

Effect of Raloxifene on Breast Cancer Cell Ki67 and Apoptosis: A Double-Blind, Placebo-controlled, Randomized Clinical Trial in Postmenopausal Patients¹

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Abstract

Purpose: Raloxifene is a selective estrogen receptor (ER) modulator approved for prevention and treatment of postmenopausal osteoporosis. This is an exploratory study of raloxifene in primary breast cancer patients.

Experimental Design: Postmenopausal women (50–80 years of age), with histological or cytological diagnosis of stage I or II primary breast cancer, were randomly assigned to 14 days of placebo, 60 mg/day raloxifene, or 300 mg twice daily (600 mg/day) of raloxifene. A core biopsy of the primary tumor was obtained before therapy, and a representative sample of the excised tumor was obtained from the operative specimen after treatment. Paired baseline and endpoint biopsies from each patient were analyzed for Ki67, apoptosis, and estrogen and progesterone receptors. Treatment group differences in efficacy measurements were primarily evaluated for baseline-to-endpoint change and percentage change using a one-way ANOVA with treatment as the fixed effect.

Results: Of 167 enrolled patients, 143 had evaluable efficacy data. Most breast cancer cases were invasive (98.6%), stage I (76.6%), and ER-positive (83.2%). In patients with ER-positive tumors, Ki67 increased 7% from baseline on placebo and decreased by 21% on 60 mg/day raloxifene ($P = 0.015$ versus placebo) and by 14% on 600 mg/day raloxifene ($P = 0.064$ versus placebo). Raloxifene did not affect apoptosis. ER

decreased significantly with 60 mg/day or 600 mg/day raloxifene compared with placebo ($P < 0.01$ for each comparison). Raloxifene had no statistically significant effects on Ki67 among patients with ER-negative tumors. There were no treatment differences in adverse events.

Conclusion: In this exploratory trial, 60 mg/day raloxifene showed a significant antiproliferative effect in ER-positive breast cancer, demonstrated by the decrease in Ki67, with no effect in ER-negative cancer. This provides support for raloxifene having a breast cancer preventive effect in postmenopausal women.

Introduction

Raloxifene is a SERM⁵ that is approved for the prevention and treatment of postmenopausal osteoporosis (1, 2). This is an exploratory study of raloxifene in patients with primary breast cancer.

An antiproliferative effect of raloxifene in the ER-positive MCF-7 human breast cancer cell line has been documented in model systems *in vitro* and *in vivo* (3, 4). In a placebo-controlled trial of the drug in postmenopausal patients with osteoporosis, breast cancer incidence was substantially decreased with raloxifene (5). The drug is now under evaluation as a preventive agent in comparison with tamoxifen in women at increased risk of developing breast cancer (Study of Tamoxifen and Raloxifene trial, Ref. 6) and in comparison with placebo in women at risk for heart disease [Raloxifene Use for the Heart trial (7)].

A small number of studies have evaluated the antiproliferative effect of antiestrogens and SERMs in primary human breast cancer by providing treatment during the short time between diagnosis and excision/mastectomy, an interval during which therapy is not conventionally given. Tamoxifen has been reported to reduce the levels of Ki67, an immunohistochemical marker of proliferation, after a median 21 days in a placebo-controlled trial of 103 patients (8). An effect in both ER-positive and ER-negative tumors was observed. The first human exposure to the steroidal pure antiestrogen ICI 182,780 (Faslodex) was in a 7-day, no-treatment controlled, presurgical study in postmenopausal women with breast cancer (9). The antiproliferative effect was confined to ER-positive tumors and was accompanied by increased levels of apoptosis (10). The tamoxifen analogue idoxifene reduces Ki67 by ~30% in postmenopausal women with ER-positive tumors, but in contrast to the study with ICI 182,780, there was no effect on apoptosis (11). The clinical relevance of these short-term biomarker studies is supported by the decrease in Ki67 levels after 2 weeks of

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⁵ The abbreviations used are: SERM, selective estrogen receptor modulator; ER, estrogen receptor; PR, progesterone receptor.

tamoxifen treatment before surgical resection of the primary tumor in patients whose tumor subsequently showed an objective response to the drug. There was no decrease in patients who failed to respond (12).

