

Comparison of the Effects of a Pure Steroidal Antiestrogen With Those of Tamoxifen in a Model of Human Breast Cancer

C. Kent Osborne, Ester B. Coronado-Heinsohn, Susan G. Hilsenbeck, Bryant L. McCue, Alan E. Wakeling, Richard A. McClelland, David L. Manning, Robert I. Nicholson*

Background: Tamoxifen, a nonsteroidal estrogen antagonist, is the most prescribed drug for the treatment of breast cancer. The use of tamoxifen is limited, however, by the development of resistance to this compound in most patients. Although tamoxifen behaves primarily as an estrogen antagonist, it has agonist (or growth-stimulatory) activity as well. ICI 182,780 is a 7 α -alkylsulfanyl analogue of estradiol lacking agonist activity. The absence of agonist activity may make this steroidal antiestrogen superior to tamoxifen in suppressing tumor cell growth. **Purpose:** We compared the inhibitory effects of ICI 182,780, tamoxifen, and estrogen withdrawal on the growth of established tumors and on tumorigenesis in a model system that uses estrogen-dependent, human MCF-7 breast tumor cells growing in athymic nude mice. We also studied the hormonal responsiveness of tumors that became resistant to the two estrogen antagonists and the effects of these drugs on estrogen-regulated gene expression. **Methods:** MCF-7 cells were injected subcutaneously into the flanks of castrated, female nude mice. The effects of repeated doses of tamoxifen and ICI 182,780 (500 μ g and 5 mg, respectively) on the growth of established tumors (8-10 mm in size) were determined after supplemental estrogen was removed. The effects of antiestrogen treatments on the process of tumorigenesis, in the absence of estrogen supplementation, were determined by initiating drug administration on

the same day as tumor cell inoculation. To evaluate the hormonal responsiveness of tumors resistant to tamoxifen and ICI 182,780, 1-mm³ segments of the tumors were transplanted onto the flanks of new recipient mice, which were then treated with estrogen or the antiestrogens—alone or in combination. Tumor growth was monitored by measuring tumor volumes twice a week. Expression of the estrogen-responsive genes, pLIV1 and pS2, in the tumors of treated animals was analyzed using blots of total cellular RNA and complementary DNA probes. **Results:** Treatment with ICI 182,780 suppressed the growth of established tumors twice as long as treatment with tamoxifen or estrogen withdrawal. Tumorigenesis, in the absence of supplemental estrogen, was delayed to a greater extent in ICI 182,780-treated mice than in tamoxifen-treated mice. ICI 182,780 was found to be more effective than tamoxifen in reducing the expression of estrogen-regulated genes. Most tumors eventually became resistant to ICI 182,780 and grew independently of estrogen. **Conclusions:** ICI 182,780 is a more effective estrogen antagonist than tamoxifen in the MCF-7 tumor cell/nude mouse model system. [J Natl Cancer Inst 87:746-750, 1995]

Tamoxifen, a nonsteroidal antiestrogen, is the most prescribed drug for the treatment of breast cancer. When used in the adjuvant setting after surgery for primary breast cancer, about one fifth of the deaths at 10 years are avoided by 2 years or more of treatment (1). Tamoxifen is also effective in inducing remissions in women with estrogen receptor (ER)-positive metastatic breast cancer. Invariably, however, tumors become resistant to tamoxifen, and tumor progression and death ensue. The evolution to tamoxifen resistance in metastatic breast cancer occurs after an average treatment duration of only 10-12 months, severely limiting the usefulness of this approach.

The mechanisms by which tumors acquire resistance to tamoxifen are poorly understood. Loss of ER from the tumor can occur by selection of an ER-negative clone or by suppression of receptor expression, but this loss explains only a minority of cases (2). Growing experi-

mental and clinical evidence suggests that resistance in some patients may be caused by the intrinsic estrogen agonist properties of tamoxifen. Although tamoxifen is predominantly an estrogen antagonist in breast cancer cells, acquisition of increasingly dominant agonist activity over time may result in clinical resistance because of the acquired ability of the drug to stimulate, rather than to inhibit, tumor growth (3-7). The mechanisms for tamoxifen-stimulated tumor growth are not clear, but these data suggest that antiestrogens with pure antagonist properties might have superior antitumor activity.

