Effects of Raloxifene After Tamoxifen on Breast and Endometrial Tumor Growth in Athymic Mice

Ruth M. O’Regan, Csaba Gajdos, Rita C. Dardes, Alex De Los Reyes, Woochan Park, Alfred W. Rademaker, V. Craig Jordan

Background: In patients with early-stage breast cancer, 5 years of treatment with the selective estrogen receptor modulator (SERM) tamoxifen reduces breast cancer recurrence and mortality, whereas more than 5 years of tamoxifen does not further reduce breast cancer recurrence and doubles the risk of endometrial cancer. We evaluated the effects on tumor growth of raloxifene, another SERM, after tamoxifen treatment in mouse models of breast and endometrial cancers. Methods: Athymic, ovariectomized mice were bitransplanted with tumors derived from human breast cancer and endometrial cancer cells that either were tamoxifen-naive or had been exposed to tamoxifen for short (6 months) or long (>5 years) terms. The effects of raloxifene (two dose levels) and tamoxifen on tumor growth in the presence and absence of low-dose estrogen were evaluated. All statistical tests were two-sided. Results: Raloxifene was less effective than tamoxifen in blocking the stimulatory effects of low-dose estrogen on the growth of tamoxifen-naive breast (P<.001) and endometrial (P = .001) tumors. Raloxifene and tamoxifen had similar inhibitory effects on the growth of short-term tamoxifen-exposed breast tumors. Raloxifene and tamoxifen had similar stimulatory effects on the growth of breast and endometrial tumors that had been exposed to at least 5 years of tamoxifen. However, neither drug blocked the stimulatory effects of estrogen on the growth of these tumors. Raloxifene was less effective than tamoxifen (P<.001) in blocking the stimulatory effects of estrogen on endometrial tumors that had been exposed to tamoxifen in the past. Conclusions: Raloxifene and tamoxifen had similar effects on these mouse models of tamoxifen-naive and tamoxifen-resistant breast and endometrial cancer. Treatment with raloxifene following 5 years of adjuvant tamoxifen may not further decrease breast cancer recurrence and may increase endometrial cancer incidence. [J Natl Cancer Inst 2002;94:274-83]

Tamoxifen is a selective estrogen receptor modulator (SERM) that improves survival in women with early-stage breast cancer (1) and is approved for use in breast cancer therapy. However, it offers no advantages over tamoxifen in the treatment of advanced breast cancer (3). Raloxifene, like tamoxifen, maintains bone density in postmenopausal women (4) and is used to prevent and treat osteoporosis in this population. Results from two pivotal osteoporosis trials (5,6) suggest that tamoxifen also prevents breast cancer. Raloxifene-like SERMs appear to be less estrogenic than tamoxifen on the endometrium, both in preclinical (7,8) and clinical (4) studies, and there has been no increase in endometrial cancer incidence noted to date in the osteoporosis trials (5). Raloxifene and tamoxifen are currently being compared in the Study of Tamoxifen and Raloxifene (STAR) trial to determine whether raloxifene is as effective as tamoxifen in preventing breast cancer in high-risk women. The STAR trial will also determine whether raloxifene, like tamoxifen, is associated with an increased risk of endometrial cancer.

The current recommendation of 5 years of tamoxifen treatment in the adjuvant setting is based on several findings. First, the Oxford Overview Analysis, an analysis of trials in which patients with early-stage breast cancer were randomly assigned to receive tamoxifen or placebo, demonstrated conclusively that 5 years of tamoxifen in the adjuvant setting results in greater reductions in breast cancer recurrence and mortality than do shorter durations of treatment (1). Second, a number of trials (9–11), including the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 trial, have shown that treatment with adjuvant tamoxifen for more than 5 years does not further improve breast cancer outcome. Third, results from NSABP B-14 also showed that the risk of endometrial cancer is doubled when tamoxifen is used for more than 5 years (9).

After 5 years of treatment with tamoxifen, postmenopausal women with early-stage breast cancer remain at risk of breast cancer recurrence (1), contralateral breast cancer (1), and osteoporosis (12). A possible strategy to address this concern would be to give these women raloxifene after 5 years of tamoxifen. However, it is unclear if such a strategy would further reduce breast cancer recurrence or increase endometrial cancer incidence in these women as has been seen with tamoxifen treatment beyond 5 years (9). Furthermore, although the safety and benefits of this strategy could be addressed by performing a clinical trial, such a trial would take at least 5 years to complete and would require the enrollment of 6000–10000 women, who would be randomly assigned to receive raloxifene or placebo after 5 years of tamoxifen.

Another strategy to address the safety of raloxifene following tamoxifen would be to examine the effects of raloxifene on the growth of tamoxifen-exposed breast and endometrial tumors in vivo. The effects of SERMs and other antiestrogens on the growth of tamoxifen-exposed breast tumors in vivo appear to mimic what has been found clinically. For example, the effects of the SERM toremifene are similar to those of tamoxifen, both in tamoxifen-exposed tumors in vivo (13) and in clinical studies

Affiliations of authors: R. M. O’Regan (Division of Hematology/Oncology), C. Gajdos, A. De Los Reyes, W. Park, A. W. Rademaker, V. C. Jordan (Robert H. Lurie Comprehensive Cancer Center), Northwestern University, Chicago, IL; R. C. Dardes, Department of Gynecology, Federal University of São Paulo, Brazil.

Correspondence to: V. Craig Jordan, Ph.D., DSc., Robert H. Lurie Comprehensive Cancer Center and Northwestern University Medical School, Olson, Rm. 8258, 303 E. Chicago Ave., Chicago, IL 60611 (e-mail: vcjordan@northwestern.edu).

See “Notes” following “References.”
tumors (ECC-1) were developed by injecting 1×10^7 ECC-1 transplantation, in athymic mice that have been treated with use of the above strategy and have since been passaged, by serial (MCF7TAM LT) were initially developed in 1988 (21).

