Effects of a New Clinically Relevant Antiestrogen (GW5638) Related to Tamoxifen on Breast and Endometrial Cancer Growth in Vivo

Rita C. Dardes, Ruth M. O’Regan, Csaba Gajdos, Simon P. Robinson, David Bentrem, Alex De Los Reyes, and V. Craig Jordan

Department of Gynecology, Federal University of São Paulo, Brazil 04023-900 [R. C. D.]; DuPont Pharmaceutical Company, Glenolden, Pennsylvania 19036 [S. P. R.]; and Robert H. Lurie Comprehensive Cancer Center [R. C. D., C. G., A. D. L. R., V. C. J.], Division of Hematology Oncology [R. M. O.], and Department of Surgery [D. B.], Northwestern University Medical School, Chicago, Illinois 60611

ABSTRACT

Purpose: Cross-resistance is an important issue for the evaluation of new antiestrogens to treat advanced breast cancer patients who have failed tamoxifen therapy. In addition, postmenopausal patients treated with long-term adjuvant tamoxifen show a 3–4-fold increase in the risk of developing endometrial cancer. Consequently, a new second line agent should be more antiestrogenic and less estrogen-like on the uterus, and be effective at controlling the growth of breast cancer after exposure to tamoxifen. The purpose was to evaluate the effects of the new tamoxifen analogue GW5638 on breast and endometrial cancer growth.

Experimental Design: Athymic mice were transplanted with an endometrial tumor model (ECC-1 E2) that is responsive to estrogen and has never been exposed to antiestrogen. In addition, we used three breast tumor models: a tamoxifen-naive tumor (T47D-E2) and two tamoxifen-stimulated tumors (MT2 TAM and MCF-7 TAM LT). The antiestrogen GW5638 (1.5 mg daily), tamoxifen (0.5 mg or 1.5 mg daily), and raloxifene (1.5 mg daily) were given p.o. The pure antiestrogen ICI182,780 (5 mg once a week) was given s.c. Western blots from MCF-7 TAM breast tumors were performed to demonstrate the regulation of estrogen receptor α expression by different ligands.

Results: Estradiol and GW5638 down-regulated the receptor compared with control. ICI182,780 completely degraded the receptor but tamoxifen had no effect. GW5638 did not promote tumor growth, and was effective in blocking the effects of postmenopausal estradiol on the growth of tamoxifen-naive breast and endometrial tumors. However, raloxifene did not completely block the effects of postmenopausal estradiol on the growth of tamoxifen-naive endometrial tumor after 14 weeks. GW5638 and ICI182,780 but not raloxifene were also effective in blocking the tamoxifen-stimulated breast tumor growth in athymic mice.

Conclusions: GW5638 is more effective than raloxifene in blocking the effect of estrogen on tamoxifen-naive endometrial cancer. More importantly, GW5638, like the pure antiestrogen ICI182,780, is able to block the growth of breast cancer stimulated by tamoxifen differently from raloxifene. GW5638 down-regulates estrogen receptor but does not completely destroy the receptor. Therefore, based on our findings, GW5638 could be developed as a second line agent for advanced breast cancer patients and an important first line agent to evaluate as an adjuvant treatment or chemopreventive.

INTRODUCTION

Tamoxifen has been used successfully for more than 3 decades and is the standard of care as an adjuvant endocrine therapy for ER3-positive breast cancer patients (1). The Oxford Overview Analysis demonstrates that 5 years of tamoxifen adjuvant therapy results in greater reductions in breast cancer recurrence and mortality compared with 1 or 2 years of treatment (1). In addition, the survival benefit is increased for at least 5 years after tamoxifen treatment is stopped. However, it appears that some breast tumors acquire resistance to tamoxifen therapy, which is a limiting factor to extending therapy and to enhancing the beneficial actions of the drug (2, 3). The ability of tamoxifen to function as an agonist in some settings (bones, uterus, and lipids) may also explain the development of drug resistance during breast cancer therapy. Tamoxifen-stimulated tumor growth can occur as an expression of the estrogen-like actions of the SERM. Compounds with no estrogen-like properties cannot support the growth of a tamoxifen-stimulated breast tumor (4, 5). When tumors acquire resistance to tamoxifen, a second line endocrine therapy can be considered because most tumors retain the ER (6). This fact has stimulated the search for new SERMs, which should not be cross-resistant with tamoxifen.