ICI 182,780 is a 7 α -alkylsulfanyl analogue of estradiol that differs substantially from tamoxifen in terms of its chemical, pharmacologic, and biologic properties. This agent has no intrinsic estrogen-agonist activity and, thus, is considered a "pure" antiestrogen (8,9). It has potent antiestrogenic activity in preclinical in vitro and in vivo model systems (10). We recently reported (7) that treating nude mice with ICI 182,780 inhibits the growth of MCF-7 human breast tumor implants that had acquired tamoxifen resistance through the mechanism of tamoxifen-stimulated growth. Similar results were obtained with another analogue, ICI 164,384, studied earlier (11). These data suggest the possibility that pure steroidal antiestrogens may be effective in some tamoxifen-resistant patients.

In the present study, we have investigated the preclinical activity of ICI 182,780 in more detail. We compared the inhibitory effects of ICI 182,780, tamoxifen, and estrogen withdrawal on the

*Affiliations of authors: C. K. Osborne, E. B. Coronado-Heinsohn, S. G. Hilsenbeck, B. L. McCue, Department of Medicine, Division of Medical Oncology, The University of Texas Health Science Center at San Antonio, San Antonio, Tex.

A. E. Wakeling, Zeneca Pharmaceuticals, Mereside Alderley Park, Macclesfield, Cheshire, England, U.K.

R. A. McClelland, D. L. Manning, R. I. Nicholson, Tenovus Cancer Research Center, University of Wales College of Medicine, Heath Park, Cardiff, Wales, U.K.

Correspondence to: C. Kent Osborne, M.D., Department of Medicine, Division of Medical Oncology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284-7884.

See "Notes" section following "References."

growth of established tumors and on tumorigenesis in a model system that uses estrogen-dependent, human MCF-7 breast tumor cells growing in athymic nude mice. We also studied the hormonal responsiveness of tumors that had become resistant to the two estrogen antagonists and the effects of these drugs on estrogen-regulated gene expression.

Materials and Methods

Nude Mouse Model System

ER-positive MCF-7 human breast cancer cells (passage 100-200) were cultured as described previously (12). The athymic nude mice used in these experiments were 4- to 5-week-old female castrated BALB/c-nu⁺/nu⁺ mice purchased from Harlan Sprague-Dawley, Inc. (Madison, Wis.). The methods for maintenance and housing of the mice and for growing MCF-7 tumors from cell suspensions and from tumor transplants have been published in detail (3,7). Animal care was in accordance with institutional guidelines.

Approximately 5×10^6 MCF-7 cells were injected subcutaneously into the flanks, just under the forelimb, of female nude mice to initiate tumor formation. Estrogen supplementation was provided in the form of a 0.25-mg estradiol (E₂) pellet (Innovative Research, Rockville, Md.) placed subcutaneously in the interscapular region of the mice. The effects of tamoxifen and ICI 182,780 on the growth of established tumors were studied after the tumors had reached a size of 8-10 mm (3-5 weeks). At this time, the animals were randomly allocated into four treatment groups: 1) continued estrogen supplementation, 2) removal of the E₂ pellet, 3) removal of the E₂ pellet plus treatment with 500- μ g tamoxifen citrate (Zeneca Pharmaceuticals, Wilmington, Del.) in peanut oil (injected subcutaneously each day, Monday through Friday), or 4) removal of the E₂ pellet and treatment with the indicated doses of ICI 182,780 (Zeneca Pharmaceuticals, Macclesfield, England) in castor oil (subcutaneous injections once a week). Initial dose-response studies with ICI 182,780 were performed in the presence of continued estrogen supplementation. Tumor growth was assessed, and tumor volumes were measured twice a week as described previously (12).

In tumorigenesis experiments, various treatments were begun on the same day tumor cells were injected. Inoculated mice were randomly allocated immediately into four treatment groups: 1) estrogen supplementation, 2) 500 μ g tamoxifen once a day, Monday through Friday, 3) 5 mg ICI 182,780 once a week, or 4) drug vehicle (peanut oil and/or castor oil). Tumor volumes were measured twice a week.

