[CANCER RESEARCH 61, 6739–6746, September 15, 2001]

Comparison of the Short-Term Biological Effects of 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5, (10)-triene-3,17β-diol (Faslodex) versus Tamoxifen in Postmenopausal Women with Primary Breast Cancer[1]

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ABSTRACT

7α-[9-(4,4,5,5,5-Pentafluoropentylsulfinyl)-nonyl]estra-1,3,5, (10)-triene-3,17β-diol (ICI 182,780; Faslodex) is a novel steroidal antiestrogen. This partially blind, randomized, multicenter study compared the effects of single doses of long-acting ICI 182,780 with tamoxifen or placebo on estrogen receptor (ERα) and progesterone receptor (PgR) content, Ki67 proliferation-associated antigen labeling index (Ki67LI), and the apoptotic index in the primary breast tumors of postmenopausal women. Previously untreated patients (stages T_1–T_3; ER-positive or -unknown) were randomized and received a single i.m. dose of ICI 182,780 50 mg (n = 39), ICI 182,780 125 mg (n = 38), or ICI 182,780 250 mg (n = 44) or oral tamoxifen 20 mg daily (n = 36) or matching tamoxifen placebo (n = 43) for 14–21 days before tumor resection surgery with curative intent. The ER and PgR H-scores, together with the Ki67LI were determined immunohistochemically in the matched pretreatment biopsy and the posttreatment surgical specimens. The apoptotic index was determined by terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling on the same samples. The effects of treatment on each of these parameters were compared using analysis of covariance. ICI 182,780 produced dose-dependent reductions in ER and PgR H-scores and in the Ki67LI. The reductions in ER expression were statistically significant at all doses of ICI 182,780 compared with placebo (ICI 182,780 50 mg, P = 0.026; 125 mg, P = 0.006; 250 mg, P = 0.0001), and for ICI 182,780 250 mg compared with tamoxifen (P = 0.024). For PgR H-score, there were statistically significant reductions after treatment with ICI 182,780 125 mg (P = 0.003) and 250 mg (P = 0.0002) compared with placebo. In contrast, tamoxifen produced a significant increase in the PgR H-score relative to placebo, and consequently, all doses of ICI 182,780 produced PgR values that were significantly lower than those in the tamoxifen-treated group. All doses of ICI 182,780 significantly reduced Ki67LI values compared with placebo (ICI 182,780 50 mg, P = 0.046; 125 mg, P = 0.001; 250 mg, P = 0.0002), but there were no significant differences between any doses of ICI 182,780 and tamoxifen. ICI 182,780 did not alter the apoptotic index when compared with either placebo or tamoxifen. Short-term exposure to ICI 182,780 reduces the ERα in breast tumor cells in a dose-dependent manner by down-regulating ER protein concentration. The reductions in tumor PgR content by ICI 182,780 demonstrate that ICI 182,780, unlike tamoxifen, is devoid of estrogen-agonist activity. Reductions in tumor cell proliferative activity (as indicated by Ki67LI) show that ICI 182,780 is likely to have antitumor activity in the clinical setting.

Received 11/9/00; accepted 7/25/01.

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INTRODUCTION

Estrogens act as endocrine growth factors for at least one-third of breast cancers (1), and their effects are mediated via the ER[3] pathway. Several approaches have been adopted to treat hormone-sensitive breast cancer. In premenopausal women these include reducing circulating estrogen by ovarian ablation or by inhibiting ovarian estrogen production. In postmenopausal women, the mainstays of therapy are the prevention of estrogen binding to its receptor using an antiestrogen or lowering estrogen levels with aromatase inhibitors. The antiestrogen tamoxifen is the most widely used hormonal treatment for all stages of breast cancer (2). However, tamoxifen possesses partial agonist activity which has positive effects on bone (3, 4) and blood lipids (5), but which also has unwanted side effects, including increased endometrial proliferation (6), a small increase in the risk of endometrial cancer (7–9), tumor flare at the start of treatment (10), and tamoxifen-mediated tumor stimulation upon progression (11).

