 to 1.52), 0.38 (95% CI, 0.15 to 0.90), and 0.96 (95% CI, 0.46 to 1.99) relative to placebo (tamoxifen × fenretinide adverse interaction $P = .03$). There was no clear association with tumor receptor type. Baseline IGF-I and mammographic density did not predict breast neoplastic events, nor did change in mammographic density.

Conclusion

Despite favorable effects on plasma IGF-I levels and mammographic density, the combination of low-dose tamoxifen plus fenretinide did not reduce breast neoplastic events compared to placebo, whereas both single agents, particularly fenretinide, showed numerical reduction in annual odds of breast neoplasms. Further follow-up is indicated.

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INTRODUCTION

Tamoxifen is US Food and Drug Administration–approved for breast cancer risk reduction in high-risk individuals and has a favorable risk:benefit ratio in premenopausal women.¹ Fenretinide, a synthetic retinoid, showed a significant reduction of second breast cancer in premenopausal women in a subgroup analysis of a phase III trial.² Synergistic efficacy between the two agents was observed in vivo in animal studies³ and their combination was safe in pilot clinical trials.⁴⁻⁶

In the present phase IIb trial, we investigated the activity and safety profile of this combination in at-risk premenopausal women. Circulating insulin-like growth factor I (IGF-I) and mammographic % density were the main outcome measures as both biomarkers are associated with premenopausal breast cancer risk⁷⁻⁹ and are modulated by either agent in clinical trials.¹⁰⁻¹² Fenretinide lowers plasma IGF-I and increases IGF-binding protein-3 (IGFBP-3),¹² and high IGF-I and low IGFBP-3 levels predicted second breast cancer risk in premenopausal women.¹³ Mammographic % density was

associated with breast cancer risk in prospective studies,¹⁴⁻¹⁶ and the greatest reduction was observed in women younger than 45 years in a phase III prevention trial showing the efficacy of tamoxifen.¹⁷ We selected a dose of 5 mg/d of tamoxifen since this reduces circulating IGF-I levels,¹⁰ and matches antiproliferative effects on breast cancer compared with 20 mg/d.¹⁸ A lower dose of tamoxifen might also reduce endometrial cancer risk^{19,20} and venous thromboembolism.¹⁸

In a preliminary analysis of this trial,²¹ we showed that the combination of low-dose tamoxifen and fenretinide was not synergistic on circulating IGF-I levels. Here we report the definitive results for plasma IGF-I and mammographic density at 2 years, provide compliance and safety data, and also report data on the rate and type of breast neoplastic events after 5.5 years.

PATIENTS AND METHODS

Participants

A detailed description of the study was previously reported.²¹ Briefly, eligible subjects were premenopausal women with either an intraepithelial neoplasia (IEN; either ductal carcinoma in situ [DCIS] or lobular carcinoma in situ [LCIS]) or a small invasive cancer (pT1c or pT1a, N₀, G1-2, estrogen receptor [ER] ≥ 10%) in the 3 years before random assignment, or a Gail 5-year-risk for breast cancer ≥ 1.3%. The study was conducted in two centers, the European Institute of Oncology, Milan, where 91% of subjects were recruited, and the Division of Medical Oncology, Vicenza, Italy.

Interventions and Study Procedures

The study was a randomized, double-blind, placebo-controlled trial with a 2 × 2 factorial design. Subjects were randomly assigned to one of the following arms: tamoxifen 5 mg/d (1 tablet) and two fenretinide and placebo capsules; tamoxifen and placebo and fenretinide 200 mg/d (two 100 mg capsules); tamoxifen and fenretinide; both placebos. Women were treated for

2 years and observed for at least 5 additional years. Blood measurements were performed every 6 months and transvaginal ultrasounds every 12 months. Adverse events were registered according to the National Cancer Institute Common Toxicity Criteria, version 2, and the menopause-related questionnaire Menopause Specific Quality of Life Questionnaire.²² Mammographic density was measured at baseline, 12, and 24 months. Compliance was measured by pill count and circulating drug levels.

