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Investigation of a New Pure Antiestrogen (ICI 182780) in Women with Primary Breast Cancer[1]

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ABSTRACT

We have conducted a clinical trial of a novel pure antiestrogen, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl]estra-1,3,5,(10)-triene-3,17β-diol (ICI 182780), to assess its tolerance, pharmacokinetics, and short term biological effects in women with primary breast cancer. Fifty-six patients were randomized to either a control group (n = 19), in which they received no preoperative treatment, or a treatment group (n = 37), in which they received daily i.m. injections of ICI 182780 at doses of 6 mg (n = 21) or 18 mg (n = 16) for 7 days prior to primary breast surgery. Serum drug concentrations, gonadotropin levels, and sex hormone-binding globulin levels were measured during the study period by radioimmunoassay. Expression of estrogen receptors (ER), progesterone receptors, the estrogen-induced protein pS2, and the cell proliferation-related antigen Ki67 was determined immunocytochemically in pre- and poststudy tumor samples.

Treatment with ICI 182780 caused no serious drug-related adverse events and had no effect on serum gonadotropin or sex hormone-binding globulin levels. Minor adverse events occurred in 5 patients receiving the 6-mg dose and 3 patients receiving the 18-mg dose. The serum concentration of ICI 182780 was dose dependent but showed variation between individuals. There was evidence of an approximately 3-fold drug accumulation over the short treatment period but steady state levels were not reached by the end of the 7 days. In patients with ER-positive tumors, treatment with ICI 182780 was associated with significant reductions in the tumor expression of ER (median ER index, 0.72 before *versus* 0.02 after treatment; $P < 0.001$), progesterone receptor (median progesterone receptor index, 0.50 before *versus* 0.01 after treatment; $P < 0.05$), and Ki67 (median Ki67 labeling index, 3.2 before *versus* 1.1 after treatment; $P < 0.05$). Treatment with ICI 182780 also resulted in a significant reduction in pS2 expression ($P < 0.05$) but this appeared unrelated to tumor ER status.

In conclusion, ICI 182780 was well tolerated after short term administration and produced demonstrable antiestrogenic effects in human breast tumors *in vivo*, without showing evidence of agonist activity. These properties identify ICI 182780 as a candidate agent with which to evaluate whether a pure estrogen antagonist offers any additional benefit in the treatment of human breast cancer over conventional nonsteroidal antiestrogens, typified by tamoxifen, which exhibit variable degrees of agonist activity.

INTRODUCTION

Estrogen acts as an endocrine growth factor for at least one third of human breast cancers. Since endocrine therapy for breast cancer was initiated in 1896 by George Beatson (1), a variety of treatment modalities have been introduced with the aim of preventing estrogen-mediated tumor growth (2). Antiestrogens achieve this directly by competing with estradiol for binding to the estrogen receptor, through which the intracellular effects of estrogens are mediated (3).

Conventional nonsteroidal antiestrogens, typified by tamoxifen, compete efficiently for estrogen receptor binding but form a complex with the receptor which retains some transcriptional activity (4). Consequently, tamoxifen exhibits a range of biological activity from full estrogen antagonism to partial agonism, depending upon the species, target tissue, and target gene response studied (5, 6).

Although some of the clinical effects of the agonist (estrogenic) activity of tamoxifen, such as reduction of serum cholesterol (7–9) and maintenance of bone mineral density (10, 11), may benefit patients receiving long term adjuvant treatment, others may be detrimental. Tamoxifen stimulates endometrial growth in animals and its use as adjuvant therapy in women with breast cancer has been found to be associated with an increased incidence of endometrial carcinoma in some but not all studies (12–14). Moreover, there is evidence, from studies using an animal model of human breast cancer and from clinical observations of tumor responses following tamoxifen withdrawal at the time of disease progression in patients with advanced breast cancer, which suggests that the agonist activity of tamoxifen may eventually stimulate breast tumor growth and be a cause of some treatment failures (15–17). It is possible, therefore, that the therapeutic efficacy of tamoxifen and other conventional nonsteroidal antiestrogens may be compromised, in comparison with that which might be achieved by complete estrogen antagonism.

ICI 182780,[3] a 7α-alkylsulfinyl analogue of estradiol, is a novel steroidal antiestrogen representative of a new class of estrogen antagonists, which differ significantly in both chemical structure and pharmacology from the conventional agents exemplified by tamoxifen. ICI 182780 is a potent pure antiestrogen; it has been shown to exhibit excellent growth-inhibitory effects in animal and *in vitro* models of human breast cancer and appears to have no demonstrable intrinsic agonist activity (18). We have conducted a clinical trial to investigate the tolerance, pharmacokinetics, and short term biological effects of seven daily doses of a short-acting formulation of ICI 182780 in postmenopausal women prior to surgery for primary breast cancer.

