# Effects of the Antiestrogens Tamoxifen, Toremifene, and ICI 182,780 on Endometrial Cancer Growth

*Ruth M. O'Regan, Angela Cisneros, Gale M. England, Jennifer I. MacGregor, Henry D. Muenzner, Vasilios J. Assikis, Malcolm M. Bilimoria, Michael Piette, Yvonne P. Dragan, Henry C. Pitot, Robert Chatterton, V. Craig Jordan*

***Background:*** **Tamoxifen has been shown to promote the growth of human endometrial tumors implanted in athymic mice, and it has been associated with a twofold to threefold increase in endometrial cancer. Toremifene, a chlorinated derivative of tamoxifen, and ICI 182,780, a pure antiestrogen, are two new antiestrogens being developed for the treatment of breast cancer. The effects of these drugs on endometrial cancer are currently unknown. Our objective was to evaluate the effects of toremifene and ICI 182,780 on the growth of human endometrial cancer in athymic mice.** ***Methods:*** **Athymic, ovariectomized mice were implanted with human endometrial tumors and treated with estrogen, tamoxifen, or the new antiestrogens.** ***Results:*** **The effects of tamoxifen and toremifene on the growth of either tamoxifen-stimulated or tamoxifen-naive endometrial tumors in athymic mice were not substantially different. ICI 182,780 inhibited the growth of tamoxifen-stimulated endometrial cancer, in both the presence and the absence of estrogen.** ***Conclusions:*** **Toremifene and tamoxifen produce identical effects in our endometrial cancer models. Therefore, it is possible that toremifene, like tamoxifen, may be associated with an increased incidence of endometrial cancer. In contrast, ICI 182,780 inhibited tamoxifen-stimulated endometrial cancer, both in the presence and in the absence of estrogen, suggesting that this drug may be safe with regard to the endometrium, even if it is used following tamoxifen, and that it may not result in an increased incidence of endometrial cancer. Indeed, it is even possible that ICI 182,780 may prove useful as an adjuvant agent in early stage endometrial cancer. [J Natl Cancer Inst 1998;90:1552–8]**

In 1988, we demonstrated that the antiestrogen tamoxifen exhibited target site-specific actions in breast and endometrial cancers *(1)*. Athymic mice were co-transplanted with the estrogen-responsive breast tumor, MCF-7, and the estrogen receptor (ER)-positive endometrial carcinoma, EnCa101. Treatment with estradiol and tamoxifen demonstrated that the antiestrogen completely inhibited the estrogen-stimulated growth of the breast tumor but stimulated growth of the endometrial carcinoma *(1)*. From these observations, we concluded that women who were being treated with long-term adjuvant tamoxifen therapy should be screened for pre-existing endometrial carcinoma, which is known to be present in five times as many women as is detected clinically *(2)*. Although tamoxifen had proven benefits in breast cancer at that time *(3)*, we suggested that pre-existing endometrial cancer would not be controlled *(1)*. Our finding of the target strated in patients. Since the original clinical report by Fornander et al. *(4)* in 1989 showing that tamoxifen significantly decreased the incidence of contralateral breast cancer but increased the incidence of endometrial cancer, the topic of the association between tamoxifen and endometrial carcinoma has been a subject of intense investigation and some controversy. Recently, we surveyed the world literature to determine the extent of the problem and to survey gynecologic recommendations based on current knowledge *(5)*. It is clear that tamoxifen causes a twofold to threefold increase in the incidence of endometrial cancer *(5)*. This increase translates to about two to three cases per thousand postmenopausal patients per annum. The disease is the same stage and grade as endometrial cancer in the general population *(5)*. As a result of the rarity of detection, no special gynecologic monitoring, other than routine annual checkups and the follow-up of suspicious spotting and bleeding, has been recommended *(6)*. Indeed, the International Agency for Research on Cancer (IARC), an agency of the World Health Organization, recently stated that no patient should stop taking tamoxifen because of concerns about the risk of endometrial cancer and that the benefits of tamoxifen use far outweigh any risks *(7)*.

Concerns about the uterine safety of tamoxifen have naturally provoked a search for agents that might control the growth of both breast and endometrial carcinomas. Toremifene (Fig. 1), a chlorinated derivative of tamoxifen, has shown promise in the treatment of advanced breast cancer in postmenopausal women *(8–10)*. The drug has been evaluated at numerous doses, ranging from 60 mg daily to 260 mg daily in postmenopausal women, and the general consensus is that responses, particularly in ER-positive breast cancer, are equivalent to those seen with tamoxifen at doses of 20 or 40 mg daily in postmenopausal women *(11)*. Based on its clinical and toxicologic profiles, toremifene at a dose of 60 mg daily has been approved by the U.S. Food and Drug Administration for the treatment of advanced breast cancer in postmenopausal women.