Objectives and Outcomes

The study was designed to determine the effects of each agent alone and to test for an interaction between tamoxifen and fenretinide on biomarkers related to premenopausal breast cancer risk.

The main outcome measures were the changes in IGF-I and mammographic percent density values from baseline to 24 months. Secondary outcome measures included changes of endometrial thickness, uterine volume and ovarian cysts. Breast neoplastic events included invasive breast cancer and DCIS, not LCIS, and were correlated with baseline IGF-I and mammographic density levels and receptor characteristics analyzed according to treatment arm.

Assay Methods

Plasma IGF-I aliquots were stored at -80°C and centrally assayed as previously described.²¹ Serum IGF-BP-3 levels were measured by chemiluminescent immunometric kits (DPC, Los Angeles, CA) designed for IMMULITE (Diagnostic Products Corporation, Los Angeles, CA). Serum tamoxifen and endoxifen, and plasma fenretinide and retinol concentrations were measured annually as previously described,^{23,24} after a median interval from the last drug intake of 23 hours for tamoxifen and 12.5 hours for fenretinide. Mammographic % density was centrally measured on analog screen films or digital scans by a single trained radiologist, blinded as to time sequence and treatment arm, using the method described by Boyd et al.^{14,25} Both analog and digital mammograms were analyzed. Forty women at baseline did not have mammographic density measured. At baseline, women with digital measurement had a breast density which was nearly 16% lower compared to those with analog film screen. The percentage changes in mammographic density during treatment

Fig 1. CONSORT diagram (participant flow diagram). AEs, adverse events.

are based on adjusting the digital density to compensate for this difference. The adjustment was based on a linear regression of % density and using the value of the parameter associated with a digital mammogram to increase the recorded digital value. A similar model was used at 12 and 24 months except that an adjustment was made for the treatment arm. At baseline the correction was 15.7%, at 12 months the correction was 14.5%, and at 24 months the correction was 16.2%. Hormone and HER2 receptor of breast neoplasms both at baseline and during follow-up were determined by immunohistochemistry in a single laboratory, as previously described.^{26,27}

Statistical Analyses

The sample size and power calculations have previously been described.²¹ A repeated measures analysis of variance model of the measurement at 6, 12, 18, 24, 30, and 36 months, adjusting for baseline value and strata, was used to estimate the main drug effects and their interaction. Log-transformations were used for IGF-I, IGF-I:IGFBP-3 ratio, and endometrial thickness. For endometrial thickness, a further adjustment included actual menopausal status and menstrual cycle phase, using days 1 through 14 or 15+ as proxies. For mammographic density the repeated measures analysis was carried out only on the women who did not have a digital measurement or for women who did have digital mammography, after inclusion of an indicator variable to adjust for the use of a digital mammogram at 12 (or 24) months, baseline density, and the interaction between baseline density and digital mammogram. All statistical testing was carried out within the statistical models with tests of interaction or main effects. Parameter estimates derived from these models are presented together with their 95% CIs.

Breast neoplastic events were analyzed using a Cox regression model. When investigating the prognostic effects IGF-I, age, mammographic density, and body mass index (BMI) were included in the model as continuous covariates though the results are presented for quartiles. The hormone and HER2 receptor status of second neoplasms in each arm was compared with the expected numbers that were based on their distribution at baseline. Correlation analysis was used at baseline to investigate the effects of age and BMI on IGFs and analog mammographic density.

RESULTS

A total of 880 subjects were registered and 586 were screened for eligibility; 190 were not eligible and 161 refused to participate, leaving 235 participants (Figure 1). The main subject characteristics have previously been reported,²¹ with no differences among groups. There were two randomization strata of the same age and BMI: at-risk women by the Gail model (23%) and women with either prior IEN (68%) or pT1mic/T1a (9%). Baseline values of the primary end point measures are reported in Table 1; subjects in the IEN/pT1mic/pT1a strata had 9.4% (95% CI, -0.6% to 20.5%) higher mean levels of

IGF-I ($P = .07$) and 9.7% higher IGF-I/IGFBP-3 (95% CI, -1.1% to 21.9%; $P = .08$) than those in the Gail strata, whereas IGFBP-3 and mammographic density had comparable values. Mammographic % density was not correlated with IGFs but negatively correlated with age ($r = -0.21$; $P = .004$) and BMI ($r = -0.38$; $P < .001$). Age was negatively correlated with IGF-I ($r = -0.25$; $P < .001$), but not with IGFBP-3 (not shown).