Endometrial tumor pieces (1 mm^2) in the opposite mammary fat pad (resulting in one breast tumor and one endometrial tumor)

Materials and Methods

Cell Culture

ECC-1 (a gift from Dr. Myles Brown, Department of Adult Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA) is a well-differentiated estrogen receptor-positive human endometrial cancer cell line developed in 1985 (19). ECC-1 cells were maintained in Dulbecco’s modified Eagle medium (Life Technologies, Inc., [Gibco BRL], Rockville, MD) supplemented with 10% fetal bovine serum, nonessential amino acids, L-glutamine, antibiotic–antimycotic, and insulin. For injection into mice, cells were harvested after trypsinization and resuspended in phosphate-buffered saline at 10^6 cells/mL.

Athymic Mouse Models of Breast and Endometrial Cancers

Breast cancer models. Tamoxifen-naive breast tumors (MCF7E) were developed by injecting 1 × 10^7 MCF7 human breast cancer cells into each mammary fat pad of ovariectomized, athymic BALB/c mice (Harlan Sprague-Dawley, Inc., Madison, WI) and implanting the mice with a silastic capsule containing estrogen as described previously (20, 21). Short-term tamoxifen-exposed breast tumors (MCF7TAM ST) were developed by treating mice bearing MCF7E tumors with tamoxifen for 6 months (20). Long-term tamoxifen-exposed breast tumors (MCF7TAM LT) were initially developed in 1988 (21) with the use of the above strategy and have since been passaged, by serial transplantation, in athymic mice that have been treated with tamoxifen.

Endometrial cancer models. Tamoxifen-naive endometrial tumors (ECC-1) were developed by injecting 1 × 10^7 ECC-1 human endometrial cancer cells into each mammary fat pad of ovariectomized, athymic mice and implanting the mice with a silastic capsule containing estrogen as described previously (20–22). The resulting ECC-1 tumors were then serially passaged in mice treated with estrogen. A tamoxifen-exposed endometrial tumor model (EnCa101 TAM) was developed in mice in 1984 from a well-differentiated human endometrial tumor (19). This tumor has been passaged for more than 5 years in mice treated with tamoxifen. A different tamoxifen-exposed endometrial tumor model, EnCa101 E, was developed by passaging the EnCa101 TAM tumors for 3 years in mice treated with estrogen alone (23).

Tumor implantation. In each experiment, 10 athymic, ovariectomized mice per treatment group were bitransplanted with breast tumor pieces (1 mm^2) in one mammary fat pad and with endometrial tumor pieces (1 mm^2) in the opposite mammary fat pad (resulting in one breast tumor and one endometrial tumor per mouse and 10 breast tumors and 10 endometrial tumors per treatment group). Established tumors were used in all experiments. Except for one experiment, the breast tumors were always implanted in the right mammary fat pad, and the endometrial tumors were always implanted in the left mammary fat pad. We implanted the following combinations of tumors in each treatment group: MCF7E and ECC-1, MCF7TAM ST and EnCa101 TAM, and MCF7TAM LT and EnCa101 TAM. In one experiment, mice were bitransplanted with ECC-1 tumors in one mammary fat pad and with EnCa101 E tumors in the opposite mammary fat pad.

Drug administration. In all experiments, mice bearing transplanted tumors were divided into groups of 10 and treated with different combinations of estrogen, tamoxifen, and raloxifene or were untreated (control group). Estrogen (Sigma Chemical Co., St. Louis, MO) was administered to the mice via 0.3-cm-long silastic estradiol capsules that were made as described previously (24), implanted subcutaneously, and replaced after 6–8 weeks of treatment. We have shown previously that these 0.3-cm estrogen capsules produce low estrogen levels in mice (mean serum level ± standard deviation = 83.8 ± 34.6 pg/mL) similar to estrogen levels found in postmenopausal women (22).

Tamoxifen was purchased from Sigma Chemical Co., and raloxifene (Evista®) tablets are commercially available (Eli Lilly Pharmaceuticals, Indianapolis, IN). Tamoxifen was suspended in a solution of 90% CMC (1% carboxymethylcellulose in double-distilled water) and 10% PEG 400/Tween 80 (99.5% polyethylene glycol 400, 0.5% polysorbate 80) at a final concentration of 10 mg/mL. Tamoxifen was administered orally 5 days each week (unless otherwise stated) at a dose of 0.5 mg (0.05 mL) per mouse per day. Raloxifene tablets (five tablets at 60 mg/tablet) were ground into a powder with the use of a mortar and pestle, and the powder was dissolved in 27 mL of double-distilled water. Three milliliters of 90% CMC and 10% PEG 400/Tween 80 was added to the raloxifene solution for a final concentration of 10 mg/mL. Raloxifene was administered orally 5 days each week (unless otherwise stated) at doses of 0.5 mg (0.05 mL) or 1.5 mg (0.15 mL) per mouse per day.

Tumors were measured weekly with calipers, and the cross-sectional area (in cm^2) was determined with the use of the formula: length (cm) × width (cm) / 4 × π. All animal procedures were approved by the Animal Care and Use Committee of Northwestern University.

Statistical Methods

The time course of tumor growth was compared across the treatment groups with the use of two-way analysis of variance, with group and time as the variables. For all experiments, the group-by-time interaction for tumor growth was statistically significant (P<0.05). These analyses were followed by time-specific comparisons of mean tumor size (at 8, 9, or 14 weeks) across groups with the use of pairwise Student’s t tests with Bonferroni corrections. The time points for analysis were chosen on the basis of the growth characteristics of the specific tumor types. Bonferroni corrections were used as follows: P<0.003 was considered to be statistically significant for the experiments with six groups of mice, and P<0.0018 was considered to be statistically significant for experiments with eight groups of mice. All statistical tests were two-sided.
RESULTS

Effects of Tamoxifen and Raloxifene on Growth of Tamoxifen-Naive Breast and Endometrial Tumors