To investigate the hormonal responsiveness of tumors that had become resistant to ICI 182,780, mice with resistant tumors were killed by cervical dislocation, and the tumors were resected and cut into 1-mm³ fragments. The fragments were then transplanted subcutaneously on the flank just under the forelimb of new 4- to 5-week-old recipient mice that were then treated with estrogen, tamoxifen, ICI 182,780, or vehicle alone.

Estrogen and Progesterone Receptor Assays

ER content was determined in tumors homogenized in 0.4 M KCl-Tris buffer, using the ER antibody kit (ER-EIA; Abbott Laboratories, North Chicago, Ill.). Progesterone receptor (PgR) levels were measured by a ligand-binding, dextran-coated charcoal method (3).

Estrogen-Regulated Gene Expression

Expression of the estrogen-responsive genes, pLIV1 and pS2, was determined by northern blot analysis, using complementary DNA (cDNA) probes labeled with [³²P]deoxycytidine triphosphate (3000 Ci/mmol; Amersham Ltd., Amersham, England, U.K.) by the random-priming method as described previously (13). Briefly, total RNA was obtained from the tumors of treated mice by cell lysis in 4 M guanidinium thiocyanate and 1% 2-mercaptoethanol and centrifugation through 5.7 M caesium chloride (Beckman L-80 ultracentrifuge, SW50 rotor, 34 000 rpm at 20 °C for 17 hours). Purified samples were stored in RNase-free water at -70 °C before electrophoresis (10 μ g/lane), blotting, and hybridization. Densitometric analysis of autoradiographs was performed using a model 620 video densitometer (Bio-Rad Laboratories, Richmond, Calif.), and values obtained were corrected for equivalence of RNA loading by comparison with the signals generated using a cDNA probe to human glyceraldehyde 3-phosphate dehydrogenase (G3PDH) (Clontech Laboratories, Inc., Palo Alto, Calif.).

Recorded densitometry values represent the area of peak values obtained, following background subtraction, from equivalently exposed autoradiographs (where x = band width in mm and y = optical density value). Hybridizations of each set of filters in the study were carried out simultaneously with the same labeled probes. The reported values represent means of groups, and at least two separate hybridizations of different filters were performed for each probe (stripping the previous probe with high-stringency washes and checking for clearance by autoradiography).

Statistical Analysis

Analyses were performed using either the Kruskal-Wallis one-way analysis of variance (when there were more than two groups) or the Wilcoxon signed rank test for two samples. All statistical tests were two-sided.

Results

ICI 182,780 Dose-Response

ICI 182,780 inhibited estrogen-induced growth of MCF-7 tumors in a dose-dependent manner. Estrogen-supplemented mice with established MCF-7 tumors were randomly allocated to receive either continued estrogen treatment or estrogen treatment plus injections of ICI 182,780 once a week in doses ranging from 0.5

mg to 10.0 mg. Inhibitory activity was modest with doses of 0.5 mg or 1.0 mg, while more dramatic—but approximately equivalent—inhibitory effects were observed with 5.0-mg and 10.0-mg doses (data not shown). For subsequent experiments, a dose of 5.0 mg per mouse, given once a week, was used.

Effect of Estrogen Withdrawal, Tamoxifen, and ICI 182,780 on MCF-7 Tumor Growth

Treatment of mice by removal of the E₂ pellet alone or with tamoxifen or ICI 182,780 significantly inhibited MCF-7 tumor growth (Fig. 1). In this experiment, tumor volumes remained stable for nearly 100 days after estrogen withdrawal before progression ensued. In contrast, tumor volumes decreased slightly with tamoxifen and ICI 182,780 treatment, and tumor size remained stable for variable periods of time. A consistent observation was the delayed time to progression that was evident in mice treated with ICI 182,780. With estrogen withdrawal alone or with tamoxifen, tumors developed resistance, and progression was evident in all mice after 3-4 months of treatment (median, 97 and 104 days, respectively). However, the median time to progression was nearly twice as long with ICI 182,780, and the growth of some tumors remained controlled for extended periods of time (median, 200 days). In fact, two of the 10 tumors from ICI 182,780-treated mice still had not progressed after 11 months and one small tumor (4 mm diameter) completely regressed and did not reappear during the course of the experiment (data not shown).

