

# Effects of Exemestane and Tamoxifen in a Postmenopausal Breast Cancer Model

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## ABSTRACT

**Purpose:** To optimize treatment strategies for postmenopausal breast cancer patients, we investigated the efficacy of the steroidal aromatase inhibitor exemestane alone or in combination with the antiestrogen tamoxifen in a xenograft model of postmenopausal breast cancer. We also determined the effects of these agents in sequential second-line therapy and the effect of the nonsteroidal aromatase inhibitor letrozole on tumors that progressed on the above treatments.

**Experimental:** Aromatase-transfected human estrogen receptor-positive breast cancer cells (MCF-7Ca) were grown as tumors in ovariectomized athymic mice. Animals received subcutaneous injection with vehicle, tamoxifen, exemestane, tamoxifen plus exemestane, and letrozole. Tumor volumes were measured weekly.

**Results:** All treatments were effective initially in suppressing tumor growth as first-line therapy compared with vehicle treatment. Exemestane suppressed tumor growth to a greater extent than tamoxifen. However, the combination of tamoxifen plus exemestane was more effective than either drug alone. After tumor volumes doubled on initial treatment, the mice were crossed over to receive exemestane or tamoxifen. Tumor growth slowed briefly in mice treated with tamoxifen and crossed over to exemestane, but tumor growth continued unabated in those changed from exemestane to tamoxifen. However, letrozole was effective in both

groups as third-line therapy for a limited period. Letrozole as initial single agent was the best overall treatment in terms of the degree of tumor suppression and the length of effectiveness of treatment.

**Conclusion:** Exemestane was more effective in controlling tumor growth than tamoxifen. In addition, the combination of exemestane plus tamoxifen was clearly more effective than sequential use of these agents in the tumor model. However, the nonsteroidal aromatase inhibitor letrozole as first-line therapy was overall the most effective treatment in controlling tumor growth.

## INTRODUCTION

Estrogen plays an important role in development and progression of breast cancers. Although the incidence of breast cancer increases with age, circulating levels of estrogen are low in postmenopausal women as the ovaries are no longer the major source of the hormone. Production of estrogens in peripheral tissue, such as adipose tissue is increased and accounts for the circulating and tissue concentrations. Despite low circulating levels of estrogens, levels in the breast are the same in postmenopausal as in premenopausal women because of hormone uptake as well as local production (1).

Current treatment for postmenopausal hormone-dependent breast cancers includes two strategies to reduce the effects of estrogen on tumor growth. One strategy is inhibition of estrogen action with antiestrogens, and the other is inhibition of estrogen synthesis with aromatase inhibitors. The antiestrogen tamoxifen has been a primary treatment for breast cancer since the 1970s (2) and has been shown to delay recurrences and contra-lateral breast cancer. The agent is well tolerated and can be taken for 5 years (3). However, tamoxifen is a partial agonist as well as an antagonist and can cause endometrial hyperplasia and occasionally endometrial cancer as well as increased risk of stroke because of its estrogenic effects (4). In addition, the compound may not be optimally effective in terms of its antagonistic actions on the tumor. For these reasons, we have investigated a different approach using inhibition of estrogen production with aromatase inhibitors (5–7). The two approaches have been compared in recent clinical trials and the results suggest that aromatase inhibitors are more effective than tamoxifen as first-line therapy for postmenopausal patients with hormone-responsive breast cancer (8–10) and also for patients with early breast cancer (11–12). Two classes of aromatase inhibitors, steroidal (formestane, exemestane), and nonsteroidal (anastrozole, letrozole) compounds have been developed. Steroidal, irreversible inhibitors (also known as suicide inhibitors) of aromatase interact with the substrate-binding site of the enzyme, binding irreversibly and causing enzyme inactivation (13). Thus, no estrogen can be synthesized until new enzyme is produced. In contrast, nonsteroidal compounds interact with the heme-iron

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group of the cytochrome P450 aromatase in a reversible reaction (13).

To investigate the effectiveness of aromatase inhibitors, our laboratory developed a xenograft tumor model using human hormone responsive (estrogen-receptor positive) breast cancer cells stably transfected with the human aromatase gene (MCF-7Ca). In this model, MCF-7Ca cells (14) are grown as tumors in the ovariectomized, immune suppressed mice (15, 16). The tumor serves as an autocrine source of estrogen that stimulates its growth, and it is sensitive to both the antiproliferative effects of antiestrogens and of aromatase inhibitors (17–19). The model simulates the postmenopausal breast cancer patient as a major source of the hormone after menopause is breast tissue and where the aromatase enzyme is not under gonadotropin regulation. As the production of adrenal androgen precursors for aromatization is deficient in these mice (20), we supplement them with androstenedione. We have shown previously that androstenedione does not affect the growth of tumors directly. Thus, growth of tumors of MCF-7 cells without aromatase transfection are not stimulated by androstenedione (15).