ICI 182,780 (Fig. 1) is an example of a pure antiestrogen, which, like tamoxifen, acts through the ER but has no demonstrated estrogen agonist effects. ICI 182,780 inhibits tamoxifen-

*Affiliations of authors:* R. M. O'Regan (Division of Hematology/Oncology), A. Cisneros, J. I. MacGregor, H. D. Muenzner, V. J. Assikis, M. Piette, V. C. Jordan (Robert H. Lurie Comprehensive Cancer Center), G. M. England, M. M. Bilimoria (Department of Surgery), R. Chatterton (Department of Obstetrics and Gynecology), Northwestern University, Chicago, IL; Y. P. Dragan, H. C. Pitot, McArdle Laboratory, University of Wisconsin, Madison.

*Correspondence to:* V. Craig Jordan, Ph.D., D.Sc., Robert H. Lurie Comprehensive Cancer Center and Northwestern University Medical School, 8258 Olson, 303, E. Chicago Ave., Chicago, IL 60611.

*See* ''Notes'' following ''References.''

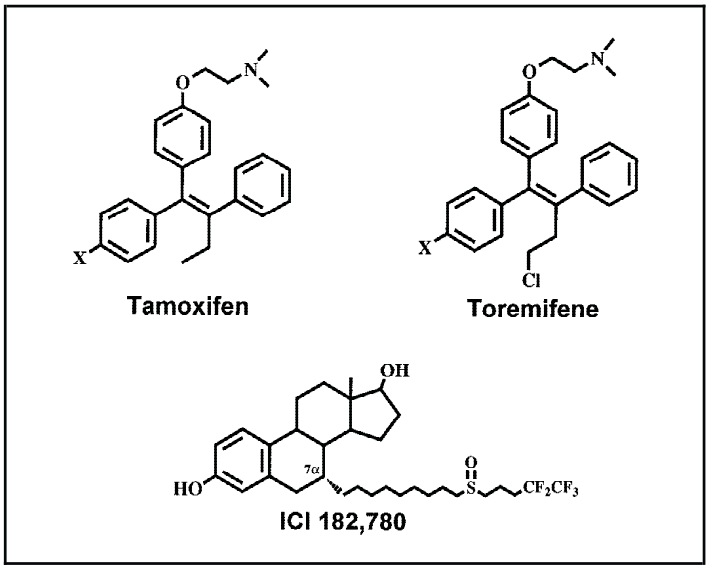


**Fig. 1.** Structures of antiestrogens—tamoxifen, toremifene, and ICI 182,780—listed in text. Toremifene and tamoxifen differ only in the presence of a chloride group. The methyl groups are designated by two open lines on nitrogen atoms in tamoxifen and toremifene. The position −4 is marked by (−X) in these structures, where hydroxyl groups are introduced during metabolism.

stimulated breast cancer growth in mice *(12).* Clinically, it must be given by depot intramuscular injection because of low oral potency. ICI 182,780 has shown promising results clinically in Europe, with high response rates of almost 70% in tamoxifen-failed, advanced breast cancer *(13),* and a large randomized, international clinical trial is under way.

However, the endometrial safety of toremifene and ICI 182,780 has not been examined satisfactorily, primarily because endometrial cancer is a rare event. Moreover, there are not the same stringent requirements for a drug that is used as a palliative therapy in advanced disease compared with drugs that are used for long-term adjuvant therapy.

As a result of claims that toremifene is safer than tamoxifen because it does not produce liver tumors in rats *(14,15),* we have used the endometrial cancer model, which was so instructive for tamoxifen, to provide information about the potential uterine safety of toremifene. Our aim was to replicate the situation seen with two clinical scenarios: 1) where toremifene will be used as first-line adjuvant therapy and 2) where toremifene will be used after adjuvant tamoxifen therapy. In addition, we have compared and contrasted the effects of tamoxifen with those of ICI 182,780 on the growth of tamoxifen-stimulated endometrial carcinomas. Clearly, an evaluation of uterine safety is important to reassure patients in clinical trials.