PATIENTS AND METHODS

Fifty-six postmenopausal women with primary breast cancer participated in the study between October 1991 and November 1992. Patients were considered eligible for the study if they were postmenopausal and had histologically or cytologically verified primary breast cancer with no overt evidence of metastases. Patients were excluded from the study if they were older than 75 years, or if they had a history of hepatic or renal impairment, insulin-dependent diabetes mellitus, or other conditions known to interfere with drug pharmacokinetics or steroid metabolism. Patients were also ineligible for the study if they weighed less than 40 kg or more than 110 kg, or if their baseline hematology, clinical chemistry, or urinalysis results were outside the range of normal values and considered to be clinically significant.

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[3] The abbreviations used are: ICI 182780, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5,(10)-triene-3,17β-diol; ER, estrogen receptor(s); PgR, progesterone receptor(s); SHBG, sex hormone-binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LI, labeling index; RT, room temperature; PBS, phosphate-buffered saline; PAP, peroxidase-antiperoxidase; DAB, diaminobenzidine tetrahydrochloride; ICA, immunochemical assay.

Study Design. After giving informed consent, patients were randomized to either a control group (n = 19), in which they received no preoperative treatment, or a treatment group (n = 37), in which they received daily i.m. injections of ICI 182780 at doses of 6 mg (n = 21) or 18 mg (n = 16) for 7 days prior to surgery. Two additional patients, randomized to receive ICI 182780, were withdrawn from the study before starting treatment because of protocol violations. The study was approved by the ethical committees of the three participating clinical units.

All patients randomized to receive ICI 182780 were visited on each treatment day by an investigator, who administered the trial medication and monitored local and systemic tolerance. ICI 182780 was administered by i.m. injection into the buttock as a short-acting formulation, containing 20 mg/ml drug in a propylene glycol-based vehicle. Blood samples were taken before and after the study period for measurement of complete blood count, prothrombin time, clinical biochemistry, and serum levels of gonadotropins and SHBG. During the study period additional blood samples were taken, immediately prior to drug administration, from patients receiving treatment with ICI 182780, for measurement of serum drug levels.

Wherever possible, pretreatment tumor samples were obtained by multiple needle core biopsies. Post-treatment tumor samples were obtained from the operative specimen at the time of primary breast surgery. Tumor samples were divided between immediate snap freezing in liquid nitrogen, for assay of steroid hormone receptors and the proliferation-related antigen Ki67, and routine fixation in formalin and paraffin wax embedding, for histological assessment and assay of the estrogen-regulated protein pS2.

Measurement of Serum Gonadotropin and Sex Hormone-binding Globulin Levels. Serum levels of gonadotropins (FSH and LH) and SHBG were measured by radioimmunoassay in the Regional Radioimmunoassay Laboratory of the University Hospital of South Manchester.

Measurement of Serum Drug Levels. The concentration of ICI 182780 in serum samples was determined by radioimmunoassay after solvent extraction. Serum samples were added to diethyl ether:hexane (1:1, v/v) and vortex mixed for 15 min. After separation the extract was added to a CN Bond-elut column which had been preconditioned with methanol and ether:hexane (1:1, v/v). After washing of the column with dichloromethane:hexane (1:1, v/v), ICI 182780 was eluted with methanol, which was reduced to dryness under nitrogen at 50°C. The residue was reconstituted in gelatin PBS containing 0.05% Nonidet P-40, to which [^{3}H]ICI 182780 and antisera were then added. The samples were incubated at 4°C for 21–24 h, after which a 1% charcoal suspension was added for 20 min before centrifugation of the samples at 2200 rpm for 10 min at 4°C. The resulting supernatant was then added to Beckman protein scintillation cocktail and counted for tritium. Concentrations of ICI 182780 were determined from a calibration curve constructed from calibration standards. The performance data for the assay were as follows: limit of detection, 0.5–1.0 ng/ml; intraassay coefficient of variation, 13%; interassay coefficient of variation, 15%.

Immunocytochemistry. The expression of steroid hormone receptors, the estrogen-regulated protein pS2, and the cell proliferation-related antigen Ki67 was determined immunocytochemically in pre- and post-treatment tumor samples.

Estrogen and Progesterone Receptor Expression. ER and PgR expression was measured in snap-frozen tumor samples using commercially available kits (ER ICA and PgR ICA; Abbott Laboratories, Diagnostics Division, North Chicago, IL), which use a sensitive PAP technique for visualization of the receptors. Details of the staining procedure for ER and validity of the results have been described previously (19). An identical procedure for staining for PgR was followed, substituting the anti-PgR antibody supplied in the Abbott PgR ICA kit. Briefly, cryostat sections (5 μm) were mounted on slides treated with tissue adhesive and were fixed in 3.7% formaldehyde in 0.01 M PBS for 15 min, followed by immersion in cold (−20°C) baths of methanol (5 min) and acetone (3 min). The slides were then rinsed in PBS and incubated with normal goat serum (blocking agent), to prevent nonspecific antibody binding, prior to application of the primary antibody for 30 min at RT. Binding of the primary antibody was revealed by the indirect PAP procedure (20). This technique uses successive applications of goat anti-rat IgG bridging antibody for 30 min at RT, rat PAP complexes for 30 min at RT, and finally a solution of the chromogenic substrate DAB, containing 0.06% (v/v) hydrogen peroxide, for 6 min. Slides were counterstained with hematoxylin, dehydrated, cleared, and mounted for examination by light microscopy.