Because both antiestrogens and aromatase inhibitors are effective in controlling tumor growth, blocking both estrogen receptors and estrogen synthesis might have an additive effect and result in better control of tumor growth. In this study, we have investigated whether the steroidal inhibitor exemestane combined with tamoxifen might be more effective than each alone. In our previous studies, we have compared the effects of combining tamoxifen with nonsteroidal aromatase inhibitors anastrozole and letrozole on tumor growth (18, 19). Although there was no benefit in combining treatment compared with the aromatase inhibitors alone, the effect of treatment with the combination was similar to that of tamoxifen alone. These data predicted the outcome of the clinical Anastrozole, Tamoxifen, and the Combination trial (11), which found that anastrozole was more effective than tamoxifen and the combination of the two agents in reducing recurrences in patients with early stage breast cancer. To date, it is not known whether combining a steroidal aromatase inhibitor with the antiestrogen tamoxifen will be more effective in slowing tumor progression compared with treatment with either of these drugs alone. We were particularly interested in examining the value of combining exemestane plus tamoxifen in this model as it had been reported previously that the combination is superior to either agent alone in the rat model with carcinogen [7,12-dimethylbenz(a)anthracene]-induced mammary tumors (21).

In the current study, we have also investigated several strategies with the goal of defining regimens that will be the basis of clinical trials to optimize treatment for postmenopausal breast cancer patients with these agents. Thus, we have investigated the effect of sequential treatment with tamoxifen and exemestane as second-line therapy and the effect of letrozole alone and in sequential therapy after tumors progressed on exemestane and tamoxifen.

## MATERIALS AND METHODS

**Materials.** MCF-7 human breast cancer cells stably transfected with the human aromatase gene (MCF-7Ca; ref. 14). Dulbecco's PBS, DMEM, penicillin/streptomycin solution, trypsin-

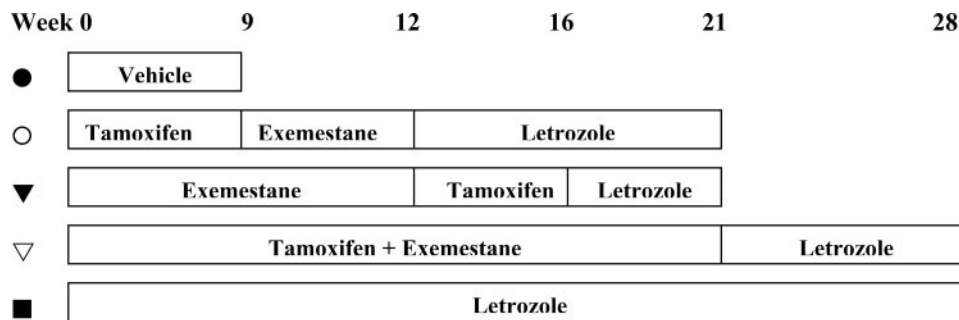
EDTA solution, and geneticin (G418) were from Invitrogen (Carlsbad, CA). Fetal bovine serum was from Hyclone (Logan, UT). Androstenedione ( $\Delta$ 4A), tamoxifen, and hydroxypropyl cellulose were obtained from Sigma Chemical Company (St. Louis, MO). Matrigel was obtained from BD Biosciences (Bedford, MA). Letrozole was kindly provided by Dr. Dean Evans (Novartis Pharma, Basel, Switzerland). Exemestane was kindly provided by Dr. Lori Hollis (Pharmacia/Pfizer, Inc.).

**Cell Culture.** MCF-7 human breast cancer cells stably transfected with the human aromatase gene (MCF-7Ca; ref. 14) were cultured in DMEM with 5% fetal bovine serum, 1% penicillin/streptomycin solution, and 750  $\mu$ g/mL G418. The culture medium was changed twice weekly.

**Ovariectomized Female Athymic Nude Mice.** Female BALB/c athymic ovariectomized mice 4 to 6 weeks old were obtained from NCI (Frederick, MD). The animals were housed in a pathogen-free environment under controlled conditions of light and humidity and received food and water *ad libitum*. Animals were allowed to acclimatize for 48 hours after shipment and before tumor inoculation was done.