Clearly, a new antiestrogen that is developed as a second

Received 11/12/01; revised 2/26/02; accepted 3/13/02.

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.

1Supported by Specialized Programs of Research Excellence CA89018-01 in breast cancer from the National Cancer Institute, by Companhia de Aperfeiçoamento de Pessoal de Nível Superior Scholarship–Research Program from the Ministry of Education-Brasilia, DF, Brazil (to R. C. D.), by the Avon Foundation (to R. C. D., R. M. O., C. G.), by a grant from DuPont, Glenolden, PA (to V. C. J.), and by the generous support of the Lynn Sage Breast Cancer Foundation of Northwestern Memorial Hospital.

2To whom requests for reprints should be addressed, at Northwestern University Medical School, Robert H. Lurie Comprehensive Cancer Center, 303 East Chicago Avenue, 8258 Olson Pavilion, Chicago, IL 60611. Phone: (312) 908-5148; Fax: (312) 908-1372; E-mail: vcjordan@nwu.edu.

3The abbreviations used are: ER, estrogen receptor; SERM, selective estrogen receptor modulator; E2, 17β-estradiol; 4-OHT, 4-hydroxytamoxifen.
line endocrine therapy for breast cancer must have a better toxicological profile than tamoxifen and not produce premature drug resistance. In addition, because tamoxifen is associated with an increased incidence in endometrial cancer in postmenopausal women (7), this becomes an important issue for consideration if the new agent is to be developed to prevent breast cancer in women at risk. Currently, raloxifene is being compared with tamoxifen in the second National Surgical Adjuvant Breast and Bowel Project prevention (P-2) trial, Study of Tamoxifen and Raloxifene, for the prevention of breast cancer in postmenopausal high-risk women. A possible advantage of raloxifene over tamoxifen is that to date there has been no reported increases in the incidence of endometrial cancer noted in any clinical trial (8–12). However, the Study of Tamoxifen and Raloxifene trial will determine the endometrial safety of raloxifene compared with tamoxifen in the same trial population. Therefore, any new antiestrogen should be more antiestrogenic or at least raloxifene-like on the uterus, because daily raloxifene therapy (30, 60, or 150 mg) for 2 years does not stimulate the endometrium (11).

GW5638 is a triphenylethylene derivative of tamoxifen (Fig. 1) with a novel carboxylic acid side chain (13). The compound is a SERM (13, 14) with estrogen-like actions to preserve bone density in ovariectomized rats but unlike tamoxifen will produce antiestrogenic actions in the rodent uterus. At the molecular level, the active metabolite of GW5638, GW7604, alters the surface of the ER complex differently than either E2 or 4-OHT (15, 16). The GW7604 complex has a novel shape that retains antiestrogenic action in breast cancer cells. Although GW5638 could have an application as a treatment for osteoporosis with a secondary action as a preventive for breast cancer (17), a recent preliminary report by Connor et al. (18) suggests a role in the treatment of breast cancer, even after the development of drug resistance to tamoxifen. However, cross-resistance among SERMs is complex and, at present, unpredictable.

Because GW5638 has a unique structure, and there is tantalizing preliminary data to suggest non-cross-resistance with tamoxifen, we chose to evaluate the pharmacology of the compound systematically in a range of human tumors grown in athymic mice.

MATERIALS AND METHODS

Endometrial Cancer Model. Antiestrogen-naïve endometrial tumors (ECC-1 E2) were developed by injecting 10 million ER-positive ECC-1 endometrial cancer cells (19) into the mammary fat pads of ovariectomized athymic mice, 4–6 weeks of age, and treating the mice with an E2 capsule (0.3 cm; see below) placed s.c.

Breast Cancer Models. Tamoxifen-naïve breast tumors (T47D E2) were developed by injecting 0.5 million ER-positive T47D A18 breast cancer cells (20) into the mammary fat pads of ovariectomized athymic mice, 4–6 weeks of age, and treating the mice with an E2 capsule (0.3 cm) placed s.c. (21). Long-term tamoxifen-stimulated breast tumors (MCF-7TAM LT) were first reported in 1988 (22), and subsequent generations have been exposed to at least 5 years of tamoxifen. The MT2 tumors were derived from MCF-7 breast cancer cells (originally from Dr. Dean Edwards, University of Texas, San Antonio, TX) that had been injected into athymic mice and serially passaged with E2 or tamoxifen capsules (23). The tumors contain a natural point mutation in the ligand domain of the ER, which results in a tyrosine for aspartate substitution at amino acid 351 (24). The Animal Care and Use Committee of Northwestern University approved all of the procedures involving animals.