Mean pill count compliance over 2 years ranged between 80% and 90% in all four arms. Figure 2 shows the blood concentrations of tamoxifen and endoxifen (upper panels), and retinol and fenretinide (lower panels) during treatment. Subjects taking tamoxifen had mean serum drug and metabolite levels around 20 and 15 ng/mL, respectively. Subjects taking fenretinide exhibited a 50% reduction in plasma retinol levels, which recovered to baseline levels at month 36.

Table 2 (upper part) shows the yearly changes in IGF-I during treatment. Tamoxifen lowered IGF-I from baseline by approximately 10% at 6 months and plateaued thereafter, with a return to baseline values after drug cessation ($P < .0001$ for the time $\times$ tamoxifen interaction), whereas IGF-I was not significantly lowered by fenretinide ($P = .35$). The combination arm induced a nearly 20% reduction of IGF-I, without evidence for a synergistic interaction ($P = .29$). Overall, tamoxifen decreased IGF-I by 16.6% (95% CI, 11.6% to 21.5%). Qualitatively similar results were noted on IGF-I:IGFBP-3 ratio (not shown). The effects on mammographic % density are presented in Table 2 (lower two parts). Tamoxifen induced a 20% reduction over 2 years, fenretinide induced a nonsignificant decline of 11%, whereas their combination induced a 22% reduction, with placebo alone a nonsignificant 12% reduction. There was no evidence of any interaction between tamoxifen and fenretinide ($P = .22$), nor of any main effect of fenretinide ($P = .43$). There was an effect of tamoxifen ($P = .003$) but this was not different according to baseline mammographic density level ($P = .16$ for the interaction). Qualitatively similar results were obtained using analog mammograms only.

The results of endometrial thickness are presented in Table 3. There was a three-way interaction between tamoxifen, menopausal status, and time on study ($P = .013$). While tamoxifen had little effect among premenopausal women at 12 months, there was a borderline increase at 24 and 36 months relative to placebo ($P = .09$ tamoxifen $\times$ time interaction). The proportion of women undergoing natural menopause during the 2 years of the study was evenly distributed among the four arms: 25%, 27%, 33%, and 28%

Table 1. Baseline Levels of the Main Outcome Measures by Disease Status

Variable	IGF-I (ng/mL)		IGFBP-3 (ng/mL)		IGF-I/IGFBP-3		Mammographic % Density*	
	Gail	IEN/T1	Gail	IEN/T1	Gail	IEN/T1	Gail	IEN/T1
Mean	136.4	150.2	3.85	3.86	0.198	0.217	49.5	46.6
Standard deviation	41.4	50.3	0.809	0.841	0.074	0.077	15.2	16.4
No. of observations	54	177	54	178	54	178	45	141

NOTE. The statistical model used was: $\log(\text{IGF-I}) \text{ strata} + \text{tamoxifen} + \text{fenretinide} + \text{tamoxifen:fenretinide}$, where strata denotes Gail or IEN/T1; tamoxifen denotes tamoxifen or its placebo; fenretinide denotes fenretinide or its placebo; and tamoxifen $\times$ fenretinide denotes the interaction between tamoxifen and fenretinide. Taken together, the main effects of tamoxifen, fenretinide, and the interaction represent the three degrees of freedom between the four treatment groups. IGFBP-3 and the ratio IGF-I:IGFBP-3 were also log-transformed, but analog mammographic density was not.

Abbreviations: IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor binding protein-3; IEN/T1, intraepithelial neoplasia or small invasive cancer (pT1mic, or pT1a).

*Only for women with analog mammography.