**Postmenopausal Intratumoral Aromatase Model.** MCF-7Ca cells were inoculated into the mice as described previously (15, 18). Subconfluent (80%) MCF-7Ca cells were washed with Dulbecco's PBS and scraped into Dulbecco's PBS. Cells were collected by centrifugation and resuspended in Matrigel (10 mg/mL). Each mouse received subcutaneous injections at two sites on each flank with 0.1 mL of cell suspension ( $2.5 \times 10^7$  cells/mL). Because athymic mice are deficient in adrenal androgens, they were supplemented with daily injections of the aromatase substrate androstenedione ( $\Delta$ 4A) 100  $\mu$ g/day for the duration of the experiment. Treatment started when tumors reached a measurable size ( $\sim 300$  mm<sup>3</sup>) 4 weeks after inoculation. Tumors were measured weekly with calipers, and volumes were calculated with the formula  $4/3\pi \times r_1^2 \times r_2$  ( $r_1 < r_2$ ), where  $r_1$  is the smaller radius. Mice were grouped for treatment (five mice per group) so that total tumor volume was similar in each group, and mice received subcutaneous injection with vehicle, nonsteroidal aromatase inhibitor letrozole (10  $\mu$ g/day), antiestrogen tamoxifen (500  $\mu$ g/day), and 5 different doses of steroidal aromatase inhibitor exemestane (50, 100, 250, 500, and 1,000  $\mu$ g/day) prepared as suspensions in 0.3% hydroxypropyl cellulose. In the second experiment, mice were treated as above with vehicle, tamoxifen (100  $\mu$ g/day), exemestane (100 and 250  $\mu$ g/day), the combination of tamoxifen and exemestane at the same doses, and letrozole (10  $\mu$ g/day). When tumors had doubled in volume during treatment, mice were crossed over to tamoxifen or exemestane for second-line treatment and then subsequently to letrozole. Mice treated with the combination were switched to letrozole treatment when their tumors doubled in volume. Animals were treated for the indicated times as shown in Scheme 1, after which they were sacrificed by decapitation, and tumors and uteri were excised, cleaned, and weighed.

**Statistical Analysis.** Linear mixed-effects models were used to estimate average tumor weight and volume across the treatment groups (22). Each experiment was analyzed separately. The mixed effect of treatment in each group and random effects for each animal within a group were estimated. The simple covariance structure for the repeated measures within a



Scheme 1 Sequence of treatment for mice in the experiment shown in Fig. 2.

subject was selected. The set of appropriate contrasts was used to compare treatment means across the groups. Data on tumor volume were grouped longitudinally and unbalanced with various treatment duration and number of measurable tumors per animal. For tumor growth, diagnostic plots suggested that models of exponential growth were appropriate to the data. Therefore, linear mixed-effects models were fitted to the natural logarithm of tumor volume over time. This approach allows the estimation of an exponential variable controlling the rate of tumor growth for each of these treatment groups. The multilevel model was more efficient and was preferred. Model with two random effects for the intercept and the slope at the mouse level and a single random effect for the intercept at tumor level (within a mouse) was chosen. The variance covariance matrix for the random effects was block diagonal. The first-order autoregressive structure was selected for these diagonal blocks.

The general linear models technique was used to analyze the uterine weight data. The treatment groups were compared with one another at 0.05 level of statistical significance. Either Tukey's or Dunnett's procedure (23, 24) was used to make the adjustments for multiple comparisons. The results of the comparisons across treatment groups are presented as the difference in growth rate, the mean difference in tumor weight, or uterine weight with a corresponding 95% confidence interval.