In the current exploratory study, postmenopausal women with primary breast cancer were randomly assigned to 14 days of one of two doses of raloxifene or placebo immediately before surgery. The lower dose of raloxifene (60 mg/day) is used for osteoporosis prevention and treatment (13), whereas the higher dose (600 mg/day) had not been assessed previously and provided a log-linear dose range. The primary endpoint was tumor cell proliferation as assessed by Ki67, a biomarker of proliferation shown previously to be suppressed by tamoxifen and the pure antiestrogen ICI182,780 (8, 9); data were also collected on apoptosis and ERs and PRs.

Materials and Methods

Study. This Phase II, double-blind, randomized, placebo-controlled trial was performed at 11 clinical trial centers in Italy and the United Kingdom. Patients were eligible for the study if they were postmenopausal, 50–80 years of age, had clinical diagnosis of stage I or II [American Joint Committee on Cancer (14)], histologically confirmed, primary breast cancer, and with tumor size adequate for core biopsy. Patients were excluded if they: had taken or received previous treatment for breast cancer, previous raloxifene therapy, any investigational agent (within 6 months of study entry), or any estrogen or progesterone therapy including nonproprietary compounds (within 3 months of study entry); were candidates for neoadjuvant therapy; had a clinical history of hepatic or renal dysfunction; or had active deep venous thromboembolic disease or were suspected of having hereditary predisposition for developing venous thromboembolic disease.

Patients were randomly assigned to receive raloxifene 60 mg once/day (marketed dose), raloxifene 300 mg twice/day, or placebo. The Eli Lilly Clinical Trials Materials Group (Indianapolis, IN) performed randomization and packaged study drug materials but was not involved in study design or patient monitoring. The sponsor produced randomly numbered kits that contained identically appearing raloxifene or placebo tablets. Trial centers dispensed the kits in numerical order to the women enrolled in the study. To maintain blinding, the 60 mg/day raloxifene group also received placebo tablets; all of the groups received the same number of tablets per day. Study drug was to be taken for 14 days between biopsy and surgical resection of the primary tumor.

Each patient gave written informed consent, and the study was approved by the ethical committees of the participating clinical centers. Additional patients were recruited to replace those found to be ineligible or nonevaluable for the primary endpoint.

Biopsy Methods. Before the start of study medication, a core-cut biopsy of the primary breast tumor was obtained using a 14-gauge needle, and at surgery a sample of the excised tumor was obtained from the operative specimen. Both specimens were fixed in 10% normal buffered formalin and embedded in paraffin wax. The embedded blocks were sent to the Royal Marsden Hospital, London, United Kingdom, for processing and analysis. Sections (3 μ m thick) of the paraffin wax blocks were cut onto positively charged slides and dried overnight at 37°C before being used for immunohistochemical analysis.

Analytical Methods. Measurement of cell proliferation was by immunohistochemical assay using the MIB1 mouse monoclonal antibody to Ki67 (15). Measurement of apoptosis was by

terminal deoxynucleotidyl transferase-mediated nick end labeling; the apoptotic index was expressed as a percentage of the total number of cells displaying apoptotic bodies (15). Tumor cells/section (3000) were counted for Ki67 and apoptotic index based on precision data published previously. ER expression was demonstrated with the DAKO 1D5 mouse monoclonal antibody (16) and PR with the Novocastra antibody NCL-PgR clone 1A6 (17). ER and PR expression were assessed semi-quantitatively by determining the percentage of tumor cells stained by the primary antibody and assessing the intensity of staining using a score of 0–3, corresponding to negative, weak, intermediate, and strong staining intensities in 10 high-powered fields. The percentage of tumor cells in each of these categories was used to calculate the overall H-score as follows: (% of cells intensity 1 \times 1) + (% of cells intensity 2 \times 2) + (% of cells intensity 3 \times 3), which provides scores ranging from 0 to 300. Tumors with a score of ≥ 20 were considered positive for either ER or PR. In all of the cases, paired baseline and endpoint samples from the same patient were stained and scored in the same assay batch. Scoring was conducted by one analyst and was subject to quality control checks by a second; if the scorers disagreed, differences were resolved by agreement after double-headed microscopic examination.

