Randomized Trial of Fenretinide to Prevent Second Breast Malignancy in Women With Early Breast Cancer

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For the Fenretinide Trial Investigators

Background: Fenretinide, a vitamin A analogue, has been shown to inhibit breast carcinogenesis in preclinical studies. We determined the efficacy of fenretinide in preventing a second breast malignancy in women with breast cancer.

Methods: We randomly assigned 2972 women, aged 30–70 years, with surgically removed stage I breast cancer or ductal carcinoma in situ to receive for 5 years either fenretinide orally (200 mg/day) or no treatment. The primary end point was the incidence of contralateral breast cancer or ipsilateral breast cancer 7 years after randomization. Other end points considered post hoc were the same outcomes stratified by menopausal status, incidence of distant metastases, overall mortality, and tumors in other organs. The hazards of breast cancer occurrence were determined by Cox proportional hazards regression analysis. Statistical tests were two-sided.

Results: At a median observation time of 97 months, there were no statistically significant differences in the occurrence of contralateral breast cancer (P = .642) or ipsilateral breast cancer (P = .177) between the two arms. However, an interaction was detected between fenretinide treatment and menopausal status in both outcomes (P for interaction in both outcomes = .045), with a possible beneficial effect in premenopausal women (contralateral breast cancer: adjusted hazard ratio [HR] = 0.66, and 95% confidence interval [CI] = 0.41–1.07; ipsilateral breast cancer: adjusted HR = 0.65, and 95% CI = 0.46–0.92) and an opposite effect in postmenopausal women (contralateral breast cancer: adjusted HR = 1.32, and 95% CI = 0.82–2.15; ipsilateral breast cancer: adjusted HR = 1.19, and 95% CI = 0.75–1.89). There were no statistically significant differences between the two arms in tumors in other organs, incidence of distant metastasis, and all-cause mortality.

Conclusions: Fenretinide treatment of women with breast cancer for 5 years appears to have no statistically significant effect on the incidence of second breast malignancies overall, although a possible benefit was detected in premenopausal women. These studies, particularly the post hoc analyses, are considered exploratory and need to be confirmed. [J Natl Cancer Inst 1999;91:1847–56]
ment in reducing the incidence of contralateral breast cancer in women with early breast cancer. The reason for studying such a cohort was twofold: 1) Patients treated for an early breast cancer have a good prognosis but have a 0.8% per year risk of developing a second malignancy in their contralateral breast (14,15), which is approximately five to six times higher than the incidence of primary breast cancer in Northern Italy and the United States (16); and 2) most of these patients were already under regular follow-up, and it was relatively easy to have them participate in a long-term study. Moreover, one could expect treatment compliance to be higher than that in the general population. Thus, similarly to tamoxifen (17), should fenretinide be effective in reducing the risk of contralateral cancer, it could then be tested as a primary preventive agent in a larger population of women at risk. It should be noted that, when the study was planned, no standard treatment was available for our cohort because the efficacy of tamoxifen as an adjuvant treatment in lymph node-negative patients had not yet been demonstrated (18). Patient entry was closed in July 1993. Since all patients completed the planned intervention period by July 1998, we report here the results of the entire study.

**Subjects and Methods**

**Subjects and Treatment**

Women with stage I breast cancer or ductal carcinoma in situ (DCIS) were enrolled from 10 Italian institutions in this multicenter phase III trial, coordinated by the Istituto Nazionale Tumori of Milan. The study received Institutional Review Board approval, and all subjects signed a written informed consent form.

All study features have been described in detail elsewhere (19). Briefly, the recruitment of patients began in March 1987 and was closed in July 1993. Eligible patients were 30–70 years old, had stage I breast cancer (T1–T2 NO) or DCIS according to the International Union Against Cancer classification (20), and had received no adjuvant systemic therapy. Women with one positive axillary lymph node at the first level were also recruited starting from July 1991; a total of 17 such women were randomly assigned. Approximately half of the eligible subjects were recruited immediately after they completed their primary treatment (i.e., surgery with or without radiotherapy). The other half were selected from patients treated with surgery with or without radiotherapy within the previous 10 years, provided that they had no recurrence of cancer. Such a retrospective recruitment was justified by the notion that the risk of contralateral breast cancer remains for at least 10 years after surgery (14,15). The details of patient numbers in both groups, their eligibility, and follow-up are described in Fig. 1.