We found previously that neither tamoxifen nor raloxifene stimulates the growth of tamoxifen-naive MCF7E breast tumors and tamoxifen-naive ECC-1 endometrial tumors in athymic, ovariectomized mice and that there is no statistically significant difference between the growth-stimulatory effects of tamoxifen and raloxifene in this tumor model (25). However, we wanted to compare the abilities of tamoxifen and raloxifene to block the stimulatory effects of low-dose estrogen in these tamoxifen-naive tumor models. We also wanted to determine whether the frequency of dosing would affect the abilities of tamoxifen and raloxifene to block the effects of low-dose estrogen in these tamoxifen-naive tumors. We, therefore, bitransplanted mice with tamoxifen-naive breast MCF7E and endometrial ECC-1 tumors and randomly assigned them to receive no treatment (control), low-dose estrogen alone (E2), or tamoxifen (0.5 mg/day) or raloxifene (1.5 mg/day) with low-dose estrogen (TAM + E2 and RAL + E2, respectively). We believe that this tumor model simulates the case of a postmenopausal woman who has never received tamoxifen and is beginning either tamoxifen therapy for breast cancer treatment or prevention or raloxifene therapy to prevent osteoporosis or breast cancer. The SERMs were administered 5 or 7 days per week for a total of 9 weeks. The 5-day-per-week treatment groups continued treatment for a total of 14 weeks. Tumor sizes were measured weekly. Based on Bonferroni corrections, P values less than .003 and less than .016 were considered to be statistically significant for the 9- and 14-week analyses, respectively.

After 9 weeks of treatment, the mean sizes of MCF7E tumors were 1.2 cm² for the E2 group, 0.38 cm² for the TAM 5 day + E2 group, 0.2 cm² for the TAM 7 day + E2 group, 0.68 cm² for the RAL 5 day + E2 group, and 0.84 cm² for the RAL 7 day + E2 group, all of which were statistically significantly larger than the mean size of MCF7E tumors (0 cm²) in the untreated (control) group—P < .001 for each; difference in mean tumor size between control and E2 = 1.2 cm² (95% confidence interval [CI] = 0.84 to 1.54 cm²), between control and TAM 5 day + E2 = 0.38 cm² (95% CI = 0.23 to 0.53 cm²), between control and TAM 7 day + E2 = 0.2 cm² (95% CI = 0.1 to 0.3 cm²), between control and RAL 5 day + E2 = 0.68 cm² (95% CI = 0.49 to 0.87 cm²), and between control and RAL 7 day + E2 = 0.84 cm² (95% CI = 0.45 to 1.24 cm²). In addition, after 9 weeks of treatment, MCF7E tumors in mice treated with low-dose estrogen alone were statistically significantly larger than those in mice treated with tamoxifen (at either dosing schedule) plus estrogen (P < .001 for each; difference in mean tumor sizes between E2 and TAM 5 day + E2 = 0.81 cm² [95% CI = 0.43 to 1.19 cm²] and between E2 and TAM 7 day + E2 = 0.99 cm² [95% CI = 0.61 to 1.37 cm²]), but were not statistically significantly larger than those in mice treated with either raloxifene dosing schedule plus estrogen (difference in mean tumor size between E2 and RAL 5 day + E2 = 0.52 cm² [95% CI = 0.14 to 0.89 cm²]; P = .01) and between E2 and RAL 7 day + E2 = 0.35 cm² [95% CI = -0.18 to 0.87 cm²; P = .183]) (Fig. 1, A). At 9 weeks, MCF7E tumors in mice receiving the tamoxifen 5-day dosing schedule were smaller than those in mice receiving either raloxifene dosing schedule, but the differences were not statistically significant (difference in mean tumor size between TAM 5 day + E2 and RAL 5 day + E2 = 0.3 cm² [95% CI = 0.06 to 0.53 cm²; P = .02] and between TAM 5 day + E2 and RAL 7 day + E2 = 0.46 cm² [95% CI = 0.04 to 0.89 cm²; P = .03]) (Fig. 1, A). However, at this same time point, mice treated with estrogen and the 7-day tamoxifen dosing schedule had statistically significantly smaller MCF7E tumors than mice treated with estrogen and the 5-day raloxifene dosing schedule (P < .001; difference in mean tumor size = 0.48 cm² [95% CI = 0.26 to 0.7 cm²]), indicating that the former treatment was more effective than the latter in blocking the stimulatory effects of low-dose estrogen on MCF7E tumor growth (Fig. 1, A). After 14 weeks of treatment, mice receiving the tamoxifen 5-day dosing schedule had a mean tumor size of 0.67 cm², which was statistically significantly smaller than that of mice receiving the ral-
oxifene 5-day dosing schedule (1.73 cm²) (P<0.001; difference in mean tumor size = 1.07 cm² [95% CI = 0.72 to 1.41 cm²]), indicating that tamoxifen was more effective than raloxifene in blocking the stimulatory effects of low-dose estrogen on MCF7E tumor growth when these SERMs were administered at the same dosing schedule (Fig. 1A). The different frequencies of administration of either tamoxifen or raloxifene did not result in statistically significant differences in the growth of MCF7E tumors after 9 weeks of treatment (difference in mean tumor size between TAM 5 day and TAM 7 day = 0.18 cm² [95% CI = -0.007 to 0.37 cm²; P = .06] and between RAL 5 day and RAL 7 day = 0.17 cm² [95% CI = -0.25 to 0.59 cm²; P = .41]) (Fig. 1A).