Statistical Methods. All of the analyses were based on assigned treatment at the time of randomization, regardless of compliance status. The original sample size of 50 patients/treatment group was intended to have 80% power to detect a 25% reduction in the post-treatment:pretreatment ratio of Ki67 comparing raloxifene with placebo (protocol-defined primary analysis), based on precision data published previously (15). During a planned interim analysis, it was discovered that this sample size calculation was flawed because of mathematical errors and that the study lacked sufficient power for the primary analysis. The incorrect sample size was reported to the data monitoring board responsible for monitoring the safety and efficacy of the study. Given the exploratory nature of the study, the data monitoring board decided not to extend the sample size at that time. Efficacy analyses of Ki67, apoptosis, ER, and PR were performed on data from 143 patients with paired baseline and endpoint data. It was *a priori* known that raloxifene was most likely to have an effect in patients with ER-positive tumors. Therefore, although data from all of the randomized patients are included here, the efficacy analyses and their interpretation focus on patients with ER-positive or ER-positive/PR-positive tumors. All of the safety analyses included data from all of the randomized patients.

Treatment-group differences between each raloxifene dose and placebo in the ratio of post-treatment:pretreatment Ki67 was evaluated using the approximate normal statistic $\sqrt{n/2} \times \log(X_n/Y_n)$, where X_n and Y_n are the mean post-treatment:pretreatment ratio for raloxifene dose group and placebo group, respectively (18). Because this protocol-defined primary analysis was underpowered, treatment group differences were also evaluated for baseline-to-endpoint change and percentage change using a one-way ANOVA, with treatment as the fixed effect. Because efficacy data were skewed and, in some cases, heterogeneity of variance was observed, ANOVA was performed on the rank-transformed data. Within-group change and percentage change were tested by a Wilcoxon signed rank statistic. Medians are presented as descriptive statistics of the variables, and the standard errors for the medians were estimated using the d-delete jackknife method (19). Treatment group differences were also analyzed in the percentage of patients whose Ki67 measurements decreased during the study

Fig. 1. Patient enrollment and follow-up. Patients were evaluable if they had paired pretreatment and post-treatment data on Ki67, apoptosis, ER, and PR. Reasons why patients had missing data include the following: no post-treatment biopsy because of early discontinuation, patient refused surgery, inadequate sample, specimen not clearly identified, or no malignancy at baseline.