Tumor Implantation and Measurements. Ovariectomized athymic mice were bilaterally implanted with 1-mm³ pieces of the relevant tumor into the mammary fat pads (10 mice/treatment group, 20 tumors) using a trochar. Tumor measurements were performed weekly using Vernier calipers. The cross-sectional area was calculated using the formula: length × width/4 × π.

Drug Administration. Silastic E2 capsules (Sigma, St. Louis, MO) were made as described previously (25), implanted s.c., and replaced every 8 weeks. The 0.3-cm E2 capsules produced a mean 83.8 pg/ml of serum E2, which simulated postmenopausal estrogen levels observed in women (26). Tamoxifen and raloxifene are commercially available, and GW5638 was a generous gift of DuPont, Glenolden, PA. Tamoxifen (Sigma) and GW5638 were first dissolved in ethanol and suspended in a solution of 90% CMC (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. Raloxifene (Evista) tablets (60 mg/tablet) were ground using a mortar (5 tablets), and powder was collected with 27 ml of double-distilled water and transferred to a 50 ml conical tube. Three ml of a solution of 90% CMC and 10% polyethylene glycol 400/Tween 80 were added to the raloxifene solution for a final concentration of 0.5 mg/0.05 ml. Tamoxifen, raloxifene, and GW5638 were administered p.o. by gavage at 0.5 or 1.5 mg/mouse/day, 5 days/week. The pure antiestrogen ICI182,780 (Astra Zeneca, Macclesfield, United Kingdom) was first dissolved in ethanol and suspended in peanut oil. ICI182,780 was administered s.c. at 5 mg/mouse once a week.

Statistical Analysis. Comparisons in mean tumor cross-sectional areas between the animal groups were analyzed by ANOVA at each week and 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).
Western Blot Analysis. Tumors were homogenized by grinding in liquid nitrogen. Tumor cell pellet was resuspended in radioimmunoprecipitation assay buffer (1 × PBS, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS), and protein concentration from supernatant was measured using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Inc., Santa Cruz, CA). Equal amounts of protein (50 μg) were run according to a standard Western blot protocol (27). The ER primary antibody used was G20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and β-actin antibody AC-15 (Sigma) was used to standardize loading. The appropriate secondary antibody conjugated with horseradish peroxidase kit (Amersham Corp., Arlington Heights, IL) was used to visualize bands using an enhanced chemiluminescence visualization kit (Amersham Corp., Arlington Heights, IL). The membrane was exposed to Kodak X-OMAT film for 10 s to 1 min.

RESULTS

Expression of ERα Levels in Tumors. Western blot analysis for expression of ERα protein (50 μg of protein) was performed on MCF-7 TAM tumors treated with estradiol (0.3-cm E2 cap), tamoxifen (1.5 mg), GW5638 (1.5 mg), or no treatment. Estradiol and GW5638-treated groups had a significantly lower levels of ERα (P < 0.05) expression compared with control group, differently from tamoxifen-treated group. More importantly, only ICI182,780 completely degraded the receptor. β-Actin was measured to ensure even loading. This figure is representative of three independent experiments.

Effects of GW5638 on the Growth of Tamoxifen-naïve Breast Tumors. We used a model to simulate the case of a postmenopausal woman who has never received tamoxifen but now will be receiving tamoxifen for breast cancer treatment or prevention. Ovariectomized athymic mice (10/group) were implanted with tamoxifen-naive (T47D E2) breast tumors and treated with estradiol (0.3-cm E2 cap), tamoxifen (1.5 mg), GW5638 (1.5 mg), a combination of SERMs with E2, or no treatment (control). At 15 weeks, the E2-treated tumors (mean tumor size at 14 weeks was 0.58 cm2) significantly greater in the estradiol and GW5638 plus estradiol groups compared with the untreated group (P < 0.05). At 14 weeks, the mean tumor size of estrogen-treatment group was 1.59 cm2, significantly larger than any other treatment group (P < 0.001). Raloxifene did not stimulate tumor growth compared with control but partially blocked the estradiol effects at 14 weeks (the mean tumor size was 0.58 cm2). GW5638 did not promote tumor growth and more importantly completely blocked estradiol tumor growth; bars, ± SD.