After 9 weeks of treatment, the mean size of the tamoxifen-naive endometrial (ECC-1) tumors in the estrogen-treated (E2) mice was 1.4 cm²; this was statistically significantly larger than the mean size of tumors in the mice that received no treatment (0 cm²) or treatment with TAM 5 day + E2 (0 cm²), TAM 7 day + E2 (0.06 cm²), RAL 5 day + E2 (0.25 cm²), or RAL 7 day + E2 (0.05 cm²) (P<0.001 for each; difference in mean tumor sizes between E2 and control = 1.4 cm² [95% CI = 1.02 to 1.79 cm²]), between E2 and TAM 5 day + E2 = 1.4 cm² [95% CI = 1.02 to 1.79 cm²], between E2 and TAM 7 day + E2 = 1.35 cm² [95% CI = 0.92 to 1.77 cm²], between E2 and RAL 5 day + E2 = 1.15 cm² [95% CI = 0.73 to 1.58 cm²], and between E2 and RAL 7 day + E2 = 1.36 cm² [95% CI = 0.97 to 1.75 cm²]) (Fig. 1B). At 9 weeks, the ECC-1 tumors in the raloxifene 5-day treatment group were larger than those in the tamoxifen 5-day treatment group (difference in mean tumor size = 0.25 cm² [95% CI = 0.07 to 0.44 cm²; P = .01]), but there were no statistically significant differences in ECC-1 tumor size between any of the treatment groups (mean difference in tumor size between TAM 5 day + E2 and RAL 7 day + E2 = 0.04 cm² [95% CI = -0.02 to 0.11 cm²; P = .16], between TAM 7 day + E2 and RAL 5 day + E2 = 0.2 cm² [95% CI = -0.03 to 0.62 cm²; P = .08], and between TAM 7 day + E2 and RAL 7 day + E2 = 0.009 cm² [95% CI = -0.12 to 0.14 cm²; P = .89]) (Fig. 1B). After 14 weeks of treatment, mice receiving the raloxifene 5-day dosing schedule had a mean tumor size of 0.77 cm²; this was statistically significantly larger than the mean tumor size of mice receiving the tamoxifen 5-day dosing schedule (0.024 cm²), indicating that the latter treatment was more effective in blocking the stimulatory effects of low-dose estrogen on ECC-1 tumor growth (P = .001; difference in mean tumor size = 0.74 cm² [95% CI = 0.35 to 1.14 cm²]) (Fig. 1B). In addition, at 14 weeks, ECC-1 tumors in mice receiving the raloxifene 5-day dosing schedule with estrogen were statistically significantly larger than those in the untreated animals (mean tumor size = 0 cm²) (P<0.001; difference in mean tumor size = 0.77 cm² [95% CI = 0.37 to 1.16 cm²]), whereas tumors in mice receiving the tamoxifen 5-day dosing schedule with estrogen were not (P = .33; difference in mean tumor size = 0.02 cm² [95% CI = -0.02 to 0.07 cm²]) (Fig. 1B). After 9 weeks, the tumors in the raloxifene 5-day dosing group were larger, though not statistically significantly so, than the raloxifene 7-day dosing group (P = .04; difference in mean tumor size = 0.2 cm² [95% CI = 0.09 to 0.4 cm²]), and the tumors in the tamoxifen 5-day dosing group were not statistically significantly different from those in the tamoxifen 7-day dosing group (P = .31; difference in mean tumor size = 0 cm² [95% CI = -0.17 to 0.06 cm²]) (Fig. 1B). These results demonstrate that tamoxifen is more effective than raloxifene in blocking the effects of low-dose estrogen on tamoxifen-naive breast and endometrial cancer growth.

**Effects of Raloxifene on Growth of Short-Term Tamoxifen-Exposed Breast Tumors**

To simulate the case of a patient with advanced breast cancer who develops progressive disease while being treated with tamoxifen, we implanted MCF7-derived human breast tumors exposed to 2 years of tamoxifen (MCF7TAM ST) into athymic, ovariectomized mice. The mice were randomly assigned to receive no treatment, low-dose estrogen, or tamoxifen (0.5 mg per day) or raloxifene (0.5 mg or 1.5 mg per day), either alone or each in combination with low-dose estrogen. The SERMs were administered for 5 days each week for 8 weeks, and tumor sizes were measured weekly. P values less than .0018 were considered to be statistically significant.

After 8 weeks of treatment, tumors in the estrogen-treated mice had a mean size of 1.0 cm² and were statistically significantly larger than tumors in the untreated mice (mean size = 0.24 cm²) (P<0.001; difference in mean tumor size = 0.71 cm² [95% CI = 0.34 to 1.08 cm²]). Tumors in the estrogen-treated mice were also larger than tumors in the SERM-treated groups, but those differences were not statistically significant (difference in mean tumor size between E2 and TAM 0.5 mg/day = 0.43 cm² [95% CI = 0.12 to 0.75 cm²; P = .01], between E2 and RAL 0.5 mg/day = 0.53 cm² [95% CI = 0.22 to 0.84 cm²; P = .002]; between E2 and RAL 1.5 mg/day = 0.53 cm² [95% CI = 0.22 to 0.84 cm²; P = .002]) (Fig. 2). There was no statistically significant difference in the growth-stimulatory ef-

![Fig. 2. Effects of tamoxifen and raloxifene on the growth of short-term tamoxifen-exposed MCF7TAM ST breast tumors. MCF7TAM ST breast tumors were implanted into the right mammary fat pads of athymic, ovariectomized mice (EnCa101 TAM tumors were implanted in the left mammary fat pad [data not shown]). The mice were divided into groups of 10 and randomly assigned to receive no treatment (control), low-dose (68 pg/mL) estrogen alone (E2), low-dose estrogen plus either tamoxifen (0.5 mg/day) or raloxifene (0.5 or 1.5 mg/day) (TAM 0.5 mg/day + E2, RAL 0.5 mg/day + E2, and RAL 1.5 mg/day + E2, respectively), or tamoxifen alone (0.5 mg/day) or raloxifene alone (0.5 or 1.5 mg/day) (TAM 0.5 mg/day, RAL 0.5 mg/day, and RAL 1.5 mg/day, respectively). The selective estrogen receptor modulators were administered for 5 days each week. Mean tumor size was determined weekly by calculating the cross-sectional area (cm²). Results shown are mean tumor size (cm²) and 95% confidence intervals (only the upper confidence limit for each point is shown). Week 0 is the point of tumor implantation.

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Effects of tamoxifen and raloxifene at either dose on MCF7TAM ST tumor growth (difference in mean tumor size between TAM 0.5 mg/day and RAL 0.5 mg/day + E2 = 0.1 cm² [95% CI = 0.27 to 0.27 cm²; P = .26] and between TAM 0.5 mg/day and RAL 1.5 mg/day + E2 = 0.1 cm² [95% CI = 0.08 to 0.27 cm²; P = .27]) (Fig. 2). Raloxifene, at both doses, and tamoxifen were only partially effective in blocking the stimulatory effects of low-dose estrogen on MCF7TAM ST tumor growth (mean difference in tumor size between E2 and TAM 0.5 mg/day + E2 = 0.47 cm² [95% CI = 0.11 to 0.82 cm²; P = .013], between E2 and RAL 0.5 mg/day + E2 = 0.49 cm² [95% CI = 0.12 to 0.87 cm²; P = .013], and between E2 and RAL 1.5 mg/day + E2 = 0.53 cm² [95% CI = 0.19 to 0.88 cm²; P = .004]), and there was no statistically significant difference between tamoxifen and raloxifene (at both doses) in blocking the effects of low-dose estrogen (difference in mean tumor size between TAM 0.5 mg/day + E2 and RAL 0.5 mg/day + E2 = 0.02 cm² [95% CI = 0.25 to 0.29 cm²; P = .85] and between TAM 0.5 mg/day + E2 and RAL 1.5 mg/day + E2 = 0.06 cm² [95% CI = 0.21 to 0.34 cm²; P = .62]) (Fig. 2). These results demonstrate that tamoxifen and raloxifene have similar effects on the growth of short-term tamoxifen-exposed breast tumors.