## Materials and Methods

### Athymic Mouse Model

Six-week-old athymic, ovariectomized mice were implanted with endometrial EnCa101 carcinomas *(1).* These tumors originated from an ER-positive, well-differentiated human endometrial tumor *(16);* they have been serially passaged in mice treated with tamoxifen and grow in response to tamoxifen (0.5 mg per animal per day) and estrogen (1-cm estrogen capsule per animal given every 6 weeks) (tamoxifen-stimulated/estrogen-responsive model). A second model (tamoxifen-naive/estrogen-responsive) was developed by passaging the tamoxifen-stimulated (0.5 mg per animal per day) endometrial EnCa101 tumors in mice and had not been exposed to tamoxifen for at least three passages. These estrogen-stimulated tumors are more responsive to estrogen for growth. Pieces of tumor (1 $mm^3$) were implanted bilaterally with a trochar into the mammary fat pads.

The mice were divided into groups of five or 10 and were treated with estrogen, antiestrogens, or the vehicle. Silastic estradiol capsules were made as described previously *(17),* implanted subcutaneously, and replaced after 6–8 weeks of treatment. Estrogen capsules were either 1 cm or 0.3 cm in length.

Tamoxifen and toremifene were each suspended in a solution of 90% CMC (1% carboxymethylcellulose in double-distilled water) and 10% PEG 400/Tween 80 (99.5% polyethyleneglygol 400 and 0.5% Tween 80). Tamoxifen was administered orally, i.e., by mouth, at a dose of 0.5 mg per mouse daily for 5 days each week. Toremifene was administered orally at a dose of 0.5, 1.5, or 5 mg per animal. ICI 182,780 was dissolved in ethanol and administered in peanut oil (following the evaporation of ethanol under $N_2$) to a final concentration of 50 mg/mL. ICI 182,780 was injected subcutaneously at a dose of 5 mg (0.1 mL peanut oil) per animal each week.

The tumors were measured weekly with calipers. The cross-sectional area was determined by use of the following formula: length × breadth/4 × π.

All procedures involving animals were approved by the Animal Care and Use Committee of Northwestern University.

### Quantitation of Antiestrogens

The mice were killed, their livers, hearts, and uteri were harvested, and serum was obtained by decapitation. Serum samples (150 μL) were deproteinated with equal volumes of 100% acetonitrile, followed by centrifugation (Model J2 HC; Beckman Instruments, Westbury, NY) at 21 200*g* for 5 minutes at 0 °C. Supernatant layers were transferred to vials. Samples were stored at −80 °C.

Tissue samples (15 mg) were homogenized in 2% acetic acid in methanol (vol/vol) and centrifuged at 502*g* for 10 minutes at room temperature, and the supernatant layer was transferred to a glass tube and dried under $N_2$ at 37 °C. The precipitates were re-extracted with 100% acetone and centrifuged at 502*g* for 10 minutes at room temperature, and the organic layer was combined with the methanolic extract and then redried. Dried samples were reconstituted in their respective mobile phases for the high-performance liquid chromatography (HPLC) assay (Waters Corporation, Milford, MA) of toremifene and tamoxifen *(18).* Samples were derivatized after separation by an in-column in-line photochemical reaction, and the highly fluorescent phenathrene derivatives were quantified by fluorescence detection. Toremifene and metabolites were separated by using the Prodigy 5-ODS3 column (Phenomenex, Torrance, CA) (0.1% diethylamine [DEA] [Fisher Chemicals, Fairlawn, NJ] in 57% acetonitrile [HPLC grade; Fisher Chemicals] in $H_2O$ for 15 minutes and 0.1% DEA in 76% acetronile in $H_2O$ at 0.1 mL/minute for 40 minutes) *(19).* Tamoxifen and metabolites were separated by column switching to a coupled analytical column (Rexchrom 5 μ-CN; Regis Chemicals, Morton Grove, IL) and eluted by reversed phase ion exchange in 34% acetontrile and 66% of 20 μ*M* potassium dibasic phosphate (HPLC grade; J. T. Baker, Phillipsburg, NJ) (pH 3.1) at 1.2 mL/minute. Both assays were conducted on Hitachi HPLC systems (Hitachi Instruments, Inc., San Jose, CA) *(20).*

### Quantitation of Estrogen

Estradiol levels were assayed in mouse serum by use of a time-resolved immunofluorescence procedure (Delphia assay; Wallac, Gaithersburg, MD). Mouse serum gives responses parallel to those of the reference preparation up to a concentration of 1300 pg/mL; thereafter, the serum responses are blunted. The intra-assay coefficient of variation was 5.2%. All samples were measured in a single assay.