Ki67 Immunoreactivity. Ki67 immunoreactivity was measured as described previously (21). Briefly, cryostat sections (5 μm) were air dried overnight, fixed in acetone at −20°C, and allowed to air dry for an additional 2 h. Endogenous peroxidase activity was blocked by incubation with 0.3% (v/v) hydrogen peroxide in PBS for 15 min at RT. Slides were then washed in PBS and incubated with 10% (v/v) normal rabbit serum in PBS before application of the mouse monoclonal antibody Ki67 (Dako Laboratories, Glostrup, Denmark) at a 1:40 dilution for 45 min at RT. Binding of the primary antibody was visualized by successive applications of a rabbit anti-mouse bridging antibody (1:25 dilution in 10%, v/v, decomplemented human serum in PBS) for 30 min at RT, mouse PAP complexes (1:100 in PBS) for 30 min at RT, and finally the chromogenic substrate DAB. Slides were counterstained with hematoxylin, dehydrated, cleared, and mounted for examination by light microscopy.

pS2 Expression. Expression of pS2 was measured in formalin-fixed, paraffin-embedded, tumor specimens. Paraffin sections (5 μm) were dewaxed in xylene and rehydrated in stepwise dilutions of ethanol. Endogenous peroxidase activity was blocked with 10% (v/v) hydrogen peroxide in PBS for 15 min, after which the sections were rinsed in PBS and incubated with normal rabbit serum for 5 min prior to incubation with a mouse monoclonal anti-pS2 antibody (22), at a dilution of 1:400, for 2 h at RT. Binding of the primary antibody was visualized by successive applications of biotinylated rabbit anti-mouse immunoglobulins (1:200) for 45 min at RT, streptavidin-horseradish peroxidase complex for 30 min, and finally the chromogenic substrate DAB. Slides were counterstained with hematoxylin, dehydrated, cleared, and mounted for examination by light microscopy.

Evaluation of Staining. In each assay, all specimens had a negative control slide (no primary antibody) of an adjacent section to assess the degree of nonspecific staining; in addition, a positive control slide of a section from a tumor known to express the appropriate antigen was also included. The specimens were evaluated under a light microscope initially at an ocular magnification of ×10, to permit localization of the malignant areas within each section and assessment of the heterogeneity of immunostaining within the tumor components. All subsequent evaluations were performed using an ocular magnification of ×40.

Estrogen and progesterone receptor expression was assessed semiquantitatively by determining the percentage of tumor cells stained by the primary antibody (minimum of 1000 tumor cells evaluated) and assessing the intensity of staining using a score of 0 to 3, corresponding to negative, weak, intermediate, and strong staining intensities. The percentage of tumor cells in each of these categories was used to calculate an index (I) for each tumor, as follows: I = [(% cells showing an intensity value of 1 × 1) + (% cells showing an intensity value of 2 × 2) + (% cells showing an intensity value of 3 × 3)]/100.

Expression of pS2 and the Ki67-related antigen was evaluated by determining the percentage of tumor cells stained by the primary antibody in multiple representative fields (minimum of 1000 tumor cells evaluated). Assessment of staining intensity was not attempted.

Definition of Steroid Receptor Status. For the purposes of this study, tumors were classed as ER/PgR positive or negative according to receptor expression in the prestudy tumor sample. Tumors with an ER/PgR index of ≥0.05 were considered receptor positive. In cases where ER/PgR expression was not evaluable in a prestudy tumor sample, the receptor status was classed as unknown.

Statistical Analysis. With the exception of serum drug level data, the results have been analyzed using nonparametric statistics. Comparison of pre- and post-treatment samples within specified patient groups has been performed using Wilcoxon's matched-pairs signed-rank test. Differences between specified patient groups have been analyzed using the Mann-Whitney U test (for analyses involving two specified groups) or the Kruskal-Wallis test (for analyses of three or more specified groups). The null hypothesis was rejected at a probability level (P) of ≤0.05.

