

Analysis of Cross-Resistance of the Selective Estrogen Receptor Modulators Arzoxifene (LY353381) and LY117018 in Tamoxifen-stimulated Breast Cancer Xenografts¹

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ABSTRACT

Purpose: Cross-resistance is the primary issue facing the evaluation of new antiestrogens to treat metastatic breast cancer because they may be tested, initially, in populations of patients that have failed long-term adjuvant tamoxifen (Tam) therapy.

Experimental Design: We have tested the benzothio-phenone derivatives, arzoxifene (Arzox; LY353381) and LY117018 in two models of Tam-stimulated tumor growth derived from either MCF-7 (M. M. Gottardis and V. C. Jordan, *Cancer Res.*, 48: 5183–5187, 1988) or T47D (J. MacGregor Schafer *et al.*, *Clin. Cancer Res.*, 6: 4373–4380, 2000) breast cancer cells.

Results: Using the MCF-7:Tam model, we found that both Arzox and LY117018 (1.5 mg/day) resulted in tumor growth and, therefore, were partially cross-resistant with Tam. Next, using the T47D:17 β -estradiol (E₂) model, we compared the antiestrogenic/antitumor properties of Arzox and LY117018 and determined that neither Arzox nor LY117018 caused T47D:E₂ tumor growth after 21 weeks. In

addition, we determined that long-term treatment does not result in failure and subsequent development of transplantable Arzox- or LY117018-stimulated tumors. To establish whether Arzox and LY117018 are cross-resistant in T47D: Tam tumors, mice were treated with Arzox or LY117018 (1.5 mg/day), and, again, we found that neither resulted in the growth of transplantable tumors. Lastly, we showed that Arzox and LY117018 were only partially able to compete with postmenopausal E₂ (0.3 cm silastic capsule) in T47D: Tam tumors. However, when T47D:E₂ tumors were treated for 7 days instead of 5 days, both Arzox and LY117018 were more effective.

Conclusions: Arzox is not cross-resistant with Tam in the T47D athymic mouse model but does exhibit cross-resistance in the MCF-7 model.

INTRODUCTION

Tam⁴ is the prototype of a class of drugs called SERMs that have antiestrogenic effects in the breast but exhibit E₂-like effects in bone and blood. Tam is the adjuvant endocrine treatment of choice for node-positive and node-negative ER-positive breast cancer (1) and is currently prescribed for the prevention of breast cancer in high-risk women (2). In addition, up to five years of adjuvant Tam treatment of patients with ER-positive disease confers a long-term survival benefit that continues for at least an additional 5 years after Tam treatment ceases (3). Unfortunately, >5 years of Tam treatment can lead to the clonal selection of metastatic breast cancers that are resistant to Tam (2). In fact, these tumors are stimulated by Tam as evidenced by the reduction in growth of tumors after Tam treatment is stopped (4–6). Most of these Tam-stimulated tumors retain expression of the ER (7) and, therefore, retain the ability to respond to second-line endocrine therapies such as the pure antiestrogen ICI182,780 (8) or an aromatase inhibitor such as anastrozole (9). A pure antiestrogen has no estrogenic properties because it causes destruction of the ER (10). Aromatase inhibitors are effective as second-line therapies because they block the synthesis of a woman's endogenous E₂ (9), which can promote tumor growth after Tam treatment fails.

A goal of current efforts in medicinal chemistry is to design better SERMs. The ideal agent would: (a) not produce premature drug resistance; (b) not be cross-resistant with Tam; and (c) have fewer side effects such as the increase in endometrial cancer incidence observed with Tam. Clearly, a new SERM that

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⁴ The abbreviations used are: Tam, tamoxifen; ER, estrogen receptor; E₂, 17 β -estradiol; Arzox, arzoxifene; Ral, raloxifene; 4-OHT, 4-hydroxytamoxifen; SERM, selective ER modulator; TGF, transforming growth factor.

