Antiestrogens: Future Prospects

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Tamoxifen is currently the endocrine therapy of choice for early and advanced breast cancer. Attempts to improve the therapeutic efficacy have included altering the triphenylethylene ring structure of tamoxifen, forming introduction

Triphenylethylene structures similar to tamoxifen (Nolvadex) were first used in the 1940s, but in comparison to synthetic and natural estrogens used at that time, they were not particularly potent antitumor agents. [1,2] Tamoxifen was introduced into the clinic in 1969. Because of its much improved toxicity profile over the estrogens and androgens in use at that time, it rapidly gained acceptance as the endocrine treatment of choice for advanced breast cancer and subsequently as adjuvant therapy.[3-5]

Three different strategies have been used to develop new antiestrogens: (1) chemical alteration of the triphenylethylene ring of tamoxifen, (2) production of new nonsteroidal ring structures (eg, the benzothiophenes), and (3) production of steroidal estradiol analogs with pure antiestrogenic activity (Figure 1).

All of the nonsteroidal antiestrogens discussed in this article are partial estrogen agonists to differing degrees. In contrast, the steroidal pure antiestrogens lack intrinsic estrogenic activity and appear to have a somewhat different mechanism of action.[6] Although both types of compounds bind to the estrogen receptor (ER), the tamoxifen analogs allow partial activation of estrogen receptors, permitting transcription of some estrogen-regulated genes, whereas the steroidal antiestrogens appear to completely inhibit transcriptional activity via the estrogen receptor.

The six nonsteroidal antiestrogens shown in Figure 1 are either marketed or in clinical trials. All but one, raloxifene, are based on the triphenylethylene molecule. In addition, pure steroidal antiestrogens (ICI 164,384 and ICI 182,780) have been developed in order to produce a state of complete estrogen deprivation. The overall goal for the development of new antiestrogens is to increase their efficacy and safety (Table 1).

This last characteristic is important because many women taking these agents are treated for long periods with adjuvant therapy. Since most breast cancer patients are unlikely to receive hormone replacement therapy due to fear of disease recurrence, new endocrine breast cancer agents with an improved side-effect profile and favorable effects on bone mineral density and the cardiovascular system would be highly valuable.

The purpose of this review is: (1) to describe the current status and ultimate potential of the nonsteroidal antiestrogen agents and (2) to introduce a new class of pure steroidal antiestrogen agents.

Nonsteroidal Antiestrogens

Tamoxifen

Tamoxifen has gained wide clinical acceptance, first for metastatic breast cancer and then for adjuvant therapy. Currently, tamoxifen is being studied for the chemoprevention of breast cancer in healthy women at high risk for the disease. The use of tamoxifen has expanded over the years because of its efficacy in prolonging overall and disease-free survival as well as reducing the incidence of contralateral breast cancer. Because tamoxifen is a mixed estrogen agonist and antagonist, it has been shown to have positive, estrogenic effects on bone and the cardiovascular system while maintaining antitumor activity via its antiestrogenic effects. The estrogenic activity of tamoxifen has been associated with a proliferative effect on the endometrium and a two- to threefold increase in the risk of endometrial carcinoma. This association has stimulated interest in the discovery of new antiestrogens with no uterotropetic activity. The methods for evaluating these compounds are shown in Table 2.
Toremifene
Toremifene (Fareston) differs from tamoxifen by only a single chlorine atom (see Figure 1). Although it produces different metabolites due to the stability of the chlorine atom,[7] toremifene has a pharmacologic profile similar to tamoxifen.

Toremifene displaced $[^3]H$estradiol binding to estrogen receptors by 50% at a concentration of 0.5 mmol/L.[7] In the ER-positive human breast carcinoma cell line MCF-7, toremifene showed a pattern of activity similar to tamoxifen: growth inhibition at low concentrations and oncolytic activity at high concentrations.[7] A concentration of $5 \times 10^{-6}$ M toremifene killed all MCF-7 cells within two days.[8] The activity of toremifene appears to be estrogen-dependent; growth inhibition of MCF-7 cells at concentrations less than $10^{-6}$ M can be reversed with estradiol.