### Statistical Methods

Differences in the mean tumor area between the treatment and control groups were measured by analysis of variance followed by unpaired Student's *t* test, performed at the last week of each experiment. Significance is reported as two-sided *P* values.

## Results

Preliminary data demonstrated that parent toremifene levels are low at 24 hours (Table 1), and we have observed that 4-

**Table 1.** Levels of toremifene in tissues from mice killed 6 or 24 hours after final dosing*

| Tissue | Time, h | |
|---|---|---|
| | 6 | 24 |
| Serum, ng/mL | 795 ± 359† | 58 ± 39† |
| Tumor, μg/g | 11.1 ± 2.1† | 2.3 ± 1.0† |
| Heart, μg/g | 6.1 ± 3.3† | <1 |
| Liver, μg/g | 13.0 ± 8.0† | <1 |

*Athymic mice (n = 10) were treated with toremifene at 1.5 mg (60 mg/kg) daily for 9 weeks. Levels of toremifene were measured 6 and 24 hours after final dosing (five mice per time point).

†Values = mean ± standard deviation.

(mean serum levels ± standard deviation for 4-hydroxy derivative at 6 hours = 2879 ± 1647 ng/mL) in athymic mice (data not shown).

To characterize the relative metabolism of tamoxifen and toremifene, we performed an experiment in which athymic, ovariectomized mice without tumors were treated with tamoxifen at a dose of 0.5 mg or 1.5 mg daily or toremifene at a dose of 0.5 mg or 1.5 mg daily for 3 weeks. Based on our preliminary data, drug levels in serum were measured 4 or 8 hours after final dosing. At both doses, serum levels of toremifene were higher than those of tamoxifen, and the major route of metabolism for both drugs (particularly toremifene) in mice appears to be 4-hydroxylation (Table 2). Results for both the 0.5-mg and the 1.5-mg doses are shown in Table 2.

At the 0.5-mg doses, parent toremifene levels were significantly higher at 4 hours ($P = .02$) but not at 8 hours ($P = .25$), compared with parent tamoxifen levels. Levels of the 4-hydroxy metabolite were significantly higher for toremifene than for tamoxifen at 4 hours ($P = .002$) and at 8 hours ($P = .001$). There was no significant difference in levels of *N*-desmethyl metabolites between tamoxifen and toremifene at 4 hours ($P = .17$) and at 8 hours ($P = .12$).

At the 1.5-mg dose, there was no significant difference between parent levels of tamoxifen and toremifene at 4 hours ($P = .27$) and at 8 hours ($P = .8$). Levels of the 4-hydroxytoremifene were significantly higher at 4 hours ($P = .02$) but not at 8 hours ($P = .15$), compared with those of tamoxifen. *P* values were calculated by analysis of variance followed by unpaired Student's *t* tests.

**Table 2.** Levels of tamoxifen, toremifene, and their metabolites in serum of mice 4 and 8 hours after final dosing*

| Drug and metabolite | Time after dose | | | |
|---|---|---|---|---|
| | 4 h | | 8 h | |
| | 0.5 mg daily† | 1.5 mg daily† | 0.5 mg daily† | 1.5 mg daily† |
| Tamoxifen, ng/mL | | | | |
| Tamoxifen | 50 ± 27 | 208 ± 81 | 58 ± 7 | 203 ± 100 |
| *N*-Desmethyltamoxifen | 60 ± 33 | 249 ± 73 | 36 ± 36 | 431 ± 107 |
| 4-Hydroxytamoxifen | 58 ± 34 | 198 ± 54 | 11 ± 19 | 236 ± 70 |
| Toremifene, ng/mL | | | | |
| Toremifene | 135 ± 77 | 302 ± 192 | 80 ± 49 | 181 ± 151 |
| *N*-Desmethyltoremifene | 106 ± 80 | 207 ± 120 | 83 ± 68 | 161 ± 100 |
| 4-Hydroxytoremifene | 566 ± 301 | 1117 ± 781 | 331 ± 174 | 708 ± 489 |

*Athymic mice (n = 10) were treated with tamoxifen or toremifene at 0.5 mg or 1.5 mg per animal (20 mg/kg or 60 mg/kg, respectively) daily for 3 weeks. Serum levels of parent drug and metabolites were measured 4 and 8 hours after final dosing.