Fig 2. Circulating levels of (A) tamoxifen (T), (B) endoxifen, (C) retinol, and (D) fenretinide (F) during the trial according to treatment arm. Data on T and F concentrations were pooled into two groups (T- and non-T-containing arms). P, placebo.

in the tamoxifen, fenretinide, combination, and placebo arms, respectively. Among women who became postmenopausal during study, tamoxifen increased endometrial thickness relative to placebo (tamoxifen $\times$ menopausal status interaction $P = .02$), which

did not persist into month 36, however. While on tamoxifen, postmenopausal women had an endometrial thickness which was 80.6% thicker than on placebo (95% CI, 46.3% to 123%). Fenretinide decreased endometrial thickness by 10.9% (95% CI, 1.9%

Table 2. Percentage Changes From Baseline in IGF-I, Mammographic % Density in All Women, and Mammographic % Density in Women With Analog Mammography Only, According to Treatment Arm

Time	Tamoxifen + Placebo			Fenretinide + Placebo			Tamoxifen + Fenretinide			Placebo + Placebo		
	Mean	95% CI	No. of Women at Each Time Point	Mean	95% CI	No. of Women at Each Time Point	Mean	95% CI	No. of Women at Each Time Point	Mean	95% CI	No. of Women at Each Time Point
Baseline IGF-I	133	124 to 143	58	144	132 to 157	59	142	131 to 155	60	139	126 to 154	58
Month 12	-12.4	-18.0 to -6.8	55	-2.2	-8.0 to 3.6	56	-20.2	-25.3 to -15.1	59	-1.7	-7.7 to 4.3	57
Month 24	-9.2	-16.2 to -2.2	54	-4.2	-9.9 to 1.6	57	-16.8	-23.8 to -9.8	59	-0.3	-7.6 to 7.0	56
Month 36	-2.4	-11.8 to 1.4	51	-2.8	-11.4 to 5.8	55	-5.2	-11.8 to 1.4	57	-8.2	-8.2 to -1.6	53
Baseline Mx density (all women)	49.9	45.4 to 54.4	47	46.4	41.5 to 51.2	49	47.2	42.8 to 51.6	53	45.8	40.9 to 50.7	46
Month 12	-9.9	-16.2 to -3.6	48	-5.9	-13.9 to 2.0	49	-17.3	-24.3 to -10.2	49	-6.5	-16.5 to 3.5	45
Month 24	-16.2	-22.6 to -9.8	43	-10.2	-20.0 to -0.04	40	-20.6	-27.7 to -13.5	51	-8.9	-19.2 to 1.5	46
Baseline Mx density (analog only)	49.5	44.8 to 54.2	45	45.6	40.7 to 50.5	47	48.0	43.6 to 52.5	50	46.0	40.9 to 51.1	44
Month 12	-13.8	-20.0 to -7.6	35	-5.3	-15.3 to 4.6	35	-14.9	-21.9 to -7.9	42	-10.8	-20.9 to -0.8	34
Month 24	-19.6	-26.3 to -12.9	25	-10.8	-23.8 to 2.1	24	-21.6	-29.0 to -14.3	28	-12.2	-25.2 to 0.9	26

NOTE. The repeated measures statistical model for IGF-I was: $\log(\text{IGF-I}) + \log(\text{IGF-I.baseline}) + \text{time} + \text{strata} + \text{tamoxifen} + \text{fenretinide} + \text{tamoxifen} \times \text{fenretinide} + \text{time} \times \text{tamoxifen}$, where IGF-I.baseline is the baseline value of IGF-I, time is a factor denoting the follow-up at 6, 12, 18, 24, 30, and 36 months; strata denotes Gail or IEN/T1; tamoxifen denotes tamoxifen or its placebo; fenretinide denotes fenretinide or its placebo; tamoxifen $\times$ fenretinide and time $\times$ tamoxifen denote interactions. The repeated measures model for mammographic density was: $\text{density} = \text{density.baseline} + \text{digital.baseline} + \text{digital} + \text{time} + \text{strata} + \text{tamoxifen} + \text{fenretinide} + \text{tamoxifen:fenretinide}$, where density.baseline is the baseline value of mammographic density; digital is an indicator variable denoting a digital measurement during follow up; digital.baseline denotes a digital mammography at baseline; time is a factor denoting the follow-up at months 12 and 24. When analyzing analog mammographic density there was no need for the two indicators terms denoting digital measurements (digital and digital.baseline).