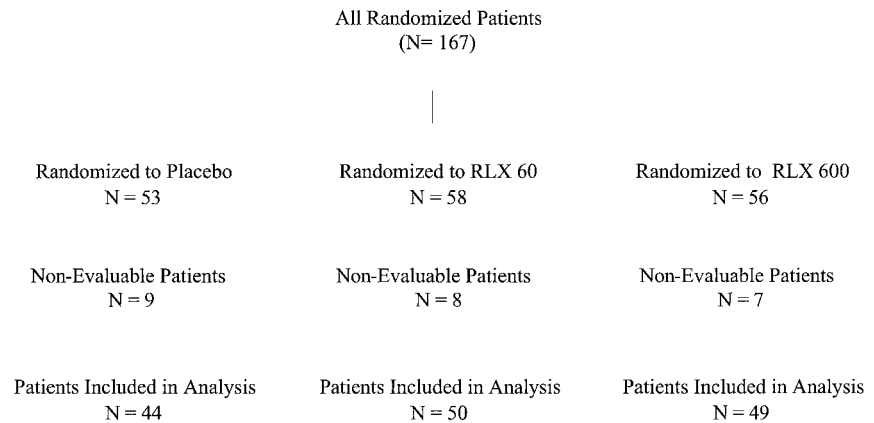


Table 1 Baseline characteristics of evaluable patients

| | Placebo | RLX 60 | RLX 600 | P |
|---|------------|------------|------------|-------|
| No. of patients | 44 | 50 | 49 | |
| Mean age (yr range) | 66 (51–79) | 66 (43–81) | 67 (51–80) | 0.920 |
| Mean yr postmenopause (range) | 16 (2–42) | 17 (2–39) | 17 (0–34) | 0.941 |
| Mean weight (kg) | 70.5 | 68.2 | 68.5 | 0.681 |
| Patients with hysterectomy (% yes) | 22.7 | 18.0 | 14.3 | 0.573 |
| Previous HRT use (% yes) | 25.0 | 22.0 | 20.4 | 0.866 |
| Tumor type [n (%)] | | | | 0.389 |
| Invasive | 43 (97.7) | 49 (98.0) | 49 (100) | |
| Other | 0 (0.0) | 1 (2.0) | 0 (0.0) | |
| Unspecified | 1 (2.3) | 0 (0.0) | 0 (0.0) | |
| Stage of diagnosis [n (%)] | | | | 0.943 |
| Stage I | 33 (75.0) | 38 (76.0) | 34 (69.4) | |
| Stage II | 9 (20.5) | 10 (20.0) | 13 (26.5) | |
| Unspecified | 2 (4.5) | 2 (4.0) | 2 (4.1) | |
| Family breast cancer history ^a (% yes) | 26.0 | 10.2 | 18.2 | 0.126 |

^a At least one primary relative (mother, sister).

using the χ^2 test. Adverse events were analyzed by the χ^2 statistic. Potential treatment-group differences in baseline characteristics were assessed by Student's *t* test for continuous variables and by the χ^2 test for categorical variables.

Results

The study randomized 167 patients (53 placebo, 58 raloxifene 60 mg/day, and 56 raloxifene 600 mg/day) with primary breast cancer, 143 of whom had paired pretreatment and post-treatment data on Ki67, apoptosis, ER, and PR (Fig. 1). The distribution was even across treatment groups: 44, 50, and 49 in the placebo, raloxifene 60 mg/day, and 600 mg/day groups, respectively. Patient characteristics of the evaluable patients are summarized in Table 1. The majority (98.6%) of breast cancer cases were invasive; 76.6% were stage I, and 23.4% were stage II. At baseline, 119 patients (83.2%) had ER-positive tumors, whereas 24 patients (16.8%) had ER-negative tumors; 107 patients (74.8%) had PR-positive tumors and 36 patients (25.2%) had PR-negative tumors; 101 tumors (70.6%) stained positively for both ER and PR. No treatment-group differences were observed for any baseline characteristic. At baseline, Ki67 was significantly higher in ER-negative patients (median = 36.3) than in ER-positive patients (median = 14.0; $P < 0.001$).

More than 96% of patients took study drug for at least 13 days; only 5 patients (1 placebo and 2 each 60 mg/day ralox-

ifene and 600 mg/day raloxifene) did not take study drug for at least 13 days.

The protocol-defined primary analysis was to compare the ratio of post-treatment:pretreatment Ki67 levels between raloxifene and placebo. For all of the randomized patients, a reduction in the mean ratio of Ki67 was observed in both raloxifene groups compared with placebo. The reduction from placebo was 31.2% in the 60-mg raloxifene group ($P = 0.070$) and 23.4% in the 600-mg raloxifene group ($P = 0.199$). In patients with ER-positive tumors, the reduction from placebo was 35.0% ($P = 0.057$) and 23.8% ($P = 0.234$) in the 60-mg and 600-mg raloxifene groups, respectively.

A more common endpoint in these types of studies is the percentage change in Ki67 from baseline to end point, measured as the difference between the two time points. When data from all of the evaluable patients were analyzed (including patients with both ER-positive and ER-negative tumors), both doses of raloxifene significantly decreased Ki67 by 15% from baseline (within-group; $P < 0.02$), corresponding to a decrease of 20% compared with placebo ($P = 0.025$ and 0.049 for 60 mg/day and 600 mg/day raloxifene, respectively).

In patients with ER-positive tumors, there was a statistically significant treatment effect for median percentage change in Ki67 (Fig. 2). Ki67 increased ~7% from baseline among patients treated with placebo, whereas it decreased by 21%

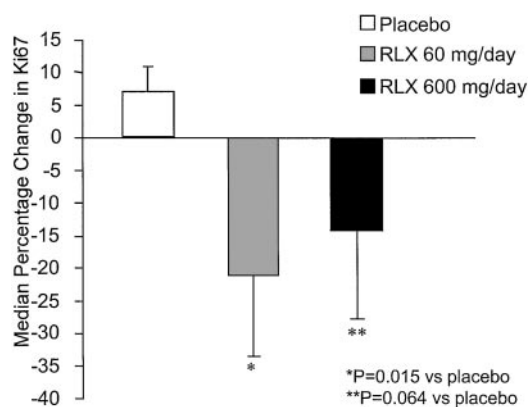


Fig. 2. Median percentage change from baseline in Ki67 among ER-positive tumors. Ki67 increased ~7% from baseline among patients treated with placebo, whereas it decreased by 21% among patients treated with 60 mg/day raloxifene ($P = 0.015$ versus placebo). There was also a reduction in Ki67 among patients treated with 600 mg/day raloxifene (14% from baseline), but this was not statistically significantly different from the change in the placebo group ($P = 0.064$).