Against dimethylbenzanthracene (DMBA)-induced rat mammary cancer, toremifene and tamoxifen showed similar antitumor activity; the one difference was that 45 mg/kg tamoxifen was toxic to the rat, whereas 45 mg/kg toremifene showed an antitumor effect.[7]

In the rat uterus, the estrogenic effects of toremifene appear to be lower than tamoxifen at low and moderate concentrations; however, the maximum estrogenic response was similar.[7] Toremifene has also demonstrated estrogenic effects on human endometrial tissue in postmenopausal breast cancer patients, including increasing endometrial thickening and proliferation.[9]

Unlike tamoxifen, toremifene did not produce liver tumors in rats at doses up to 48 mg/kg for one year.[7]

Toremifene has been studied in a comparative trial with tamoxifen in 648 perimenopausal or postmenopausal women with metastatic breast cancer (hormone receptor positive or unknown receptor status).[10] The three treatment arms consisted of tamoxifen (20 mg/d), toremifene (60 mg/d), or toremifene (200 mg/d). In the intent-to-treat analysis, the frequency of objective response (complete or partial) was 19% for tamoxifen, 21% for 60-mg toremifene, and 23% for 200-mg toremifene ($P$ not significant between treatments). The median response durations were 19.1 months, 16.9 months, and 18.4 months, respectively ($P$ not significant between treatments). Likewise, there were no significant differences among the three treatments in the median time to progression or median overall survival.

Overall, the type and frequency of adverse effects were similar among the three groups, including the incidence of tumor flare and thromboembolic and cardiac events; however, the 200-mg toremifene dose was associated with a greater frequency of elevated aspartate aminotransferase (AST) levels and nausea.

At this time, there does not appear to be any benefit of toremifene over tamoxifen. The clinical experience with toremifene will need to be expanded substantially to determine if there is any benefit of the drug with respect to rare adverse events such as endometrial cancer.

Droloxifene
Droloxifene (3-hydroxytamoxifen) is currently undergoing clinical trials in advanced breast cancer. Unlike tamoxifen, droloxifene is itself the active moiety and thus does not require metabolism for activation.[11] Droloxifene shows a higher binding affinity for ER compared with tamoxifen.[12] The IC$_{50}$ for the displacement of 17-beta-estradiol from ER is $1 \times 10^{-8}$ M. In rats, droloxifene exhibits higher antiestrogenic activity and lower estrogenic activity than tamoxifen.[12]

Droloxifene is more effective than tamoxifen in inhibiting the growth of ER-positive breast cancer cells, even at therapeutic concentrations (0.1 to 0.4 mM), and its activity is related to ER content.[13] Interestingly, short-term (1-hour) exposure to droloxifene in vitro produced maximum growth inhibition, leading investigators to conclude that the agent may be suitable for intermittent therapy. Moreover, droloxifene produced a greater antitumor effect than tamoxifen against DMBA-induced breast tumors in rats. Droloxifene (at doses up to 200 mg/kg/d for 6 months or doses up to 90 mg/kg/d for 24 months) is not hepatocarcinogenic in the rat.[12]

A large phase II trial of droloxifene was conducted in centers located in Europe, Canada, and Brazil.[14] The purpose of the study was to compare three daily doses (20 mg, 40 mg, and 100 mg) in postmenopausal women with advanced breast cancer. Eligible patients had no prior exposure to systemic hormonal therapy and had positive or unknown hormone-receptor status; of 369 randomized patients, 268 were evaluable for response.

Complete or partial responses were seen in 30% of the 20-mg group, 47% of the 40-mg group, and 44% of the 100-mg group. The differences were significant between the 20-mg group and the 40-mg group ($P = .02$) and between the 20-mg group and the 100-mg group ($P = .04$). Half of the responses were seen within the first two months of starting treatment.

The median duration of response was 12 months for 20 mg, 15 months for 40 mg, and 18 months for 100 mg. Again, the 40-mg ($P = .02$) and the 100-mg ($P = .01$) groups had significantly better results...
than the 20-mg group. Side effects reported by more than 20% of patients included hot flashes, lassitude, and nausea; the frequency of these effects did not appear to be dose related. Thromboembolic events did not occur more frequently than with other antiestrogens.