Women were randomly assigned to receive either no treatment or fenretinide (a gift from R. W. Johnson Pharmaceutical Research Institute, Springhouse, PA), given orally at a dose of 200 mg/day for 5 years (two capsules, 100 mg each, taken at dinner). A placebo control was not incorporated into the study design because of the long duration of intervention, the large size of the capsule, and the objective nature of the main end point. Moreover, all women were followed by use of the same procedures throughout the study period, and the radiologists who examined the mammograms were blinded to the allocated treatment arm. Since fenretinide treatment is associated with a statistically significant decrease in plasma retinol levels (21), a 3-day drug interruption at the end of each month was introduced to minimize diminished adaptation to darkness. The above dosage and schedule were adopted on the basis of the findings from a previous randomized phase I trial (22).

Random assignment of patients was centrally managed by the coordinating center in Milan. After the clinical investigator telephoned the data manager, the criteria for inclusion and exclusion were checked. Eligible patients were recorded and randomly assigned to one of the two treatment arms according to a computer-generated randomization list, stratified by the center. This procedure was done in such a way that the investigators did not know the allocation prior to its actual occurring.

The women were assessed every 6 months with a complete medical examination, liver function tests (serum aspartate aminotransferase, serum alanine aminotransferase, alkaline phosphatases, and total bilirubin), biochemistry tests (total cholesterol and triglycerides), and blood hematology (hemoglobin, white blood cells, and platelets) as previously described (19). The occurrence of adverse events was assessed by questioning the patient, and a specific questionnaire was adopted for visual disturbances (19). In case of a positive or a doubtful answer to the questionnaire, the patient underwent a complete ophthalmologic consultation and evaluation, including electroretinograms if needed (23) and, in a subgroup of patients, dark-adaptometry tests (24). Adverse events were defined as subjective complaints or abnormalities at physical examination or any disease not related to tumor recurrence. Their severity was graded with the use of the World Health Organization toxicity criteria with slight modifications (19).

We evaluated patient compliance to treatment by counting the unused capsules. Serial measurements of plasma levels of fenretinide, its main metabolite N-(4-methoxyphenyl)retinamide, and retinol were also obtained in subjects treated in Milan, representing approximately 60% of the whole study population, by use of methods previously described (21).

![Fig. 1. Study profile and design: recruitment, eligibility, and follow-up status of breast cancer patients and control subjects enrolled in the fenretinide efficacy study.](image-url)
Every participant underwent a mammogram and a chest x-ray once every year and a bone scan every 18 months.

**Study End Points for Determination of Drug Efficacy and Sample Size**

The primary end point of the study to evaluate drug efficacy was the occurrence of contralateral breast cancer as the first malignant event. Another main end point was the incidence of ipsilateral breast cancer reappearance, defined as a local recurrence in the same quadrant or the occurrence of a second breast malignancy in different quadrants from the primary tumor. The use of this end point was considered to be appropriate for a preventive intervention because it is at least in part due to the suppression of premalignant or early-malignant cells. Conversely, occurrences of distant metastasis (including regional relapse) and death were recorded, but they were not considered to be end points for drug efficacy insomuch as fenretinide was not thought to be active in the late phases of breast carcinogenesis. The time to disease recurrence was computed from the date of randomization to the date when the diagnosis of recurrence was made or the last follow-up assessment available for women who remained disease free. Time to second primary cancer in organs other than the breast or death with no evidence of disease was regarded as a censored observation.

The required sample size for the trial was 3500 women, equally divided in the two treatment arms. The above figure was computed according to the procedure of Wu et al. (25), which was based on the following assumptions: a 0.8% yearly incidence rate of contralateral breast cancer in the control group, a 5% type I error probability level (for a two-sided test) and a 90% power to detect a 50% rate reduction in the treatment arm, a 3-year linear lag to obtain a full intervention effect, a 7-year follow-up period, and a 10% cumulative dropout rate. With an actual sample of 2867 assessable patients from 2972 randomly assigned, as described in the “Results” section, we estimated that the power of the study was close to the nominal level (87%).

**Statistical Analysis**

All of the recorded events were included in the analysis, regardless of treatment duration and compliance levels, according to the intention-to-treat principle.