Effects of Raloxifene Treatment on Breast and Endometrial Tumors After 5 Years of Tamoxifen Exposure

The current recommended duration of tamoxifen treatment for women with breast cancer in the adjuvant and preventive settings is 5 years. However, after 5 years of tamoxifen therapy, these women remain at risk of breast cancer relapse, contralateral breast cancer, and other diseases related to menopause, including osteoporosis. One treatment strategy for such women would be to prescribe raloxifene after 5 years of tamoxifen treatment to maintain bone density and possibly protect against contralateral breast cancer and recurrence of the primary breast cancer. We assessed this strategy by examining the effects of tamoxifen and raloxifene on the growth of breast and endometrial tumors that had been exposed to tamoxifen for more than 5 years.

Athymic, ovariectomized mice were bitransplanted with long-term tamoxifen-exposed breast (MCF7TAM LT) tumors and long-term tamoxifen-exposed endometrial (EnCa101 TAM) tumors. These breast and endometrial tumors each have been exposed to at least 5 years of tamoxifen by serial transplantation in athymic mice. The mice were randomly assigned to receive no treatment, low-dose estrogen, or tamoxifen (0.5 mg/day) or raloxifene (0.5 mg/day or 1.5 mg/day), alone or in combination with low-dose estrogen. The SERMs were administered for 5 days each week for 8 weeks, and tumor sizes were measured weekly. P values less than .0018 were considered to be statistically significant.

After 8 weeks of treatment, the MCF7TAM LT tumors in all treatment groups, except the groups receiving estrogen alone or raloxifene alone at 1.5 mg/day, were statistically significantly larger than the tumors in the untreated (control) group (difference in mean tumor sizes between control and E2 = 0.48 cm² [95% CI = 0.19 to 0.77 cm²; P = .003], between control and TAM 0.5 mg/day = 0.83 cm² [95% CI = 0.61 to 1.05 cm²; P < .001], between control and RAL 0.5 mg/day = 0.26 cm² [95% CI = 0.12 to 0.39 cm²; P < .001], between control and RAL 1.5 mg/day = 0.11 cm² [95% CI = -0.03 to 0.26 cm²; P = .12], between control and TAM 0.5 mg/day + E2 = 0.82 cm² [95% CI = 0.6 to 1.04 cm²; P < .001], between control and RAL 0.5 mg/day + E2 = 0.68 cm² [95% CI = 0.5 to 0.87 cm²; P < .001], and between control and RAL 1.5 mg/day + E2 = 0.64 cm² [95% CI = 0.47 to 0.83 cm²; P < .001]) (Fig. 3, A and B). Tamoxifen alone stimulated MCF7TAM LT tumor growth statistically significantly more than raloxifene alone at either dose (difference in mean tumor size between TAM 0.5 mg/day and RAL 0.5 mg/day = 0.57 cm² [95% CI = 0.33 to 0.82 cm²; P < .001] and between TAM 0.5 mg/day and RAL 1.5 mg/day = 0.72 cm² [95% CI = 0.46 to 0.98 cm²; P < .001]). In the absence of treatment with low-dose estrogen, there was no statistically significant difference between the effects of the two raloxifene panels A and B. The selective estrogen receptor modulators were administered for 5 days each week. Mean tumor size was determined weekly by calculating the cross-sectional area (cm²). Results shown are mean tumor size (cm²) and 95% confidence intervals (only the upper confidence limit for each point is shown). Week 0 is the point of tumor implantation.

Fig. 3. Effects of tamoxifen and raloxifene on the growth of long-term tamoxifen-exposed MCF7TAM LT breast tumors. MCF7TAM LT breast tumors were implanted into the right mammary fat pads of athymic, ovariectomized mice (EnCa101 TAM tumors were implanted in the left mammary fat pad). The mice were divided into groups of 10 and randomly assigned to receive no treatment (control), low-dose (68 pg/mL) estrogen alone (E2), or either tamoxifen (0.5 mg/day) or raloxifene alone (0.5 or 1.5 mg/day) (TAM 0.5 mg/day, RAL 0.5 mg/day, and RAL 1.5 mg/day, respectively; panel A) or low-dose estrogen plus either tamoxifen (0.5 mg/day) or raloxifene (0.5 or 1.5 mg/day) (TAM 0.5 mg/day + E2, RAL 0.5 mg/day + E2, and RAL 1.5 mg/day + E2, respectively; panel B). The selective estrogen receptor modulators were administered for 5 days each week. Mean tumor size was determined weekly by calculating the cross-sectional area (cm²). Results shown are mean tumor size (cm²) and 95% confidence intervals (only the upper confidence limit for each point is shown). Week 0 is the point of tumor implantation.
doses on MCF7TAM LT tumor growth ($P = .102$; difference in mean tumor size between RAL 0.5 mg/day and RAL 1.5 mg/day = 0.15 cm$^2$ [95% CI = –0.04 to 0.33 cm$^2$]) (Fig. 3, A). Tamoxifen and raloxifene at both doses were ineffective at blocking the stimulatory effects of low-dose estrogen on MCF7TAM LT tumor growth (difference in mean tumor size between E2 and TAM 0.5 mg/day + E2 = 0.34 cm$^2$ [95% CI = –0.04 to 0.72 cm$^2$; $P = .07$], between E2 and RAL 0.5 mg/day + E2 = 0.21 cm$^2$ [95% CI = –0.14 to 0.55 cm$^2$; $P = .22$], and between E2 and RAL 1.5 mg/day + E2 = 0.16 cm$^2$ [95% CI = –0.16 to 0.47 cm$^2$; $P = .31$]) (Fig. 3, B). There was no statistically significant difference between raloxifene at either dose and tamoxifen in blocking the effects of low-dose estrogen on MCF7TAM LT tumor growth (difference in mean tumor size between TAM 0.5 mg/day + E2 and RAL 0.5 mg/day + E2 = 0.14 cm$^2$ [95% CI = –0.08 to 0.44 cm$^2$; $P = .16$]) (Fig. 3, B).