Effect of ICI 182,780 on Tumorigenesis

ICI 182,780 also had a greater impact on tumor formation in mice in which drug treatments were begun on the day of tumor cell inoculation (Fig. 2). Tumors grew rapidly in mice treated with estrogen. Tumor growth was substantially delayed in mice treated with tamoxifen, but after 2 months, the growth rate increased. Tumors grew very slowly, or not at all, in mice treated with ICI 182,780—similar to the growth pattern observed in estrogen-deprived mice (12). By day 70, barely measurable tumors were present in the majority of mice. In another experiment (data not shown), three of six mice

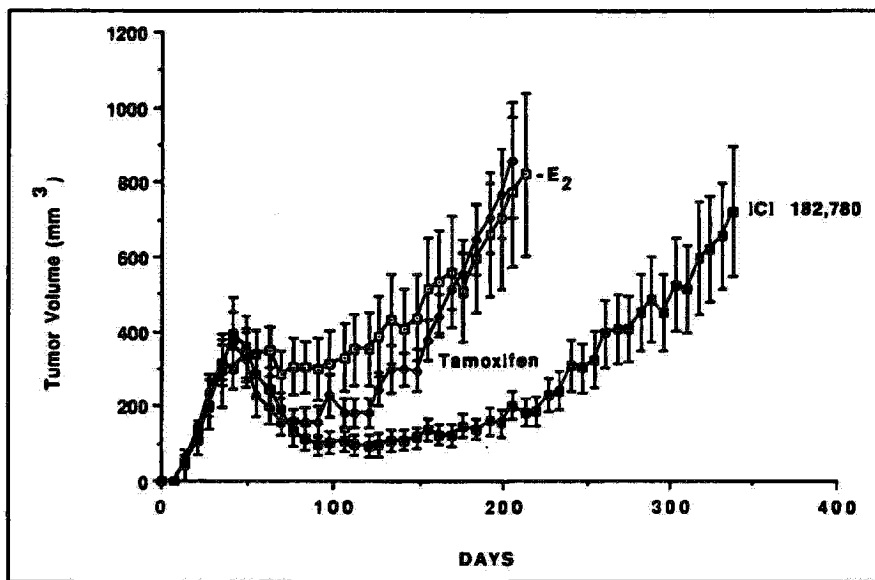


Fig. 1. Effects of estrogen (estradiol [E₂]) withdrawal, tamoxifen, and ICI 182,780 on MCF-7 tumor growth. Estrogen-supplemented mice were inoculated with MCF-7 cells. On day 36 when tumors had formed, mice were randomly allocated to treatment by withdrawal of estrogen (-E₂; -○-); withdrawal of estrogen and treatment with 500 μg tamoxifen given once a day, Monday through Friday (-◆-); or 5 mg ICI 182,780 given once a week (-■-). Tumor volumes were determined at the times shown. n = 10 mice per group; means ±SE.