To compare the hazard of occurrence of contralateral breast cancer in the two treatment arms, the Cox proportional hazards regression analysis model was adopted. The analysis was performed both with and without adjustment for the following three covariates: 1) menopause at the time of randomization (before or after menopause), 2) primary tumor site (outer quadrant or inner/central quadrant), and 3) lobular histology (no or yes). Menopause was defined as the absence of menstruation for at least 1 year. Hysterectomized patients without bilateral salpingo-oophorectomy were considered to be premenopausal if they were younger than 51 years at the time of randomization. The above covariates were chosen because they constitute statistically significant risk factors for contralateral breast cancer (26). Evaluating estrogen receptors (ERs) and progesterone receptors could not be considered in the present analysis, mostly because these receptors were not assessed in all of the patients and, in instances when they were measured, the measurements were often made by use of different methods. Moreover, the interval from the appearance of the primary tumor to the date of randomization was not included among the covariates, since preliminary exploration (not shown here in detail) showed no influence of this factor on occurrence of contralateral breast cancer. This finding further supports the adoption of a retrospective accrual. By contrast, we investigated the intervention effect according to menopause by including in the Cox model the interaction term between these two factors. Such a decision, not specified in the original protocol, was made on the basis of previous studies (27,28) on subgroups of women participating in the trial, where menopausal status and age statistically significantly modified the effect of treatment on the change in circulating insulin-like growth factor-I. It is noteworthy that circulating insulin-like growth factor-I levels are currently considered to be an important risk factor and a promising surrogate biomarker for breast cancer in premenopausal women (29).

Treatment, the selected covariates, and the interaction term between treatment and the menopausal status were entered into the Cox model by means of 0–1 indicator variables. Checking of the proportional hazards assumption implied by the Cox model relied on the analysis of scaled Schoenfeld residuals (30). Furthermore, we applied the Gail–Simon test (31) to assess whether the interaction being investigated was of a qualitative type.

Since many patients were still under observation well beyond the 7-year period foreseen by the protocol, we repeated both the unadjusted and adjusted analyses after censoring the observation time at 7 years.

Since the time of menopause onset was not systematically recorded during the study in all premenopausal subjects, insight into the dynamic nature of this factor could not be accounted for in the primary analysis. As a surrogate, we investigated the effect of the patient’s current age on the occurrence of contralateral breast cancer. For this purpose, a logistic model was fitted in which the occurrence of the event within each year of the woman’s age during the study was used as the dependent variable (0 = no event; 1 = event), and the corresponding age period was used as the predictor variable. The effect of age was modeled through a four-knot-restricted cubic spline (32). In general, the higher the number of knots, the better the model fit to the sample data. Since a high number of knots may imply a loss in model generalization ability, we used a restricted cubic spline with four knots, which is generally regarded as a good compromise between model flexibility and the risk of overfitting (33).

The analyses of ipsilateral breast cancer reappearance, distant metastasis, and mortality also relied on the use of the Cox models, along the same lines described for contralateral breast cancer. In addition to menopausal status, the following covariates were considered: pathologic tumor size (<1.0 cm, 1.1–2.0 cm, or ≥2.1 cm), histology (infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating carcinoma with extensive intraductal component, or all other), and type of surgery for the primary tumor (only for ipsilateral breast cancer reappearance: quadrantectomy or radical mastectomy) plus all of the first-order interaction terms between treatment and the covariates. The interval between the appearance of the primary tumor and the date of randomization was also included among the predictor variables (through a four-knot-restricted cubic spline), since this factor proved to be statistically significantly associated with the incidence of ipsilateral breast cancer.

All statistical tests were two-sided at the conventional 5% significance level. Computations were made by use of the SAS™ software (34) and the S-Plus Design library developed by Harrell (33).

**RESULTS**

The study was started on March 1987, and accrual was closed prematurely on July 1993 for a number of concomitant reasons, including the Medical Alert issued by the U.S. National Cancer Institute that recommended adjuvant treatment in all breast cancer patients (positive or negative for axillary lymph nodes). A total of 2972 women were randomly assigned in this study. The chart diagram of the study profile is illustrated in Fig. 1. Details of the accrual process are reported in a separate publication (35). Briefly, 5187 women were screened. Among those women, 827 proved not to be eligible, while 1388 refused to participate in the trial. One hundred five randomly assigned subjects were excluded because of ineligibility (39 in the fenretinide arm and 33 in the control arm) or because of withdrawal soon after randomization (25 in the fenretinide arm and eight in the control arm), thus leaving a sample of 2867 assessable women (1432 in the fenretinide arm and 1435 in the control arm). The main characteristics of assessable subjects are shown in Table 1. The two groups were well matched for the entire complement of host and tumor characteristics considered. The high percentage of premenopausal women at randomization (48%) was not sought, but it can be explained by the fact that most of the participating centers are reference centers for the treatment of breast cancer in the country and, thus, a higher proportion of younger women tends to be referred there. The prevalence of women who underwent breast-conserving surgery (63% overall) was higher for premenopausal patients (67%) than for postmenopausal patients (58%), although it was almost evenly distributed between arms (68% in the fenretinide arm and 66% in the control arm). The characteristics of the 105 excluded women overlapped those of the assessable patients, with the exception of age and menopausal status. Specifically, the women excluded from the analysis were younger and, consequently, were more frequently pre-
Table 1. Characteristics of breast cancer patients and control subjects enrolled in the study of fenretinide efficacy and information regarding the type of disease and treatment follow-up

