

Additive Antitumor Effect of Aromatase Inhibitor Letrozole and Antiestrogen Fulvestrant in a Postmenopausal Breast Cancer Model

Danijela Jelovac,¹ Luciana Macedo,¹ Olga G. Goloubeva,² Venkatesh Handratta,¹ and Angela M.H. Brodie¹

¹Department of Pharmacology and Experimental Therapeutics, School of Medicine and ²Division of Biostatistics, Greenebaum Cancer Center, University of Maryland, Baltimore, Maryland

Abstract

Blocking estrogen receptors with antiestrogens and blocking estrogen synthesis with aromatase inhibitors are two strategies currently being used for reducing the effect of estrogen in postmenopausal estrogen receptor-positive breast cancer patients. To optimize these treatment strategies, we have investigated whether tumor progression can be delayed by combining the pure antiestrogen fulvestrant with the nonsteroidal aromatase inhibitor letrozole. These studies were done in ovariectomized, athymic mice bearing tumors of estrogen receptor-positive human breast cancer cells stably transfected with the aromatase gene (MCF-7Ca). Groups of mice with equivalent tumor volumes were injected s.c. daily with vehicle (control; $n = 6$), fulvestrant (1 mg/d; $n = 7$), letrozole (10 $\mu\text{g}/\text{d}$; $n = 18$), or letrozole (10 $\mu\text{g}/\text{d}$) plus fulvestrant (1 mg/d; $n = 5$). All treatments were effective in suppressing tumor growth compared with controls ($P < 0.001$). Tumor volumes of the fulvestrant-treated group had doubled in 10 weeks. After 19 weeks of letrozole (10 $\mu\text{g}/\text{d}$) treatment when tumors had nearly doubled in volume, mice ($n = 18$) were assigned to second-line therapy with letrozole (100 $\mu\text{g}/\text{d}$; $n = 6$), tamoxifen (100 $\mu\text{g}/\text{d}$; $n = 6$), or remained on letrozole treatment (10 $\mu\text{g}/\text{d}$; $n = 6$). However, tumors continued to increase in volume in these groups. Tumors of animals treated with the combination of letrozole plus Faslodex regressed over 29 weeks of treatment by 45%. Thus, the combination of letrozole plus fulvestrant was more effective in suppressing tumor growth than either letrozole or fulvestrant alone or sequential therapies with tamoxifen or a higher dose of letrozole (100 $\mu\text{g}/\text{d}$). (Cancer Res 2005; 65(12): 5439-44)

Introduction

Estrogens play an important role in the development and progression of breast cancers (1). 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 estrogen synthesis. Production of estrogens in postmenopausal women occurs mainly via conversion of adrenal androgens mediated by aromatase in peripheral tissue, such as adipose tissue (2). Concentrations of estrogens in the breast are higher than in the circulation and equivalent to those

in premenopausal women (3). Current treatments for postmenopausal hormone-dependent breast cancer patients include two strategies to reduce the effects of estrogens on tumor growth. One method involves blocking estrogen from binding to estrogen receptors with antiestrogens and the other inhibits estrogen synthesis with aromatase inhibitors. The antiestrogen tamoxifen has been used since the 1970s for the treatment of breast cancer (4) and has been shown to delay recurrences and contralateral breast cancer. However, tamoxifen exhibits both estrogen agonist and antagonist effects, depending on its target tissue. In the breast, tamoxifen acts primarily as an antagonist, whereas in bone, liver, and the uterus, it acts predominantly as an estrogen agonist. As a result of its estrogenic activity, women treated with tamoxifen are at greater risk of endometrial cancer as well increased risk of strokes (5). In addition, patients' tumors may eventually progress on tamoxifen. Thus, tamoxifen in the adjuvant setting was found to be beneficial for 5 years but not for a longer time. The search for antiestrogens without agonist activity ultimately resulted in the development of the antiestrogen fulvestrant ("Faslodex"). Fulvestrant, like tamoxifen, binds to estrogen receptor competitively; but in contrast to tamoxifen, its binding to estrogen receptor leads to degradation and down-regulation of estrogen receptor (6).

A different approach to antiestrogen therapy was taken by us in the early 1970s to avoid the agonist properties of tamoxifen and improve efficacy and safety for patients (7, 8). Aromatase inhibitors were identified that block conversion of androgens to estrogens and are without agonist effects. Two classes of aromatase inhibitors, steroidal (e.g., exemestane) and nonsteroidal (e.g., anastrozole and letrozole), are now available for treatment of hormone-dependent breast cancer patients. Recent results from the clinical trials suggest that aromatase inhibitors are more effective than tamoxifen as first-line therapy for postmenopausal patients with hormone-responsive breast cancer (9-11).

To investigate the effectiveness of various strategies for using aromatase inhibitors and antiestrogens, 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). The model simulates the postmenopausal breast cancer patient as the source of estrogen is from nonovarian tissue (12, 13) and where estrogen synthesis is not under gonadotropin regulation. In this model, MCF-7Ca cells are grown as tumors and serve as an autocrine source of estrogen in the ovariectomized, immune-suppressed mice (14, 15). The resulting tumor xenografts are sensitive to both the antiproliferative effects of antiestrogens and aromatase inhibitors (14-16).

Blocking both estrogen receptors and estrogen synthesis with a combination of an antiestrogens and an aromatase inhibitor might have an additive effect and better control over tumor

Requests for reprints: Angela M.H. Brodie, Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Health Science Facilities 1, Room 580, 685 West Baltimore Street, Baltimore, MD 21201. Phone: 410-706-3137; Fax: 410-706-0032; E-mail: abrodie@umaryland.edu.