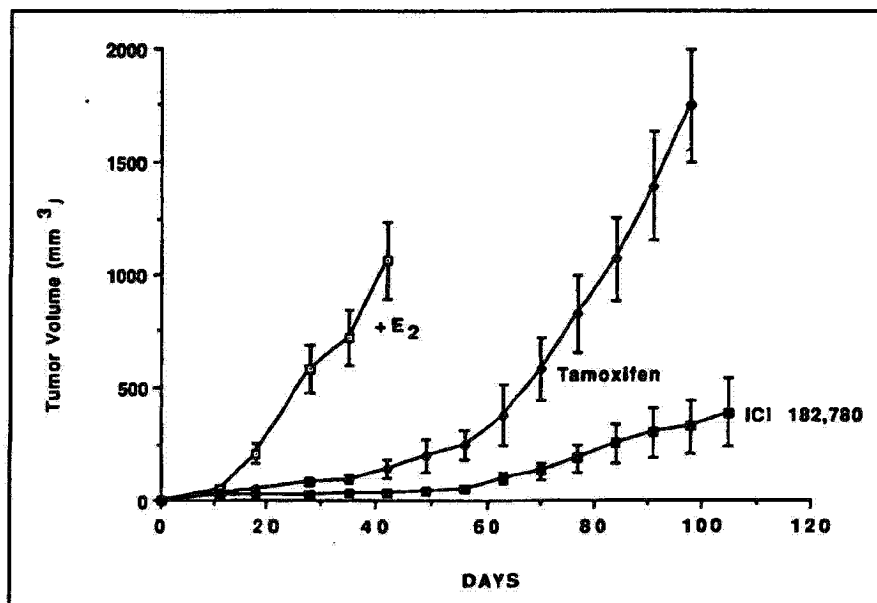


Fig. 2. Effect of estrogen, tamoxifen, and ICI 182,780 on MCF-7 tumorigenesis. Mice were inoculated with MCF-7 cells on day 0 and randomly allocated immediately to receive treatment with a 17β estradiol (E₂) pellet (+E₂; -○-); 500 μg tamoxifen given once a day, Monday through Friday (-◆-); or 5 mg ICI 182,780 given once a week (-■-). Tumor volumes were determined at the times shown. n = 8 mice per group; means ±SE.

treated with ICI 182,780 failed to grow measurable tumors even after 6 months of treatment.

ICI 182,780-Resistant Tumors

As indicated above, tumor resistance eventually occurred in most, but not all,

mice treated with ICI 182,780. This resistance was manifested by regrowth of tumors, usually after many months of treatment. To investigate the hormonal sensitivity of these resistant tumors, fragments of a tumor that had progressed after months of treatment with

ICI 182,780 were transplanted into new castrated, recipient mice that were then treated with estrogen, tamoxifen, ICI 182,780, tamoxifen plus ICI 182,780, or vehicle alone. This experiment was conducted five times with different tumor transplants, and a representative result is shown in Fig. 3. Transplanted tumor fragments grew well in all mice, even those treated with vehicle alone (-E₂), suggesting estrogen independence. However, in four of five experiments, tumor growth was slightly increased by estrogen treatment (+E₂), indicating continued sensitivity to the hormone. As expected, growth of these transplanted ICI 182,780-resistant tumors was also observed in recipient mice treated with ICI 182,780.

Interestingly, in four of the five experiments, treatment of recipient mice with tamoxifen alone or tamoxifen plus ICI 182,780 resulted in a slight retardation of tumor growth compared with treatment using vehicle alone or ICI 182,780 alone, although the observed differences in the individual experiments were modest and not statistically significant. A total of six of the 25 mice in these experiments showed slower tumor growth with tamoxifen treatment, indicating some heterogeneity among the transplanted fragments in response to tamoxifen. However, most mice resistant to ICI 182,780 showed cross-resistance to tamoxifen.

Resistance to ICI 182,780 was not due to a complete loss of tumor ER, although treatment with this drug reduced expression of both ER and PgR. Tumors harvested 4 weeks after initiating treatment with ICI 182,780 (ER = 37 ± 3 fmol/mg protein; PgR = 27 ± 7 fmol/mg protein) as well as those harvested at the time of resistance to ICI 182,780 (ER = 16 ± 4 fmol/mg protein; PgR = 17 ± 8 fmol/mg protein) expressed both ER and PgR at markedly reduced levels compared with estrogen-treated controls (ER = 208 ± 81 fmol/mg protein; PgR = 103 ± 20 fmol/mg protein) (*P* = .024).

Expression of two estrogen-responsive genes, pS2 and pLIV1, was also measured in these tumors (Table 1). pS2 and pLIV1 messenger RNA (mRNA) expression was reduced by 20%-74% in tumors from tamoxifen-treated mice (*P* = .013). It is interesting that pS2 and pLIV1 expression remained suppressed even after