<table>
<thead>
<tr>
<th></th>
<th>Fenretinide group (n = 1432)</th>
<th>Control group (n = 1435)</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
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<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>30–35</td>
<td>20</td>
<td>1.4</td>
<td>20</td>
<td>1.4</td>
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<tr>
<td>36–40</td>
<td>102</td>
<td>7.1</td>
<td>108</td>
<td>7.5</td>
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<td>41–45</td>
<td>196</td>
<td>13.7</td>
<td>214</td>
<td>14.9</td>
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<tr>
<td>46–50</td>
<td>336</td>
<td>23.5</td>
<td>354</td>
<td>24.7</td>
</tr>
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<td>51–55</td>
<td>305</td>
<td>21.3</td>
<td>289</td>
<td>20.1</td>
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<tr>
<td>56–60</td>
<td>255</td>
<td>17.8</td>
<td>222</td>
<td>15.5</td>
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<td>61–65</td>
<td>186</td>
<td>13.0</td>
<td>189</td>
<td>13.2</td>
</tr>
<tr>
<td>66–70</td>
<td>32</td>
<td>2.2</td>
<td>39</td>
<td>2.7</td>
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<tr>
<td>Menopausal status</td>
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<td></td>
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<tr>
<td>Premenopausal</td>
<td>663</td>
<td>46.3</td>
<td>711</td>
<td>49.5</td>
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<td>Postmenopausal</td>
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<td>53.7</td>
<td>724</td>
<td>50.5</td>
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<td>Primary tumor site</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Outer quadrant</td>
<td>906</td>
<td>63.3</td>
<td>924</td>
<td>64.4</td>
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<tr>
<td>Inner/central quadrant</td>
<td>516</td>
<td>36.0</td>
<td>501</td>
<td>34.9</td>
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<tr>
<td>Not reported</td>
<td>10</td>
<td>0.7</td>
<td>10</td>
<td>0.7</td>
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<td>Primary tumor stage*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>1006</td>
<td>70.2</td>
<td>1060</td>
<td>73.9</td>
</tr>
<tr>
<td>pT2</td>
<td>335</td>
<td>23.4</td>
<td>304</td>
<td>21.2</td>
</tr>
<tr>
<td>pT1–pT2</td>
<td>26</td>
<td>1.8</td>
<td>26</td>
<td>1.8</td>
</tr>
<tr>
<td>pTX</td>
<td>44</td>
<td>3.1</td>
<td>31</td>
<td>2.2</td>
</tr>
<tr>
<td>pTis</td>
<td>21</td>
<td>1.5</td>
<td>14</td>
<td>1.0</td>
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<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal carcinoma (IDC)</td>
<td>968</td>
<td>67.6</td>
<td>936</td>
<td>65.2</td>
</tr>
<tr>
<td>IDC with predominant</td>
<td>73</td>
<td>5.1</td>
<td>61</td>
<td>4.3</td>
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<tr>
<td>intraductal component</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Infiltrating lobular carcinoma (ILC)</td>
<td>217</td>
<td>15.1</td>
<td>244</td>
<td>17.0</td>
</tr>
<tr>
<td>IDC + ILC</td>
<td>61</td>
<td>4.3</td>
<td>68</td>
<td>4.7</td>
</tr>
<tr>
<td>Other infiltrating histotypes</td>
<td>92</td>
<td>6.4</td>
<td>112</td>
<td>7.8</td>
</tr>
<tr>
<td>Intraductal carcinoma</td>
<td>21</td>
<td>1.5</td>
<td>14</td>
<td>1.0</td>
</tr>
<tr>
<td>Primary tumor treatment</td>
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<td></td>
</tr>
<tr>
<td>Breast-conserving surgery</td>
<td>900</td>
<td>62.8</td>
<td>895</td>
<td>62.4</td>
</tr>
<tr>
<td>Radical mastectomy</td>
<td>532</td>
<td>37.2</td>
<td>540</td>
<td>37.6</td>
</tr>
<tr>
<td>Years from surgery to randomization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>718</td>
<td>50.1</td>
<td>691</td>
<td>48.1</td>
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<tr>
<td>1–3</td>
<td>419</td>
<td>29.3</td>
<td>489</td>
<td>34.1</td>
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<tr>
<td>4–6</td>
<td>230</td>
<td>16.1</td>
<td>185</td>
<td>12.9</td>
</tr>
<tr>
<td>≥7</td>
<td>65</td>
<td>4.5</td>
<td>70</td>
<td>4.9</td>
</tr>
</tbody>
</table>

*International Union Against Cancer tumor-node–metastasis (TNM) classification (20). pTX = primary tumor cannot be assessed; pTis = carcinoma in situ.