Issues that remain to be resolved for droloxifene include its effects on the uterus, bone, and the cardiovascular system as well as its potential for cross-resistance with tamoxifen. Droloxifene has been shown to have estrogenic effects on bone in the rat,[15] and clinically, has produced decreases in plasma cholesterol without affecting cholesterol synthesis.[16] Although these results look promising, as with toremifene, droloxifene's ultimate clinical profile will only be determined in trials comparing it with tamoxifen.

**Raloxifene**

Raloxifene is a benzothiophene derivative with a high binding affinity for ER, reportedly 2.9-fold greater than that of estradiol.[17] In the rat, raloxifene has exhibited antiestrogenic activity in the breast and uterus and estrogen agonist activity on bone and lipids.[18]

In contrast to tamoxifen, raloxifene showed a lack of uterotrophic effect after four days of treatment in ovariectomized rats.[18] Near complete antagonism of estrogen-induced uterotrophic activity was observed at 1 mg/d.[17] Moreover, three days of raloxifene was able to block the uterotrophic action of estradiol for 10 subsequent days. Raloxifene was unable to reverse the uterotrophic response to tamoxifen. Raloxifene dose-dependently inhibited estrogen-stimulated proliferation of MCF-7 cells.[18]

Sato et al[19] compared the effects of raloxifene and tamoxifen on bone, cholesterol, and the uterus in six-month-old, ovariectomized rats. The effect of raloxifene on bone mineral density was dose-dependent, with an ED50 of 0.3 mg/kg/d (35 days of treatment), while the ED50 for tamoxifen was 0.1 mg/kg/d. At doses of 0.1-10 mg/kg, raloxifene dose-dependently reduced cholesterol levels to 51% to 62% of ovariectomized controls. The results were similar for tamoxifen. In contrast, uterine epithelial thickness increased by 250% with tamoxifen therapy, compared with only 60% seen with raloxifene.

Raloxifene (100 mg orally, two times a day) was studied in 14 patients with disseminated breast cancer refractory to tamoxifen.[20] There was one minor response in a patient with soft-tissue disease and in five patients with stable disease. Side effects included hot flashes (n = 4), fatigue (n = 3), leg cramps (n = 1), and mild nausea (n = 3).

Although the antitumor activity of raloxifene needs further evaluation, the drug may have a role in the prevention of osteoporosis in postmenopausal women. In this population, raloxifene doses 50 or more mg/d have significantly reduced total serum and low-density lipoprotein (LDL) cholesterol as well as serum markers of bone turnover (eg, osteocalcin, alkaline phosphatase).[18]

**TAT-59**

TAT-59 is a triphenylethylene derivative in early development in Japan. Like tamoxifen, TAT-59 is converted to an active metabolite, 4-OH-TAT-59.[21,22] TAT-59 shows a binding affinity to ER similar to tamoxifen.[21] The IC50 for the inhibition of 5 × 10^-9 M estradiol binding to rat uterine ER was 5.37 × 10^-9 M for 4-OH-TAT-59 and 3.63 × 10^-9 M for 4-OH-tamoxifen.

Tominaga et al[23] compared the activity of 10^-6 M concentrations of TAT-59, droloxifene, toremifene, and tamoxifen against MCF-7 cells incubated with 10^-8 M estradiol in vitro. The following order of potency (from greatest to least) was reported after both 48 and 120 hours of incubation: TAT-59, droloxifene, tamoxifen, toremifene.

Toko et al[22] reported that TAT-59 was 2.9- to 5.5-fold more potent than tamoxifen in inhibiting the uterotrophic effect of ovariectomy in immature rats. Against MCF-7 cells transplanted into nude mice, TAT-59, unlike tamoxifen, was able to suppress tumor growth at a dose of 0.1 mg/kg/d (P < .01). However, the effects of both agents were similar at 0.3 mg/kg/d.[22] Compared with tamoxifen, TAT-59 was 10-fold more active against DMBA-induced mammary tumors in rats and demonstrated more activity against tumors with low ER content.[22] Preliminary clinical data indicate that TAT-59 has activity similar to tamoxifen.[6]

**Idoxifene**

Idoxifene is a triphenylethylene derivative designed to have a greater affinity for ER and to be more resistant to metabolism than tamoxifen.[24] In vitro, idoxifene shows 2.5-fold greater binding affinity for ER than tamoxifen.