At 8 weeks of treatment, the mean size of EnCa101 TAM endometrial tumors in the estrogen-treated mice was 2.53 cm$^2$; this was statistically significantly larger than the mean size of tumors in the untreated (control) mice (0.2 cm$^2$) (Fig. 4). Tamoxifen and raloxifene at 0.5 mg/day or at 1.5 mg/day, in either the presence or absence of low-dose estrogen, stimulated EnCa101 TAM tumor growth more than no treatment ($P < .001$ for each; differences in mean tumor size between control and TAM 0.5 mg/day = 2.33 cm$^2$ [95% CI = 1.13 to 3.53 cm$^2$]) (Fig. 4, A). There were no statistically significant differences between the mean sizes of the EnCa101 TAM tumors in mice treated with estrogen alone and in mice treated with any SERMs alone (difference in mean tumor size between E2 and TAM 0.5 mg/day = 0.72 cm$^2$ [95% CI = –0.7 to 2.15 cm$^2$; $P = .3$], between E2 and RAL 0.5 mg/day = 0.68 cm$^2$ [95% CI = –0.67 to 2.04 cm$^2$; $P = .3$], and between E2 and RAL 1.5 mg/day = 0.33 cm$^2$ [95% CI = –1.22 to 1.89 cm$^2$; $P = .66$]) (Fig. 4, A). Neither tamoxifen nor raloxifene at either dose blocked the stimulatory effects of low-dose estrogen on EnCa101 TAM tumor growth (difference in mean tumor size between E2 and TAM 0.5 mg/day + E2 = 1.14 cm$^2$ [95% CI = –0.47 to 2.77 cm$^2$; $P = .15$], between E2 and RAL 0.5 mg/day + E2 = 0.27 cm$^2$ [95% CI = –1.22 to 1.77 cm$^2$; $P = .7$], and between E2 and RAL 1.5 mg/day + E2 = 0.16 cm$^2$ [95% CI = –1.27 to 1.59 cm$^2$; $P = .82$]) (Fig. 4, B). There were no statistically significant differences between the mean sizes of EnCa101 TAM tumors in mice treated with tamoxifen and both raloxifene doses when the SERMs were administered alone (difference in mean tumor size between TAM 0.5 mg/day and RAL 0.5 mg/day = 0.04 cm$^2$ [95% CI = –0.92 to 1.01 cm$^2$; $P = .93$] and between TAM 0.5 mg/day and RAL 1.5 mg/day = 0.39 cm$^2$ [95% CI = –0.76 to 1.55 cm$^2$; $P = .48$]) (Fig. 4, A) or in the abilities of tamoxifen and raloxifene at both doses to block the stimulatory effects of low-dose estrogen on the growth of EnCa101 TAM tumors (difference in mean tumor size between TAM 0.5 mg/day + E2 and RAL 0.5 mg/day + E2 = 0.87 cm$^2$ [95% CI = –0.38 to 2.12 cm$^2$; $P = .16$] and between TAM 0.5 mg/day + E2 and RAL 1.5 mg/day + E2 = 0.98 cm$^2$ [95% CI = –0.17 to 2.14 cm$^2$; $P = .09$]) (Fig. 4, B).

These results demonstrate that there is no statistically significant difference between tamoxifen and raloxifene on the growth of long-term tamoxifen-exposed human breast and endometrial tumors in athymic mice when the SERMs are co-administered with low-dose estrogen. In addition, there was no statistically significant difference in the effects of tamoxifen and raloxifene
alone on the growth of long-term tamoxifen-exposed human endometrial cancers in athymic mice.

Effects of Raloxifene on Endometrial Tumors Previously Exposed to Tamoxifen

Postmenopausal women who have a history of breast cancer are generally advised not to take estrogen replacement therapy for the remainder of their lifetime, but as a result they remain at risk for osteoporosis and fractures. Raloxifene, which maintains bone density in postmenopausal women while having antiestrogenic effects on the breast, would be a reasonable treatment strategy to prevent osteoporosis in women with a history of breast cancer. However, it is unclear how such a strategy would affect the risk of endometrial cancer in such women. We have attempted to address this issue by evaluating the effect of raloxifene on the growth of EnCa101E endometrial tumors, which were developed by passaging tamoxifen-exposed EnCa101 TAM endometrial tumors in athymic mice treated for 3 years with low-dose estrogen. We believe that this animal model simulates the case of a patient who completed 5 years of adjuvant tamoxifen therapy some years ago and is now considering raloxifene for osteoporosis prevention. EnCa101 E endometrial tumors were implanted into the mammary fat pads of athymic, ovariectomized mice. The mice were divided into groups of 10 and randomly assigned to receive no treatment (control) or treatment with low-dose estrogen alone or tamoxifen (0.5 mg/day) or raloxifene (0.5 or 1.5 mg/day) alone, either alone or in combination with low-dose estrogen. The SERMs were administered for 5 days each week for 9 weeks, and tumor sizes were measured weekly. In this experiment, a P value less than .0018 was considered to be statistically significant.