Table 2. Number of events during the study of fenretinide efficacy and results of the Cox models evaluating the end points of the study

<table>
<thead>
<tr>
<th></th>
<th>No. of events</th>
<th>Hazard ratio</th>
<th>95% confidence interval</th>
<th>Wald’s statistic, two-sided P</th>
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<tr>
<td>Contralateral breast cancer</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Unadjusted analysis</td>
<td>65</td>
<td>0.92</td>
<td>0.66–1.29</td>
<td>.642</td>
</tr>
<tr>
<td>Adjusted analysis*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal women</td>
<td>27</td>
<td>0.66</td>
<td>0.41–1.07</td>
<td>.045†</td>
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<td>Postmenopausal women</td>
<td>38</td>
<td>1.32</td>
<td>0.82–2.15</td>
<td></td>
</tr>
<tr>
<td>Ipsilateral breast cancer reappearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unadjusted analysis</td>
<td>100</td>
<td>0.83</td>
<td>0.64–1.09</td>
<td>.177</td>
</tr>
<tr>
<td>Adjusted analysis‡</td>
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<tr>
<td>Premenopausal women</td>
<td>58</td>
<td>0.65</td>
<td>0.46–0.92</td>
<td>.045†</td>
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<tr>
<td>Postmenopausal women</td>
<td>42</td>
<td>1.19</td>
<td>0.75–1.89</td>
<td></td>
</tr>
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</table>

*Adjusted for primary tumor site, lobular histology, menopausal status, and interaction between treatment and the menopausal status.

†P value for testing interaction between treatment and menopausal status.

‡Adjusted for primary tumor site, size, and histology, type of surgery, interval from surgery to randomization, menopausal status, and interaction between treatment and menopausal status.

menopausal (58%) than the remaining subjects (48%). However, there was no imbalance in terms of age and menopausal status between the two treatment arms.

In the fenretinide group, premature treatment discontinuation was recorded in 546 women for the following reasons: tumor recurrence, 221 case patients; adverse events, 147 case patients; and voluntary withdrawals, 178 case patients. These latter withdrawals were due to psychological reasons (57 case patients), difficulty in swallowing the capsule (40 case patients), or unwillingness to adhere to follow-up procedures, external advice, or the need for concomitant treatments (29 case patients); in the remaining 52 case patients, no specific reason was ascertained.

Treatment compliance as assessed by pill counting was very high (median value = 98%; interquartile range = 95%–100%) and mostly stable over time. A high level of compliance was maintained throughout the 5-year intervention period. The degree of compliance could not be inferred by plasma drug levels because of the large intrasubject variability of plasma drug levels described in a previous report (36). However, plasma drug levels were undetectable only in a negligible number of subjects allocated to fenretinide treatment (0.6%), and none of the control subjects had detectable plasma drug levels as expected.

As of September 1, 1998, the median follow-up duration was 97 months. Twenty-six (0.9%) patients, nine in the fenretinide group (four during intervention and five after treatment completion) and 17 in the control arm, were lost to follow-up.

Malignant events recorded during the study and the results obtained with the Cox models regarding the treatment effect on efficacy outcomes are shown in Table 2. As already mentioned, all of the subjects were included in the analysis, regardless of treatment duration and compliance levels, according to the intention-to-treat principle, with the exception of 105 patients who were not eligible or withdrew shortly after randomization.

**Constralateral Breast Cancer**

The comparison between the two trial arms (Table 2) without taking into account covariate information showed no evidence of an overall treatment effect (65 events in the fenretinide arm versus 71 in the control arm; hazard ratio [HR] = 0.92; 95% confidence interval [CI] = 0.66–1.29; P = .642). When taking into account all covariates (treatment, menopausal status at randomization, primary tumor site, lobular histology, and the inter-
action between treatment and menopausal status), an interaction of borderline statistical significance was detected between treatment and menopausal status ($P = .045$). Specifically, a protective effect of fenretinide was apparent in premenopausal women (fenretinide group, 27 events; control group, 42 events; adjusted HR = 0.66; 95% CI = 0.41–1.07), whereas an opposite trend was observed in postmenopausal women (fenretinide group, 38 events; control group, 29 events; adjusted HR = 1.32; 95% CI = 0.82–2.15). These findings suggest a “qualitative” interaction, which, however, failed to reach statistical significance ($P < .20$ by the Gail–Simon test (31)]. By restricting the analysis to the first 7 years of follow-up (99 contralateral cancers, corresponding to 73% of all observed events), the above results were substantially unaffected.