among patients treated with 60 mg/day raloxifene ($P = 0.015$ versus placebo). There was also a reduction in Ki67 among patients treated with 600 mg/day raloxifene (14% from baseline), but this was not statistically significantly different from the change in the placebo group ($P = 0.064$; Table 2). Similar results were observed among the subset of patients with ER-positive/PR-positive tumors: median percentage change was +8%, -25%, and -13% in the placebo, 60 mg/day raloxifene, and 600 mg/day raloxifene groups, respectively. There was no significant effect of raloxifene in ER-negative tumors.

The percentage of all of the evaluable patients with ER-positive tumors of which the Ki67 measurements decreased below baseline during treatment additionally supports the effectiveness of raloxifene to reduce Ki67: 69.1% and 63.4% of patients assigned to 60 mg/day and 600 mg/day raloxifene, respectively, compared with 38.9% assigned to placebo ($P = 0.008$ and 0.032 for 60 mg/day and 600 mg/day raloxifene versus placebo, respectively). Similar results were observed among the subset of patients with ER-positive/PR-positive tumors.

Among all of the evaluable patients or just those with ER-positive or ER-positive/PR-positive tumors, there was no significant effect of raloxifene on apoptosis (Table 2).

Patients with initial ER-positive tumors receiving either dose of raloxifene or placebo demonstrated a significant decrease in tumor ER immunoreactivity (H-score) during the two-week treatment period (Table 2). However, patients receiving either 60 mg/day or 600 mg/day raloxifene experienced significantly greater decreases in ER H-score (21.2% and 28.8%, respectively) during therapy as compared with those receiving placebo (8.9%; $P \leq 0.010$ for each comparison). The effect of therapy on tumor ER levels was similar for patients with initial ER-positive/PR-positive tumors. H-score for PR remained stable in all three of the groups. There was no significant correlation between change in ER and change in Ki67.

Raloxifene was generally safe and well tolerated. Treatment-emergent adverse events were reported by 51 patients (30.7%; 18 on placebo, 19 on 60 mg/day raloxifene, and 14 on 600 mg/day raloxifene); only 2 patients (1 each on placebo and 600 mg/day raloxifene) discontinued the trial because of an adverse event. The most commonly reported adverse event was

vasodilatation (hot flashes), reported by 10.2% of all patients (9.4%, 10.5%, and 10.7% in the placebo, 60 mg/day raloxifene group, and 600 mg/day raloxifene group, respectively). One patient (assigned to 60 mg/day raloxifene) died after surgery because of myocardial infarction; the investigator assessed that this was not attributable to study drug. There were no statistically significant treatment-group differences in the incidence of adverse events overall or in the incidence of any individual adverse event.

Discussion

In this 14-day exploratory study in postmenopausal women with primary breast cancer, 60 mg/day raloxifene significantly decreased Ki67 but did not affect apoptosis. Both doses of raloxifene (60 mg/day and 600 mg/day) significantly decreased ER compared with placebo.

In a trial of 7705 postmenopausal women with osteoporosis, raloxifene (pooled 60 and 120 mg/day doses) was found to reduce the risk of newly diagnosed invasive breast cancer by 76% (95% confidence interval: 56–86%) and ER-positive breast cancer by 90% (95% confidence interval: 76–96%) compared with placebo without significantly affecting the risk of ER-negative breast cancer (5). Because follow-up in that study was for a median of only 40 months, and breast cancer generally takes several years to grow to a clinically or radiographically detectable size (20), it may be that some of the risk reduction represented suppression or regression of subclinical cancer. It seems likely that the response of such ER-positive subclinical disease will be comparable with that of the early stage ER-positive primaries as included in the present study. Overall, the results presented here support raloxifene having an antiproliferative effect on ER-positive but no effect on ER-negative cancers.