After three days of incubation, 10^-6 M idoxifene inhibited growth of MCF-7 cells in vitro by 58.7% compared with 38.7% for the same concentration of tamoxifen. Moreover, idoxifene was 1.5-fold
more potent than tamoxifen in inhibiting estradiol-induced proliferation of MCF-7 cells ($P = .006$). The inhibitory effect of idoxifene was reversible by estradiol, and the drug was not active against an ER-negative cell line.[24] Idoxifene's uterotropic activity is reported to be 1.4-fold lower than that of tamoxifen.[24]

Idoxifene showed similar reduction in tumor volume as with tamoxifen in the N-nitrosomethylurea-induced rat mammary cancer model, but was active in a greater percentage of rats (92% vs 75%).[24]

A phase I study of idoxifene was conducted in 20 postmenopausal women with advanced breast cancer.[25] Groups of five patients each received either 10, 20, 40, or 60 mg idoxifene daily for two weeks. However, some patients continued to receive the drug at a dosage of 20 mg/d until disease progression occurred.

Idoxifene was associated with significant reductions in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels with no difference observed in estradiol and sex hormone binding globulin (SHBG) levels. Fourteen patients experienced side effects that were not dose related. These effects included mild nausea, anorexia, vomiting, and fatigue. In 14 patients who remained on the drug for up to 384 days, there were two partial responses and four patients with stable disease lasting 1.5 to 14 months.

As idoxifene is in the early stages of clinical development, it will be interesting to see how its antitumor efficacy compares with that of tamoxifen in more advanced clinical trials.

**Pure Steroidal Antiestrogens**

Pure steroidal antiestrogens have been developed by Alan Wakeling and Jean Bowler at Zeneca Pharmaceuticals that lack the intrinsic estrogenic activity of the tamoxifen analogs (see Figure 1). Instead of altering the triphenylethylene ring, these researchers decided to work with the estradiol molecule itself to try to design a potent, steroidal antiestrogen. The Zeneca investigators added a long alkyl side chain at the 7-alpha position of the B ring of the steroid.[26]

The first candidate, ICI 164,384, was shown to be a potent antiestrogen. However, it was hydrophobic and thus showed reduced bioavailability. ICI 164,384 was modified by attaching fluorine atoms to the alkyl side chain. The new compound, ICI 182,780, has affinity for the ER equivalent to that of estradiol and has now entered clinical development.

In vitro, ICI 182,780 displaced 17-beta-[3H]estradiol binding to the ER with an IC$_{50}$ of $0.94 \times 10^{-8}$ M.[27]

ICI 182,780 dose-dependently blocked the uterotrophic effect of estradiol in immature female rats; complete estrogen antagonism was observed at a dose of 0.5 mg/kg/d subcutaneously.[27] Peroral administration of ICI 182,780 reduced its efficacy by one order of magnitude.

In vitro, ICI 182,780 inhibited the growth of MCF-7 cells with an IC$_{50}$ of 0.29 nM.[27] When the activity of ICI 182,780 was compared with tamoxifen against this cell line after three to five days of treatment, only 7% of cells exposed to 10 nM ICI 182,780 were capable of cell division, compared with 37% of those exposed to 4 mM tamoxifen. The concentrations tested were considered optimally antiestrogenic but not cytotoxic.

Against MCF-7 cells xenografted into nude mice, a single 5-mg subcutaneous injection of ICI 182,780 was equivalent to the antitumor efficacy of four weeks of daily tamoxifen (10 mg/kg/d orally).[22] ICI 182,780 has been effective against tamoxifen-resistant human breast cancer cell lines,[28,29] indicating a lack of cross-resistance with tamoxifen and suggesting the possibility for its use as second-line therapy after tamoxifen failure.