With regard to the effect of the covariates, the estimated HRs were in the expected direction, a greater risk being associated with premenopausal status in the control arm (HR = 1.56; 95% CI = 0.97–2.50; $P = .067$) and lobular histology in both groups (HR = 1.33; 95% CI = 0.90–1.96; $P = .154$).

Fig. 2 shows the cumulative hazard curves for the occurrence of contralateral breast cancer according to treatment, separately for premenopausal (left panel) and postmenopausal (right panel) women. A clear separation between the curves was evident for premenopausal women; it is interesting that the difference in effect was maintained over time. The difference between the two curves was not stable over time for postmenopausal women.

The incidence of contralateral breast cancer according to the current age of women receiving fenretinide or no treatment is presented in Fig. 3. For the control arm, the curve was centered on the anticipated value of the annual incidence rate of 0.8% and showed a trend toward a reduction up to the age of 60 years, followed by a slight increase in the subsequent years. In the fenretinide group, the incidence was substantially stable up to age 50 years but tended to increase thereafter. The two curves crossed at ages 55–56 years, before which fenretinide treatment appeared to be protective; by contrast, after this age span, a trend to a detrimental effect of fenretinide may not be excluded, even though the unstable behavior of contralateral breast cancer in the control group renders this comparison difficult to interpret.

Ipsilateral Breast Cancer

In terms of ipsilateral breast cancer reappearance (Table 2), the between-group difference in the unadjusted analysis was not statistically significant (fenretinide group = 100 events; control group = 121 events; HR = 0.83; 95% CI = 0.64–1.09; $P = .177$). Most events ($n = 173, 78\%$ of 221) occurred in women undergoing quadrantectomy. Of these 173 women, 123 (71% of 173; 51 in the fenretinide arm and 72 in the control arm) were premenopausal at randomization. Taking into account covariate information (treatment, menopausal status at randomization, primary tumor site, histology, interval from primary tumor to randomization, and the interaction between treatment and menopausal status), only the interaction between treatment and...
menopausal status was statistically significant \( (P = .045) \). Among premenopausal women, there were 58 events in the fenretinide arm versus 87 events in the control arm \( (HR = 0.65; 95\% \ CI = 0.46–0.92) \); among postmenopausal women, there were 42 events in the fenretinide arm versus 34 events in the control arm \( (HR = 1.19; 95\% \ CI = 0.75–1.89) \). The cumulative hazard curves for ipsilateral breast cancer reappearance according to menopausal status are shown in Fig. 4. Notably, as observed for contralateral breast cancer, a greater risk of ipsilateral breast cancer reappearance was associated with premenopausal status in the control arm \( (HR = 2.6; 95\% \ CI = 1.74–3.91; P < .0001) \).

**Distant Metastases and Deaths**

A total of 326 (11.4\%) of 2867 subjects developed distant metastases. No statistically significant treatment effect was detected after covariate adjustment \( (HR = 0.98; 95\% \ CI = 0.79–1.22; P = .858) \). The cumulative hazard curves for distant metastases are shown in Fig. 5. Likewise, no statistically significant difference in overall mortality was observed after covariate adjustment \( (HR = 1.16; 95\% \ CI = 0.92–1.48; P = .215) \).

**Tumors in Other Organs**

The frequency of a second primary tumor as a first event in organs other than the breast was equivalent in the two arms (38 in the fenretinide arm versus 40 in the control arm, of which nine versus 14 were in premenopausal women in the fenretinide and control groups, respectively). The most frequent tumors in the fenretinide versus control groups, respectively, were lung tumors (nine versus five cases), renal cell tumors (four versus six), ovarian tumors (three versus six), colorectal tumors (three versus five), and endometrial tumors (five versus two).

As previously reported (37), the incidence of ovarian cancer during the 5-year intervention period was statistically significantly lower in the fenretinide arm than in the control arm \( (0 \) versus six patients), whereas three cases of ovarian cancer occurred in women in the fenretinide arm after treatment cessation for 9, 27, and 38 months, respectively.

**Adverse Events**

Laboratory data were assessed with homogeneous criteria in both arms. The number of women with abnormal values in both groups is reported in Table 3. No difference was observed between the two arms.