At 9 weeks of treatment, the mean size of EnCa101 E tumors in mice treated with estrogen alone was 1.0 cm²; this was statistically significantly larger than the mean size of tumors in the control group (0.07 cm²) (P < .001; difference in mean tumor size = 0.92 cm² [95% CI = 0.48 to 1.37 cm²]) (Fig. 5). There was no statistically significant difference between the mean size of tumors in the estrogen-treated mice and the mean size of tumors in the mice treated with either tamoxifen (0.46 cm²) or raloxifene at 0.5 mg/day (0.21 cm²) or 1.5 mg/day (0.4 cm²) alone (difference in mean tumor size between E2 and TAM 0.5 mg/day = 0.53 cm² [95% CI = 0.03 to 1.04 cm²]; P = .04), between E2 and RAL 0.5 mg/day = 0.78 cm² [95% CI = 0.28 to 1.28 cm²]; P = .004), and between E2 and RAL 1.5 mg/day = 0.59 cm² [95% CI = 0.12 to 1.06 cm²; P = .02]). There were also no statistically significant differences between the mean size of EnCa101 E tumors in mice treated with tamoxifen and the mean size of tumors in mice treated with raloxifene (at either dose) (differences in mean tumor size between TAM 0.5 mg/day and RAL 0.5 mg/day = 0.25 cm² [95% CI = -0.04 to 0.54 cm²; P = .09] and between TAM 0.5 mg/day and RAL 1.5 mg/day = 0.06 cm² [95% CI = -0.22 to 0.34 cm²; P = .66]) when these SERMs were administered without low-dose estrogen (Fig. 5, A). Neither tamoxifen nor raloxifene at either dose was effective in blocking the stimulatory effects of low-dose estrogen on EnCa101 E tumor growth (differences in mean tumor size between E2 and TAM 0.5 mg/day + E2 = 0.51 cm² [95% CI = 0.03 to 0.99 cm²; P = .04], between E2 and RAL 0.5 mg/day + E2 = 0.41 cm² [95% CI = -0.09 to 0.93 cm²; P = .14], and between E2 and RAL 1.5 mg/day + E2 = 0.21 cm² [95% CI = -0.33 to 0.75 cm²; P = .41]) (Fig. 5, B). There was also no statistically significant difference in the ability of tamoxifen or raloxifene at 0.5 mg/day to block the stimulatory effects of low-dose estrogen on EnCa101E tumor growth (P = .35; mean difference in tumor size between TAM 0.5 mg/day + E2 and RAL 0.5 mg/day + E2 = 0.7 cm² [95% CI = -0.22 to 0.41 cm²]) However, raloxifene at 1.5 mg/day was statistically significantly less effective than tamoxifen at blocking the effects of low-dose estrogen on EnCa101E tumor growth (P < .001; difference in mean tumor size between RAL 1.5 mg/day + E2 and TAM 0.5 mg/day + E2 = 0.72 cm² [95% CI = 0.38 to 1.06 cm²]) (Fig. 5, B). These results demonstrate that tamoxifen
and raloxifene had similar effects on the growth of human endometrial tumors that have been previously exposed to tamoxifen. Moreover, in this tumor model, tamoxifen was superior to raloxifene at blocking the effects of low-dose estrogen on endometrial tumor growth.

**Discussion**

Raloxifene is used extensively to prevent osteoporosis in postmenopausal women and is being evaluated as a breast cancer preventive agent. However, clinical trials that evaluated the efficacy of raloxifene in the treatment of advanced breast cancer have yielded disappointing results (3,26). Consequently, raloxifene is not being developed as a treatment for breast cancer and there are, therefore, no data on the use of raloxifene as an adjuvant treatment for early-stage breast cancer. Raloxifene, unlike tamoxifen, has not been associated with an increased incidence of endometrial cancer in the osteoporosis trials (5) and appears less estrogenic on the endometrium in preclinical (7,8) and clinical (4) studies. However, if it is considered that the association between tamoxifen use and risk of endometrial cancer became evident only after tamoxifen had been widely used for 10 years (21), it cannot be assumed from the current available data that raloxifene will not increase the risk of endometrial cancer. The ongoing STAR trial should provide a definitive answer to this question.

We have evaluated the effects of raloxifene and tamoxifen on tumor growth in mouse models of tamoxifen-naive breast and endometrial cancers. We found previously that neither of these SERMs, when administered without estrogen, stimulated tumor growth in these animal models (27). However, tamoxifen, at one third the dose of raloxifene, was statistically significantly more effective than raloxifene in blocking the stimulatory effects of low-dose estrogen on the growth of breast and endometrial tumors that had never previously been exposed to tamoxifen. Raloxifene partially antagonized the effects of low-dose estrogen on the tamoxifen-naive endometrial tumors for the first 9 weeks of treatment, after which tumor growth was no longer controlled. By contrast, tamoxifen continued to block the stimulatory effects of low-dose estrogen on endometrial tumor growth through 14 weeks of treatment. These results suggest that tamoxifen may be more effective than raloxifene in controlling the growth of both breast and endometrial tumors in women who are receiving these SERMs for the first time. The STAR trial will definitely address this issue.

The oral bioavailability of raloxifene is only 2% (27). Even though the doses of tamoxifen and raloxifene used in our mouse experiments mimic the dose ratios currently recommended for the treatment and prevention of breast cancer with tamoxifen and the prevention of osteoporosis by raloxifene, as well as those used in the STAR trial, it is possible that higher doses of raloxifene than those used in our experiments may be more effective at controlling breast and endometrial tumor growth in mouse tumor models and perhaps in humans, compared with what we observed. This possibility is supported by clinical data that suggest that high doses of raloxifene (e.g., 150 mg twice daily, 15 times the currently used dose of tamoxifen) may be effective in the treatment of advanced breast cancer in women (26). Results from the osteoporosis prevention trials also suggest that high-dose raloxifene (120 mg daily) is effective in preventing breast cancer (5,6). Finally, in other clinical trials, high-dose raloxifene (150 mg daily) appears antiestrogenic on the endometrium (28,29). However, the doses used in our experiments mimic the dose ratio currently recommended both for the treatment and prevention of breast cancer with tamoxifen and for the prevention of osteoporosis and in the STAR trial with raloxifene.