The use of immunohistochemical methodology limits the quantitative interpretation of the data. However, the scoring systems used in our earlier studies of this type have revealed changes for each of the parameters measured (9–11). Therefore, these systems may be expected to reveal meaningful changes elicited by raloxifene.

Previous studies have indicated that two clinically active antiestrogens, tamoxifen and ICI 162,780, both have antiproliferative effects within 2 weeks of initiating therapy (10, 12, 21), and importantly, for tamoxifen, such changes relate to subsequent response to therapy (12). It is not appropriate to compare the degree of change in Ki67 in those studies with the present one, because different types of biopsy (smaller frozen core-cut or fine needle aspiration) and antibodies were used. However, it has been reported (11) in a study using identical methodology to that used in this study that idoxifene (40 mg/day) reduced Ki67 from mean pretreatment levels of 20.1% to 14.3% after 14–21 days in ER-positive tumors. These effects appear to be slightly greater than those found with raloxifene, but the results were obtained in a different study, so this apparent difference would need to be confirmed in a direct, randomized comparison.

The key question in this study was whether raloxifene had any antiproliferative activity. At the time this study was designed, there were no data on the effects of raloxifene in either established tumors or in the prevention setting. A comparison against tamoxifen was not undertaken, because it would have required a much larger number of patients to accommodate a fourth study arm. In addition, the 7–10 day half-life of tamoxifen means that a steady state is not reached for several weeks (22), which would have compromised interpretation of the data.

Table 2 Median change and median percentage change during treatment in patients with ER-positive breast cancer

| Tests (unit) | Treatment | n ^a | Baseline (median ± SE) | Median change (median ± SE) | Median % change (median ± SE) | ANOVA P ^b | |
|--------------------------------|-----------|----------------|---------------------------|--------------------------------|----------------------------------|----------------------|--------------------------------|
| | | | | | | Overall | Raloxifene dose vs. placebo |
| Ki67 (% of labeled cells) | Placebo | 36 | 13.35 ± 3.01 | 0.75 ± 0.44 | 7.09 ± 3.81 | 0.042 | 0.015 |
| | RLX 60 | 42 | 13.35 ± 1.82 | -2.75 ± 0.74 ^c | -21.0 ± 12.53 ^d | | |
| | RLX 600 | 41 | 14.60 ± 2.12 | -2.30 ± 1.63 ^d | -14.2 ± 13.68 ^d | | |
| Apoptosis (% of labeled cells) | Placebo | 36 | 0.60 ± 0.05 | 0.05 ± 0.09 | 4.54 ± 15.24 ^d | 0.462 | 0.064 |
| | RLX 60 | 42 | 0.60 ± 0.09 | 0.10 ± 0.06 | 16.7 ± 11.64 ^d | | |
| | RLX 600 | 41 | 0.70 ± 0.06 | 0.00 ± 0.07 | 0.00 ± 9.20 | | |
| ER (H-score) | Placebo | 36 | 207.50 ± 8.04 | -20.5 ± 8.08 ^c | -8.92 ± 3.41 ^c | 0.002 | 0.010 |
| | RLX 60 | 42 | 206.00 ± 8.12 | -46.00 ± 9.46 ^c | -21.2 ± 3.71 ^c | | |
| | RLX 600 | 41 | 211.00 ± 9.86 | -65.00 ± 11.75 ^c | -28.8 ± 4.11 ^c | | |
| PR (H-score) | Placebo | 36 | 107.00 ± 30.96 | -0.55 ± 5.23 | -0.44 ± 5.46 | 0.718 | <0.001 |
| | RLX 60 | 42 | 106.00 ± 14.34 | 0.70 ± 4.44 | 1.19 ± 7.33 | | |
| | RLX 600 | 40 | 134.00 ± 15.99 | -9.00 ± 7.54 | -5.77 ± 3.40 | | |

^a RLX 60, raloxifene HCl 60 mg/day; RLX 600, raloxifene HCl 600 mg/day; n, no. of patients with paired data.

^b Between-group P based on ANOVA for the rank-transformed percentage change.

^c Within-group P ≤ 0.001.

^d Within-group P < 0.05.