Abbreviations: Mx, mammographic; IGF, insulin-like growth factor; IEN/T1, intraepithelial neoplasia or small invasive cancer (pT1mic, or pT1a).

Table 3. Endometrial Thickness (mm) According to Treatment Arm, Separately for Women Remaining Premenopausal (upper part) and Women Becoming Postmenopausal (lower part) During the Trial

Time	Tamoxifen + Placebo			Fenretinide + Placebo			Tamoxifen + Fenretinide			Placebo + Placebo		
	Geometric Mean	95% CI	No. of Observations	Geometric Mean	95% CI	No. of Observations	Geometric Mean	95% CI	No. of Observations	Geometric Mean	95% CI	No. of Observations
Premenopausal												
baseline	6.6	5.7 to 7.5	52	7.4	6.5 to 8.3	47	7.1	6.3 to 8.0	49	7.1	6.1 to 8.4	49
12 months	7.8	6.6 to 9.4	43	7.6	6.8 to 8.6	39	7.9	6.7 to 9.3	38	8.1	7.1 to 9.3	44
24 months	8.7	7.3 to 10.3	36	7.2	6.2 to 8.3	37	6.9	6.1 to 7.9	35	6.7	5.6 to 8.0	36
36 months	9.4	8.2 to 10.8	32	6.8	5.6 to 8.3	29	7.5	6.0 to 9.4	29	6.8	5.4 to 8.6	27
Peri/postmenopausal												
baseline	8.2	1.2 to 56.8	2	3.7	2.4 to 5.8	5	3.5	2.5 to 5.0	5	4.7	2.1 to 10.7	4
12 months	4.7	3.0 to 7.5	7	2.9	1.9 to 4.3	8	5.1	3.2 to 8.1	13	3.9	2.2 to 7.0	5
24 months	7.0	5.0 to 9.7	12	2.6	2.0 to 3.4	14	4.0	3.2 to 5.1	17	3.0	2.1 to 4.3	14
36 months	3.6	2.5 to 5.3	14	2.7	2.0 to 3.8	18	2.9	2.3 to 3.7	18	3.3	2.4 to 4.7	16

NOTE. The numbers of premenopausal women decrease and the numbers of peri- or postmenopausal women increased during the study as menopausal status changed. The repeated measures statistical model for endometrial thickness (ET) was: $\log(ET) + \log(ET_{baseline}) + \text{time} + \text{strata} + \text{menopausal status cycle} + \text{tamoxifen} + \text{fenretinide} + \text{tamoxifen} \times \text{fenretinide} + \text{menopausal status cycle} \times \text{tamoxifen} + \text{menopausal status cycle} \times \text{time} + \text{menopausal status cycle} \times \text{tamoxifen} \times \text{time}$, where $ET_{baseline}$ is baseline endometrial thickness; time is a factor denoting the follow-up at 12, 24, and 36 months; strata denotes Gail or IEN/T1; tamoxifen denotes tamoxifen or its placebo; fenretinide denotes fenretinide or its placebo; menopausal status cycle is a factor taking three levels (premenopausal days 1-14 of menstrual cycle, premenopausal days 15+ of menstrual cycle, and postmenopausal); tamoxifen $\times$ fenretinide, tamoxifen $\times$ time, menopausal status cycle $\times$ time, menopausal status cycle $\times$ tamoxifen, and menopausal status cycle $\times$ tamoxifen $\times$ time denote interactions.

Abbreviation: IEN/T1, intraepithelial neoplasia or small invasive cancer (pT1mic or pT1a).

to 19.0%; $P = .021$) compared to placebo up to month 36 and counteracted tamoxifen-induced endometrial thickness increase, although the effect modification was not significant ($P = .15$ for the tamoxifen $\times$ fenretinide interaction). Qualitatively similar results were obtained on uterine volume, whereas there was no effect of either agent on ovarian cysts (data not shown).