Because of the lack of a placebo control group, a comparison between the two study arms for other adverse events was not deemed appropriate. Of a total of 1432 treated subjects, the most frequently reported adverse events were diminished dark adaptation \( (n = 221 \ [15.4\%]) \) and dermatologic disorders \( (n = 234 \ [16.3\%]) \), including skin or mucosal dryness \( (n = 82 \ [5.7\%]) \) and
n = 59 [4.1%, respectively], pruritus (n = 44 [3.1%]), urticaria (n = 37 [2.6%]), and dermatitis (n = 33 [2.3%]). Gastrointestinal symptoms (n = 159 [11.1%]) were mainly due to dyspeptic syndrome (n = 87 [6.1%]) or nausea (n = 44 [3.1%]). Disorders of the ocular surface (n = 119 [8.3%]) included ocular dryness (n = 44 [3.1%]), lacrimation (n = 34 [2.4%]), and conjunctivitis (n = 34 [2.4%]).

A total of 63 subjects (4.4%) had to stop intervention because of severe adverse events. Eighty-four (5.9%) additional patients stopped treatment for events that were considered to be unrelated to treatment.

DISCUSSION

The primary objective of this study was to evaluate the possibility that fenretinide, a synthetic retinoic acid derivative with a remarkable antitumor activity and low toxicity in animal models (17), could reduce the incidence of contralateral breast cancer in women treated for an early breast cancer. Should fenretinide be effective, similar to the effectiveness of tamoxifen on contralateral breast cancer (17), it could then be tested in healthy women at increased risk for breast cancer. This finding would open a new avenue toward primary prevention of breast cancer.

The average annual incidence rate of 0.8% of contralateral breast cancer observed in the control group and the comparability between prospectively and retrospectively selected patients indicate that the assumptions made when planning the study were fulfilled. The reasonably large sample size and the high compliance to treatment obtained throughout the trial were such to warrant a high study power. Overall, no statistically significant effect of fenretinide was observed on contralateral breast cancer. However, when menopausal status was taken into account, premenopausal women were shown to benefit from fenretinide treatment, which was contrary to what was observed in postmenopausal subjects. Importantly, the age of menopause onset for women who were still menstruating at the time of randomization and changed their status during the study was not systematically recorded; therefore, our analysis could not directly take into account the dynamic nature of this factor. We could, however, investigate the effect of fenretinide on the occurrence of contralateral breast cancer by using the patient’s current age as a surrogate of this hormonal change. This analysis showed that women treated with fenretinide, compared with untreated control subjects, were at lower risk up to the age of about 56 years and possibly at slightly greater risk thereafter, even though this comparison is difficult to interpret, given the unstable course of contralateral breast cancer in the control group.

The above findings might suggest the existence of a “qualitative” interaction between fenretinide intervention and menopausal status (or age, which is obviously related) that should, however, be interpreted with caution from the statistical viewpoint. In fact, the search for such an interaction was not specified in the original protocol, but an interaction was suggested later on the basis of biologic considerations. The reason for this analysis has been explained in the “Statistical Analysis” section. Furthermore, in spite of the statistically significant findings with the use of the Cox model-derived test, the Gail–Simon test (31) failed to support the hypothesis of a qualitative interaction.

From the biologic viewpoint, the different treatment effects observed in the present study depending on menopausal status are consistent with the results of previous studies. We previously showed that fenretinide affects the levels of circulating insulin-like growth factor-I differently in premenopausal and postmenopausal women; compared with findings in untreated control subjects, insulin-like growth factor-I levels decreased in premenopausal women and not in postmenopausal women during fenretinide treatment (27,28). Importantly, higher circulating insulin-like growth factor-I levels have recently been shown to be associated with a greater risk of developing subsequent breast cancer in a large prospective study in the subgroup of premenopausal women (29). One possible explanation for the distinct effect in premenopausal versus postmenopausal women might thus be related to the presence or absence of adequate circulating estrogen levels, which seem to dictate negative or positive control of gene expression by fenretinide.

The existence of complex hormonal interactions in breast cancer biology is also supported by other observations. For example, the complex incidence pattern of breast cancer over age, as illustrated by the curve inflection across menopause (the Clemmensen’s hook) and the dual effect of body size on breast cancer risk depending on age, has been regarded as evidence of...
two different diseases based on menopausal status (38–41). Indeed, in our control group, as well as in previous observations (14,15,42), the incidences of contralateral or ipsilateral breast cancer were higher in premenopausal women than in postmenopausal women.