Currently, there are two other clinically available nonsteroidal, mixed agonist/antagonist antiestrogens, toremifene, which is used in the treatment of breast cancer (12), and raloxifene, which is being used in the management of osteoporosis (13). These two agents, together with tamoxifen, comprise a group of compounds that are described as SERMs (14). No new SERM has yet provided significant advantages over tamoxifen in the treatment of breast cancer in terms of either efficacy or tolerability, and all SERMs discovered to date show some degree of partial agonist activity. Furthermore, cross-resistance between the new SERMs and tamoxifen may limit their application in advanced disease after adjuvant tamoxifen treatment (15). Despite the potential advantages of the partial agonist properties of the SERMs, a drug that acts as a nonagonist (pure) antiestrogen may be an important step toward improving breast cancer treatment (16).

Fulvestrant (Faslodex), formerly known as ICI 182,780, is a novel estrogen antagonist that, unlike tamoxifen, has no estrogen-agonist activity (Fig. 1). Preclinical and early clinical studies (17–40) suggest that ICI 182,780 has biological effects indicative of improved clinical efficacy in the treatment of breast cancer. The main features are ER down-regulation, antiproliferative activity, induction of apoptosis, lack of cross-resistance with tamoxifen, and the absence of ER-agonist activity.

ICI 182,780 has a binding affinity for the ER that is ~100 times

[3] The abbreviations used are: ER, estrogen receptor(s); SERM, selective estrogen receptor modulator; ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoropentylsulfinyl)-nonyl]estra-1,3,5, (10)-triene-3,17β-diol; PgR, progesterone receptor(s); Ki67LI, Ki67 proliferation-associated antigen labeling index; AI, apoptotic index; DAB, diaminobenzidine tetrahydrochloride; ANCOVA, analysis of covariance; ICA, immunocytochemical assay; NRS, normal rabbit serum.

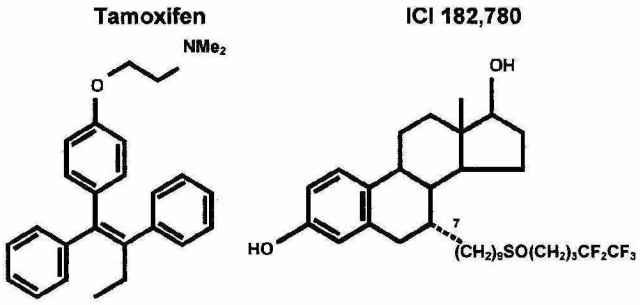

Fig. 1. Chemical structures of the nonsteroidal SERM, tamoxifen, and of the novel nonagonist (pure) antiestrogen, ICI 182,780.

greater than that of tamoxifen (17), and in animal models, ICI 182,780 markedly attenuates the ability of the ER to activate or inhibit gene transcription (20–22). Several different mechanisms may underlie this effect, including impaired dimerization, increased ER turnover, and disrupted nuclear localization (23–25). ICI 182,780 treatment blocks the uterotrophic effects of ER agonists (estrogens) and of partial agonists such as tamoxifen (26–28) and raloxifene (29) and reduces ER levels in the tumors of women with primary breast cancer (30). Therefore, ICI 182,780 seems to act as an ER down-regulator, because it functionally blocks the ER and reduces cellular ER levels such that the receptor is rendered unavailable or unresponsive to estrogen or estrogen agonists.

The PgR gene is an estrogen-regulated gene (34), so drugs with estrogenic activity will increase its expression. Accordingly, tamoxifen has been shown to increase PgR levels (35), whereas initial work on primary breast tumors found that a short-acting formulation of ICI 182,780 reduced PgR levels (30), suggesting that it is devoid of estrogen-agonist activity and may have a different mechanism of action to that of tamoxifen. Additional evidence that ICI 182,780 and tamoxifen have different underlying modes of action comes from studies showing that tamoxifen-resistant tumors remain sensitive to ICI 182,780 treatment *in vitro* (18, 19), *in vivo* (36, 37), and in the clinic (38–40).

ICI 182,780 has antiproliferative effects, as assessed by immunohistochemical detection of the Ki67 proliferation-associated antigen (30–32). Previous small clinical studies have suggested that both tamoxifen and ICI 182,780 increase apoptosis in primary human breast cancer (33).

The study reported here represents the first direct randomized comparison of the short-term biological effects of ICI 182,780 (50 mg, 125 mg, or 250 mg as a single i.m. injection) with tamoxifen (20 mg/day p.o. for 14–22 days) and tamoxifen placebo in women with primary breast cancer. It is also the first investigation of any dose-response effect of ICI 182,780 and the first time that the biological effects of the clinical trials formulation (250 mg) have been assessed. The end points of the trial were ERα (referred to as ER for the remainder of this paper) and PgR H-scores, Ki67LI, and the AI.