Adverse events were not significantly different among groups, including menopausal symptoms and endometrial polyps (Appendix Table A1, online only). Grade 1 cardiac arrhythmia (mainly palpitations) were slightly more frequent in the tamoxifen arm ($n = 6$) versus the other three arms ($n = 1, 1$, and 2 , respectively; $P = .02$). There were three serious adverse events, one instance of deep vein thrombosis, and one instance of optic neuritis on tamoxifen, and one instance endometrial cancer on fenretinide.

As at November 1, 2007, after a median follow-up of 5.5 years (range, 0 to 9.2 years), there were 48 women with breast neoplasms (plus three cases of LCIS): 11 with tamoxifen (mean $\pm$ standard deviation annual rate = $3.5\% \pm 1.0\%$), seven with fenretinide ($2.1\% \pm 0.8\%$), 14 with the combination ($5.1\% \pm 1.3\%$), and 16 with placebo ($4.7\% \pm 1.3\%$). All events but one ($n = 47$; 98%) were second breast neoplasms in women with prior IEN ($n = 46$) or pT1mic/T1a ($n = 2$), 19 were second IEN (DCIS), one with sarcoma and 28 stage I breast cancers, 37 were ipsilateral and 11 contralateral neoplasms. The Cox regression analysis in Table 4 shows a trend to a reduced hazard of breast neoplasms for each single agent, particularly fenretinide, relative to placebo, but a similar effect for their combination (HR, 0.96; 95% CI, 0.46 to 1.99), with an antagonistic interaction for the addition of fenretinide to tamoxifen compared with tamoxifen alone ($P = .03$).

Table 4. Multivariate Cox Regression Analysis of Breast Neoplastic Events

Variable	All Women			No. of Breast Cancer Events
	HR	95% CI	P	
Gail strata	1.0			1
IEN/pT1mic/pT1a strata	11.9	1.64 to 86.73	.014	47
Placebo	1.0	—		16
Tamoxifen	0.70	0.32 to 1.52	.36	11
Fenretinide	0.38	0.15 to 0.90	.03	7
Tamoxifen and fenretinide combination	0.96*	0.46 to 1.99	.91	14
Baseline IGF-I quartile, ng/mL			.71	
Lowest (50-113)	1.00	—		11
Middle low (114-139)	0.80	0.34 to 1.85		11
Middle high (140-180)	0.93	0.40 to 2.16		11
Top (181-345)	1.29†	0.59 to 2.85		15

Abbreviations: IEN/pT1mic/pT1a, intraepithelial neoplasia or small invasive cancer (pT1mic or pT1a); IGF-1, insulin-like growth factor I.

* $P = .03$ for the interaction test for a common effect of tamoxifen at each of the two levels of fenretinide.

† P for trend = .08, where the trend was estimated by replacing the quartile groups with the measured IGF-I values and the trend test is a test that the coefficient of IGF-I is equal to 0.

Table 5. Receptor Characteristics of Second Breast Neoplasms During the Trial Among Women in the IEN/pT1mic/pT1a Strata Only

Parameter	Tamoxifen + Placebo		Fenretinide + Placebo		Tamoxifen + Fenretinide		Placebo + Placebo	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
ER+ or PgR+	9	7.6	6	4.9	8	8.6	14	10.9
IEN/pT1mic/pT1a, No.	5	4	1	5	2	6	6	8
HER-2+	1	1.2	1	1.6	6	4.9	1	3.8
IEN/pT1mic/pT1a, No.	1	0	0	1	3	3	0	1
Triple negative	0	1.2	0	0.4	0	0.4	0	0.4

NOTE. Expected numbers in each arm are based on IEN/pT1mic/pT1a receptor distribution at baseline; $n = 46$ because one patient had missing information. Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; IEN/invasive, intraepithelial neoplasia or small invasive cancer (pT1mic or pT1a).