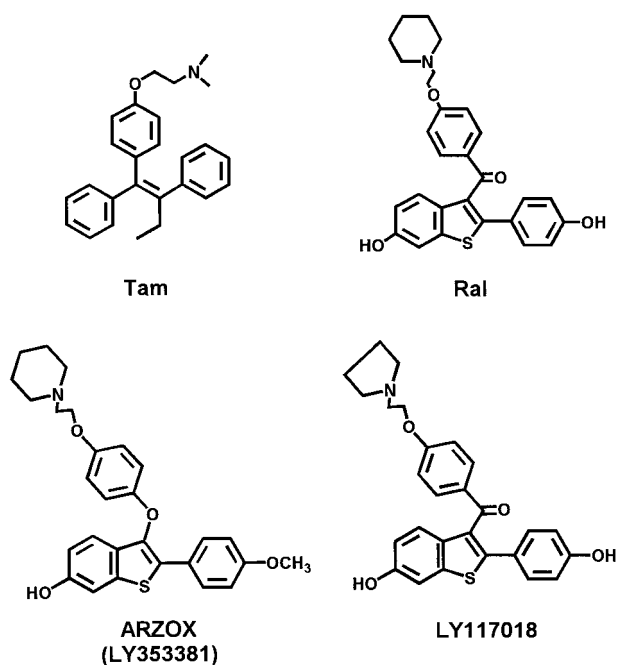


Fig. 1 Chemical structures of Tam, Ral, Arzox (LY353381), and LY117018.

could be used for >5 years and exhibit a better toxicological profile than Tam would provide great benefit as both a treatment and preventive agent.

Any new SERM would be directly compared with Tam and must not only be equally good but show a significant advantage over Tam. An agent under investigation would first be tested in a population of women with recurrent breast cancer that have failed Tam during adjuvant therapy; therefore, there is a high likelihood that these cancers would be resistant to Tam. Thus, any new agent should first be tested for cross-resistance to Tam in the laboratory. These data are extremely important, because they will be part of the clinical drug development decision-making paradigm that could influence the advancement of a drug for use as an adjuvant therapy in Tam-naïve women.

New SERMs are being sought to improve the efficacy of breast cancer treatment and decrease side effects. The benzothiophene antiestrogens were first described in 1980 (11). The compound LY117018 (Fig. 1) demonstrated significantly lower uterotrophic activity compared with Tam and, in fact, blocked increases in Tam-stimulated rat uterine weight (12, 13). A successor compound LY156758, known as keoxifene (Ref. 14; Fig. 1), has antitumor activity in laboratory models (15–17). The drug was not developed as a breast cancer therapy; the drug, renamed Ral, was subsequently developed clinically for the prevention (18) and treatment (19) of postmenopausal osteoporosis, with the additional effect of reducing the incidence of primary breast cancer (20). Although Ral is being evaluated as a preventive for breast cancer, it is not being promoted as a treatment for breast cancer. Bioavailability is too low at 2% of the administered dose (21) so there are concerns about effective continuous antitumor activity. As a result, the new agent, Arzox (22) has been designed to improve bioavailability and provide

sustained antiestrogenic blockade (Fig. 1) so that it can be used to treat breast cancer.

The athymic mouse model that is routinely used to predict the potential clinical success of a new antiestrogen in a breast cancer clinical trial, is the ER-positive MCF-7 tumor xenograft that grows in response to E_2 (23, 24). Tam blocks E_2 -stimulated growth (25). The model has also been used to study the development of drug resistance to antiestrogens (26–28).

MCF-7 cells express wild-type p53 (29), which represents only 50% of breast cancers (30). Therefore, for these studies, we also use a novel human breast tumor line derived from T47D cells (31), which express a mutant p53 that is nonfunctional (32). After 10 weeks of Tam treatment, T47D tumors became Tam-stimulated (T47D:Tam) and remained E_2 -responsive. This occurs at a faster rate than MCF-7 tumors, which suggests that the differences between these tumor lines directly impact the development of Tam-stimulated growth (31).

MATERIALS AND METHODS

Athymic Mouse Model. The MCF-7 and T47D tumors used in these parallel experiments were developed as described previously (31). Ovariectomized 4–5-week-old athymic mice (Harlan Sprague Dawley, Madison, WI) were given bilateral transplants s.c. in the axillary mammary fat pads with 1-mm³ pieces of MCF-7 or T47D tumor using a trocar. The Animal Care and Use Committee of Northwestern University approved all of the procedures involving animals.