An important clinical finding in our study is the reduced incidence of ipsilateral breast cancer in premenopausal women treated with fenretinide. Again, the benefit was not found in postmenopausal women, who showed in fact a slight trend toward a higher number of events. This observation further suggests that the preventive effect of fenretinide is associated with the presence of circulating sex steroids in the premenopausal range. The mechanism of action that leads to a better result in the surgically treated breast is not easy to interpret. In patients treated with breast-conserving surgery, ipsilateral breast cancer reappearance is partly attributable to the proliferation of residual cancer cells and partly attributable to the progression of premalignant lesions that may already be present in the surrounding areas of the primary tumor. This consideration suggests that fenretinide is active also in a later phase of breast carcinogenesis. Importantly, this potential benefit justifies further investigation of young women with breast cancer treated with breast-conserving surgery, in whom the incidence of ipsilateral breast cancer reappearance is relatively high (42). In contrast, the lack of an effect on distant metastases suggests that progression to a more malignant phenotype renders the cell resistant to fenretinide, possibly as a result of the loss of retinoid receptor expression (43).

In our study, accurate evaluation of fenretinide toxicity is hampered by the lack of a placebo control group. However, the frequency of patients with abnormal laboratory values did not differ between the two treatment arms. Dermatologic, gastrointestinal, visual (retinal), and other ophthalmologic events were relatively frequent, but exact quantification of the fraction actually related to treatment is not possible. Nevertheless, only 63 (4.4%) of 1432 subjects experienced adverse events that prompted premature treatment discontinuation, and no life-threatening events were observed.

The temporal pattern of occurrence of visual and other ophthalmologic complaints and their possible association with plasma retinol levels were previously investigated in subsets of women accrued in this trial (44). We showed that the 5-year cumulative incidence of diminished dark adaptability was approximately 20%. These symptoms occurred more frequently at the start of intervention, but they tended to recover spontaneously even without treatment discontinuation. The probability of developing visual disturbances was related to a greater reduction in plasma retinol levels following fenretinide administration. Other ophthalmologic symptoms, such as eye dryness or lacrimation, were less frequent, their cumulative incidence being approximately 8%, and were more frequent in older patients (44).

Like other retinoids, fenretinide may be teratogenic, although it is not stored in the human embryo (45). Thus, appropriate contraceptive measures must be adopted by premenopausal women while on treatment and for up to at least 6 months after its cessation, when drug plasma levels are at the limit of detectability (21).

Our findings have several implications. From the biologic viewpoint, further fundamental research should be addressed to elucidate the interaction between fenretinide and steroid hormones at the cellular and the molecular levels. On a clinical basis, the beneficial trend for contralateral breast cancer and ipsilateral breast cancer reappearance observed in premenopausal women supports a prevention trial in young, healthy women at increased risk for breast cancer. A combination treatment with tamoxifen is also suggested. Fenretinide and tamoxifen have an enhancing effect in preventing mammary tumor development in animal models (46). The recent findings of the National Surgical Adjuvant Breast and Bowel Project P-1 prevention trial (7) showed that tamoxifen was efficacious in inhibiting the development of ER-positive tumors. In breast cancer cell lines, fenretinide, in contrast to retinoic acid, induces apoptosis both in ER-positive and in ER-negative cells, although it is more effective in ER-positive cells (47). Thus, the addition of fenretinide to tamoxifen could be able to prevent a certain number of ER-negative tumors, the proportion of which is higher in premenopausal women than in postmenopausal women. To shed light on this issue, a pathologic review of the hormone receptor status of primary and contralateral or ipsilateral breast cancers is currently under way in the single reference laboratory reviewing all histologic sections.

In a previous report on this trial, we showed that fenretinide treatment was associated with a statistically significant inhibition of ovarian cancer incidence in women aged 50 years or younger while on intervention, with six cases being observed in the control group versus no cases in the fenretinide group \(P = .016; (37)\). While this effect was partially lost in the follow-up period, the concomitant inhibition of contralateral breast cancer and ovarian cancer in young women might also suggest a beneficial effect in women with a high probability of carrying a BRCA1 mutation (48). Results in breast cancer cell lines support the concepts of increasing BRCA1 protein expression by hormonal agents as a strategy to compensate for the impaired function associated with breast and ovarian carcinogenesis (49,50). Additional studies are necessary to provide further insight into this potential effect of fenretinide.

In conclusion, we observed a different effect of fenretinide on the risk of contralateral breast cancer and ipsilateral breast cancer reappearance, depending on menopausal status or age, with a beneficial effect in premenopausal women and a reversed trend in postmenopausal women. These studies are considered exploratory, and the results should be confirmed. Further studies are necessary to evaluate the efficacy of this drug in reducing the risk of breast cancer in young women. In addition, this agent has the potential to decrease the rate of local recurrence after breast-conserving surgery in premenopausal patients with breast cancer.

APPENDIX

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References

(20) International Union Against Cancer. TNM classification of malignant tumours. 4th ed. Berlin (Germany); 1987.


NOTES

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