## RESULTS

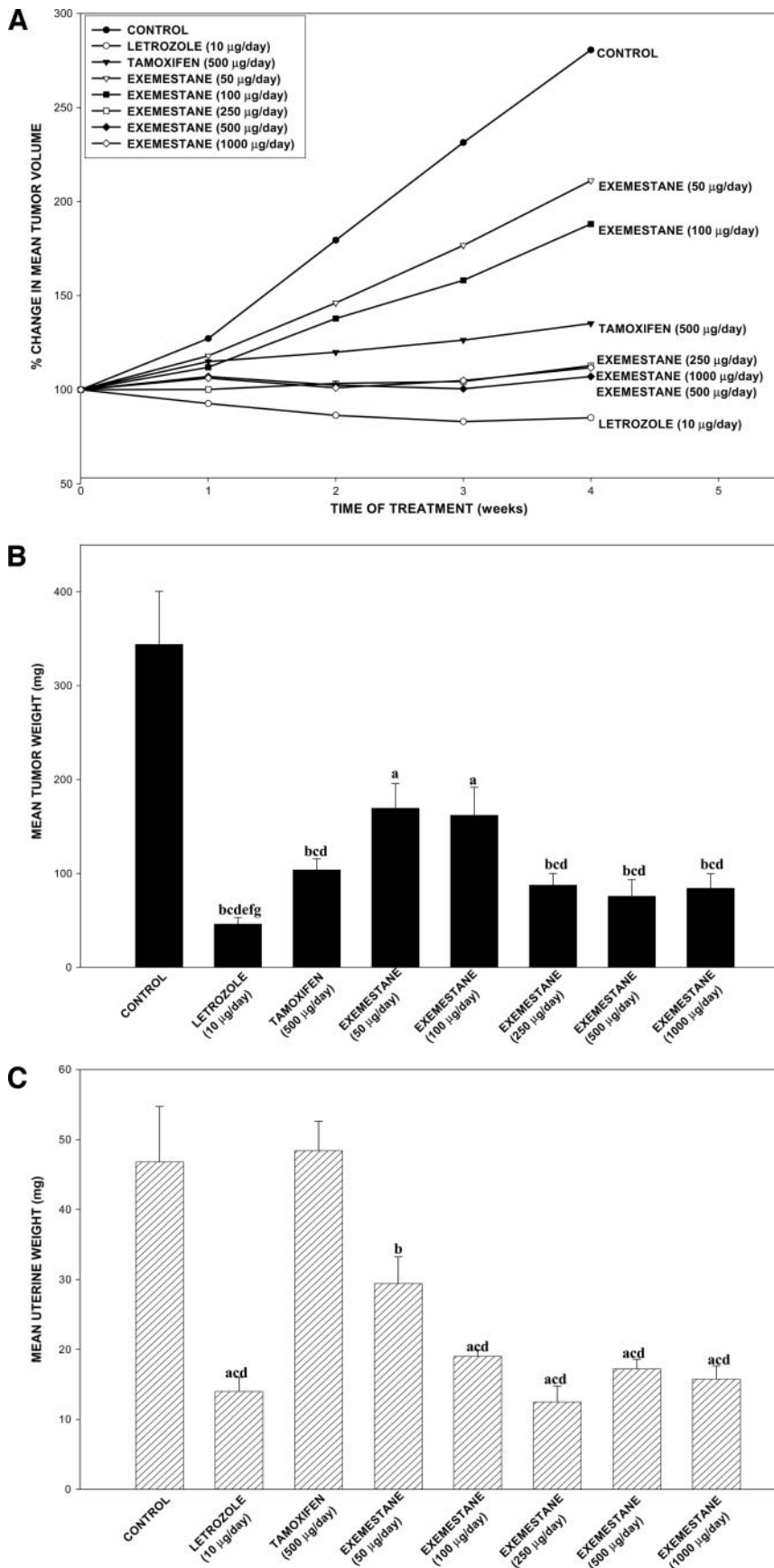
**Dose Response to Exemestane Compared with Letrozole and Tamoxifen.** To identify the optimal dose of exemestane on tumor growth *in vivo*, several doses of the inhibitor were evaluated in comparison to treatment with letrozole, tamoxifen, and vehicle (control). All of the treatments were effective at suppressing tumor growth compared with the vehicle-treated tumors (Fig. 1A). The tumor volumes in the letrozole-treated animals regressed by 15% over the 4 weeks of treatment, whereas the tamoxifen-treated tumors increased slightly by 35%. Nevertheless, although the tamoxifen-treated tumors did not regress, their growth was significantly reduced compared with the control tumors that increased by 2.8-fold during the 4 weeks of treatment. The estimated difference in the weekly growth rate was 0.15 mm<sup>3</sup> with the 95% confidence interval from 0.08 to 0.21 mm<sup>3</sup> (Fig. 1A). The inhibition of tumor growth by exemestane was shown to be dose-dependent. At doses of 50 and 100 µg/day, tumor volumes increased by 1.1 and 0.9-fold, respectively, over 4 weeks. In the animals treated

with exemestane at doses of 250, 500, and 1,000 µg/day, tumor volumes remained close to pretreatment levels indicating that these doses of exemestane effectively inhibited tumor growth to a maximum extent. Exemestane at doses of 250 µg/day was superior to tamoxifen (500 µg/day;  $P = 0.037$ ). When exemestane was compared with letrozole, there was no statistically significant difference between the tumor growth rates of the mice treated with higher dose (250 µg/day) of exemestane compared with those treated with letrozole (10 µg/day).