Hormone and Drug Treatments. Mice were divided into groups of 10 and were treated with E_2 (Sigma Chemical Co., St. Louis, MO), antiestrogens, or combinations. Silastic E_2 capsules (0.3 cm or 1 cm in length) made as described previously (33) were implanted s.c. and were replaced every 8–10 weeks. The 0.3-cm E_2 capsules produced a mean 83.8 pg/ml of serum E_2 , whereas 1.0-cm E_2 capsules produced a mean 379.5 pg/ml of serum E_2 (34). Each was designed to represent the low or high E_2 levels observed in post- or premenopausal women, respectively. Tam (Sigma Chemical Co.), LY117018, or Arzox (generous gifts of Eli Lilly, Indianapolis, IN; Fig. 1) were first dissolved in ethanol and suspended in a solution of 90% carboxymethylcellulose (1% carboxymethylcellulose in double-distilled water) and 10% polyethylene glycol 400/Tween 80 (99.5% polyethylene glycol 400 and 0.5% Tween 80). Ethanol was evaporated under nitrogen before use. Tam, LY117018, or Arzox was administered p.o. by gavage at a dose of either 0.5 or 1.5 mg per mouse per day, 5 days/week unless otherwise indicated.

Tumor Measurements. Tumor measurements were performed weekly using Vernier calipers. The cross-sectional area was calculated using the formula: length \times width/4 \times π . Mean tumor cross-sectional area was determined by measuring tumors at all of the implantation sites.

Statistical Analysis. Comparisons in mean tumor cross-sectional area between the animal groups were analyzed by ANOVA at each week followed by unpaired Student's *t* test. The two-tailed *P* of the last week of each experiment was reported using StatMost 2.5 (Datamost Corp., Salt Lake City, UT).

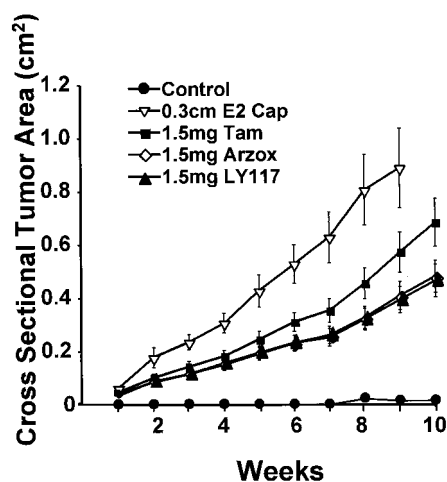


Fig. 2 Comparison of Tam-stimulated MCF-7: Tam tumor growth in response to Tam, or Arzox, or LY117018 treatment (mean \pm SE). Fifty ovariectomized athymic mice were bilaterally transplanted with MCF-7: Tam tumors (10/group) and were treated for 10 weeks with 0.3 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control.

RESULTS

We used the Tam-stimulated MCF-7 tumor model (MCF-7: Tam) to determine whether differences in tumor characteristics play a role in cross-resistance to Tam. The MCF-7: Tam tumors were developed as described previously (31). Fifty athymic mice bitransplanted with MCF-7: Tam tumors were treated as follows: 0.3 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control (Fig. 2). In addition to the above-described treatments, mice were treated with 1.5 mg Arzox+0.3 cm E₂ capsule or 1.5 mg LY117018+0.3 cm E₂ capsule, but these data were omitted from the figure for clarity. As expected, after 10 weeks, the E₂ and Tam groups grew maximally. The growth of all of the groups compared with control was significant ($P < 0.0001$). However, the growth of E₂ versus Tam was not significant ($P = 0.25$). The growth of the 1.5 mg Arzox ($P = 0.019$) and 1.5 mg LY117018 ($P = 0.016$) tumor groups were significantly less than that of the E₂ group. However, the growth of the 1.5 mg Arzox and LY117018 tumor groups compared with Tam, although less, only approached significance ($P = 0.06$). This demonstrates that Arzox and LY117018 are partially cross-resistant with Tam in the MCF-7 model. The growth of the 1.5 mg Arzox+E₂ and LY117018+E₂ tumor groups was not significantly different from the E₂ or Tam groups ($P > 0.09$; data not shown).