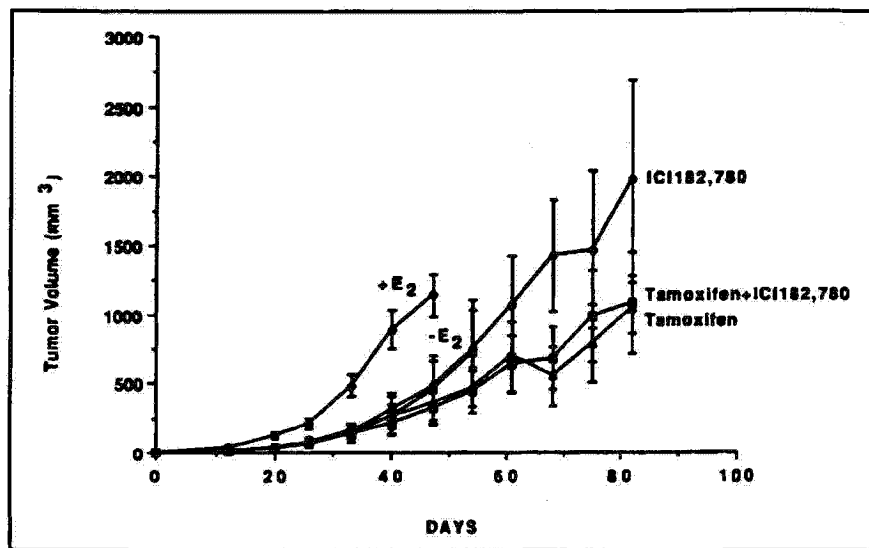


Fig. 3. Hormonal sensitivity of ICI 182,780-resistant tumors. Fragments of a tumor that had developed resistance in a mouse treated with ICI 182,780 were transplanted into new recipient female castrated nude mice. The recipient mice were then randomly allocated to receive vehicle alone (-E₂; -□-), an estradiol (E₂) pellet (+E₂; -◇-); tamoxifen alone (-▲-); ICI 182,780 alone (-◆-), or a combination of tamoxifen plus ICI 182,780 (-■-). Tumor volumes were calculated on the days shown. n = 6 mice per group; means ± SE.

evolution to tamoxifen resistance when the drug was stimulating tumor growth (3). In fact, pS2 was significantly lower in tamoxifen-resistant tumors than in tamoxifen-sensitive tumors ($P = .012$). This finding suggests that the agonist activity of tamoxifen, if responsible for tamoxifen-stimulated tumor growth, may be specific to genes associated with cell proliferation, while its antagonist activity continues to suppress the activity of genes less crucial for tumor survival. In contrast to the results obtained with tamoxifen, mRNA expression was nearly

abrogated by treatment with ICI 182,780 ($P < .004$), and there was no difference between sensitive and resistant tumors. It is unlikely, therefore, that ICI 182,780 resistance is caused by metabolic conversion of the drug to E₂, since expression of these estrogen-regulated genes remained low.

Discussion

Clinical data demonstrate that, in some patients, the current endocrine therapies for breast cancer result in temporary tumor regression or growth stabilization, followed by tumor regrowth, usually within 6-18 months of treatment. We have developed an experimental *in vivo* model that mimics this clinical scenario. Our data suggest that, in this experimental model system, ICI 182,780 possesses a greater ability to suppress estrogen-sensitive gene expression and greater antitumor activity than the partial estrogen antagonist tamoxifen. In addition, MCF-7 tumorigenesis was significantly delayed by ICI 182,780 when compared with tamoxifen. Moreover, a proportion of treated mice failed to develop tumors even after prolonged follow-up, an event rarely encountered in our experience treating mice with tamoxifen. ICI 182,780 also suppressed growth of established tumors for a significantly longer duration

than treatment by estrogen withdrawal alone or with tamoxifen. Finally, expression of the estrogen-regulated genes pS2 and pLIV1 was nearly abolished by treatment with ICI 182,780.

Previous reports by us and by other investigators (7,11,14-16) have also shown that the growth of tumors with acquired tamoxifen resistance can be inhibited or blocked by treatment with a pure antiestrogen such as ICI 182,780, suggesting that the pure antiestrogens work by a different mechanism of action than tamoxifen and other similar antiestrogens. Tamoxifen resistance in our model system is associated with drug-induced tumor growth stimulation that occurs after an initial period of growth suppression (3). The ability of tamoxifen alone to stimulate the growth of these tumors is less than that of estrogen. Interestingly, when combined with estrogen, tamoxifen can still inhibit estrogen-stimulated growth, indicating that it continues to possess both estrogen-agonist and antagonist properties (7). The increasingly dominant agonist properties of tamoxifen that develop after prolonged treatment can be blocked by the addition of pure antiestrogens (7,11). Evidence for tamoxifen-stimulated tumor growth as a mechanism for acquired tamoxifen resistance in patients has also been presented (5,6,17). On the basis of these preclinical studies, it has been suggested that treatment with ICI 182,780 might induce tumor regression in some patients who have developed tamoxifen resistance. One recent study (18) has shown that short-term ICI 182,780 treatment of patients who have ER-positive tumors causes statistically significant reductions in the Ki67 labeling index and reductions in the expression of estrogen-regulated genes such as PgR and pS2. In addition, remissions have now been reported in tamoxifen-resistant patients treated with this drug (19).