©2005 American Association for Cancer Research.

growth. In our previous studies, we have compared the effects of combining tamoxifen with the nonsteroidal aromatase inhibitors anastrozole and letrozole (17, 18). Although there was no benefit of the combined treatment compared with the effects of these aromatase inhibitors alone, the combination was similar to tamoxifen alone, possibly revealing a weak agonist effect of tamoxifen in the face of low estrogen levels. These data predicted the outcome of the clinical Arimidex and Tamoxifen Alone or in Combination trial (19), which found that anastrozole was more effective than tamoxifen and the combination of the two agents. However, as the antiestrogen fulvestrant differs from tamoxifen by causing estrogen receptor degradation, we hypothesized that the combination of letrozole with this antiestrogen to inhibit estrogen production would be more effective in slowing tumor progression compared with treatment with either agent alone.

In the current study, we have attempted to optimize treatment strategies that would be effective for postmenopausal breast cancer patients. We have investigated the effect of (a) fulvestrant alone, (b) letrozole alone, (c) the combination of the nonsteroidal aromatase inhibitor letrozole with the pure antiestrogen fulvestrant, and (d) second-line therapy with tamoxifen and with a higher dose of letrozole (100 µg/d) after tumors progress on the therapeutically effective dose of letrozole (10 µg/d).

Materials and Methods

Materials. MCF-7 human estrogen receptor-positive breast cancer cells stably transfected with the human aromatase gene (MCF-7Ca) were kindly provided by Dr. S. Chen (City of Hope, Duarte, CA; ref. 20). Dulbecco's PBS, DMEM, penicillin/streptomycin solution, trypsin-EDTA solution, and geneticin (G418) were from Life Technologies, Inc. (Grand Island, NY). Fetal bovine serum (FBS) was from Hyclone (Logan, UT). Androstenedione, 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. D. Evans (Novartis Pharma, Basel, Switzerland). Fulvestrant was kindly provided by Dr. A. Wakeling (AstraZeneca, Wilmington, DE).

Cell culture. MCF-7Ca were cultured in DMEM with 5% FBS, 1% penicillin/streptomycin solution, and 750 µg/mL G418. The culture medium was changed twice weekly. 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).

Postmenopausal intratumoral aromatase model. All animal studies were done according to the guidelines and approval of the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine. Female BALB/c athymic ovariectomized mice, 4 to 6 weeks old, were obtained from National Cancer Institute-Frederick Cancer Research and Development Center (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 before tumor inoculation was done.

MCF-7Ca cells were inoculated into the mice as previously described (14, 15). Each mouse received a s.c. injection at one site on each flank with 0.1 mL of cell suspension (2.5×10^7 cells/mL). As athymic mice were deficient in adrenal androgens (21), they were supplemented with daily s.c. injections of the aromatase substrate androstenedione (100 µg/d) for the duration of the experiment.

First-line treatment with fulvestrant, letrozole, or letrozole plus fulvestrant. When the tumors reached a measurable size (~ 300 mm³), ~ 6 weeks after MCF-7Ca cells were inoculated, animals were assigned to four groups with equivalent tumor volumes and injected s.c. daily with androstenedione supplement (100 µg/d) and vehicle (control; $n = 6$), or androstenedione along with one of the following treatments: fulvestrant (1 mg/d; $n = 7$), letrozole (10 µg/d; $n = 18$), or letrozole (10 µg/d) plus

fulvestrant (1 mg/d; $n = 5$). The doses of letrozole and fulvestrant used had been previously determined to be maximally effective in reducing tumor growth (15, 22). All drugs were prepared in 0.3% hydroxypropyl cellulose. Tumors were measured weekly with calipers and volumes were calculated using the formula $(4/3)\pi \times r_1^2 \times r_2$ (where $r_1 < r_2$). Animals were treated for the indicated times (Fig. 1); after which they were sacrificed by decapitation and tumors and uteri were excised, cleaned, weighed, and stored at -80°C .

Second-line therapies with a higher dose of letrozole (100 µg/d) or tamoxifen for tumors proliferating during letrozole (10 µg/d) treatment. Following initial regression, tumors of animals ($n = 18$) treated with a therapeutically effective dose of letrozole (10 µg/d) were growing by week 19. These 18 mice were then assigned to the following three treatment groups: group 1 continued on treatment with letrozole (10 µg/d; $n = 6$); group 2 was given a second-line treatment with a higher dose of letrozole (100 µg/d; $n = 6$), and group 3 was treated with tamoxifen (100 µg/d; $n = 6$). The dose of tamoxifen used was chosen because in previous studies it caused optimal tumor growth suppression as first-line treatment (22, 23). Tumors were measured weekly and tumor volumes were calculated. Tumor growth rate was estimated over 29 weeks and compared across the following four groups: letrozole (10 µg/d), letrozole (100 µg/d), tamoxifen (100 µg/d), and letrozole (10 µg/d) plus fulvestrant (1 mg/d; Fig. 2). The experiment was terminated at week 29. Tumor volume and weight, as well as uterine weight, were measured.