We compared these findings with the T47D breast cancer tumor model (31). Previously, we showed that the T47D: E₂ tumors grew robustly with pre- and postmenopausal levels of E₂, and that Tam was able to block this growth (31). We tested Arzox and LY117018 in this model. T47D: E₂ tumors were bitransplanted into 50 athymic mice and treated with 1 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control (Fig. 3). After 21 weeks, the E₂-treated tumors grew rapidly, whereas the LY117018 and control groups did not grow at all. Tam produced stimulated tumors compared with either 1.5 mg Arzox ($P = 0.004$) or of LY117018 ($P < 0.0001$). The

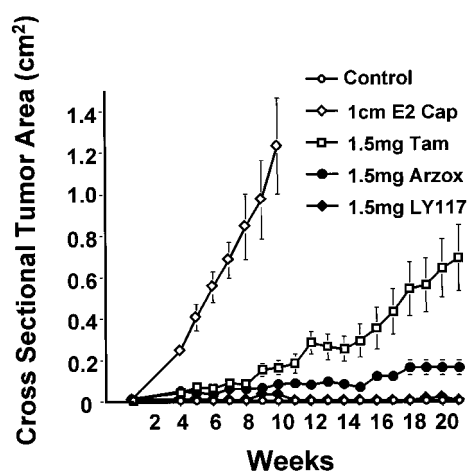


Fig. 3 Growth of T47D: E₂ tumors in ovariectomized athymic mice (mean \pm SE). Fifty mice (10/group) were bilaterally transplanted with T47D: E₂ tumors and were treated for 21 weeks with 1 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control.

Tam group began to grow as expected at about 15 weeks demonstrating the acquisition of Tam-stimulated growth. The Arzox group grew minimally with two tumors measuring ~ 0.5 cm²; these tumors were harvested and retransplanted but did not grow at all after 10 weeks of treatment (data not shown). This demonstrates that these tumors did not experience the phenotypic switch that the Tam-treated tumors experience and, therefore, did not become Arzox-stimulated.

We used a different experimental strategy in an attempt to produce a line of Arzox-stimulated tumors. We transplanted T47D: E₂ tumors and treated all of the mice with 1 cm E₂ capsules to establish a consistent tumor take. After 8 weeks, once the tumors had reached an average size of 0.3 cm², the 1 cm E₂ capsules were removed. The mice were subsequently treated for 4 weeks as follows: 1 cm E₂ capsule, 0.5 mg Tam, 1.5 mg Arzox, or control (Fig. 4). By the end of week 12, the E₂ group had grown significantly more ($P < 0.05$) than the 0.5 mg Tam group, 1.5 mg Arzox group, or control. Interestingly, both the 0.5 mg Tam and 1.5 mg Arzox treatments reduced tumor size compared with control, although Arzox was more effective. After an additional 10 weeks of treatment, the E₂ group grew maximally ($P < 0.001$) compared with 0.5 mg Tam group, 1.5 mg Arzox, and control which did not grow (Fig. 5). We have shown previously, that low-dose (0.5 mg) Tam does not result in the development of Tam-stimulated tumor growth (31), and 1.5 mg Tam daily is necessary to produce Tam-stimulated tumors (Fig. 3).

Cross-resistance of the benzothiofenenes and Tam was investigated using a Tam-stimulated T47D tumor line (T47D: Tam) whose growth is stimulated by both E₂ and Tam (1.5 mg daily; Ref. 31). The T47D: Tam tumors were bitransplanted and were treated with 1 cm E₂ capsule, 0.5 mg Tam, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control (Fig. 6). After 18 weeks, as expected, both the E₂ and the Tam groups grew maximally and were not significantly different from each other ($P > 0.05$) but were significantly different from control ($P < 0.001$), whereas the 0.5 mg Tam treatment group grew at about

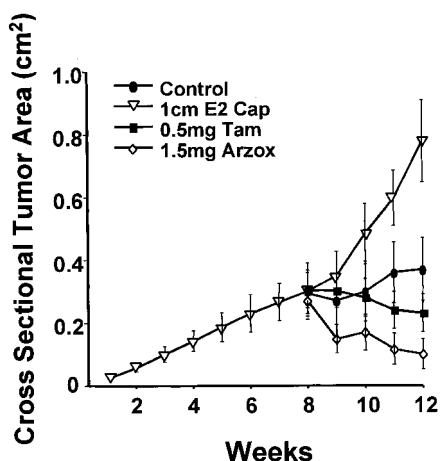


Fig. 4 Inability to develop Arzox-stimulated tumors in T47D:E₂ tumors in ovariectomized athymic mice (mean ± SE). Forty mice were bilaterally transplanted with T47D:E₂ tumors and were treated for 8 weeks with 1 cm E₂ capsule. Mice were then separated into groups of 10 and treated for an additional 4 weeks with 1 cm E₂ capsule, 0.5 mg Tam, 1.5 mg Arzox, or control.