RESULTS

Patient Demography. The details of patients in the three treatment groups are summarized in Table 1. The three groups were well matched with respect to patient age, tumor size, T stage, and axillary lymph node status. However, the group treated with ICI 182780 at the

Table 1 *Patient characteristics*

	Treatment group		
	Control	ICI 182780, 6 mg/day	ICI 182780, 18 mg/day
n	19	21	16
Age (years)			
Median	61	66	63
Range	51–74	49–74	54–71
Tumor size (cm)			
Median	2.2	2.5	2.3
Range	0.5–5.5	1.5–5.0	0.7–4.5
Tumor T stage			
T_2	18 (95%)	17 (81%)	15 (94%)
T_3	1 (5%)	4 (19%)	1 (6%)
Histological type			
ILC	1 (6%)	2 (10%)	4 (25%)
IDC	17 (94%)	18 (90%)	12 (75%)
Histological grade			
I	6 (35%)	2 (11%)	5 (42%)
II	4 (24%)	5 (28%)	5 (42%)
III	7 (41%)	11 (61%)	2 (16%)
Axillary lymph node status			
Positive	10 (59%)	9 (45%)	8 (53%)
Negative	7 (41%)	11 (55%)	7 (47%)
Not known	2	1	1
ER status			
Positive	10 (63%)	6 (40%)	12 (86%)
Negative	6 (37%)	9 (60%)	2 (14%)
Not known	3	6	2
PgR status			
Positive	7 (44%)	4 (25%)	9 (64%)
Negative	9 (56%)	12 (75%)	5 (36%)
Not known	3	5	2

6-mg dose contained a greater proportion of histological grade III, steroid receptor-negative tumors than did the control and 18-mg dose groups.

Drug Tolerability. Treatment with ICI 182780 caused no serious drug-related adverse events, and no patients were withdrawn from the study because of drug toxicity. Minor systemic adverse events were reported by 5 patients receiving the 6-mg dose and 3 patients receiving the 18-mg dose (Table 2). The majority of these events were considered to be unrelated to the trial treatment, in the opinion of the investigating clinician. In addition, the short-acting formulation of ICI 182780 used in this study was well tolerated locally at the site of injection, with only 1 patient developing any significant local reaction.

No clinically significant changes in the laboratory parameters measured (biochemical profile, complete blood count, prothrombin time, and urinalysis) were seen in patients receiving ICI 182780.

One death occurred among the patients randomized to receive ICI 182780. The patient received 7 days of treatment at the 18-mg dose prior to undergoing wide local excision of her primary breast tumor, from which she made an uneventful postoperative recovery. Because of concern about satisfactory tumor clearance, the patient underwent a second operation 3 weeks later (*i.e.*, 21 days after completing treatment with ICI 182780). The surgery was again uneventful but the patient collapsed 2 days postoperatively with a fatal pulmonary embolus. There had been no changes in the patient's platelet count or coagulation parameters following treatment with ICI 182780 and in the view of the surgeon concerned this event was unrelated to the use of the drug.

Table 2 *Adverse events reported for patients treated with ICI 182780*

Dose (mg)	Adverse event	Duration	Severity	Relationship to drug
6	Headache	6 h	Mild	No opinion
6	Dyspepsia		Moderate	Probably unrelated
6	Headache	3 h	Moderate	Probably unrelated
6	Headache	5 h	Mild	Probably unrelated
6	Headache	3 h	Mild	Probably unrelated
	Hypertension	16 h	Moderate	Definitely unrelated
18	Facial swelling	2.5 h	Mild	No opinion
	Vaginal spotting		Mild	No opinion
	Hyperglycemia		Moderate	Probably unrelated
18	Headache	5 h	Moderate	Probably related
18	Headache	1 day	Moderate	Definitely unrelated

Pharmacokinetics. The mean serum drug levels *versus* time achieved in patients receiving the 6-mg and 18-mg daily doses of ICI 182780 are shown in Fig. 1. The serum concentration of ICI 182780 was dose dependent but showed some variation between individual patients. An approximately 3-fold drug accumulation was seen over the 7-day dosing period, although steady state serum levels were not reached by the end of the study.

Endocrinology. Paired pre- and poststudy endocrinology measurements were available for 33 (59%) of the 56 subjects. No significant changes in the serum levels of LH, FSH, or SHBG were seen in any of the treatment groups during the study period (data not shown).

Steroid Receptor Expression. Paired pre- and poststudy measurements of ER and PgR expression were available for 45 (80%) of the 56 subjects, of which 28 (62%) were ER positive and 20 (45%) were PgR positive, as measured in prestudy tumor samples. Among patients receiving the 6-mg dose of ICI 182780, 40% had ER-positive tumors, compared to 86% of patients receiving the 18-mg dose and 63% of controls. Although these differences were not statistically significant, they could potentially have affected the distribution of other ER-related parameters within the treatment groups.

Among the ER-positive tumors, there were no significant differences between the treatment groups with respect to the prestudy ER or PgR values. Of the 16 tumors identified as ER negative in prestudy needle core biopsy specimens, 2 were identified as weakly ER positive in poststudy samples (ER index values of 0.05 and 0.15) and 2 were identified as PgR positive (PgR index values of 0.55 and 0.70).