Statistical analysis. Data on tumor volume and weight, as well as uterine weight, were analyzed separately. Linear mixed-effect models (24) were used to estimate growth rate and average tumor volume and weight across treatment groups. Data on tumor volume were longitudinal and unbalanced. The duration of treatment varied across treatment groups. Mice receiving vehicle were sacrificed at week 7, receiving fulvestrant alone at week 17; animals in the remaining groups were treated until week 29. For tumor volume, diagnostic plots suggested that models of exponential growth were appropriate to the data. Therefore, linear mixed-effect models were fit to the natural logarithm of tumor volume over time. This approach allows the estimation of an exponential variable controlling the tumor growth rate for each treatment group. Responses from different animals were assumed to be statistically independent whereas those within an animal were correlated. Via model diagnostics, the first-order autoregressive covariance structure was chosen as the most appropriate for the data. Weight of the multiple tumors per subject and uterine weight were obtained for each mouse after it was sacrificed. The general linear model approach was used to analyze uterine weight data.

All hypothesis tests were two sided. Adjustment for multiple comparisons was made by using Holm's procedure. All treatment groups were compared at the 0.05 level of significance.

Results

Growth responses to the treatments with fulvestrant, letrozole, or letrozole plus fulvestrant *in vivo*. After 6 weeks, mice were assigned to four groups so that there was no statistically significant difference in tumor volume among the groups at the beginning of treatment (week 0). In addition to androstenedione (100 µg/d) supplement, animals were injected s.c. daily with vehicle (control), fulvestrant (1 mg/d), letrozole (10 µg/d), or letrozole (10 µg/d) plus fulvestrant (1 mg/d). The percentage change in tumor volume is plotted in Fig. 1. Tumors in the control group doubled in their mean initial volume after 3 weeks (Table 1) and increased about 6-fold in volume after 7 weeks (Fig. 1). By week 7, all treatments were effective in suppressing tumor growth when compared with the control group (all three $P < 0.001$). These animals were sacrificed at week 7 due to large tumor size, and the estimated mean tumor weight was 885.71 ± 128.28 mg. The mean uterine weight of the vehicle-receiving group was 66.83 ± 7.54 mg. As the average weight of the atrophic uterus in ovariectomized mice is ~ 10 mg, the greater uterine weight of mice receiving

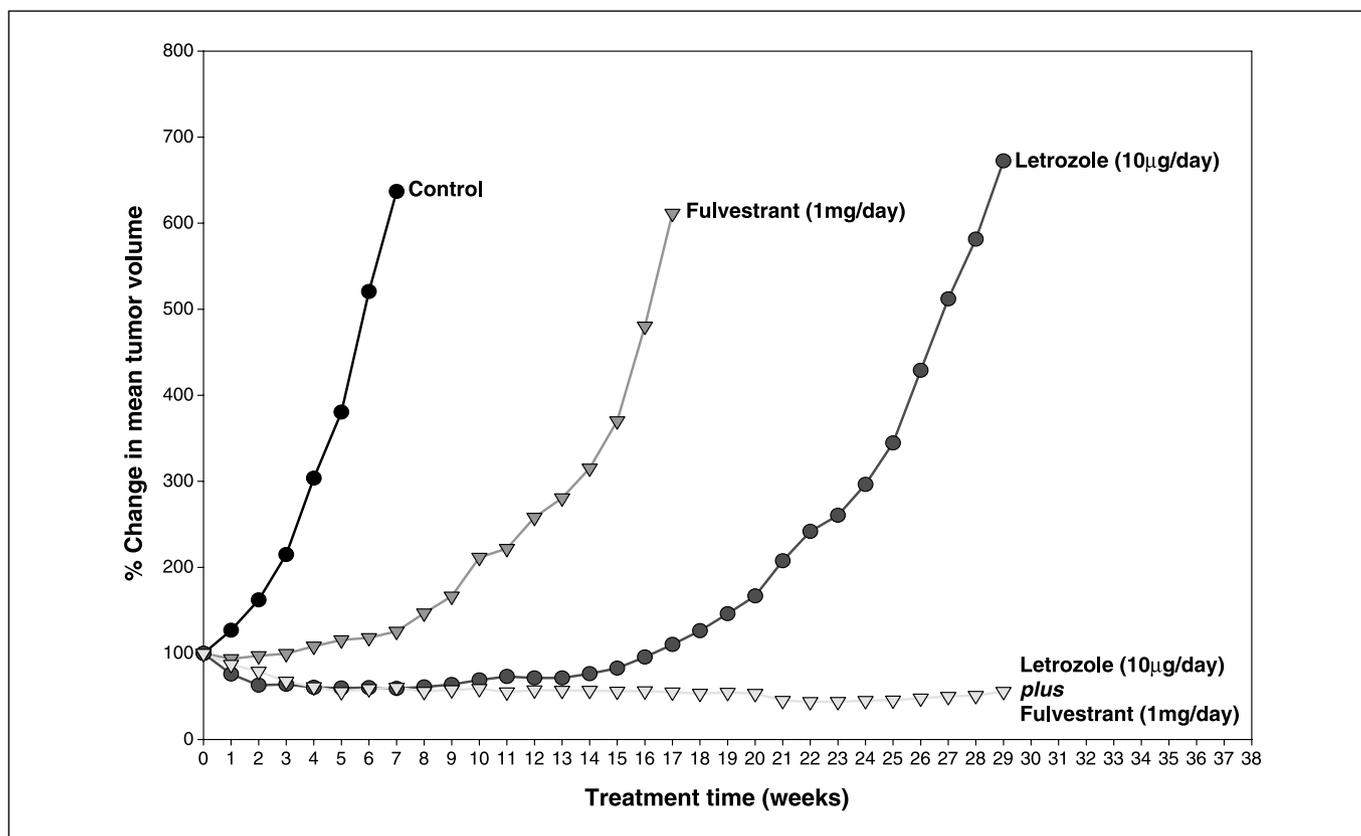


Figure 1. The effect of letrozole (10 µg/d) and fulvestrant (1 mg/d) alone or in the combination on the growth of MCF-7Ca breast tumor xenografts in female ovariectomized athymic nude mice. When tumors reached ~300 mm³, animals were divided into four groups and injected s.c. daily with vehicle (control; *n* = 6), fulvestrant (1 mg/d; *n* = 7), letrozole (10 µg/d; *n* = 18), or letrozole (10 µg/d) plus fulvestrant (1 mg/d; *n* = 5). Tumor volumes were measured weekly and expressed as the percent change in mean tumor volume relative to the initial size at week 0.

androstenedione indicates that aromatase in the tumors is producing enough estrogens to maintain the uterine weight similar to intact mice in diestrus.