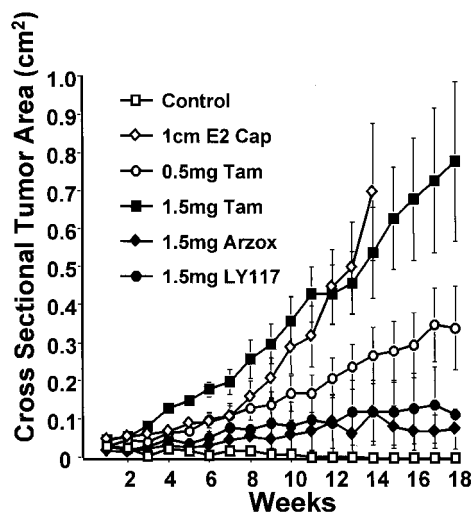


Fig. 6 Arzox and LY117018 do not stimulate growth in T47D:Tam tumor compared with E₂ and Tam in ovariectomized athymic mice (mean ± SE). T47D:Tam tumors were bitransplanted into 60 athymic mice and were treated as follows: 1 cm E₂ capsule, 0.5 mg Tam, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg LY117018, or control.

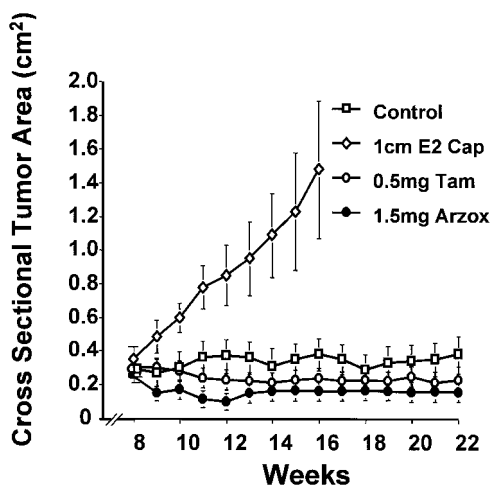


Fig. 5 Long-term attempt to develop Arzox-stimulated tumors in T47D:E₂ tumors in ovariectomized athymic mice (mean ± SE). Forty mice bearing T47D:E₂ tumors were treated with 1 cm E₂ capsule until the tumors reached an average size of 0.3 cm². At this point, mice were separated into groups of 10 and treated for an additional 16 weeks with 1 cm E₂ capsule, 0.5 mg Tam, 1.5 mg Arzox, or control.

50% of the 1.5 mg Tam rate. Neither the Arzox nor the LY117018 groups differed significantly from control ($P > 0.05$). This shows that both Arzox and LY117018 are not cross-resistant with Tam in the T47D:Tam tumor model. This is an important observation, but the real question is whether, when combined with E₂ in the T47D:Tam tumor model, Arzox and LY117018 continue to act as antiestrogens.

To address this question, we bitransplanted T47D:Tam tumors into athymic mice and treated as follows: 0.3 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg Arzox+0.3 cm E₂ capsule, 1.5 mg LY117018, 1.5 mg LY117018+0.3 cm E₂

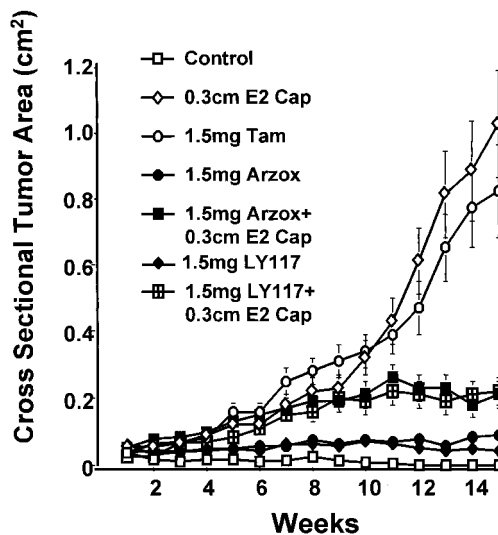


Fig. 7 Arzox and LY117018 are not cross-resistant to Tam in T47D:Tam tumors grown in ovariectomized athymic mice (mean ± SE). Seventy ovariectomized athymic mice (10/group) were bilaterally transplanted with T47D:Tam tumors and treated for 15 weeks with 0.3 cm E₂ capsule, 1.5 mg Tam, 1.5 mg Arzox, 1.5 mg Arzox+0.3 cm E₂ capsule, 1.5 mg LY117018, 1.5 mg LY117018+0.3 cm E₂ capsule, or control.