ER Expression. Among the patients in the control group with ER-positive tumors, there was a significant tendency for the prestudy needle core biopsy sample to underestimate the level of ER expression, as measured in the poststudy surgical tumor specimen (median pre- and poststudy ER indices of 0.50 and 0.95, respectively; $P < 0.05$, Wilcoxon matched-pairs signed-rank test) (Fig. 2*A*). Despite this find-

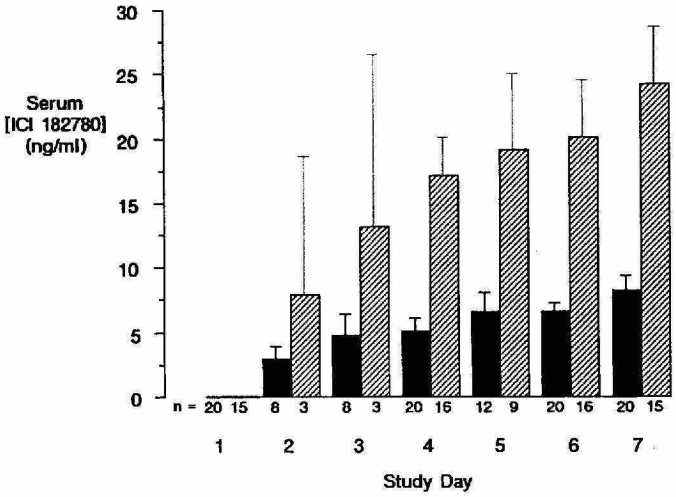

Fig. 1. Serum concentrations of ICI 182780 during the 7-day treatment period. *Columns*, means; *bars*, 2 SEM. Patients received daily i.m. injections of 6 mg (■) or 18 mg (▨) ICI 182780. *Numbers below columns*, number of drug level measurements available on individual treatment days.

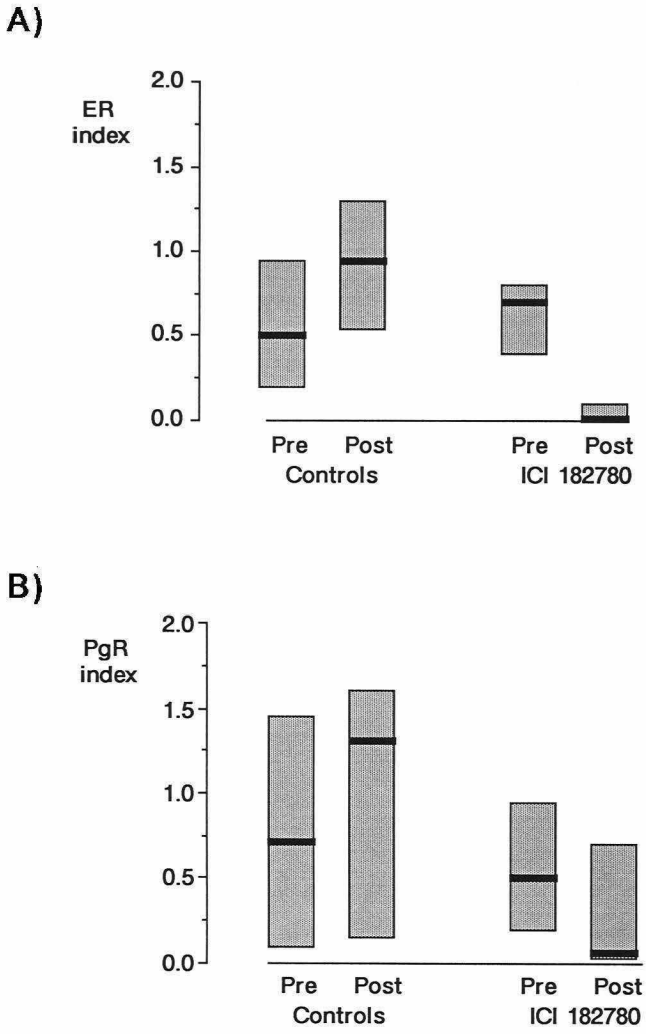

Fig. 2. Pre- and poststudy tumor ER expression (*A*) and PgR expression (*B*) in controls and treated patients with ER-positive tumors. *Columns*, interquartile ranges; *bars*, medians.

ing, treatment with ICI 182780 caused a significant reduction in the median ER index of ER-positive tumors, from 0.73 before treatment to 0.02 after treatment ($P < 0.001$). When the patients receiving ICI 182780 were further subdivided according to the dose received, significant reductions in the median ER indices of ER-positive tumors were evident at both the 6-mg and 18-mg dose levels, decreasing from 0.60 to 0.06 in the 6-mg dose group ($P < 0.05$) and from 0.73 to 0.01 in the 18-mg group ($P < 0.01$).