At the end of treatment, animals were sacrificed and tumors and uteri were dissected and weighed. The results for tumor volumes correlated well with tumor weights. Thus, tumor weights (Fig. 1B) in the control group ( $344 \pm 56$  mg) were significantly greater than in all of the treatment groups ( $P = 0.05$ ). The mean weight of letrozole-treated tumors ( $48 \pm 7$  mg) was significantly lower than that of tamoxifen-treated tumors ( $P < 0.001$ ). There was a statistically significant difference in tumor volumes between letrozole and exemestane-treated tumors at doses of 250 µg/day ( $P = 0.03$ ). Also, the tumor weights of the letrozole-treated group were significantly less. The difference in treatment means is 29.1 mg with the 95% confidence interval (3.5–64.7 mg). The weight of tumors treated with the lowest dosage, 50 mg of exemestane, was significantly lower than in the control group ( $P = 0.05$ ) but was not significantly different from tamoxifen.

The mouse uterus is very sensitive to the effects of estrogen and can be used as a bioassay of the estrogenic effects of compounds. In the tumor model, sufficient estrogen is produced by the tumors to maintain uterine weight comparable with that of the mouse in diestrus. Treatment with aromatase inhibitors letrozole and exemestane reduced uterine weights significantly compared with those of the control and tamoxifen-treated mice (Fig. 1C). For instance, the estimated difference in treatment means between the control and the group treated with 50 mg of exemestane was 17.4 mg with the 95% confidence interval (6.7–27.8 mg). This result suggests that the two aromatase inhibitors cause marked reduction in estrogen levels. In contrast, tamoxifen did not cause any significant difference in uterine weight compared with control. This finding is consistent with previous reports that this antiestrogen acts as an estrogen on the uterus (25).

**The Effect of Sequential Treatment on Tumors Progressing on Exemestane and Tamoxifen Alone and in Combination.** In the previous experiment, we identified submaximal (100 µg/day) and maximally (250 µg/day) effective doses



*Fig. 1* A, the dose effect of exemestane compared with letrozole and tamoxifen on the growth of MCF-7Ca breast tumor xenografts in ovariectomized athymic mice. All animals were inoculated with MCF-7Ca aromatase-transfected human breast cancer cells at two sites per flank and supplemented with androstenedione ( $\Delta 4A$ , 100  $\mu\text{g}/\text{day}$ ) for the duration of the experiment. When tumor volumes reached  $\sim 500 \text{ mm}^3$ , animals were grouped ( $n = 5$ ) for treatment with vehicle, letrozole (10  $\mu\text{g}/\text{day}$ ), tamoxifen (500  $\mu\text{g}/\text{day}$ ), and exemestane at different doses (50, 100, 250, 500, and 1,000  $\mu\text{g}/\text{day}$ ). Tumor volumes were measured weekly and expressed as the percentage change from the initial tumor volume. B, the dose effect of exemestane compared with letrozole and tamoxifen on the tumor weights of MCF-7Ca breast tumor xenografts in ovariectomized athymic mice. After 4 weeks of the indicated treatments, the mice were sacrificed, and tumors were removed and weighed. The overall effect of treatment on tumor weight was different across the treatment groups ( $P = 0.005$ ). Tumor weights that were significantly lower are shown: a,  $P < 0.05$  versus control; b,  $P < 0.001$  versus control; c,  $P < 0.05$  versus exemestane at 50  $\mu\text{g}/\text{day}$ ; d,  $P < 0.05$  versus exemestane at 100  $\mu\text{g}/\text{day}$ ; e,  $P < 0.05$  versus exemestane at 250  $\mu\text{g}/\text{day}$ ; and f,  $P < 0.001$  versus tamoxifen. C, the dose effect of exemestane compared with letrozole and tamoxifen on the uterine weights of the above ovariectomized athymic mice. After 4 weeks of the indicated treatments, the mice were sacrificed, and uteri were removed, cleaned, and weighed. The overall effect of treatment on uterine weight across eight groups was  $P < 0.001$ . Uterine weights that were significantly lower are shown: a,  $P < 0.05$  versus control; b,  $P < 0.01$  versus tamoxifen; c,  $P < 0.005$  versus tamoxifen; and d,  $P < 0.05$  versus exemestane at 50  $\mu\text{g}/\text{day}$ .