To confirm that the 1-cm and 0.3-cm estrogen capsules resulted in levels of estradiol approximating premenopausal and postmenopausal levels, we performed a separate experiment in which athymic, ovariectomized mice without tumors were untreated or were implanted with 1-cm or 0.3-cm estradiol capsules for 2 weeks (Fig. 2). Mean estradiol levels ± standard errors were 379.5 ± 101.2 pg/mL and 83.8 ± 34.6 pg/mL for the 1-cm and 0.3-cm capsules, respectively (Fig. 2). The 1-cm capsule produces serum estradiol levels approximating those in premenopausal women, which vary throughout the menstrual cycle, between 150 pg/mL and 350 pg/mL *(21)*. The 0.3-cm capsule results in levels similar to those in postmenopausal women, in whom the majority of circulating estrogen is in the form of estrone, which is secreted at an average of 35 μg/day to 40 μg/day *(22)*. Although these levels are much higher than physiologic estrogen levels in mice, we wanted to provide levels similar to levels in premenopausal and postmenopausal women because the tumors implanted were of human origin.

For the evaluation of the impact of estradiol and toremifene on the growth of a tamoxifen-stimulated endometrial tumor, mice were treated with vehicle, with estrogen (1-cm capsule), with tamoxifen at a dose of 0.5 mg daily, or with toremifene at a dose of 0.5 mg, 1.5 mg, or 5 mg daily. A broad range of toremifene doses was used to cover the range used clinically relative to tamoxifen, i.e., three to 10 times the dose of tamoxifen.

There was no significant difference between tamoxifen and toremifene (at all three doses) on tumor growth at 9 weeks ($P = .438$) (Fig. 3). Both antiestrogens stimulated tumor growth compared with that in the untreated animals ($P<.05$) but to a lesser extent than estrogen ($P = .02$) (Fig. 3).

We had observed that toremifene produces higher serum levels than tamoxifen in mice that had not been implanted with tumors (Table 2). It was, therefore, possible that lower serum

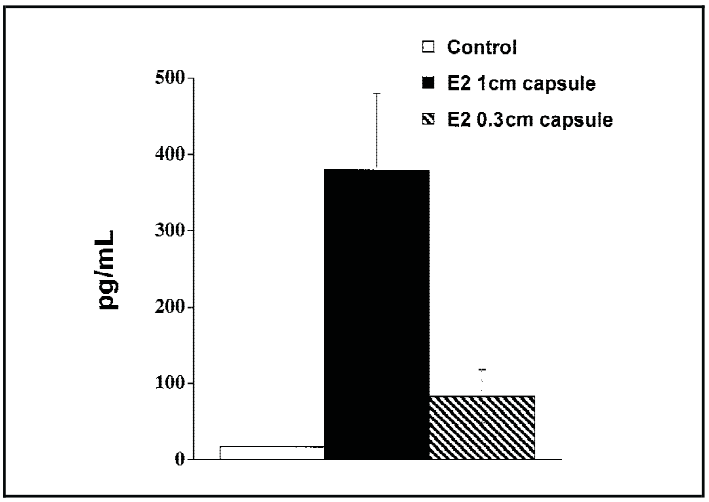


**Fig. 2.** Serum estrogen levels were measured for different capsule sizes. The estrogen level (mean ± standard error) for the 1-cm and 0.3-cm estrogen (E2) capsules were 379.5 ± 101.2 pg/mL and 83.8 ± 34.6 pg/mL, respectively, after implantation of the capsules for 2 weeks in athymic, ovariectomized mice (n =