Effects of GW5638 on the Growth of Antiestrogen-naïve Endometrial Tumors (ECC-1 E2). We compared the antagonistic effects of raloxifene with GW5638 on human endometrial cancer implanted into athymic mice. Tumor cell pellet was resuspended in radioimmunoprecipitation assay buffer (1 × PBS, 1% NP40, 0.5% sodium deoxycholate, and 0.1% SDS), and protein concentration from supernatant was measured using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Inc., Santa Cruz, CA). Equal amounts of protein (50 μg) were run according to a standard Western blot protocol (27). The ER primary antibody used was G20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and β-actin antibody AC-15 (Sigma) was used to standardize loading. The appropriate secondary antibody conjugated with horseradish peroxidase kit (Amersham Corp., Arlington Heights, IL) was used to visualize bands using an enhanced chemiluminescence visualization kit (Amersham Corp., Arlington Heights, IL). The membrane was exposed to Kodak X-OMAT film for 10 s to 1 min.

Fig. 2 ERα protein (50 μg) expression in MCF-7 TAM tumors treated with estradiol (0.3-cm E2 cap), tamoxifen (1.5 mg), GW5638 (1.5 mg), or no treatment. Estradiol and GW5638-treated groups had a significantly lower levels of ERα (P < 0.05) expression compared with control group, differently from tamoxifen-treated group. More importantly, only ICI182,780 completely degraded the receptor. β-Actin was measured to ensure even loading. This figure is representative of three independent experiments.

Fig. 3 Effects of GW5638 on the growth of antiestrogen-naïve endometrial cancer (ECC-1 E2). ECC-1 E2 endometrial tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol alone (0.3 cm E2 cap), raloxifene (Ral; 1.5 mg), GW5638 (GW; 1.5 mg), or antiestrogens with postmenopausal estradiol. At 14 weeks, ECC-1 E2 tumor growth was significantly greater in the estradiol and raloxifene plus estradiol groups compared with the untreated group (P < 0.05). At 14 weeks, the mean tumor size of estrogen-treatment group was 1.59 cm2, significantly larger than any other treatment group (P < 0.001). Raloxifene did not stimulate tumor growth compared with control but partially blocked the estradiol effects at 14 weeks (the mean tumor size was 0.58 cm2). GW5638 did not promote tumor growth and more importantly completely blocked estradiol tumor growth; bars, ± SD.
GW5638 and Hormone-responsive Cancer Growth

Postmenopausal estradiol alone (0.3 cm E₂ cap) divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol alone (0.3 cm E₂ cap), tamoxifen (Tam; 1.5 mg), GW5638 (GW; 1.5 mg), or antiestrogens with postmenopausal estradiol. At 15 weeks, T47D E₂ tumor growth was significantly greater in the estradiol group (mean tumor size was 1.22 cm²) compared with the untreated group or any other treatment group (P < 0.001). Tamoxifen and GW5638 did not stimulate tumor growth compared with control and completely blocked the estradiol effects on the tumor growth at 15 weeks; bars, ± SD.

Effects of GW5638 on the growth of tamoxifen-naive breast tumors (T47D E₂). T47D E₂ breast tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol alone (0.3 cm E₂ cap), tamoxifen (Tam; 1.5 mg), GW5638 (GW; 1.5 mg), or antiestrogens with postmenopausal estradiol. At 15 weeks, T47D E₂ tumor growth was significantly greater in the estradiol group (mean tumor size was 1.22 cm²) compared with the untreated group or any other treatment group (P < 0.001). Tamoxifen and GW5638 did not stimulate tumor growth compared with control and completely blocked the estradiol effects on the tumor growth at 15 weeks; bars, ± SD.

Effects of GW5638 on the growth of tamoxifen-stimulated breast tumors (MCF-7 TAM LT). MCF-7 TAM LT breast tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol (0.3 cm E₂ cap), tamoxifen (Tam; 1.5 mg), or GW5638 (GW; 1.5 mg). At 10 weeks, MCF-7 TAM LT tumor growth was significantly greater in the tamoxifen group compared with the untreated group (P < 0.001). The mean tumor size at 10 weeks of the tamoxifen-treatment group was 0.6 cm², significantly larger than any other treatment group (P < 0.001). GW5638 did not stimulate tumor growth compared with control; bars, ± SD.