of exemestane (Fig. 1A). These doses and a submaximal dose of tamoxifen (100  $\mu\text{g}/\text{day}$ ) were evaluated as single agents and in combination. After tumors reached a measurable size, animals were assigned to seven groups of five mice each for first-line treatment with vehicle, letrozole (10  $\mu\text{g}/\text{day}$ ), tamoxifen (100  $\mu\text{g}/\text{day}$ ), exemestane (100  $\mu\text{g}/\text{day}$ ), exemestane (250  $\mu\text{g}/\text{day}$ ), tamoxifen (100  $\mu\text{g}/\text{day}$ ) plus exemestane (100  $\mu\text{g}/\text{day}$ ), and tamoxifen (100  $\mu\text{g}/\text{day}$ ) plus exemestane (250  $\mu\text{g}/\text{day}$ ; Fig. 2).

After 9 weeks, the control tumors had increased by 4.4-fold. Tamoxifen-treated tumors grew slowly over the period of 9 weeks, and increased in size by 2.3-fold. Exemestane at 250  $\mu\text{g}/\text{day}$  was more effective than at 100  $\mu\text{g}/\text{day}$ , and tumors increased in size by 1.8- and 3.5-fold, respectively, by 9 weeks. However, treatment with tamoxifen (100  $\mu\text{g}/\text{day}$ ) combined with exemestane in doses of 100 and 250  $\mu\text{g}/\text{day}$  was more effective in suppressing the growth rate than either tamoxifen ( $P = 0.02$ ) or exemestane ( $P = 0.04$ ) alone and was not significantly different from letrozole. Letrozole treatment initially caused tumors to regress by 20% in the first 4 weeks. Tumors then slowly resumed growth and reached the pretreatment volume by 9 weeks.

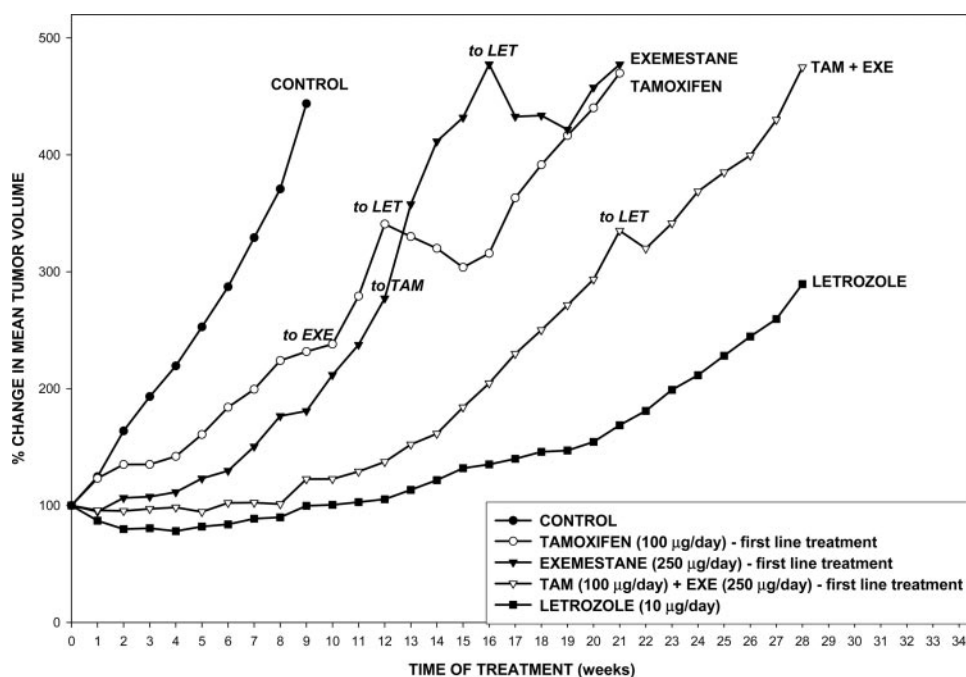
To determine the effect of these agents in sequential therapy, mice were changed to second-line treatment when tumor volume had increased by 2- to 3-fold. Control tumors doubled their initial volume after <4 weeks (Table 1). Tamoxifen-treated tumors had doubled after 8 weeks of treatment. Tumors treated with exemestane at the dose of 250  $\mu\text{g}/\text{day}$  doubled