PgR Expression. There was a similar, although nonsignificant, tendency for the prestudy needle core biopsy specimen to underestimate the expression of PgR in untreated controls with ER-positive tumors (median pre- and poststudy PgR indices of 0.7 and 1.3, respectively; $P = 0.07$, Wilcoxon matched-pairs signed-rank test) (Fig. 2*B*). In patients with ER-positive tumors treated with ICI 182780, there was a significant reduction in the median PgR index, from 0.50 before treatment to 0.01 after treatment ($P < 0.05$). The reduction in PgR expression did not achieve statistical significance when the effects of individual dose levels of ICI 182780 were analyzed separately.

pS2 Expression. Paired pre- and poststudy measurements of pS2 were available for 37 (66%) of the 56 patients (Fig. 3). There were no significant differences between the treatment groups with respect to the prestudy level of pS2 expression (median pS2 expression of 24% for controls *versus* 7% for treatment group; $P = 0.76$, Mann-Whitney *U* test) and there was no significant change in pS2 expression between the pre- and poststudy tumor samples in the control group. Treatment with ICI 182780 resulted in a significant reduction in pS2 levels (median pre- and post-treatment pS2 expression of 7% and 1%, respectively; $P < 0.05$, Wilcoxon matched-pairs signed-rank test). However, there was no relationship with increasing drug dose and no difference according to tumor ER status prior to treatment.

Ki67 Labeling Index. Paired pre- and post-treatment measurements of the Ki67 LI were available for 44 (79%) of the 56 subjects (Fig. 4). There were no significant differences between the treatment groups with respect to the prestudy tumor Ki67 LI. Among invasive ductal carcinomas, there was a trend for the pretreatment Ki67 LI to increase with increasing histological grade. This increase was significant between grades I and III (median Ki67 LI of 1.8% *versus* 5.5%, respectively; $P < 0.05$, Mann-Whitney *U* test). In addition, the median Ki67 LI was higher among ER-negative than ER-positive tumors (6.0% *versus* 3.6%) but this difference was not statistically significant ($P = 0.7$; Mann-Whitney *U* test).

Following treatment with ICI 182780 there was a significant reduction in the median Ki67 LI of ER-positive tumors, from 3.2% before treatment to 1.1% after treatment ($P < 0.05$%; Wilcoxon matched-pairs signed-rank test). However, there were no significant changes in the Ki67 LI of ER-negative or control tumors. When the ER-positive tumors were further subdivided according to the dose of ICI 182780 received, a significant reduction in the Ki67 LI was evident only with patients treated with the 18-mg daily dose, for whom the median Ki67 LI decreased from 4.0% before treatment to 1.1% after treatment ($P < 0.05$). With patients receiving the 6-mg dose

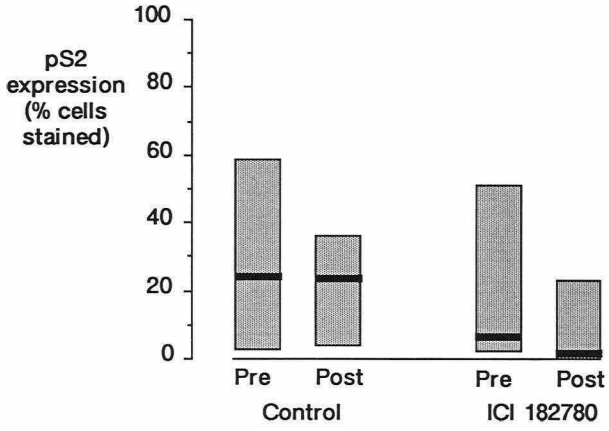

Fig. 3. Pre- and poststudy tumor pS2 expression in controls and treated patients irrespective of ER status. *Columns*, interquartile ranges; *bars*, medians.

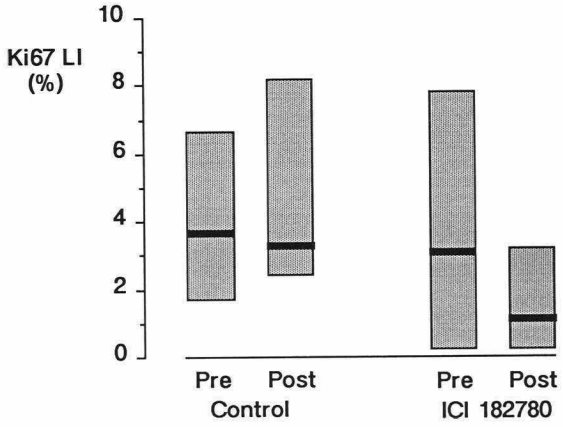

Fig. 4. Pre- and poststudy tumor Ki67 LI in controls and treated patients with ER-positive tumors. *Columns*, interquartile ranges; *bars*, medians.

there was a smaller and nonsignificant decrease in the Ki67 LI, from 1.8% before treatment to 0.8% after treatment ($P = 0.3$).