Although ICI 182,780 controls MCF-7 tumor growth for longer durations than tamoxifen, eventual resistance to this agent is common. MCF-7 tumors that progress after prolonged treatment are estrogen-independent (grow in the absence of estrogen supplementation) although they are still estrogen-sensitive (growth is enhanced by estrogen). The mechanisms by which resistance to

Table 1. Expression of estrogen-sensitive genes*

Treatment group (No. of blots analyzed)	Gene, relative mRNA level	
	pS2	pLIV1
Estrogen (4)	12.2 ± 0.7	12.2 ± 0.6
Tamoxifen-sensitive (5)	9.8 ± 0.5	6.0 ± 1.5
Tamoxifen-resistant (5)	3.2 ± 0.4	7.5 ± 1.8
ICI-sensitive (5)	0.3 ± 0.05	0 ± 0
ICI-resistant (8)	0.6 ± 0.23	2.3 ± 1.3

*mRNA expression was measured by northern blot analysis of total RNA extracted from MCF-7 tumors taken from mice treated with estrogen (controls), tamoxifen for 3 weeks (tamoxifen-sensitive), tamoxifen until the time of tumor progression (tamoxifen-resistant), ICI 182,780 for 4 weeks (ICI-sensitive), or ICI 182,780 until tumor progression (ICI-resistant). Values shown are the means ± SE of scanning densitometry units corrected for RNA loading.

ICI 182,780 develops are not clear, but reduced levels of ER and reduced expression of estrogen-regulated genes (compared with tamoxifen-sensitive or with tamoxifen-resistant tumors) are evident. Reduced ER levels have also been seen in tumors from patients treated with ICI 182,780, in cultured breast cancer cells, and in mouse uterine tissue following the administration of the prototype pure antiestrogen ICI 164,384 (18-20). Other data suggest that the pure antiestrogen-ER complex may be more fragile and more susceptible to receptor degradative pathways (16). In contrast, ER levels are high in tamoxifen-resistant tumors obtained with our model system (3). On the basis of our data, we would predict that most patients with ICI 182,780-resistant tumors would not respond well to subsequent treatment with tamoxifen.

Even if pure antiestrogens are shown to have superior antitumor activity in women with breast cancer, they may not be the optimal antiestrogens for clinical use. The estrogenic properties of tamoxifen in bone and on blood lipids may help to reduce bone loss and prevent cardiovascular disease, which are added benefits when treating breast cancer patients for prolonged periods after surgery for primary tumors or for breast cancer prevention (21,22). The effect of ICI 182,780 on these parameters is not yet known, but it might be deleterious given its lack of estrogenic qualities. However, treatment with ICI 182,780 might not be associated with the increased risk of endometrial cancer recently attributed to tamoxifen (23). Further clinical study of pure antiestrogens in tamoxifen-resistant and in tamoxifen-naive patients is clearly indicated.