Fulvestrant (1 mg/d)-treated tumors were static for the first 4 weeks of treatment. Thereafter, these tumors started to proliferate and had doubled after 10 weeks of treatment (Fig. 1; Table 1). At week 17, tumor volumes were significantly larger in the group treated with fulvestrant alone compared with letrozole ($P < 0.001$). Also, treatment with letrozole plus fulvestrant was superior to fulvestrant alone ($P < 0.001$). Fulvestrant-treated mice were sacrificed at week 17 due to large tumor size. Mice receiving fulvestrant had significantly smaller uteri (10.71 ± 1.90 mg) than mice in the control group (66.83 ± 7.54 mg; $P < 0.0001$). These data indicate that fulvestrant can effectively block the uterotrophic activity of estrogens produced by peripheral aromatization of androstenedione.

As previously reported (16, 17, 25), letrozole treatment initially induced marked regression of MCF-7Ca tumors in ovariectomized nude mice (Fig. 1). In the present study, tumor volume was reduced by 40% over the first 8 weeks of treatment. These tumors slowly returned to their initial size after 17 weeks and had doubled their initial volume after 21 weeks of treatment (Fig. 1; Table 1).

Treatment with the combination of letrozole plus fulvestrant also caused an initial regression of tumors (similar to letrozole alone for the first 8 weeks of treatment), but was able to maintain tumor growth inhibition by 45% over 29 weeks of treatment (Fig. 1).

Effect of second-line therapies with a higher dose of letrozole (100 µg/d) or tamoxifen on tumors proliferating during letrozole (10 µg/d) treatment compared with the combination of letrozole plus fulvestrant. As the tumors started to proliferate during letrozole (10 µg/d) treatment, second-line therapies were initiated at week 19. Animals (*n* = 18) were assigned to three different groups so that there was no difference in total tumor volume across these groups. One group of mice continued on treatment with letrozole (10 µg/d; *n* = 6); another group received second-line treatment with a higher dose of letrozole (100 µg/d; *n* = 6); and the third group received tamoxifen (100 µg/d; *n* = 6; Fig. 2). The tumor growth rate over 29 weeks was compared across the following four groups: letrozole (10 µg/d), letrozole (100 µg/d), tamoxifen (100 µg/d), and letrozole (10 µg/d) plus fulvestrant (1 mg/d). The experiment was terminated at week 29 when tumor volumes and weights, as well as uterine weights, were measured.

Letrozole (10 µg/d) alone for 29 weeks was less effective than letrozole plus fulvestrant in controlling tumor growth on week 29 ($P = 0.0005$). Also, tumor volumes were statistically significantly larger in the letrozole treatment group than in the combination ($P < 0.0001$).

Second-line treatment with tamoxifen (100 µg/d), as previously reported (18), was not effective after letrozole treatment. The percentage change in tumor volume is shown in Fig. 2. Tumors of mice switched to tamoxifen treatment continued to proliferate, and had a similar estimated growth rate (exponential variable $\beta = 0.064 \pm 0.016$) as tumors of mice continued on letrozole at 10 µg/d for 29 weeks ($\beta = 0.036, \pm 0.008; P = 0.116$), but grew faster