Baseline plasma IGF-I did not significantly predict breast neoplasms ($P = .08$), nor did IGF-I during treatment ($P = .64$). Neither IGFBP-3 ($P = .98$), nor IGF-1:IGFBP-3 ratio ($P = .39$), nor baseline mammographic density ($P = .45$), nor its change ($P = .40$) predicted breast neoplasms. Older women had a lower risk of breast neoplasm ($P = .01$), whereas BMI had no effect ($P = .79$). Adjusting for IGF-I and treatment group, women age 44 or older were less likely to have an event (HR, 0.64, 95% CI, 0.36 to 1.14) compared to women age 30 to 43 years.

Tumor receptor characteristics occurring during treatment are presented in Table 5. The rates of disease were similar according to major receptor status at baseline as there were nine of 34 initial HER2-positive neoplasms and 40 ER/progesterone receptor (PgR) positive events of 139 in the initial neoplasms (assuming all unknowns are ER/PgR positive). The antagonistic interaction trend between tamoxifen and fenretinide was not explained by ER/PgR and HER2 status of the original neoplasm as there was no statistical evidence of imbalance in ER/PgR positive neoplasms (χ^2 , 1.36; $P = .71$), nor in HER2-positive neoplasms over the four treatment groups (χ^2 , 4.1; $P = .25$).

DISCUSSION

The results of this trial indicate that IGF-I levels were significantly lowered by low-dose tamoxifen alone or combined with fenretinide, without evidence for a synergistic interaction. Like with sex-steroids under antiandrogens or aromatase inhibitors,^{28,29} the biomarker levels recovered as intervention ceased, whereas they continued to decline with time (aging) in the placebo arm. Baseline IGF-I and IGF-I/IGFBP-3 levels were higher in women with prior disease than in unaffected women, consistent with epidemiological studies.⁷⁻⁹ However, the levels of IGF-I at baseline were only weakly associated with neoplastic risk, and their decline did not explain the favorable trend of tamoxifen on breast events. In a previous trial, a 13% decline of IGF-I under fenretinide was associated with a 35% lower risk of second breast cancer in premenopausal women.¹³ We have no clear explanation why fenretinide did not significantly decrease IGF-I levels in this trial.

Both single agents and their combination decreased mammographic density, a risk biomarker of breast cancer¹⁴⁻¹⁶ and a promising surrogate end point.¹⁷ Mammographic density showed no difference between affected and unaffected women, nor there was a correlation with IGFs, at variance with previous studies.³⁰ The reduction attained with 2 years of low-dose tamoxifen alone or combined with fenretinide is similar to the 20% relative reduction observed after 18 months of

tamoxifen 20 mg/d in premenopausal women in the International Breast Cancer Intervention Study, which was associated with a 40% decrease of breast cancer.¹⁷ Studies have shown that a decrease in density of 1% translates into a nearly 2% lower risk of developing breast cancer,^{14,17} and the 30% to 35% risk reduction of breast neoplasms on low-dose tamoxifen is in line with the 15% decrease of mammographic density observed in our study. At variance, the 10% decrease in density with fenretinide alone was lower than the 50% cancer risk reduction achieved by the retinoid, and the 20% decrease in density in the combination arm contrasts with the adverse interaction on clinical events. Further follow-up may help to shed light into the role of mammographic density as surrogate biomarker of breast cancer prevention.

The results of pill count and circulating drug levels indicate that treatment compliance was high. Levels of tamoxifen were comparable to our previous studies in women in the same age range treated at 5 mg/d.^{18,31} Fenretinide concentrations were slightly lower than in previous studies, where the mean steady-state level was around 300 to 350 ng/mL,²³ possibly because of the younger age, a factor associated with lower concentrations.³² However, fenretinide induced a comparable 50% decrease of circulating retinol,^{23,33} which returned to baseline values 1 year after treatment cessation.

The results of endometrial thickness at 3 years confirm our preliminary finding that low-dose tamoxifen increased endometrial thickness only in women who became postmenopausal during the trial.²¹ Moreover, this effect lasted for as long as tamoxifen was used and returned to baseline after treatment cessation. Notably, there was no increased incidence of endometrial polyps and ovarian cysts. Unexpectedly, fenretinide induced a significant 10% decrease in endometrial thickness and uterine volume up to year 3. In vitro, this retinoid exerts antiproliferative effects on uterine tissue which is mediated by different mechanisms, including aromatase inhibition via ceramide, receptor independent mechanisms,^{34,35} induction of apoptosis at target cells,³⁶ and modulation of retinoic acid receptors at target tissue.³⁷ Further studies are warranted to determine the clinical implications of these findings for endometrial proliferative disorders.