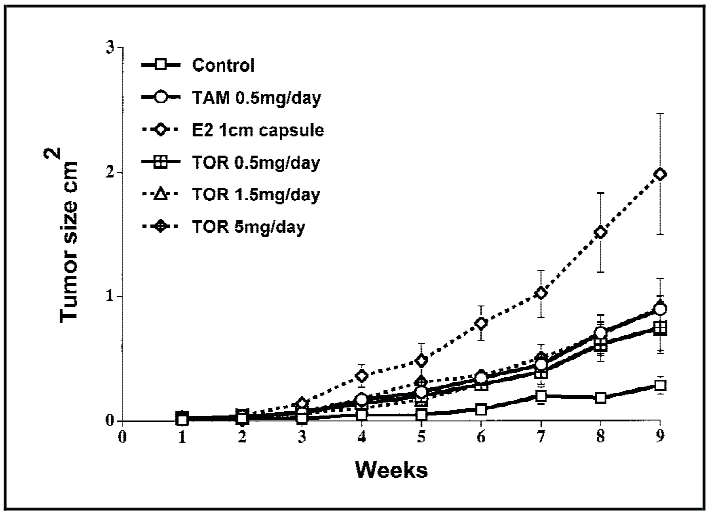


**Fig. 3.** Athymic, ovariectomized mice were divided into groups of 10, implanted with tamoxifen-stimulated endometrial tumors (1-mm$^2$ tumor piece [one piece] per mammary fat pad per animal), and treated with estrogen (E2) (1-cm capsule), tamoxifen (TAM) at a dose of 0.5 mg (20 mg/kg) daily, or toremifene (TOR) at a dose of 0.5 mg, 1.5 mg, or 5 mg daily (20 mg/kg, 60 mg/kg, or 200 mg/kg, respectively) or untreated (control). Tamoxifen and toremifene at all three doses significantly stimulated tumor growth ($P<.05$), although to a lesser extent than estrogen ($P = .02$), compared with control. There was no significant difference between tamoxifen and toremifene at all three doses ($P = .438$). The results are expressed as means ± standard error. The results were analyzed by analysis of variance test followed by unpaired two-sided Student's *t* test.

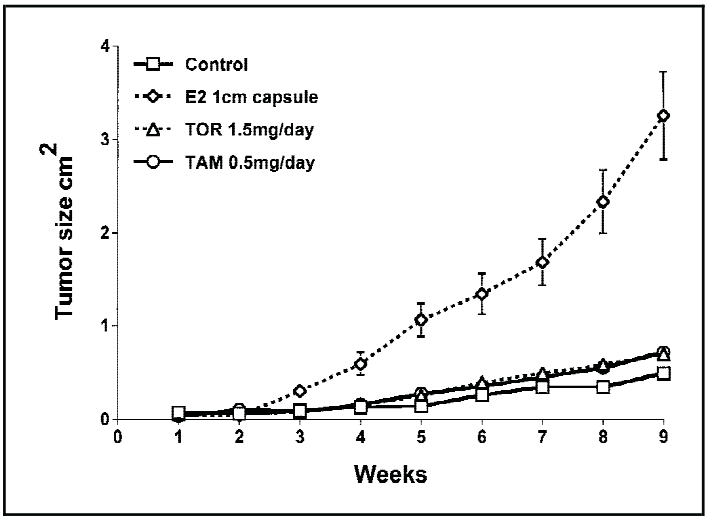


**Fig. 4.** Athymic, ovariectomized mice were divided into groups of 10, implanted with tamoxifen-naive/estrogen-responsive endometrial tumors (1-mm$^2$ tumor piece [one piece] per mammary fat pad per animal) and were treated with estrogen (E2) (1-cm capsule), tamoxifen (TAM) at a dose of 0.5 mg (20 mg/kg) daily, or toremifene (TOR) at a dose of 1.5 mg (60 mg/kg) daily or untreated (control). Neither tamoxifen nor toremifene significantly stimulated tumor growth compared with control (two-sided $P = .09$ for tamoxifen; two-sided $P = .06$ for toremifene). The results are expressed as means ± standard error. Error bars are not shown for the toremifene, tamoxifen, and control groups because the values were too low. The results were analyzed by analysis of variance test followed by unpaired two-sided Student's *t* test.

levels of toremifene may be associated with less tumor growth than lower serum levels of tamoxifen. To examine this possibility further, we performed an experiment in which athymic mice were implanted with tamoxifen-stimulated/estrogen-responsive endometrial tumors and treated daily with tamoxifen at a dose of either 0.5 mg or 1.5 mg. The tumor area was measured weekly, and serum levels of tamoxifen and metabolites were assayed 4 hours after the last dosing (Table 3). We were surprised to observe that the 1.5-mg dose resulted in less tumor growth than the 0.5-mg dose, despite higher serum levels (Table 3).

To evaluate the action of tamoxifen or toremifene on the growth of tamoxifen-naive/estrogen-responsive endometrial tumors, we treated the mice with vehicle, with estrogen (1-cm capsule), with tamoxifen (0.5 mg daily), or with toremifene (1.5 mg daily). A ratio of 1 : 3 of tamoxifen to toremifene was chosen because clinical trials have demonstrated that 60 mg of toremifene is equivalent in efficacy to 20 mg of tamoxifen *(9)*. There was no significant difference in tumor growth between tamoxifen and toremifene after 9 weeks of treatment ($P = .833$) (Fig. 4). Estrogen significantly stimulated tumor growth compared with control ($P = .0002$); however, in contrast to the tamoxifen-stimulated/estrogen-responsive model, neither antiestrogen significantly stimulated tumor growth compared with control ($P = .09$ for tamoxifen; $P = .06$ for toremifene) (Fig. 4).