The effects of ICI 182,780 on the human endometrium were studied in 30 premenopausal women requiring hysterectomy for benign conditions.[30] ICI 182,780 (12 mg/d intramuscularly) or no treatment was given for seven days prior to surgery. Treated patients had significantly lower ER levels in myometrial cells and reduced Ki67 (proliferation-associated nuclear antigen) in the endometria. The treatment had no effect on progesterone receptor (PR) levels.

In another study using the same dosage and duration,[31] endometrial proliferation was measured using ultrasound during the follicular phase of the menstrual cycle prior to hysterectomy. In contrast to the control group, treated patients showed no increase in endometrial thickness throughout the study, indicating a potent antiestrogenic effect of ICI 182,780 on the endometrium.

A clinical trial of ICI 182,780 was conducted in 56 postmenopausal women with primary breast cancer.[32] Patients were given either no treatment ($n = 19$) or ICI 182,780 6 mg ($n = 21$) or 18 mg ($n = 16$) for seven days prior to primary breast surgery. Treatment had no significant effect on serum levels of LH, FSH, or SHBG. Compared with pretreatment levels, ICI 182,780 produced...
significant reductions in the expression of ER (P < .01), PR (P < .05), and Ki67 (P < .05) in ER-positive breast tumors. When the data on Ki67 were separated by dose, the reduction was only significant for the 18-mg group. ICI 182,780 also reduced expression of an estrogen-regulated protein (p52) (P < .05). Adverse effects, primarily headaches, were reported by five patients receiving 6 mg and three patients receiving 18 mg ICI 182,780, and most effects were considered unrelated to treatment. The injections were well tolerated, with only one local reaction.

A phase I study of ICI 182,780 was conducted in 19 patients with advanced breast cancer resistant to tamoxifen.[33] The first four patients received 100 mg as a monthly intramuscular injection, increased to 250 mg from the second month onward. The remaining 15 patients received 250 mg during the entire study. Patients were treated until progression occurred; the median duration was more than 18 months. Thirteen patients responded to treatment (seven partial responses and six with stable disease). There were no serious drug-related adverse events and no reports of new hot flashes, vaginal dryness, or altered libido during the study.

In summary, ICI 182,780 is a potent, pure antiestrogen lacking the partial estrogen agonist activity of the nonsteroidal antiestrogens like tamoxifen. ICI 182,780 lacks proliferative activity on the endometrium, suggesting an advantage over tamoxifen with respect to the development of endometrial cancer. The lack of cross-resistance with tamoxifen seen so far indicates that tamoxifen-resistant tumors would be likely to respond to second-line therapy with pure antiestrogens.

**Discussion**

There are currently a variety of antiestrogens in development, including triphenylethylene derivatives, a benzothiophene derivative, and pure steroidal antiestrogens. New antiestrogens will have to compete with the long-established track record of tamoxifen, including its reduced recurrence of breast cancer and mortality rates as well as its favorable side-effect profile. The activity of toremifene in vitro and in vivo appears to be very similar to that of tamoxifen, and no apparent benefit to the new agent is seen at this time. A number of candidates, including droloxifene, TAT-59, idoxifene, and ICI 182,780, have demonstrated good preclinical and clinical activity and will need to be tested in phase III trials against tamoxifen. Although raloxifene exhibits favorable ER-binding affinity and estrogen antagonist activity, published data on its antitumor activity are lacking. Raloxifene may prove to be a successful therapy for the prevention of osteoporosis.

All of the investigational drugs discussed in this article require further evaluation. However, it should be noted that even after nearly 20 years of clinical use, the full clinical potential of tamoxifen may not yet be totally realized. Trials of intermittent and alternating tamoxifen therapy need to be conducted to determine whether tamoxifen resistance can be avoided. For the new compounds, an evaluation of their potential for cross-resistance with tamoxifen as well as their effects on the endometrium, bone mineral density, and lipid profiles is essential. Only after these data become available will the ultimate potential of these agents be evident.

**References:**


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