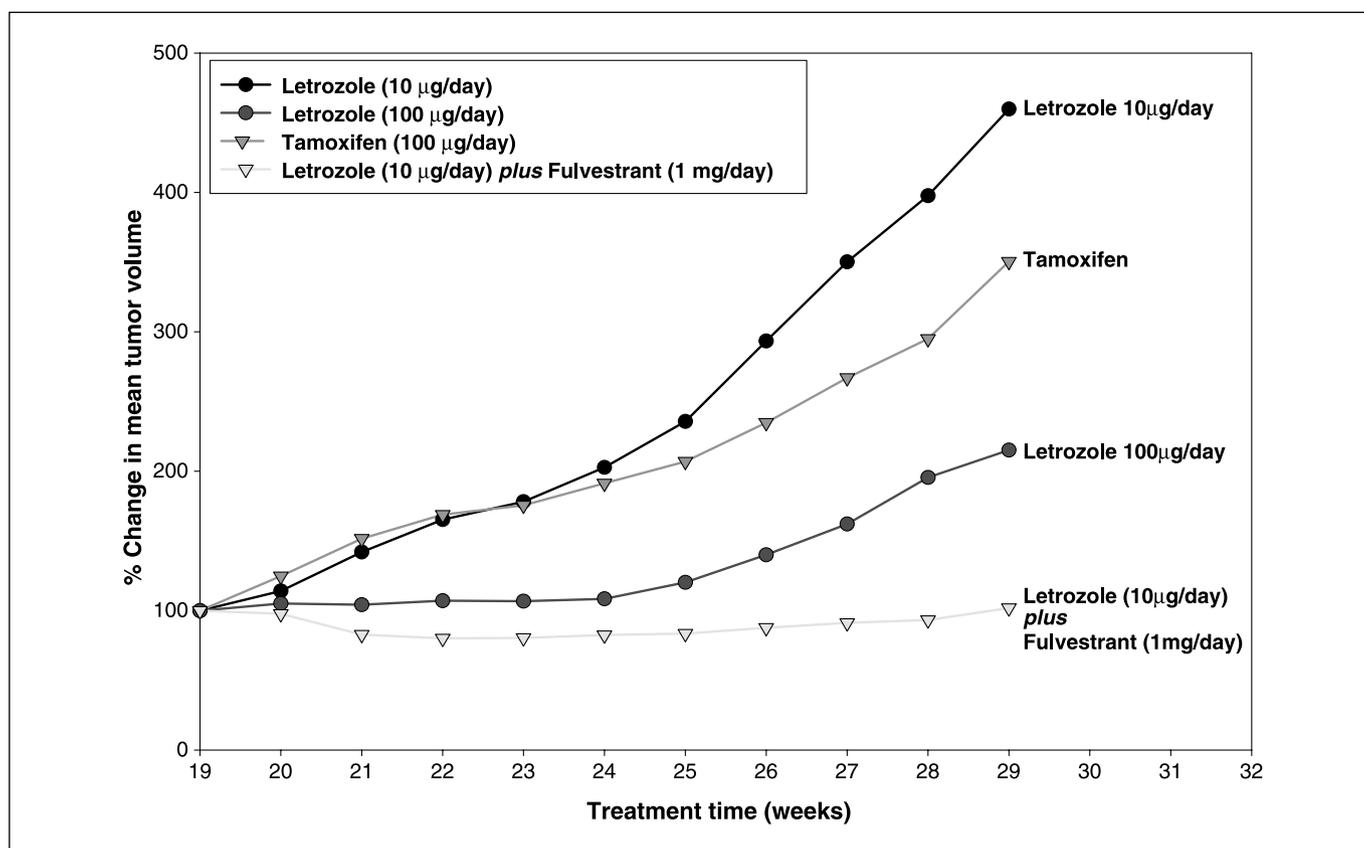


Figure 2. The effect of second-line treatments with tamoxifen and a higher dose of letrozole (100 µg/d) on the growth of MCF-7Ca tumors progressing on treatment with letrozole (10 µg/d). After 19 weeks, the tumors were proliferating during the letrozole (10 µg/d) treatment. Animals ($n = 18$) were assigned to three groups so that total tumor volumes were similar in each group. Groups were continued on letrozole treatment (10 µg/d; $n = 6$), given tamoxifen (100 µg/d; $n = 6$) or a higher dose of letrozole (100 µg/d; $n = 6$). Second-line treatment lasted for 10 weeks. Tumor volumes were measured weekly and expressed as the percent change in mean tumor volume relative to the initial size at week 19.

than the tumors of the group switched to a higher dose of letrozole (100 µg/d; $\beta = 0.002 \pm 0.016$; $P = 0.007$). The tamoxifen group had a larger estimated exponential variable governing growth rate than tumors treated with letrozole plus fulvestrant ($P < 0.0001$). Also, tumor volume was significantly larger in the tamoxifen-treated group than in the letrozole plus fulvestrant group ($P = 0.003$). None of the tumors in the group receiving the combination increased in volume during treatment.

Tumors of animals switched to letrozole 100 µg/d as a second-line treatment had a lower estimated growth rate than letrozole (10 µg/d)-treated tumors, but this was marginally statistically significant ($P = 0.059$). There was no statistically significant difference in tumor weights between letrozole (10 µg/d), tamoxifen, and letrozole (100 µg/d), indicating that the tumors were not responsive to these therapies. However, the mean tumor weight of mice treated with letrozole plus fulvestrant was 98.8 ± 40.39 mg, which was significantly lower than the tumor weight of all other groups (851.82 ± 101.28 mg; $P = 0.0001$).

As expected, the mean uterine weight of tamoxifen-treated mice was significantly heavier [46.0 ± 7.16 (SE) mg] compared with letrozole 10 µg/d [12.33 ± 1.20 (SE) mg], second-line treatment with letrozole 100 µg/d [11.0 ± 0.73 (SE) mg], or letrozole plus fulvestrant [10.25 ± 1.11 (SE) mg; Fig. 3; $P < 0.0001$]. Also, tamoxifen-treated mice had larger uteri than the mice treated with the pure antiestrogen fulvestrant. These findings are consistent with reports of the estrogenic effects of tamoxifen on the uterus.

However, mice in the control group had significantly heavier ($P = 0.0004$) uterine weights compared with the group treated with tamoxifen as a second-line therapy. In previous studies in this model, uteri of tamoxifen-treated mice were similar to controls. The present findings might be explained by the effect of the first-line therapy with letrozole for 19 weeks which inhibited estrogen production and likely decreased the size of the uteri compared with the control group.

Table 1. The effect of letrozole and fulvestrant alone or in combination on time taken for tumors to double in volume in ovariectomized nude mice

Treatment	Time to increase tumor volume by 2-fold (wk)
Control	3
Fulvestrant (1 mg/d)	10
Letrozole (10 µg/d)	21
Letrozole (10 µg/d) plus fulvestrant (1 mg/d)	ND

NOTE: All groups received androstenedione (100 µg/d) and vehicle (control) or the treatment indicated. ND, not determined.