DISCUSSION

This study is the first investigation of short term administration of ICI 182780 to women with primary breast cancer. We have found the short-acting formulation of ICI 182780 used in this study to be well tolerated at the two doses studied over a 7-day treatment period and to be associated with minimal local or systemic toxicity. These findings are in agreement with earlier studies in healthy human volunteers, who received single doses of 2–60 mg of the short-acting formulation of ICI 182780 by i.m. injection.[4] The two drug dose levels used in the present study were selected to be within the expected dose range required to produce antiestrogenic efficacy and were derived from the results of previous studies in primates.[4]

The most frequent adverse event observed in the present study was mild to moderate headache. The absence of comparable adverse event data from the control group makes it difficult to assess whether these events were drug related. This difficulty is compounded because the patient population in this study included women who had only recently been informed of their diagnosis of breast cancer and of their need for breast surgery and who might therefore be expected to exhibit a number of stress-related symptoms, including headaches. A daily placebo injection would have been a better comparison for the control group but was rejected prior to the study was started, on ethical grounds.

The adverse events observed in this study were mostly considered to be unrelated to administration of ICI 182780, in the opinion of the attending physician. However, similar adverse events, particularly headaches, were also reported in healthy volunteer studies with the same short-acting drug formulation.[4] It is possible, therefore, that these adverse events were related either to the drug itself or to the propylene glycol-based vehicle used in the short-acting formulation. This question will be addressed in future studies which are planned with a different, long-acting, formulation of ICI 182780 contained in a castor oil-based vehicle.

More serious toxicity has not been seen in any of the previous human volunteer or animal studies, and in particular there has been no evidence of altered coagulation or thrombogenicity after treatment with ICI 182780, even when it was administered at much higher doses than were used in the present study. It therefore seems unlikely that the fatal pulmonary embolus that occurred in a patient who received the 18-mg dose of ICI 182780 was drug related. However, since such complications are rare following breast operations, it is important that blood thrombogenicity be appropriately monitored in future studies with ICI 182780. Conventional nonsteroidal antiestrogens like tamoxifen do have a potentially thrombogenic effect via reduction of antithrombin III levels (7, 23–25), although this effect has not been shown to be clinically significant and has been attributed to the agonist activity of these drugs, which does not appear to be shared by ICI 182780 (18). However, the pure antagonist profile of activity of ICI 182780 in human subjects will need to be confirmed in future clinical studies.

Measurement of serum drug levels in this study showed that there was some interpatient variation in the serum concentration of ICI 182780 achieved. The mean pre-dose concentration was dose dependent and increased over the 7-day dosing period, showing an approximately 3-fold accumulation. However, the serum concentrations of ICI 182780 failed to reach a plateau by day 7, indicating that steady state drug levels were not achieved by the end of the study period. Animal studies have demonstrated considerable interspecies variability in the elimination half-life of ICI 182780, with a half-life of about 4 h in rats and 2 days in dogs after i.m. administration.[4]

No attempt was made in the present study to assess the distribution and metabolism of ICI 182780. Animal studies have shown that the drug is rapidly released from the injection site and is distributed to most tissues, with the exception of the brain and spinal cord, within 2 h after i.m. dosing. All species studied produce a large number of metabolites, which have yet to be identified. The principal route of drug elimination appears to be in bile.[4]

The maximum serum drug levels achieved in this short term study were on the order of 10 nM, which is 100 times lower than the typical serum level of tamoxifen achieved with standard doses of 20 mg p.o. daily. However, because the ER binding affinity of ICI 182780 is approximately 100 times greater than that of tamoxifen (18), it is likely that ICI 182780 will be at least as effective as an estrogen antagonist at these lower serum levels.

In addition to studying the tolerance and pharmacokinetics of ICI 182780, we have also looked for evidence of biological activity during short term administration, either in primary breast tumors or at remote sites. The absence of any changes in the serum gonadotropin or SHBG levels following treatment with ICI 182780 must be interpreted with caution, firstly because of the short treatment period and secondly because, due to sampling omissions by one participating clinical center, data were available for only 59% of the subjects. It is unlikely that any antiestrogenic activity that ICI 182780 might exhibit at the pituitary level would have been detectable in this study, since this activity would tend to increase serum FSH and LH levels, which are already significantly elevated in postmenopausal women as a result of their reduced serum estrogen levels. The lack of any demonstrable reduction in serum gonadotropin levels after treatment with ICI 182780 does, however, provide preliminary evidence of a lack of agonist activity of this agent at the pituitary level. This is in contrast to the effect of tamoxifen, which reduces serum LH and FSH levels in postmenopausal women as a result of its agonist activity at the pituitary, albeit after rather more prolonged administration (5).

Similarly, the lack of effect of treatment with ICI 182780 on serum SHBG levels suggests that the drug was without agonist or antagonist activity in the liver. This again contrasts with the estrogen-like action of tamoxifen in increasing serum SHBG levels as a result of increased hepatic SHBG synthesis (24, 26). We emphasize, however, that to our knowledge there have been no comparable published studies of the systemic endocrine effects of tamoxifen used with such short term administration.