References

- (1) Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group [see comment citations in Medline]. *Lancet* 339:1-15, 71-85, 1992
- (2) Encarnacion CA, Ciocca DR, McGuire WL, et al: Measurement of steroid hormone receptors in breast cancer patients on tamoxifen. *Breast Cancer Res Treat* 26:237-246, 1993
- (3) Osborne CK, Coronado E, Allred DC, et al: Acquired tamoxifen resistance: correlation with reduced breast tumor levels of tamoxifen and isomerization of trans-4-hydroxytamoxifen [see comment citation in Medline]. *J Natl Cancer Inst* 83:1477-1482, 1991
- (4) Gottardis MM, Jordan VC: Development of tamoxifen-stimulated growth of MCF-7 tumors in athymic mice after long-term antiestrogen administration. *Cancer Res* 48:5183-5187, 1988
- (5) Pritchard KI, Thomson DB, Myers RE, et al: Tamoxifen therapy in premenopausal patients with metastatic breast cancer. *Cancer Treat Rep* 64:787-796, 1980
- (6) Hoogstraten B, Gad-el-Mawla N, Maloney TR, et al: Combined modality therapy for first recurrence of breast cancer. A Southwest Oncology Group Study. *Cancer* 54:2248-2256, 1984
- (7) Osborne CK, Jarman M, McCague R, et al: The importance of tamoxifen metabolism in tamoxifen-stimulated breast tumor growth. *Cancer Chemother Pharmacol* 34:89-95, 1994
- (8) Wakeling AE, Bowler J: Novel antioestrogens without partial agonist activity. *J Steroid Biochem* 31:645-653, 1988
- (9) Wakeling AE, Dukes M, Bowler J: A potent specific pure antiestrogen with clinical potential. *Cancer Res* 51:3867-3873, 1991
- (10) Wakeling AE: Steroidal pure antiestrogens. In *Regulatory Mechanisms in Breast Cancer* (Lippman M, Dickson R, eds). Boston: Kluwer Acad Publ, 1991, pp 239-257
- (11) Gottardis MM, Jiang SY, Jeng MH, et al: Inhibition of tamoxifen-stimulated growth of an MCF-7 tumor variant in athymic mice by novel steroidal antiestrogens. *Cancer Res* 49:4090-4093, 1989
- (12) Osborne CK, Hobbs K, Clark GM: Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. *Cancer Res* 45:584-590, 1985
- (13) Manning DL, McClelland RA, Gee JM, et al: The role of four oestrogen-responsive genes, pLIV1, pS2, pSYD3 and pSYD8, in predicting responsiveness to endocrine therapy in primary breast cancer. *Eur J Cancer* 29A:1462-1468, 1993
- (14) Br nner N, Frandsen TL, Holst-Hansen C, et al: MCF7/LCC2: a 4-hydroxytamoxifen resistant human breast cancer variant that retains sensitivity to the steroidal antiestrogen ICI 182,780. *Cancer Res* 53:3229-3232, 1993
- (15) Lykkesfeldt AE, Sorensen EK: Effect of estrogen and antiestrogens on cell proliferation and synthesis of secreted proteins in the human breast cancer cell lines MCF-7 and a tamoxifen resistant variant subline, AL-1. *Acta Oncol* 31:131-138, 1992
- (16) Parker MG: Action of pure antiestrogens in inhibiting estrogen receptor action. *Breast Cancer Res Treat* 26:131-137, 1993
- (17) Wiebe VJ, Osborne CK, Fuqua SA, et al: Tamoxifen resistance in breast cancer. *Crit Rev Oncol Hematol* 14:173-188, 1993
- (18) DeFriend DJ, Howell A, Nicholson RI, et al: Investigation of a new pure antiestrogen (ICI 182780) in women with primary breast cancer. *Cancer Res* 54:408-414, 1994
- (19) DeFriend DJ, Blamey RW, Robertson JF, et al: Response to the pure antiestrogen ICI 182780 after tamoxifen failure in advanced breast cancer. *Breast Cancer Res Treat* 17:136, 1993
- (20) Gibson MK, Nemmers LA, Beckman WC Jr, et al: The mechanism of ICI 164,384 antiestrogenicity involves rapid loss of estrogen receptor in uterine tissue. *Endocrinology* 129:2000-2010, 1991
- (21) Love RR, Mazess RB, Barden HW, et al: Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer [see comment citation in Medline]. *N Engl J Med* 326:852-856, 1992
- (22) Love RR, Newcombe PA, Wiebe DA, et al: Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer [see comment citation in Medline]. *J Natl Cancer Inst* 82:1327-1332, 1990
- (23) Fornander T, Hellstr m AC, Moberger B: Descriptive clinicopathologic study of 17 patients with endometrial cancer during or after adjuvant tamoxifen in early breast cancer. *J Natl Cancer Inst* 85:1850-1855, 1993

Notes

Supported in part by Public Health Service grants CA30251, CA30195, CA58183, and CA54174 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Manuscript received September 19, 1994; revised December 27, 1994; accepted March 6, 1995.

***Need the latest information on
clinical trials for HIV and AIDS?***

***Call the AIDS Clinical Trials
Information Service:***

1-800-TRIALS-A

(1-800-874-2572) Monday through Friday, 9 a.m. to 7 p.m. eastern time.