PATIENTS AND METHODS

Two hundred and one women with primary breast cancer participated in a multicenter, randomized, partially blinded study. The administration of tamoxifen and tamoxifen placebo was double blind, and the administration of ICI 182,780 (at one of three doses) was open. Postmenopausal women with histologically proven primary breast cancer awaiting tumor resection were recruited to the study from June 1997 to May 1999. Each woman gave written informed consent and underwent an initial eligibility screen in the week before randomization. Prestudy assessments included past medical history, concomitant therapy, demography, current medical conditions, hematology, and biochemistry screening.

Patients were included if they were postmenopausal (>12 months since the last menstrual period and/or had castrate levels of follicle-stimulating hormone >40 IU/liter) and had a clinically staged, histologically confirmed T_1, T_2 or T_3 primary breast cancer. They had to be fit for surgery within 1 month and have a tumor large enough to provide sufficient biopsy samples. Patients were ER-positive or -unknown at entry to the trial. The study was approved by the Ethics Committees of all centers.

Patients were not eligible for the study if they had evidence of metastatic disease or had received any prior treatment for their primary tumor. Other exclusion criteria were: (a) treatment with hormone replacement therapy within 4 weeks of starting the trial; (b) baseline hematology or clinical chemistry outside the normal range; (c) risk of human immunodeficiency virus, hepatitis B, or hepatitis C transmission; (d) history of disease affecting steroid metabolism; (e) bleeding diathesis or thrombocytopenia (platelets $<100 \times 10^9$/liter); or any other reason that could jeopardize the protocol. Treatment with drugs known to affect sex hormone status could not be commenced during the trial (e.g., ketoconazole or prednisolone), although the patient could continue to receive such drugs if they were being taken before the study and the patient's hormone status was stable.

Patients were randomized to one of the following treatments: single i.m. dose of ICI 182,780 50 mg (n = 40), 125 mg (n = 40), and 250 mg (n = 41); tamoxifen, 20 mg, once daily p.o. for 14–21 days (n = 37); or tamoxifen placebo, once daily p.o. for 14–21 days (n = 43). Patients were scheduled for tumor resection surgery with curative intent between day 15 and day 22 after the start of treatment. On the day of surgery, patients were reassessed for concomitant therapy, concomitant conditions, hematology, and biochemistry. All patients returned for postsurgical assessment on day 57.

Tumor Sampling

The Tru-cut/core biopsy taken at the first clinic attendance for diagnostic purposes was used as the prerandomization tumor sample. Where possible, a minimum of three cores was taken, sufficient to provide material for the three laboratories. The posttreatment specimen was obtained at definitive surgical resection. All of the tissue samples were fixed in 3.7% formalin immediately after removal, then embedded in paraffin wax for sectioning and subsequent analysis of biological markers.

Drug Administration

Long acting ICI 182,780 (AstraZeneca, Macclesfield, United Kingdom) was administered by i.m. injection into the gluteus maximus muscle. Patients were randomized to receive 50 mg of ICI 182,780 (1 ml), 125 mg of ICI 182,780 (2.5 ml), or 250 mg of ICI 182,780 (5 ml). Tamoxifen was supplied as Nolvadex tablets containing 20 mg of tamoxifen (AstraZeneca) and administered at a dose of 20 mg/day. The tamoxifen placebo tablet (AstraZeneca) matched the 20 mg tamoxifen tablet. Both tamoxifen and tamoxifen placebo were administered p.o.

Adverse Events Monitoring

Adverse events (defined as the development of a new medical condition or the deterioration of a preexisting medical condition subsequent to or during exposure to the trial medications) were monitored throughout the study. Patients were followed up for adverse events for 57 days postdosing.