We considered the possibility that the superiority of tamoxifen to raloxifene in blocking the effects of low-dose estrogen on the growth of the tamoxifen-naive tumors could be due to the short half-life of raloxifene. We, therefore, compared the abilities of the SERMs administered on a 5- or 7-day dosing schedule to block the effects of low-dose estrogen on tumor growth and found that there was no statistically significant difference between the two dosing schedules for either SERM on the growth of tamoxifen-naive breast and endometrial tumors. Raloxifene at either dosing schedule was unable to block the effects of low-dose estrogen on tamoxifen-naive breast tumors, whereas tamoxifen at either dosing schedule was. It is interesting that we recently obtained similar results with LY117018, a raloxifene analogue that is ineffective in blocking the stimulatory effects of low-dose estrogen on the growth of a different tamoxifen-naive human breast tumor model (T47D) (30). In contrast, we found that tamoxifen blocks estrogen-stimulated growth of this tamoxifen-naive T47D human breast tumor model. These results may reflect the relatively long serum half-life of tamoxifen, of about 7 days (31), which results in stable levels of its active 4-hydroxy metabolite (32) and the efficient inhibition of the estrogen receptor in breast cancer cells. These results are consistent with our recent demonstration that arzoxifene, a raloxifene-like SERM with improved oral bioavailability, completely blocks the effects of low-dose estrogen on tamoxifen-naive T47D breast tumors in athymic mice when administered on a 7-day dosing schedule (30). Because there was no statistically significant difference between the 5- and 7-day raloxifene dosing schedules in controlling estrogen-stimulated growth of tamoxifen-naive endometrial tumors in the present study, we conclude that dosing frequency is not a major factor in controlling the growth of these tamoxifen-naive tumors when given for short durations (e.g., <3 months), but it becomes more relevant when the SERMs are given for longer durations. Thus, the poor bioavailability of raloxifene and the fact that tamoxifen accumulates suggest that incomplete compliance with raloxifene may ultimately render chemoprevention with raloxifene less effective than that with tamoxifen in postmenopausal women. The STAR trial will definitely address this issue.

Results from two clinical studies have indicated that raloxifene is not useful in the treatment of tamoxifen-refractory breast cancer. In the first study (3), women with advanced breast cancer who were treated with standard doses of raloxifene after previous treatment with tamoxifen had unimpressive response rates. In a second study (26), high-dose raloxifene demonstrated some efficacy as a first-line therapy in women with advanced breast cancer, but the small number of women in this trial who had received prior adjuvant tamoxifen did not appear to benefit. On the basis of these studies, raloxifene is not considered to be useful in the treatment of tamoxifen-refractory breast cancer. We confirmed these findings in our mouse model of short-term tamoxifen-exposed breast cancer, where we found no statistically significant difference in the growth-stimulatory effects of the SERMs or in their abilities to block the stimulatory effects of low-dose estrogen on tumor growth.

Continuing tamoxifen beyond 5 years in women with early-stage breast cancer does not appear to further reduce the risk of osteoporosis and in the STAR trial with raloxifene.
breast cancer recurrence (9). At present, it is unclear what the best therapeutic strategy is for women who have completed 5 years of adjuvant tamoxifen therapy and remain at risk for breast cancer recurrence and osteoporosis. One possible strategy would be to give these women raloxifene; therefore, the main objective of our study was to determine whether raloxifene would be an effective therapy to prevent breast cancer recurrence after adjuvant tamoxifen. We found that neither raloxifene nor tamoxifen was effective in blocking the stimulatory effects of low-dose estrogen in a mouse model of human breast cancer exposed to tamoxifen for more than 5 years. Therefore, we believe that in women completing 5 years of tamoxifen therapy, raloxifene may not further reduce the risk of breast cancer recurrence.

Tamoxifen is associated with an increased incidence of endometrial cancer in postmenopausal women (2), which may, at least in part, be caused by its estrogen-like effects on pre-existing endometrial cancer. The NSABP-B14 trial has demonstrated a twofold increase in endometrial cancer incidence in women who used tamoxifen for more than 5 years compared with those who used tamoxifen for 5 years (9). There are no clinical data on the effects of raloxifene on the risk of endometrial cancer in women completing 5 years of tamoxifen therapy. To address this issue, we examined the effects of raloxifene on the growth of human endometrial tumors exposed to 5 years of tamoxifen in athymic mice. Both tamoxifen and raloxifene stimulated the growth of these endometrial tumors, and there was no statistically significant difference in the growth-stimulatory effects of the two SERMs. Neither tamoxifen nor raloxifene was able to control the effects of low-dose estrogen, and there was no difference between the SERMs. These findings suggest that raloxifene, like tamoxifen (9), may also increase the incidence of endometrial cancer in women who previously have taken tamoxifen for 5 years.

Lastly, we wanted to examine the effects of raloxifene in an endometrial cancer model that mimics the case of a woman who has stopped taking tamoxifen and then, some years later, begins treatment with raloxifene. We found no statistically significant difference between the growth-stimulatory effects of raloxifene and tamoxifen on the growth of endometrial tumors previously exposed to tamoxifen. Raloxifene, at clinically relevant doses, was statistically significantly less effective than tamoxifen in controlling the growth-stimulatory effects of estrogen in this endometrial cancer model. We believe that these results suggest that women who use raloxifene who have ever been treated with tamoxifen should be monitored for the development of endometrial cancer as they would if they were being treated with tamoxifen.

In conclusion, we have found that raloxifene and tamoxifen have similar effects in all of our tamoxifen-exposed breast and endometrial cancer models. These results support the idea that raloxifene has no value in the treatment of advanced, tamoxifen-resistant breast cancer (3). Moreover, these results suggest that raloxifene treatment that follows 5 years of tamoxifen treatment may not further reduce breast cancer recurrence and may increase endometrial cancer incidence.

Ongoing clinical trials are currently addressing whether the use of aromatase inhibitors may be a valuable treatment option for breast cancer patients who have completed 5 years of tamoxifen therapy and who require continued control of their breast cancer. Aromatase inhibitors are effective agents in tamoxifen-resistant advanced breast cancer (33–35) and may, therefore, offer an advantage over SERMs in women who have completed 5 years of adjuvant tamoxifen therapy. In addition, aromatase inhibitors exhibit no estrogen-like properties and are, therefore, unlikely to increase the incidence of endometrial cancer. This lack of agonistic effects is, however, unlikely to result in beneficial effects on bone density, and alternative agents, such as bisphosphonates, may be of use in this setting.

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NOTES

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