Finally, mice (five per group) were implanted with tamoxifen-stimulated/estrogen-responsive endometrial tumors. The mice were treated with vehicle, with postmenopausal levels of estrogen (provided by a 0.3-cm estrogen capsule), or with ICI 182,780 at a dose of 5 mg weekly, with and without estrogen (0.3-cm capsule). As can be seen in Fig. 5, estrogen stimulated tumor growth compared with control at 10 weeks. However, ICI 182,780 inhibited tumor growth in the presence of estrogen compared with control (Fig. 5), and ICI 182,780 when given alone did not stimulate tumor growth.

## Discussion

Tamoxifen is an effective therapy approved for all stages of breast cancer. Toremifene, or chlorotamoxifen, shows efficacy in the treatment of endocrine therapy-naive, postmenopausal patients with advanced disease *(9);* however, it demonstrates cross-resistance with tamoxifen, even when high doses (as high as 10 times the dose of tamoxifen) are administered *(23)*.

**Table 3.** Tumor growth and serum levels of tamoxifen and metabolites in mice receiving 0.5-mg or 1.5-mg doses per animal per day for 7 weeks*

| | | Tamoxifen and metabolite† | | |
|---|---|---|---|---|
| Dose, mg | Tumor area, cm$^2$† | Tamoxifen, ng/mL | *N*-Desmethyltamoxifen, ng/mL | 4-Hydroxytamoxifen, ng/mL |
| 0.5 | 1.1 ± 0.9 | 50.9 ± 23 | 27.6 ± 13.8 | 64.4 ± 50.4 |
| 1.5 | 0.6 ± 0.7 | 334.3 ± 60.7 | 337.7 ± 74.9 | 477.8 ± 127.9 |

*Athymic mice (n = 10) were treated with tamoxifen at a dose of 0.5 mg per animal (20 mg/kg) or 1.5 mg per animal (60 mg/kg) daily. The tumor area at 7 weeks and levels of tamoxifen and metabolites, 4 hours after final dosing, are shown.

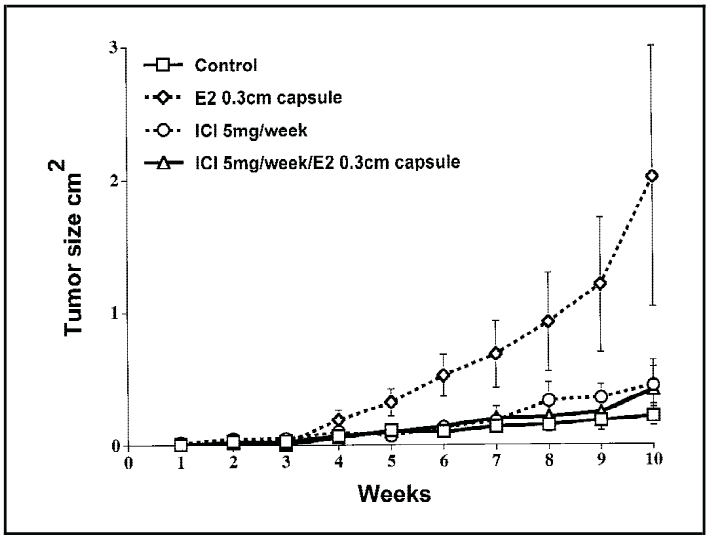


**Fig. 5.** Athymic, ovariectomized mice were divided into groups of five, implanted with tamoxifen-stimulated endometrial tumors, and treated with estrogen (E2) (0.3-cm capsule) or ICI 182,780 (ICI) at a dose of 5 mg (200 mg/kg) given as a single subcutaneous dose once weekly, with or without estrogen (0.3-cm capsule) or untreated (control). ICI 182,780 inhibited tumor growth both in the presence (two-sided $P = .10$) and in the absence (two-sided $P = .17$) of estrogen, although this did not reach statistical significance because of small numbers of animals per group. The results were analyzed by analysis of variance test followed by two-sided unpaired Student's $t$ test.

The major route of metabolism for both antiestrogens (tamoxifen and toremifene) in the mouse appears to be 4-hydroxylation. It is interesting that tamoxifen seems to be cleared more rapidly than toremifene in the mouse, resulting in the lower tissue and serum levels seen in mice with and without tumors.