**Table 1** The effect of exemestane alone or in combination with tamoxifen on delaying the progression of MCF-7Ca breast tumor growth in postmenopausal hormone-dependent breast cancer model

Treatment	Tumor-doubling time (weeks)
Control ( $\Delta 4A$ )	<4
Tamoxifen (100 $\mu\text{g}/\text{day}$ )	8
Exemestane (100 $\mu\text{g}/\text{day}$ )	6
Exemestane (250 $\mu\text{g}/\text{day}$ )	10
Tamoxifen (100 $\mu\text{g}/\text{day}$ ) plus exemestane (100 $\mu\text{g}/\text{day}$ )	13
Tamoxifen (100 $\mu\text{g}/\text{day}$ ) plus exemestane (250 $\mu\text{g}/\text{day}$ )	16
Letrozole (10 $\mu\text{g}/\text{day}$ )	24

between 9 and 10 weeks whereas those treated with the combination of tamoxifen and exemestane (250  $\mu\text{g}/\text{day}$ ) doubled in 16 weeks. Thus, the time of tumor progression was significantly delayed compared with either drug alone. Letrozole-treated tumors did not double until 24 weeks of treatment.

After 9 weeks of treatment with tamoxifen when tumor volumes had increased 2.3-fold increased compared with their initial size, animals were switched to treatment with exemestane (250  $\mu\text{g}/\text{day}$ ; Fig. 2). Tumors became static for a brief period with exemestane and then continued to grow. After 3 weeks of treatment with exemestane, tumor volume had increased up to



**Fig. 2** The effect of sequential treatment on MCF-7Ca breast tumors that progressed on exemestane or tamoxifen alone or the combination of exemestane and tamoxifen in ovariectomized female athymic mice.  $\circ$ , mice treated with tamoxifen (100  $\mu\text{g}/\text{day}$ ) as first-line therapy were switched after 9 weeks to treatment with exemestane (250  $\mu\text{g}/\text{day}$ ) for 3 weeks to week 12, and then to letrozole (10  $\mu\text{g}/\text{day}$ ) as a third-line treatment until week 21;  $\blacktriangledown$ , mice treated with exemestane (250  $\mu\text{g}/\text{day}$ ) as first-line therapy were switched after 12 weeks to tamoxifen (100  $\mu\text{g}/\text{day}$ ) as a second-line treatment for 4 weeks until week 16, and then to third-line treatment with letrozole (10  $\mu\text{g}/\text{day}$ ) until week 21;  $\triangle$ , mice treated with the combination of tamoxifen (100  $\mu\text{g}/\text{day}$ ) plus exemestane (250  $\mu\text{g}/\text{day}$ ) as first-line treatment were switched after 21 weeks to second-line therapy with letrozole (10  $\mu\text{g}/\text{day}$ ) and continued to week 28;  $\blacksquare$ , mice were treated with letrozole (10  $\mu\text{g}/\text{day}$ ) for 28 weeks.

3.4-fold. In a further effort to control tumor growth, mice were switched to letrozole treatment. However, tumors regressed for 3 weeks but then tumor growth resumed. When mice initially treated with exemestane (250  $\mu\text{g}/\text{day}$ ) were switched to treatment with tamoxifen at week 12, tumor growth continued unabated until week 16. The mice were then switched to letrozole treatment, which slowed tumor growth for 3 weeks before growth resumed. By week 21, the two groups started on exemestane or tamoxifen and receiving three sequential treatments had similar tumor volumes. Also, a second group of animals treated with exemestane (100  $\mu\text{g}/\text{day}$ ) for 9 weeks (data not shown) followed by second-line treatment with tamoxifen and third-line treatment with letrozole showed a similar growth pattern as tumors treated initially with the higher dose of exemestane. Combining tamoxifen plus exemestane prolonged the time of tumor doubling to 16 weeks. However, these tumors were unresponsive to second-line treatment with letrozole after 1 week. Overall, letrozole treatment alone produced the best results in delaying tumor progression (tumors did not double for 24 weeks). The weekly growth rate was less with continuous treatment with letrozole only compared with the tumor growth rate of mice treated first line with exemestane (250  $\mu\text{g}/\text{day}$ ) plus tamoxifen and then switched to letrozole ( $P = 0.046$ ; Fig. 2).