capsule, or control (Fig. 7). In this experiment, after 15 weeks, the growth of both postmenopausal E₂ and Tam was not significantly different ($P = 0.4$), whereas the Arzox and the LY117018 groups were similar to the control ($P > 0.05$). Interestingly, when combined with postmenopausal levels of E₂, both the Arzox and the LY117018 were able to significantly block growth compared with E₂ and Tam alone ($P < 0.01$); however, they did not completely block growth. This

can be explained in two ways. Firstly, Arzox and LY117018 at the doses tested are not capable of fully blocking E_2 -stimulated growth, *i.e.*, higher doses are required. Secondly, mice are fed Arzox and LY117018 *p.o.* once/day, 5 days/week, whereas the E_2 capsules are implanted resulting in dosing of postmenopausal E_2 24 h/day, 7 days/week. Because the related compound, Ral, is poorly bioavailable and is cleared rapidly (21), the blood levels of Arzox and LY117018 may drop below efficacious levels and are, therefore, unable to block the growth-stimulatory effects of E_2 .

To address the issue of continuous drug treatment, we examined the efficacy of 5 *versus* 7 days of Arzox and LY117018 combined with postmenopausal levels of E_2 in the T47D: E_2 model. We bitransplanted T47D: E_2 tumors into mice and treated them as follows: 0.3 cm E_2 capsule, 1.5 mg Tam (5 days)+0.3 cm E_2 capsule, 1.5 mg Arzox (5 days)+0.3 cm E_2 capsule, 1.5 mg Arzox (7 days)+0.3 cm E_2 capsule, 1.5 mg LY117018 (5 days)+0.3 cm E_2 capsule, 1.5 mg LY117018 (7 days)+0.3 cm E_2 capsule, or control (Fig. 8A). As expected, the E_2 group grew significantly more than all of the antiestrogen-treated groups ($P < 0.0001$).

LY117018 was less effective than Arzox at either 5 day ($P < 0.001$) or 7 day ($P < 0.0001$) dosing. Additionally, 5 day treatment with Arzox was not significantly different from 5 day treatment with Tam, and both were superior to 5 day treatment with LY117018. Treatment for 7 days with Arzox was more effective than that for 5 days ($P < 0.02$). Most importantly, 7 day treatment with Arzox completely blocks E_2 action to the level observed in the untreated control group. The comparison of the tumor areas after 12 weeks of antiestrogen treatment is shown in Fig. 8B.

DISCUSSION

The development of the T47D Tam-resistant breast cancer model has introduced a new dimension into the investigation of drug resistance to SERMs (31). We have found that the MCF-7 model and the T47D breast cancer model are distinctly different in their response to SERMs, and this may have important implications for the sequential use of these compounds clinically.

Arzox and LY117018 are partially cross-resistant with Tam in the MCF-7:Tam tumor model but not in the T47D:Tam tumor model. This is distinct from our findings with the triphenylethylene compound, idoxifene, which was cross-resistant to Tam in the MCF-7:Tam and T47D:Tam tumor models (35). This suggests that the differences in the estrogen-like properties and mechanism of action of SERMs in laboratory assays (36) is now demonstrated in the signal transduction pathways of two Tam-stimulated breast tumor models.

Possible differences in coregulator expression may play a role in dictating response of the tumors to different classes of antiestrogens. During the progression of MCF-7: E_2 tumors from Tam-sensitive to Tam-stimulated, which occurs after ~8 months of Tam treatment, changes occur under selection pressure that may result in the down-regulation of corepressor(s). [The acquisition of Tam-resistance in a mouse model of human breast cancer correlates with decreased levels of the corepressor N-CoR (37).] If this is the case, treatment with a different