Analysis of Tumor Marker Expression

ER. ERα expression was assessed at the Tenovus Centre for Cancer Research, Cardiff, Wales, on sections cut from the formalin-fixed, paraffin-embedded tissue specimens described above. All mounted sections were dried overnight at 60°C before being dewaxed and rehydrated to PBS (pH 7.2–7.4). Endogenous peroxidase activity was quenched by incubation in hydrogen peroxide (0.5% in methanol) for 10 min and then rinsing in running tap water for 5 min and in PBS for 5 min. Then sections were enzyme-digested in a bath of 0.02% Pronase E (Sigma Chemical Co., Poole, United Kingdom) in PBS at 37°C before being rinsed as described previously. To block the nonspecific staining, a blocking reagent, comprising 20% normal swine serum (Dako Ltd., Glostrup, Denmark) in PBS was applied to the sections and then "tapped off"

Table 1 ER and PgR status of tumors—per-protocol patients

Characteristic		Placebo	ICI 182,780 50 mg	ICI 182,780 125 mg	ICI 182,780 250 mg	Tamoxifen
ER status	Positive	29 (69.0)	33 (86.8)	34 (89.5)	32 (74.4)	27 (81.8)
n (%)	Negative	8 (19.0)	4 (10.5)	1 (2.6)	6 (14.0)	4 (12.1)
	Unknown	5 (11.9)	1 (2.6)	3 (7.9)	5 (11.6)	2 (6.1)
PgR status	Positive	28 (66.7)	29 (76.3)	29 (76.3)	29 (67.4)	21 (63.6)
n (%)	Negative	10 (23.8)	7 (18.4)	5 (13.2)	9 (20.9)	9 (27.3)
	Unknown	4 (9.5)	2 (5.3)	4 (10.5)	5 (11.6)	3 (9.1)

before incubation overnight at room temperature with the primary antibody (diluted 1:2), which was the rat antihuman ERα antibody (Clone H222) supplied in the ER-ICA kit by Abbott Laboratories (North Chicago, IL). Sections were washed in PBS (5 × 4 min) and then a secondary biotinylated sheep antirat immunoglobulin (Amersham Life Science Ltd., Amersham, United Kingdom) diluted 1:500 in 20% normal swine serum was applied for 60 min. Sections were washed again in PBS (5 × 4 min) before the avidin-biotin-horseradish peroxidase complex (Dako Ltd.) diluted 1:120 in PBS was added for 60 min with additional washing afterward in PBS (5 × 4 min). Then the DAB chromogen was applied (as supplied in the Abbott ER-ICA kit) to the sections and left for 10 min before rinsing in distilled water (2 × 3 min). Staining was enhanced by treating the sections with 0.5% copper sulfate in 0.85% sodium chloride for 8 min and rinsing in distilled water (2 × 3 min). The sections were counterstained with 0.5% methyl green for 5 min, washed in distilled water (2 × 3 min), dehydrated, cleared, and mounted for examination by light microscopy.

ERα immunopositivity appeared clearly as a brown nuclear signal in tumor epithelial cells against a background of green nuclear counterstain. Tumor epithelial cell ER content in the pre- and posttreatment specimens for each patient was assessed by the consensus of two people (J. M. W. G. and R. I. N.) using the dual viewing attachment of a light microscope. Overall staining was assessed at ×10, and a representative area was viewed at ×40 to assess the number of positive tumor cell nuclei and staining intensity. The percentages of positive tumor epithelial cells in each staining intensity category (i.e., negative −/−; very weak +/−; weak +; moderate ++; and strong +++) were recorded for each sample, and positive-control breast cancer samples of known ER positivity were included in every assay to monitor assay performance. Results were expressed as the ER H-score where: H-score = [(0.5 × % +/−) + (1 × % +) + (2 × % ++) + (3 × % +++)]. A value of >0 implies an ER-positive state with a range of 0–300.

PgR Expression. Levels of PgR in sections from the same samples were also assessed by the Tenovus Centre for Cancer Research, Cardiff, Wales. The assay procedure was similar to that used to detect ER, except that the primary anti-PgR antibody (Clone KD68) was that supplied by Abbott Laboratories in the PgR-ICA kit, as was the DAB chromogen. In this assay the primary antibody was diluted 1:4, and no enzyme retrieval was used. Results were expressed as the PgR H-score, using the same equation as that used to calculate the ER H-score.