Discussion

Our data indicate that when the two drugs, fulvestrant inhibiting estrogen action and letrozole inhibiting estrogen synthesis, were combined, there was a significantly greater effect on tumor growth than treatment with either letrozole or fulvestrant alone. The combined treatment suppressed tumor growth resulting in tumor regression which was maintained in all tumors throughout the 29-week treatment period, suggesting that the combination of fulvestrant with letrozole could be more effective in breast cancer patients than these drugs administered separately. The combination of letrozole plus fulvestrant was significantly more effective than fulvestrant alone at week 17 or letrozole alone at week 29 in suppressing tumor growth. Animals treated with letrozole plus fulvestrant had the lowest tumor growth rate and estimated average tumor volume at week 29 compared with groups treated with letrozole (10 $\mu\text{g}/\text{d}$), or switched from letrozole to tamoxifen as second-line treatment. Also, treatments with letrozole (10 $\mu\text{g}/\text{d}$), or switched from letrozole to tamoxifen or a higher dose of letrozole (100 $\mu\text{g}/\text{d}$), were less effective in reducing tumor weight than treatment with letrozole plus fulvestrant from the beginning.

The effect of letrozole (10 $\mu\text{g}/\text{d}$) on tumor growth in the MCF-7Ca xenograft model suggests that this aromatase inhibitor is more

effective than the pure antiestrogen fulvestrant (1 mg/d) in controlling tumor growth and delaying the time of tumor progression. The percentage change in tumor volume had increased 2-fold in 10 weeks on fulvestrant and in 21 weeks on letrozole.

When treatment was switched at week 19 from letrozole to tamoxifen, tumors continued to proliferate with a growth rate similar to letrozole (10 $\mu\text{g}/\text{d}$), indicating no benefit from the second-line treatment with tamoxifen (Fig. 2). Our data show that the group of mice switched to a higher dose of letrozole had a significantly lower growth rate than animals switched to tamoxifen ($P = 0.006$). However, there was no statistically significant difference in tumor weights between groups continued on letrozole (10 $\mu\text{g}/\text{d}$), switched to tamoxifen, or to a higher dose of letrozole (100 $\mu\text{g}/\text{d}$). Although increasing the dose of letrozole did slow tumor growth rate, the effect on tumor volume and weight was not significant. These data indicate that switching the animals from the letrozole (10 $\mu\text{g}/\text{d}$) to tamoxifen, or increasing the dose of letrozole, might not be an optimal treatment choice for the patients with tumors progressing on therapeutically effective dose of letrozole. Further studies are needed to determine the optimal second-line therapy for patients whose tumors progress during letrozole treatment.