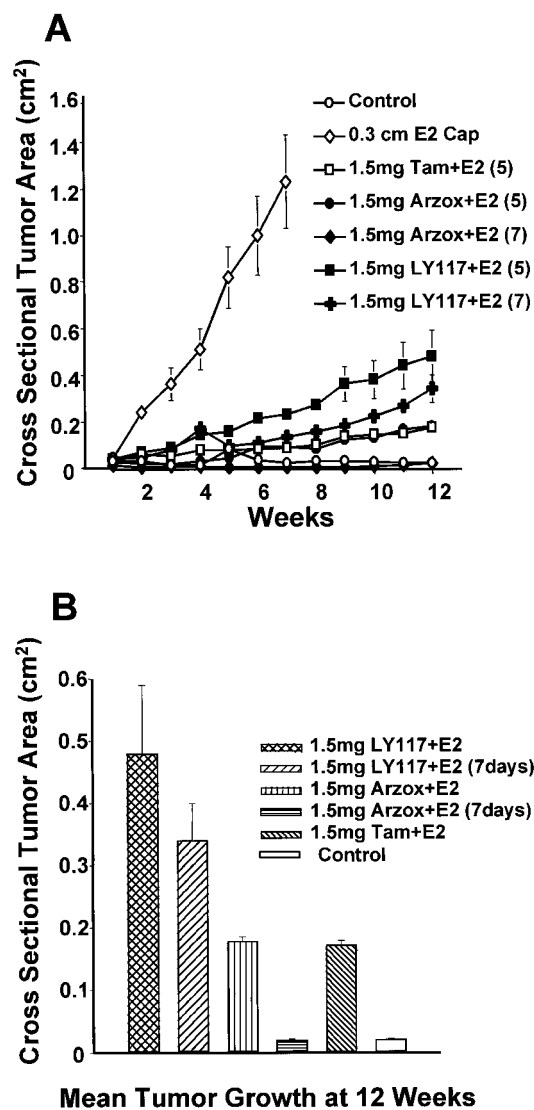


Fig. 8 Comparison of 5 and 7 day dosing of Arzox or LY117018 in T47D: E_2 tumors grown in ovariectomized athymic mice (mean \pm SE). A, seventy ovariectomized athymic mice (10/group) were bilaterally transplanted with T47D: E_2 tumors and treated for 12 weeks with 0.3 cm E_2 capsule, 1.5 mg Tam (5 day)+0.3 cm E_2 capsule, 1.5 mg Arzox (5 day)+0.3 cm E_2 capsule, 1.5 mg Arzox (7 day)+0.3 cm E_2 capsule, 1.5 mg LY117018 (5 day)+0.3 cm E_2 capsule, 1.5 mg LY117018 (7 day)+0.3 cm E_2 capsule, or control. B, mean tumor growth of mice treated with antiestrogens at week 12.

SERM such as Arzox would not be advantageous because the down-regulation of corepressor(s) has already occurred. However, there are mechanistic distinctions between different SERM:ER complexes (38, 39) that may result in the binding of different corepressors, which may be the reason cross-resistance does not necessarily occur.

In addition, these differences in SERM:ER complexes may also affect coactivator binding (40). The development of T47D:Tam tumors from T47D: E_2 tumors occurs very rapidly (31), after ~3 months. A different mechanism of resistance may evolve that results in an increase in coactivator(s) that leads to

Table 1 A classification of agents that either stimulate or block Tam-resistant tumor growth (MCF-7:Tam or T47D:Tam)

The tumor growth characteristics are compared with the SERM classification system based on the stimulation or blockade of the *TGF α* gene in the ER-negative MDA-MB-231 breast cancer cell line stably transfected with the wild-type ER cDNA (36). The numbers in parentheses are the references.

	MCF-7:Tam	T47D:Tam	TGF α assay
E ₂	Stimulate (26)	Stimulate (31)	Stimulate (52)
Tam-like	Stimulate (35)	Stimulate (35)	Stimulate (45, 53)
Ral-like	Stimulate (Fig. 2)	Block (Fig. 7)	Block (43)
Pure antiestrogen	Block (35, 54)	Block ^a	Block (44)

^a Unpublished observations.

Tam-stimulated growth. Because distinct SERM:ER complexes are formed, it is possible that different coactivators specifically activate transcription of specific complexes. For example, L7/SPA (the only antagonist-specific coactivator defined to date) (41) increases transcription of the Tam:ER complex but not of the pure antiestrogen ICI164,384:ER complex, which proves that this coactivator specifically up-regulates based on the ligand (42).