## DISCUSSION

The effect of exemestane on tumor growth in the MCF-7Ca xenograft model suggests that this aromatase inhibitor at optimal doses (250, 500, and 1,000  $\mu\text{g}/\text{day}$ ) is similar to or slightly better than the maximal dose of tamoxifen (500  $\mu\text{g}/\text{day}$ ) in reducing tumor weight after 4 weeks of treatment. Furthermore, aromatase inhibitors did not have estrogenic effects on the uterus unlike the antiestrogen tamoxifen. In the second experiment, a submaximal dose of tamoxifen (100  $\mu\text{g}/\text{day}$ ) was used in combination with 100 and 250  $\mu\text{g}/\text{day}$  of exemestane to determine whether the two agents acting by different mechanisms have additive effects. There was a significantly greater reduction in tumor growth with both doses of exemestane (100 and 250  $\mu\text{g}/\text{day}$ ) used in the combined treatment than with either doses of exemestane alone or tamoxifen alone. A similar result was reported previously by Zaccheo *et al.*, (21) who found that growth of carcinogen [7,12-dimethylbenz(a)anthracene]-induced tumors of the rat were inhibited to a greater extent by the combination of exemestane and tamoxifen than with either alone. The combined treatment, which inhibited estrogen action and estrogen synthesis, delayed tumor doubling for nearly 16 weeks, twice as long as with tamoxifen alone. This suggests that combining tamoxifen with this steroidal aromatase inhibitor may be more effective in breast cancer patients than the drugs administered separately. The results in the mouse model are in contrast to our previous findings with combinations of nonsteroidal aromatase inhibitors and antiestrogens (18, 19). However, whereas the exemestane-tamoxifen combination was more effective than either agent alone, it was less effective than the aromatase inhibitor letrozole only. Thus, tumors were significantly larger after 28 weeks of the combined treatment than those treated with letrozole.

The xenograft model bearing tumors of MCF-7Ca human breast cancer cells has predicted the results of clinical trials

evaluating tamoxifen and aromatase inhibitors (17, 19). For example, letrozole was superior to tamoxifen in the mouse model (17, 19) and in time to progression and time to treatment failure in breast cancer patients (8). Anastrozole also significantly increased time to progression compared with tamoxifen in patients ( $P = 0.005$ ; refs. 8 and 9). In addition, results of the Anastrozole, Tamoxifen, and the Combination trial (11) confirmed our findings of combining nonsteroidal aromatase inhibitors with tamoxifen in the mouse model (17, 19). Letrozole plus tamoxifen in the xenograft model was less effective than letrozole alone, and the combination was not significantly different in delaying tumor doubling than tamoxifen alone. One possible explanation for the difference in results between the two types of aromatase inhibitors is that letrozole is more potent than exemestane (Fig. 1A). The slight reductions reported in serum levels of nonsteroidal aromatase inhibitors (27 and 38%) when combined with tamoxifen seems unlikely to influence their activity because estrogen concentrations remain maximally suppressed (19, 26). Clearance rates of exemestane and tamoxifen in combination compared with those of the drugs alone are reported not to be altered in breast cancer patients (27, 28). Additional studies are therefore needed to explore whether these or other mechanisms explain the difference between the two classes of aromatase inhibitors.

When tamoxifen was used in sequence followed by exemestane, tumor growth was arrested briefly. This finding in our model of advanced breast cancer is consistent with reports that patients who progressed on tamoxifen responded to subsequent exemestane treatment (10). Although the period of response was relatively brief in the mouse, recent studies indicate better results are achieved in early breast cancer. Thus, women who switched from tamoxifen to exemestane after 2 to 3 years had 32% fewer recurrences than those on tamoxifen (12).

Tumors that were initially treated with exemestane and crossed over to tamoxifen grew continuously without interruption. Although this finding is not consistent with response to these drugs in patients, tumor growth was arrested for a short time (3 weeks) when mice were switched to letrozole treatment. However, when tumors were initially exposed to tamoxifen plus exemestane in combination, their response time was even shorter (1 week) to subsequent treatment with letrozole as a second-line treatment. Nevertheless, growth of tumors treated with the combination initially was reduced, and tumors did not reach the same volume as those of groups treated with three sequential therapies, for 7 additional weeks. In conclusion, exemestane was more effective in controlling tumor growth than tamoxifen. In addition, the combination of exemestane plus tamoxifen was clearly more effective than sequential use of these agents in the tumor model. However, the nonsteroidal aromatase inhibitor letrozole as first-line therapy was overall the most effective treatment in controlling tumor growth.

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