The pharmacokinetics of raloxifene are not linear, but the 600 mg/day dose would be expected to result in plasma levels ~8-fold higher than those seen with 60 mg/day. However, there was no dose-relationship in the change in Ki67 in this study. Thus, it would appear that antiproliferative effects of raloxifene are maximal at the exposure levels achieved with the 60 mg/day dose, the dose approved for prevention and treatment of osteoporosis and under investigation for prevention of breast cancer and heart disease. This lack of a dose relationship might be explained by saturation of the ER at the lower dose, but we do not have data to address whether this is the case. The data provide no support for exploring doses of raloxifene above 60 mg/day in future chemoprevention studies.

It is notable that reduced antitumor effectiveness was observed previously at higher doses of raloxifene (and three other nonsteroidal antiestrogens including tamoxifen) in 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats (23). It is unclear whether these observations in animals with intact ovarian function have mechanistic similarities to those made in the postmenopausal breast cancer patients in the present study.

Raloxifene had no appreciable effect on apoptosis in this study. A number of studies have shown that apoptosis can be induced by estrogen withdrawal or by treatment with an antiestrogen, both *in vitro* and in animal model systems (17, 24–26). In an earlier small study, apoptosis was also found to be significantly higher in patients treated with ICI 182,780 compared with controls; in patients treated with tamoxifen, there was a small change in apoptosis with borderline statistical significance in patients with ER-positive tumors (10). Thus, the absence in this study of a significant increase in apoptotic index after treatment with raloxifene was unexpected. However, it was recently noted that idoxifene did not increase apoptosis in primary breast tumors (11) despite doing so in a xenograft model (25). Thus, it is possible that despite effects seen in model systems there may be no significant effect on apoptosis by SERMs in patients, at least during the first few weeks of therapy.

The measurement of ER and PR content is of importance when evaluating the likely response to endocrine therapy, because it has been well established that response occurs almost exclusively in steroid receptor-positive disease. The greater decrease in ER levels in the raloxifene-treated patients than in

the placebo group is consistent with some, but not all, earlier data with tamoxifen (8, 21) and with idoxifene (11) but is not as great an effect as that seen with ICI 182,780 (9, 27), which decreases stability of the ER protein (28). The decrease in ER in the placebo group emphasizes the importance of placebo controls in this sort of study. This decrease may have occurred as a result of differences in histological fixation between core-cuts and excision biopsies or systematic bias because of a differential effect of tissue heterogeneity on scoring of ER between core-cuts and excision biopsies. Other possible explanations are that the taking of a core-cut might itself induce a change in ER in the tumor (as a result of the local release of healing/growth factors) or that systematic differences in scoring between core-cuts and excisions might occur despite efforts to avoid this. An additional consideration in the interpretation of the greater decrease seen in the raloxifene-treated tumors is that the change in the ER H-score may result from a conformational change in ER induced by the binding of raloxifene (29), which could influence the binding to the antibody.

Additional studies would be necessary to determine whether the decrease in ER expression persists with continued treatment. However, it is important to recognize that if this occurred, it may lead to incorrect assignment of ER status in patients taking raloxifene for the prevention or treatment of osteoporosis or who are in breast cancer chemoprevention trials. As noted above (5), the decreased breast cancer incidence seen in osteoporosis trials with raloxifene was restricted to those tumors categorized as ER positive. This interpretation may require additional consideration in the light of raloxifene suppression of ER.

PR is an estrogen-induced gene, and its expression may denote an intact estrogen response mechanism (30). Thus, decreases in PR expression may be considered indicative of an antiestrogenic effect as seen with ICI 182,780 (9, 27). In contrast, the early increases seen in PR after initiating tamoxifen treatment are considered indicative of an early predominance of an agonist effect of tamoxifen (at least on the PR gene; Ref. 12). There was no change in PR expression in this study, which suggests that raloxifene lacks an estrogenic effect on the PR gene.

In conclusion, in this exploratory trial, 60 mg/day raloxifene significantly decreased growth of ER-positive breast cancer as shown by the decrease in the Ki67 labeling index, with

no effect in ER-negative cancers. It is likely that 60mg/day raloxifene will have a similar effect on subclinical disease and thereby a prophylactic effect on breast cancer incidence in postmenopausal women.

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