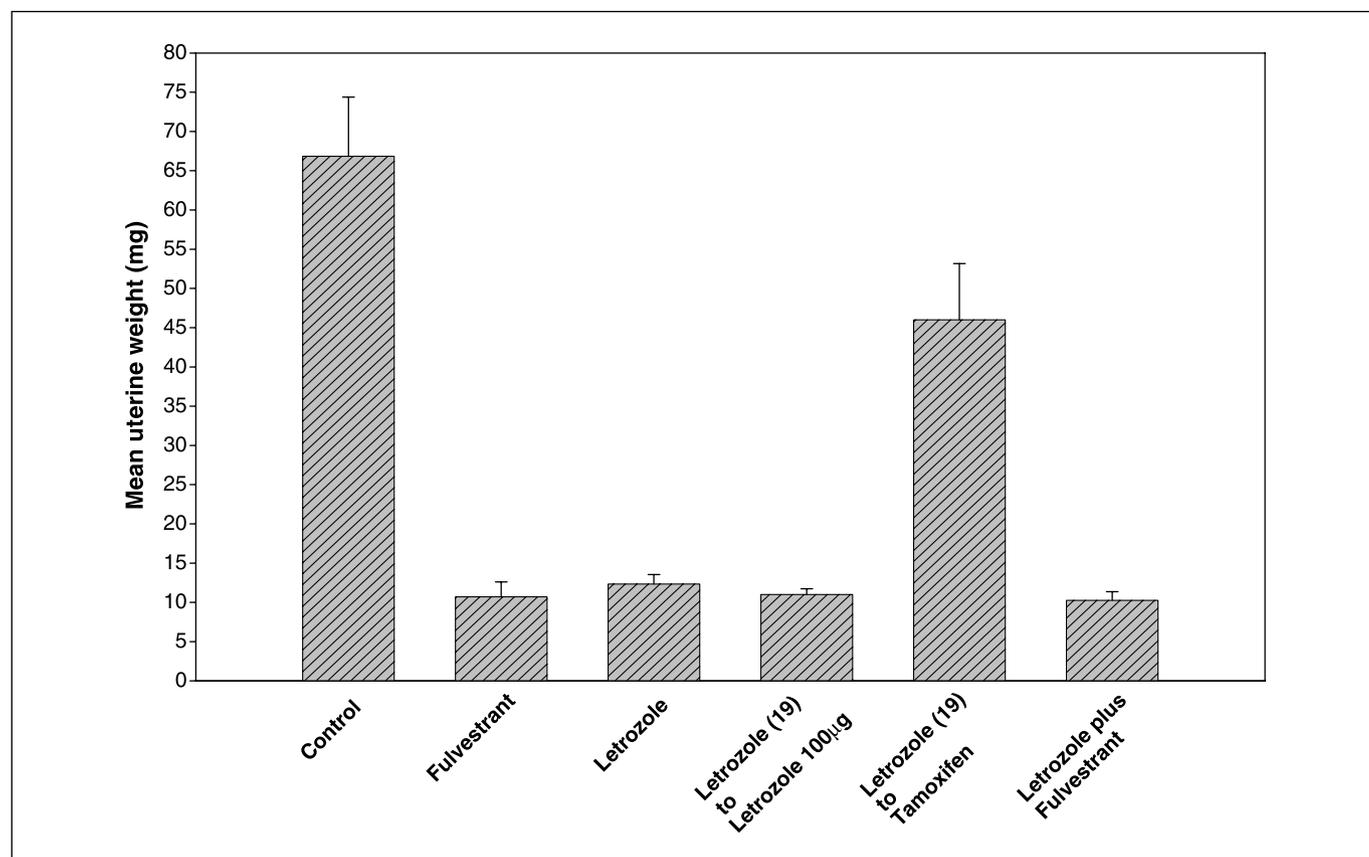


Figure 3. The effect of letrozole (10 $\mu\text{g}/\text{d}$) and fulvestrant (1 mg/d) alone or in combination on uterine weight in female ovariectomized athymic nude mice bearing MCF-7Ca breast tumors. After the tumors reached a measurable size, animals were assigned to four groups with similar tumor volumes and injected s.c. daily with vehicle (control; $n = 6$), fulvestrant (1 mg/d; $n = 7$), letrozole (10 $\mu\text{g}/\text{d}$; $n = 18$), or the combination of letrozole (10 $\mu\text{g}/\text{d}$) plus fulvestrant (1 mg/d; $n = 5$). All animals were supplemented with androstenedione (100 $\mu\text{g}/\text{d}$) for the duration of the experiment. Mice which received vehicle and animals treated with fulvestrant alone were sacrificed at week 7 and week 17, respectively, due to large tumor size. At week 19, animals treated with letrozole (10 $\mu\text{g}/\text{d}$) were assigned to three groups for second-line treatment with a higher dose of letrozole (100 $\mu\text{g}/\text{d}$; $n = 6$), tamoxifen (100 $\mu\text{g}/\text{d}$; $n = 6$), or continued on letrozole (10 $\mu\text{g}/\text{d}$; $n = 6$). At week 29, the remaining mice were sacrificed, and the uteri were removed, cleaned, and weighed. Animals in the control and tamoxifen-treated groups had statistically significant larger uterine weights compared with all other groups ($P < 0.0001$). Also, the control group had a significantly larger uterine weight compared with the group switched to tamoxifen as second-line therapy ($P = 0.0004$).

The effect of the treatments on the uterine weight was determined as a measure of estrogenic activity of the compounds (Fig. 3). Control mice had uteri similar to intact animals in diestrus due to the effect of estrogen produced by conversion of androstenedione by the tumor cells. Animals switched to tamoxifen had significantly larger uterine weights than the mice continued on letrozole (10 µg/d), or switched to a higher dose of letrozole (100 µg/d), showing estrogenic effects of tamoxifen on the uteri. Uterine weights of mice treated with the combination were not statistically significantly different from the uteri of mice treated with either drug alone. Although mice were not sacrificed at the same time, uterine weights of the groups treated with letrozole plus fulvestrant, or with these drugs separately, were significantly smaller than those of control group. These results indicate successful inhibition of estrogen synthesis by letrozole and also effective blockade of estrogen action on uterus by fulvestrant. Tamoxifen, on the other hand, only partially blocked the effects of estrogen, whereas neither letrozole nor fulvestrant showed any estrogenic effects on the mouse uterus and were clearly superior to tamoxifen in this regard. This suggests that these drugs, unlike tamoxifen, may not cause endometrial hyperplasia in patients.

The xenograft model bearing MCF-7Ca human breast cancer cells has predicted the results of clinical trials evaluating antiestrogen tamoxifen and aromatase inhibitors (17, 18). In our previous studies, letrozole plus tamoxifen and anastrozole plus tamoxifen in the xenograft model were less effective than letrozole alone and the

combination was similar to tamoxifen alone. The above results were subsequently confirmed in the Arimidex and Tamoxifen Alone or in Combination trial (19). Recent clinical trials have shown that fulvestrant had a similar efficacy as tamoxifen in postmenopausal women with estrogen receptor-positive and advanced breast cancer (26). In contrast, letrozole was shown to be more effective than tamoxifen (9). In the present study, the better antitumor effects of letrozole as a single agent compared with fulvestrant might be explained by the high potency of letrozole as an aromatase inhibitor in reducing estrogen production. However, the additive effect in tumors treated with the combination of these two compounds suggests that some transcription via the estrogen receptor may occur with fulvestrant treatment alone and is not completely blocked by the antiestrogen. Further studies into the mechanism of the effects are currently in progress.

In conclusion, the combination of nonsteroidal aromatase inhibitor letrozole and antiestrogen fulvestrant was clearly more effective than either one of these agents in the tumor model and maintained tumors without growth throughout the course of 29 weeks of treatment.

Acknowledgments

Received 8/4/2004; revised 11/2/2004; accepted 2/3/2005.