Ki67 Proliferation-associated Antigen Expression. Ki67 antigen was assessed on sections of the pre- and posttreatment tissue specimens at the Christie Hospital, Manchester, United Kingdom, using the MIB-1 anti-Ki67 antibody supplied by Coulter Electronics (Luton, United Kingdom). Briefly, slides were dewaxed and rehydrated to PBS (pH 7.6). Endogenous peroxidase was quenched using hydrogen peroxide (0.2%) in methanol for 10 min. The sections were then rinsed in water and PBS and microwaved (800 W) in 10 mM citrate buffer (pH 6.0) at power 7 for 15 min after boiling point was reached. After cooling for 20 min, sections were washed in PBS and nonspecific binding was blocked with 10% NRS in 0.5% casein/PBS containing 4 drops/ml of the avidin block supplied by Vector Laboratories (Peterborough, United Kingdom) for 15 min. The primary antibody was then applied at a dilution of 1:50 in 10% NRS/0.5% casein/PBS containing 4 drops/ml of biotin block (Vector Laboratories), and the sections were incubated for 80 min at room temperature. After washing in PBS (2 × 5 min), the secondary biotinylated rabbit antimouse antibody (DAKO E413; Dako Ltd., Ely, United Kingdom) was applied at a dilution of 1:300 in 10% NRS/0.5% casein/PBS for 40 min, and after washing in PBS (2 × 5 min), the avidin biotinylated enzyme complex reagent (Vectastain ABC Elite kit; Vector Laboratories) was applied for 40 min. After the final PBS wash (2 × 5 min), incubation with the DAB chromogen ("SigmaFast" 3,3-diaminobenzidine tablet set; Sigma Chemical Co.-Aldrich Company, Poole, United Kingdom) was performed for 8 min at room temperature before a wash in distilled water. Samples were counterstained with 20% hematoxylin for 3–5 min, dehydrated, cleared, and mounted for examination by light microscopy. Results were expressed as the Ki67LI (the percentage of positively stained nuclei calculated after counting at least 1000 tumor cells).

AI. The AI was measured using the terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay at the Royal Marsden Hospital, London, United Kingdom. After dewaxing and rehydration to deionized water, endogenous peroxidases were quenched with hydrogen peroxide (1%) in PBS for 15 min and washing three times in deionized water. Then sections were digested in 0.5% pepsin (pH 2) for 30 min at 37°C in a humidified chamber. Digestion was terminated, and sections were rinsed for 1 min and washed five times for 5 min each in deionized water. Then sections were washed twice in Tris-buffered saline (pH 7.6) for 5 min and incubated for 1 h at 37°C in 100 μl/slide of a reaction mixture containing 0.75 μl of terminal deoxynucleotidyltransferase, 0.50 μl of biotinylated 16dUTP, 10 μl of 50 mM cobalt chloride, and 20 μl of reaction buffer (1 M sodium cacodylate + 125 mM Tris-HCl + 1.25 mg/ml BSA in deionized water). After washing twice in deionized water and three times in PBS, sections were incubated with horseradish peroxidase-conjugated streptavidin (Dako Ltd.) diluted 1:4000 in PBS + 1% BSA + 0.5% Tween 20. Another three washes in PBS/Tween 20 preceded development with 0.05% DAB and 0.07% imidazole for 30 s and then 10 min of incubation with 100 μl of 1% hydrogen peroxide. Sections were washed in running tap water for 5 min and then immersed in 0.5% copper sulfate plus 0.9% sodium chloride in deionized water for 1 min. DAB was then inactivated with chloros and the sections were washed in running tap water,

Table 2 Demographic characteristics of ER-positive per-protocol patients

Characteristic		Placebo	ICI 182,780 50 mg	ICI 182,780 125 mg	ICI 182,780 250 mg	Tamoxifen
Age (yr)	n	29	33	34	32	27
	Mean	65.9	69.2	68.7	66.1	68.7
	SD	9.2	8.4	7.3	8.3	8.4
Clinical disease staging	T_1	5 (17.2)	2 (6.1)	5 (14.7)	2 (6.3)	6 (22.2)
n (%)	T_2	10 (34.5)	11 (33.3)	10 (29.4)	12 (37.5)	9 (33.3)
	T_3	1 (3.4)	3 (9.1)	1 (2.9)	0 (0.0)	1 (3.7)
	Not T_4[a]	13 (44.8)	17 (51.5)	18 (52.9)	18 (56.3)	11 (40.7)
Tumor grade at surgery[b]	G1	6 (20.7)	6 (18.2)	8 (23.5)	9 (28.1)	6 (22.2)
n (%)	G2	14 (48.3)	16 (48.5)	15 (44.1)	16 (50.0)	13 (48.1)
	G3	7 (24.1)	9 (27.3)	9 (26.5)	6 (18.8)	7 (25.9)
	GX	2 (6.9)	2 (6.1)	2 (5.9)	1 (3.1)	1 (3.7)

[a] Unable to categorize, but definitely not T_4.
[b] G1, well-differentiated; G2, moderately differentiated; G3, poorly differentiated; GX, unassessable.