In previous studies, we have described an *in vitro* antiestrogen classification system that differentiates antiestrogens through the use of mutant ERs (43–45). These ERs are stably transfected into ER-negative MDA-MB-231 cells and contain mutations at amino acid 351: D351 (wild type), D351Y, and D351G. A TGF α assay system is used to classify antiestrogens as Tam-like, Ral-like, or pure antiestrogen. As shown in Table 1, there are differences between the growth responses of MCF-7:Tam tumors and T47D:Tam tumors to E₂ and antiestrogens. When TGF α is measured in the ER-expressing MDA-MB-231 cells, the response is identical to that of the T47D:Tam tumors. This is a significant observation because these models reflect the inherent differences between breast cancer cells that dictate responses to antiestrogens. At this point, the most likely candidates for future studies are coactivators and corepressors. Studies are in progress to determine the relative levels of known coregulators in our models *in vivo* and *in vitro*.

Many tumors that first respond to Tam will ultimately develop acquired Tam resistance (7). However, approximately two-thirds of the tumors that become resistant to Tam continue to express ER (46, 47); therefore, many of these respond to second-line endocrine therapies. Consequently, cross-resistance to Tam is an important issue in the development of novel SERMs for the treatment of breast cancer.

Tam is a prodrug being converted to the active metabolite 4-OHT, which blocks estrogen binding to the ER (48). Serum levels of 4-OHT during long-term adjuvant Tam (150 ng/ml) are ~2–5 ng/ml (49). Thus, the prolonged serum half-life of Tam (7 days; Ref. 50) and the stable levels of 4-OHT provide adequate blockade of the breast cancer ER in patients. Arzox is designed to improve bioavailability over the 2% observed with Ral (21). A recent Phase I clinical trial of Arzox in heavily treated postmenopausal breast cancer patients showed no objective responses, but Phase II studies are evaluating the efficacy if the drug in patients who failed prior Tam (51). Nevertheless, a

pharmacokinetic evaluation of Arzox, the parent drug, show steady-state levels between <1–20 ng/ml for daily dosing regimens between 10 and 100 mg Arzox. The levels of the desmethyl active metabolite of Arzox, LY335362, were below the level of detection (<0.05 ng/ml). Clearly, the polyphenolic nature of the benzothiophene antiestrogens results in rapid clearance and emphasize the need for strict compliance in high-estrogen environments.

The experimental doses of Arzox and LY117018 were selected to directly compare the ratios to the doses of Tam used in the clinic. Tam is used at 20 mg daily (1), whereas Ral is recommended for the treatment of osteoporosis at a dose of 60 mg daily (three times the Tam dose; Ref. 19). The increased metabolic stability and oral bioavailability of Arzox at the doses tested were illustrated when Arzox and LY117018 (a raloxifene analogue) were directly compared in the T47D:Tam model (Fig. 7). In this situation, 5 days of Arzox or LY117018 treatment did not stimulate growth but when combined with E₂ capsule, which released E₂ 7 days week, they were both unable to completely block E₂-stimulated growth. We found a difference between the two feeding schedules and profound differences between Arzox and LY117018 when 5 *versus* 7 days of treatment was compared (Fig. 8A). Arzox completely inhibited E₂-stimulated tumor growth on a 7 day treatment schedule, whereas both Arzox and LY117018 were much less effective on a 5 day treatment schedule. Tam is known to accumulate to high levels in patients (1). This is beneficial in the treatment of breast cancer to produce continuous ER blockade. Clearly, the issue of bioavailability and compliance will become very important when evaluating the test results of new, rapidly excreted SERMs. It is clear from these data that the low bioavailability of polyhydroxylated SERMs (21) will be reduced further by sporadic dosing schedules. Compliance will be crucial for maintaining the effectiveness of Arzox in premenopausal breast cancer patients or if Arzox is evaluated as a preventative in premenopausal women.

In conclusion, the primary goal of the study was to evaluate the SERMs Arzox and LY117018 in E₂- and Tam-stimulated breast cancer models to determine whether they are cross-resistant with Tam. These findings demonstrate that the differences in tumor characteristics between breast cancers can dictate the effectiveness of antiestrogen therapies and reflect the putative differences in the mechanisms of actions of SERMs. Because Arzox is not cross-resistant in the T47D:Tam tumor model but is cross-resistant in the MCF-7: Tam tumor model, this SERM may be effective in some patients who have failed Tam therapy. However, the disparate actions of Arzox in the two models suggests that sequential treatment with Arzox after Tam may not provide an optimal treatment option. Nevertheless, these studies suggest that clinical studies should be pursued not only in advanced breast cancer but also in adjuvant therapy. The observation that Arzox appears to be less susceptible to the development of drug resistance compared with Tam indicates that longer adjuvant treatment schedules may be possible.

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