The question we wanted to address was whether toremifene and ICI 182,780 can support the growth of EnCa101 endometrial cancer in athymic mice. This model was used previously to justify clinical studies to detect an association between tamoxifen and endometrial carcinoma in patients receiving adjuvant therapy; therefore, the current evaluation is important at the outset of exposure of patients to any new agent because endometrial cancer is so rare and, as has been noted with tamoxifen, only rigorous studies can detect even a modest association. Indeed, numerous early studies from the U.K. *(24,25)* showed no association between tamoxifen and endometrial cancer; therefore, well-designed preclinical studies are essential to avoid patients being inadvertently uninformed of the risks. Clearly, a woman should not be led to believe that no risks exist because inadequate and early clinical studies are being reported.

In our tamoxifen-naive endometrial cancer model, which simulates the antiestrogen-naive woman, neither tamoxifen nor toremifene significantly stimulated tumor growth compared with control. We used the higher dose of toremifene, since the recommended dose is three times that of tamoxifen in the treatment of breast cancer and we had observed that higher doses of tamoxifen, paradoxically, result in less tumor growth (Table 3). Our results suggest that, in women who have not been treated with tamoxifen, either antiestrogen would be safe, at least initially, even if she has pre-existent endometrial cancer. It is likely that tumor growth would occur eventually because of clonal

Tamoxifen and toremifene clearly have cross-resistance for EnCa101 growth. We chose a broad range of oral doses of toremifene to ensure that the large doses that have been used clinically were not, in fact, inhibitory for endometrial cancer *(9,26)*. Equivalent, three times, and 10 times the daily dose of tamoxifen all supported the growth of the EnCa101 tumors; however, in all cases, growth was not as rapid as that observed with estradiol. Clearly, the known estrogen-like properties of toremifene *(27)* in animals translate to estrogen-like effects to support the accelerated growth of pre-existing endometrial cancer.

Much has been made of a potential link between DNA adduct formation and the carcinogenesis of high doses of tamoxifen in rat liver and the potential for carcinogenesis in humans *(28–30)*. Toremifene has not been demonstrated to form DNA adducts in rat liver *(14)*, and it was thought, therefore, that it would be less likely than tamoxifen to result in an increased risk for endometrial cancer. Our data suggest that this theory is not the case and that toremifene stimulates endometrial tumor growth in athymic mice to the same extent as tamoxifen.

However, there is little evidence for a link between liver tumorigenesis in rats and endometrial cancer in women with tamoxifen. First, extensive investigations of human metabolism and adduct formation have demonstrated that there are fundamental differences between rats and humans *(31)*. Second, studies of DNA adduct formation with tamoxifen in human liver *(32)* and human uterus *(33)* have been negative, although an intriguing study from Scandinavia *(34)* suggests uterine adduct formation during tamoxifen therapy. Obviously, on the face of it, this theory would seem to be of concern, but it is inconsistent with the known genesis of human cancer. If the DNA adduct hypothesis is correct, endometrial cancer would be predicted to occur after several years of tamoxifen exposure. Initiation and promotion of human cancer may require even a decade. However, this is inconsistent with the facts. Nearly all tamoxifen-associated endometrial cancers occur within the first 5 years of exposure, and half of them are detected after fewer than 2 years of treatment. We have suggested that this is consistent with the activation and detection of pre-existing disease *(5)*. The model would be that estrogen-induced endometrial cancer undergoes clonal selection during tamoxifen or toremifene treatment and is subsequently detected on follow-up of gynecologic symptoms. In addition, a recent report *(35)* noted similar chromosome changes and gene rearrangements in tamoxifen-associated and control polyps. If tamoxifen is a carcinogen and if endometrial hyperplasia and polyps are part of a stepwise process resulting in cancer, tamoxifen-associated polyps should have genomic abnormalities different from those of polyps occurring in patients not receiving tamoxifen *(35)*. Our data suggest that any woman exposed to tamoxifen, who had a pre-existing endometrial cancer, would have continued growth of disease during toremifene treatment. This theory is consistent with the similar estrogen-like effects of tamoxifen and toremifene on the human uterus *(36)*.

In contrast, ICI 182,780 inhibited tamoxifen-stimulated endometrial growth in the presence of postmenopausal levels of estradiol, and, when administered alone, it did not increase the growth of endometrial cancer. This observation suggests that,

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