Fig. 4 Effects of GW5638 on the growth of tamoxifen-naive breast tumors (T47D E₂). T47D E₂ breast tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol alone (0.3 cm E₂ cap), tamoxifen (Tam; 1.5 mg), GW5638 (GW; 1.5 mg), or antiestrogens with postmenopausal estradiol. At 15 weeks, T47D E₂ tumor growth was significantly greater in the estradiol group (mean tumor size was 1.22 cm²) compared with the untreated group or any other treatment group (P < 0.001). Tamoxifen and GW5638 did not stimulate tumor growth compared with control and completely blocked the estradiol effects on the tumor growth at 15 weeks; bars, ± SD.

Fig. 5 Effects of GW5638 on the growth of long-term tamoxifen-stimulated breast tumors (MCF-7 TAM LT). MCF-7 TAM LT breast tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol (0.3 cm E₂ cap), tamoxifen (Tam; 1.5 mg), or GW5638 (GW; 1.5 mg). At 10 weeks, MCF-7 TAM LT tumor growth was significantly greater in the tamoxifen group compared with the untreated group (P < 0.001). The mean tumor size at 10 weeks of the tamoxifen-treatment group was 0.6 cm², significantly larger than any other treatment group (P < 0.001). GW5638 did not stimulate tumor growth compared with control; bars, ± SD.

Effects of GW5638 on the growth of a tamoxifen-stimulated breast tumor with a single natural point mutation, Asp351Tyr, in the ER (MT2 TAM). We used a different tamoxifen-stimulated breast tumor model, MT2 TAM (23), which contains a natural point mutation at amino acid 351 (aspartate by tyrosine; Ref. 24), which is known to enhance the estrogen-like actions of the SERMs tamoxifen and raloxifene (29, 30). These experiments were conducted to verify the efficacy of GW5638 as an antitumor agent in a second tamoxifen-resistant model. Ovariectomized athymic mice (10/group) were implanted with tamoxifen-stimulated (MT2 TAM) breast tumors and treated with E₂ (0.3-cm E₂ cap), tamoxifen (1.5 mg), GW5638 (1.5 mg), or no treatment (control). As we have demonstrated previously, E₂ does not statistically promote tumor growth of MCF-7 TAM LT tumors in athymic mice compared with control (28). At 10 weeks, MCF-7 TAM LT tumor size was significantly greater in the tamoxifen-treated group (mean tumor size = 0.6 cm²) compared with the untreated mice (P < 0.001; Fig. 5). Most importantly, the tumors in animals treated with GW5638 exhibited little or no growth. These data indicate that tamoxifen and GW5638 were not cross-stimulatory in this model of drug resistance.

DISCUSSION

Tamoxifen is currently recommended as the most effective adjuvant therapy for treating ER-positive breast cancers. However, the effectiveness of this therapy is limited by the development of resistance to tamoxifen during extended treatment. Even in the absence of ER mutations or significant alterations in the metabolism of tamoxifen, the majority of patients with advanced disease eventually develop resistance to tamoxifen.
GW5638 did not promote tumor growth compared with control; bars, ± SD.

Fig. 6  Effects of GW5638 on the growth of tamoxifen-stimulated breast tumor that contains a natural mutated ERα (MT2 TAM). MT2 TAM (tamoxifen-stimulated) breast tumors were bilaterally implanted into mammary fat pads of ovariectomized athymic mice. Mice were divided into groups of 10 and were untreated (control), or treated with postmenopausal estradiol (0.3 cm E2 cap), tamoxifen (Tam; 1.5 mg), or GW5638 (GW; 1.5 mg). At 8 weeks, MT2 tumor growth was significantly greater in the tamoxifen group compared with the untreated group (P < 0.05). The mean tumor size at 8 weeks of the tamoxifen-treatment group was 0.32 cm2, significantly larger than any other treatment group (P < 0.05). GW5638 did not promote tumor growth compared with control; bars, ± SD.