Safety and tolerability data were reassuring, given the very few serious adverse events and the lack of significant excess of menopausal symptoms and visual and dermatological effects. The lower dose of tamoxifen may explain the lack of excess of hot flashes and vaginal discharge compared with 20 mg/d,³⁸ whereas the use of placebo may have prevented the overdetection of dark-adaptation alterations and skin dryness previously associated with fenretinide use in unblinded trials.³⁹ There was a slight trend to more G1-cardiac arrhythmia in the

tamoxifen single arm which is in line with the findings of our phase III trial of tamoxifen 20 mg/d, where the risk of arrhythmia and atrial fibrillation was slightly increased (HR, 1.73; 95% CI, 1.01 to 2.98).³⁸ Previous studies showed that high tamoxifen doses (> 80 to 100 mg/m² twice per day) may induce QT prolongation,^{40,41} and this may lead to arrhythmic disorders.

While still preliminary, our findings support the hypothesis generated from a subgroup analysis of a previous trial² that fenretinide on its own can reduce the risk of second breast cancer in premenopausal women. In contrast, the finding of an adverse interaction in the combination arm *vis-à-vis* a favorable effect of either agent alone was totally unexpected. Given the low power of the finding, these results may be a chance effect, also because they are not consistent with the beneficial modulation of IGF-I and mammographic density observed in the combination arm. The adverse effect was not totally explained by the steroid and HER2-receptor distribution of prior IEN although there were more HER2-positive neoplasms than expected in the combination arm. Clearly, the association between treatment and tumor receptor type is a topical issue given the lack of effective agents for prevention of ER-negative disease. In a previous study,⁴² there was evidence for a preventive effect of fenretinide both in ER-positive and ER-negative second breast cancers in premenopausal women, although fenretinide was more effective in ER-positive disease, in line with *in vitro* studies.⁴³ Unfortunately, the numbers are too small to draw any reliable conclusion, and further follow-up is necessary to address this important issue.

We conclude that despite favorable effects on plasma IGF-I levels and mammographic density, the combination of low-dose tamoxifen plus fenretinide did not reduce breast neoplastic events compared to placebo, whereas both single agents, particularly fenretinide, showed

numerical reduction in annual odds of breast neoplasms. Further follow-up is warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Andrea Decensi, Chris Robertson

Financial support: Karen A. Johnson

Administrative support: Aliana Guerrieri-Gonzaga, Serena Mora
Provision of study materials or patients: Andrea Decensi, Davide Serrano, Massimiliano Cazzaniga, Marcella Gulisano, Viviana Galimberti, Enrico Cassano, Simona M. Moroni, Giuseppe Pelosi, Bernardo Bonanni

Collection and assembly of data: Aliana Guerrieri-Gonzaga, Davide Serrano, Massimiliano Cazzaniga, Serena Mora, Marcella Gulisano, Harriet Johansson, Simona M. Moroni, Franca Formelli, Ernst A. Lien, Giuseppe Pelosi

Data analysis and interpretation: Andrea Decensi, Chris Robertson, Aliana Guerrieri-Gonzaga, Harriet Johansson, Franca Formelli, Ernst A. Lien, Bernardo Bonanni

Manuscript writing: Andrea Decensi, Chris Robertson, Aliana Guerrieri-Gonzaga, Karen A. Johnson

Final approval of manuscript: Andrea Decensi, Chris Robertson, Aliana Guerrieri-Gonzaga, Davide Serrano, Massimiliano Cazzaniga, Serena Mora, Marcella Gulisano, Harriet Johansson, Viviana Galimberti, Enrico Cassano, Simona M. Moroni, Franca Formelli, Ernst A. Lien, Giuseppe Pelosi, Karen A. Johnson, Bernardo Bonanni

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