Grant support: National Cancer Institute, NIH, grant CA-62483 (A. Brodie).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

- James VHT, Reed MJ. Steroid hormones and human cancer. *Prog Cancer Res Ther* 1980;14:471-87.
- Hemsell DL, Gordon J, Breuner PF, Siiteri PK, MacDonald PC. Plasma precursor of estrogen. II. Correlation of extent of conversion of plasma androstenedione to estrone with age. *J Clin Endocrinol Metab* 1974;38:476-9.
- Szymczak J, Milewicz A, Thijssen JHH, Daroszewski J. Concentration of sex steroids in adipose tissue after menopause. *Steroids* 1998;63:319-21.
- Cole MP, Jones CTA, Todd IDH. A new antiestrogenic agent in late breast cancer: an early appraisal of ICI 46,474. *Br J Cancer* 1971;25:270-5.
- Fisher B, Constantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J Natl Cancer Inst* 1994;86:527-37.
- Howell A, Osborne CK, Morris C, Wakeling AE. ICI 182,780 (Faslodex): development of a novel, "pure" antiestrogen. *Cancer* 2000;89:817-25.
- Schwarzel WC, Kruggel W, Brodie HJ. Studies on the mechanism of estrogen biosynthesis. VII. The development of inhibitors of the enzyme system in the human placenta. *Endocrinology* 1973;92:866-80.
- Brodie AMH, Schwarzel WC, Shaikh AA, Brodie HJ. The effect of an aromatase inhibitor, 4-hydroxy-4-androstene-3,17-dione, on estrogen dependent processes in reproduction and breast cancer. *Endocrinology* 1977;100:1684-94.
- Mouridsen H, Gershanovich M, Sun Y, et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. *J Clin Oncol* 2003;21:2101-9.
- Nabholtz JM, Buzdar A, Pollak M, et al. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. *J Clin Oncol* 2000;18:3758-67.
- Paridaens R, Dirix L, Lohrisch C, et al. Mature results of a randomized phase II multicenter study of exemestane versus tamoxifen as first-line hormone therapy for postmenopausal women with metastatic breast cancer. *Ann Oncol* 2003;14:1391-8.
- Miller WR, Forrest APM. Oestradiol synthesis by a human breast carcinoma. *Lancet* 1974;2:866-8.
- Lu Q, Nakamura J, Savinov A, et al. Expression of aromatase protein and mRNA in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancer. *Endocrinology* 1996;137:3061-8.
- Yue W, Zhou DJ, Chen S, Brodie AMH. A new nude mouse model for postmenopausal breast cancer using MCF-7 cells transfected with the human aromatase gene. *Cancer Res* 1994;54:5092-5.
- Yue W, Wang J, Savinov A, Brodie A. Effect of aromatase inhibitors on the growth of mammary tumors in a nude mouse model. *Cancer Res* 1995;55:3073-7.
- Lu Q, Wang J, Liu Y, Long B, Brodie A. The effects of aromatase and antiestrogens in the nude mouse model. *Breast Cancer Res Treat* 1998;50:63-71.
- Lu Q, Liu Y, Long BJ, Grigoryev D, Gimbel M, Brodie A. The effect of combining aromatase inhibitors with antiestrogens on tumor growth in a nude mouse model for breast cancer. *Breast Cancer Res Treat* 1999;57:183-92.
- Long BJ, Jelovac D, Handratta V, et al. Therapeutic strategies using the aromatase inhibitor letrozole and tamoxifen in breast cancer model. *J Natl Cancer Inst* 2004;96:456-65.
- ATAC Trialists' Group. Pharmacokinetics of anastrozole and tamoxifen alone, and in combination, during adjuvant endocrine therapy for early breast cancer in postmenopausal women: a sub-protocol of the "Arimidex and tamoxifen alone or in combination" (ATAC) trial. *Br J Cancer* 2001;85:317-24.
- Zhou D, Pompon D, Chen S. Stable expression of human aromatase complementary DNA in mammalian cells: a useful system for aromatase inhibitor screening. *Cancer Res* 1990;50:6949-54.
- Rebar RW, Morandini IC, Erickson GF, Petze JE. The hormonal basis of reproductive defects in athymic mice: diminished gonadotropin concentration in prepubertal females. *Endocrinology* 1981;108:120-6.
- Brunner N, Frandsen TL, Holst-Hansen C, et al. MCF7/LCC2: a 4-hydroxytamoxifen resistant human breast cancer variant that retains sensitivity to the steroidal antiestrogens ICI 182,780. *Cancer Res* 1993;53:3229-32.
- Brunner N, Bronzert D, Vindelov LL, Rygaard K, Spang-Thomsen M, Lippman ME. Effect of growth and cell cycle kinetics of estradiol and tamoxifen on MCF-7 human breast cancer cells grown *in vitro* and in nude mice. *Cancer Res* 1989;49:1515-20.
- Pinheiro JC, Bates DM. Mixed-effects models in S and S-plus. New York (NY): Springer-Verlag; 2000. p. 21-45, 174-96.
- Long BJ, Jelovac D, Thiantanawat A, Brodie AM. The effect of second-line antiestrogen therapy on breast tumor growth after first-line treatment with the aromatase inhibitor letrozole: long-term studies using the intratumoral aromatase postmenopausal breast cancer model. *Clin Cancer Res* 2002;8:2378-88.
- Howell A, Robertson JFR, Abram P, et al. Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial. *J Clin Oncol* 2004;22:1605-13.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Additive Antitumor Effect of Aromatase Inhibitor Letrozole and Antiestrogen Fulvestrant in a Postmenopausal Breast Cancer Model

Danijela Jelovac, Luciana Macedo, Olga G. Goloubeva, et al.

Cancer Res 2005;65:5439-5444.

Updated version Access the most recent version of this article at:
<http://cancerres.aacrjournals.org/content/65/12/5439>

Cited articles This article cites 25 articles, 12 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/65/12/5439.full.html#ref-list-1>

Citing articles This article has been cited by 20 HighWire-hosted articles. Access the articles at:
</content/65/12/5439.full.html#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.