We tested GW5638 in our two tamoxifen-stimulated tumor models to establish that GW5638 is more antiestrogenic on the breast and presents a distinct mechanism of action to tamoxifen that may avoid cross-resistance. We have used a long-term (5 years) tamoxifen-stimulated tumor (MCF-7 TAM LT; Refs. 22, 28) and a second tamoxifen-stimulated breast tumor (MT2 TAM; Ref. 23) model, which contains a single natural point mutation, Asp351Tyr (24), in the ER that enhances estrogen-like proprieties of tamoxifen and raloxifene (29, 30). We used the MCF-7 TAM LT breast cancer model to simulate the case of a patient with advanced breast cancer who has developed progressive disease as a result of long-term tamoxifen exposure. In addition, we used the second tamoxifen-stimulated breast tumor model, MT2 TAM, to establish whether GW5638 could block the tumor growth in the presence of a mutant ER. Our results show that GW5638 does not promote tumor growth in either tamoxifen-stimulated breast tumor models. These results support and extend previous findings with a MCF-7 (short-term) tamoxifen-stimulated tumor model (18). Our laboratory has tested many antiestrogens (triphenylethlene or benzothiophene derivatives) in diverse tamoxifen-stimulated tumor models in vivo (33, 34), and the only other compound that was not cross-resistant with tamoxifen in any tamoxifen-stimulated tumor grown in athymic mice was the pure antiestrogen ICI182,780 (33). In the present study, raloxifene was cross-resistant with tamoxifen (Fig. 7), whereas GW5638 and ICI182,780 were not.

Tumors that initially respond to tamoxifen undergo specific alterations that allow them to recognize tamoxifen as an agonist and display a selective growth advantage over their unaltered neighboring cells (35). There are many hypotheses that try to explain why some tamoxifen-treated patients develop resistance to the drug but the actual shape of SERM-ER complexes may be critical for signal transduction. Evidence for this hypothesis has been presented by Wijayaratne et al. (15) who developed a set
of peptides using phage display that recognize different surfaces on ERα. These workers (15) demonstrated that after binding to ERα, each SERM induces a distinct ERα-ligand conformation. Using computer-assisted molecular models of ER complexes, our previous study showed that the antiestrogens, 4-OHT and GW7604, are distinct because the antiestrogenic side chain of 4-OHT weakly interacted with the surface amino acid 351 (aspartate), but the carboxylic acid of GW7604 caused a strong repulsion of aspartate 351 (16).

Tamoxifen is not a pure antiestrogen, and has both agonist and antagonist properties. The development of tamoxifen resistance could be associated with an increase in its agonist-like properties, resulting in loss of antagonist effects through inappropriate tumor stimulation. The direction of transcription by antagonist-occupied steroid receptors may be controlled by the ratio of coactivators and corepressors recruited to the transcription complex by promoter-bound receptors. One alternative explanation is that decreased levels of nuclear receptor corepressor, a corepressor protein, is associated with the acquisition of tamoxifen-resistant MCF-7 breast cancer in the laboratory (36). Tamoxifen-stimulated breast cancers could also grow because the SERM steroid receptor complex preferentially bound by an excess of coactivators (37, 38).

However, drug resistance to tamoxifen cannot be simply a loss of the corepressor nuclear receptor corepressor or silencing mediator of retinoid and thyroid receptor (36), because no other SERM would be effective in a tamoxifen-stimulated MCF-7 tumor. GW5638 and IC1182,780 (a pure antiestrogen) are active (Figs. 5–7; Refs. 18, 33), so coactivator changes and receptor degradation must also play a part in subverting tamoxifen-stimulated drug resistance. We demonstrate down-regulation of ER in vivo in a tamoxifen-stimulated tumor (Fig. 2) by GW5638, which is consistent with data in vitro (16). The decrease in ER noted with GW5638 and IC1182,780 (Fig. 2) is distinct from the effect of tamoxifen on ERα that remains unaffected. Recent studies on the ubiquitination of the ER complex and subsequent destruction by proteasomes illustrates that the tamoxifen ERs complex is ubiquitinated to a lesser extent than either the GW5638 or IC1182,780 complexes (39).

In conclusion, we have found that GW5638 is an effective antitumor agent and antiestrogen in tamoxifen-naïve breast and endometrial tumors. These results support the previous studies in vitro (15, 16) and the idea that GW5836 could be useful for the treatment of advanced breast cancer. More importantly, these data suggest that GW5836 may be more refractory to the early development of drug resistance as a first line therapy. The SERM could also be developed as an effective chemopreventive because the pharmacokinetics suggest a better blockade of estrogen-stimulated tumor growth than raloxifene but with potentially similar beneficial effects on bones and the uterus.

REFERENCES