In contrast to the results described above, ICI 182780 produced a significant decline in the expression of ER and PgR in primary breast cancers, as determined by immunohistochemistry on pre- and post-treatment tumor samples. This effect was demonstrable despite the apparent tendency for the needle core biopsies to underestimate pre-treatment steroid receptor expression, as judged by the results for the untreated control patients. This may have resulted in the magnitude of decrease in receptor expression seen after treatment with ICI 182780 being underestimated. For the sake of clarity, however, no attempt has been made to adjust for these underestimations by using statistically derived correction factors.

A similar decrease in ER concentrations has been demonstrated in mouse uterine tissue following the administration of the prototype pure antiestrogen ICI 164384; ER levels fell to 5% of control levels within 4 h after treatment (27). Since cycloheximide did not affect this response and no change in receptor mRNA was detected, it appears that the pure antiestrogen-ER complex becomes more fragile and perhaps more susceptible to the normal processes involved in receptor degradation.

[4] Zeneca Pharmaceuticals, unpublished observations.

This mechanism is unlikely to explain the decrease in PgR levels seen after treatment with ICI 182780, because this agent is structurally distinct from progesterone and is unlikely to possess significant binding affinity for the PgR. Rather, the decreased detection of this estrogen-regulated protein is more likely to be the result of (*a*) the competitive actions of ICI 182780 on the ER, preventing ER binding and activation by endogenous estrogens, and (*b*) the severely attenuated transcriptional activity of the pure antiestrogen-ER complex. The inhibitory activity of ICI 182780 on PgR expression is in contrast to the reported *in vivo* stimulatory effects of conventional nonsteroidal antiestrogens like tamoxifen (28), a property which has been ascribed to their partial estrogen-like activity.

The estrogen-regulated protein pS2 has been found to be a good indicator of patient response to endocrine treatment and in combination with ER and PgR levels enables a group with an extremely good prognosis to be defined (22, 29). Like PgR, it would be unexpected for pS2 to be expressed frequently in tumors which do not have an intact estrogen signaling mechanism and pS2 levels would be expected to fall in the presence of estrogen antagonism, as was found to be the case in this study of ICI 182780. We did find, however, that pS2 was expressed in some ER-negative tumors and that the fall in pS2 occurred in these tumors as well as in ER-positive tumors. It is notable that the ER, PgR, and Ki67 analyses were performed on separate tissue specimens from those used for pS2 measurement and it is possible that this factor contributed to the apparant lack of relationship between pS2 expression and ER status. There have been no reports of the short term effects of tamoxifen on pS2 expression *in vivo* with which comparisons with the present study can be made. However, it has recently been reported that pS2 levels in breast cancers are reduced during long term treatment with tamoxifen.[5]

The mouse monoclonal antibody Ki67 recognizes a proliferation-associated nuclear antigen present in the late G_1, S, G_2, and M, but not G_0, phases of the cell cycle (30, 31). Previous studies have found that the Ki67 LI is positively correlated with histological grade (21, 32) and negatively correlated with estrogen receptor content (33, 34). Data from the present study are in agreement with these findings. In addition, like other measures of tumor proliferation the Ki67 LI has been found to be related to prognosis (35).

In a previous study, Clarke *et al.* (21) were able to show that short term tamoxifen treatment for a median period of 14 days prior to surgery caused a significant reduction in the Ki67 LI of primary breast tumors. In the present study we observed a significant reduction in the Ki67 LI of ER-positive tumors from women treated with ICI 182780. The absence of a similar change in the Ki67 LI of ER-negative tumors treated with ICI 182780 or of controls suggests that this effect was drug related and estrogen receptor dependent. In addition, the reduction in Ki67 LI appeared also to be dose dependent, because a significant decrease was demonstrable only in patients treated with the higher 18-mg dose. Although this may have been due in part to the relatively small number of patients treated with the 6-mg dose of ICI 182780 who had ER-positive tumors and also to the relatively low pretreatment Ki67 LI in this subgroup of tumors, it is of note that previous studies of ICI 182780 in human breast cancer cell lines have clearly demonstrated dose-dependent antiproliferative effects *in vitro* (18). The observed reduction in Ki67 LI in the present study does provide preliminary evidence that treatment with ICI 182780 may achieve the desired endpoint of antiestrogen therapy in breast cancer, *i.e.*, inhibition of tumor cell proliferation.

In summary, ICI 182780 is one of a new generation of potent pure antiestrogens and is the first therapeutic agent to be investigated in clinical trials with the potential to completely deprive breast tumors of estrogenic stimulation. This small study has shown ICI 182780 to be well tolerated after short term administration and has produced preliminary evidence to suggest that this novel agent does exhibit biological activity as an estrogen antagonist in primary breast tumors, without producing demonstrable agonist effects. Phase II trials with a long-acting formulation of this agent are now in progress.

[5] Dowsett *et al.*, personal communication.

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