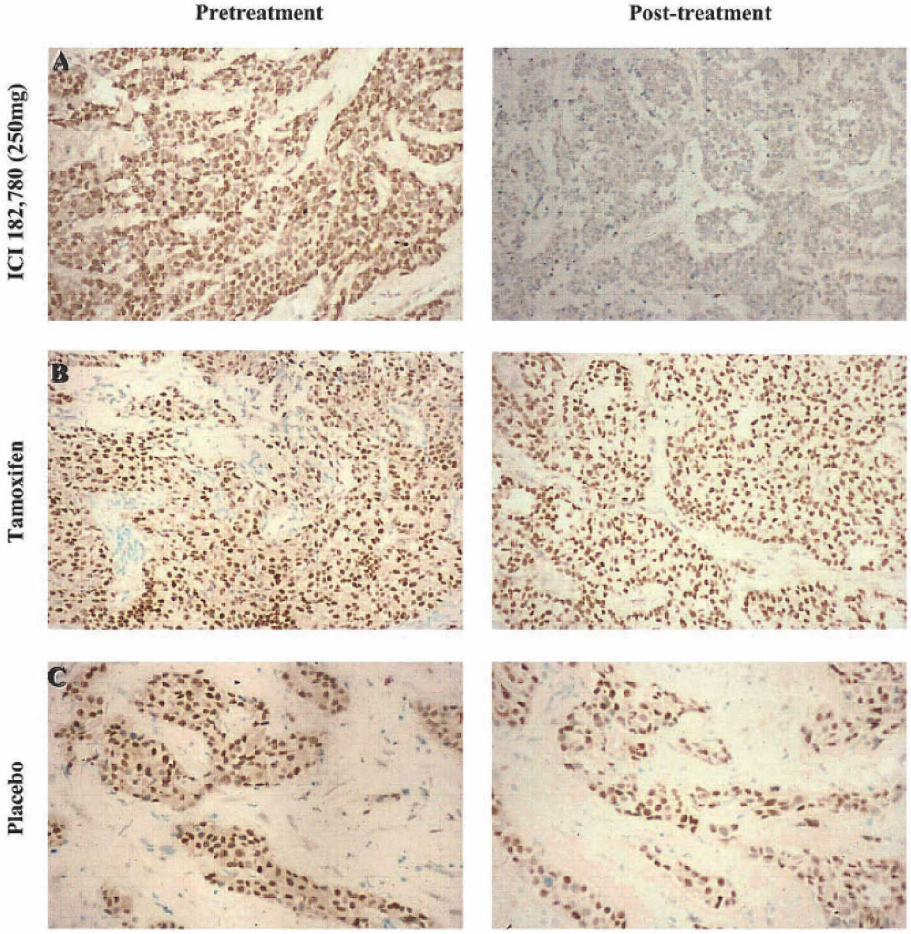

Fig. 2. Comparison of ER expression in a biopsy sample taken pretreatment with that from a sample taken from the same tumor after treatment with ICI 182,780 (250 mg; A), tamoxifen (B), and tamoxifen placebo (C). ER immunopositivity appears as a brown nuclear signal against a background of the green nuclear counterstain. Photographs supplied by R. I. N.

counterstained with hematoxylin in blued tap water (30 s), dehydrated, cleared, and mounted for examination by light microscopy. Results were expressed as the percentage of apoptotic cells in 3000 tumor cells.

Statistical Analysis

This trial was an exploratory trial, so the minimum power required for statistical testing was set at 80%. The four end points (surrogate tumor tissue markers) were considered equally important, so all were classed as primary end points. The secondary end points were tolerability and pharmacokinetic data (pharmacokinetic data are not presented in this paper). This "per protocol" analysis included only those patients who received the full course of treatment, completed the end of treatment assessment for the primary end point, and had no significant protocol deviations or violations. All analyses were carried out by the Biometrics Group, AstraZeneca.

It was calculated that ~30 patients/group were needed to detect the following differences between ICI 182,780 and the comparator with 80% power using a two-sided significance level of 5%: 0.3 for ER H-score; 0.4 for PgR H-score; 4.5 for Ki67; and 0.2 for apoptosis. To allow for ER-/PgR-negative tumors, a total of 201 patients were recruited and ~40 were randomized to each treatment group.

The primary end point data were assessed statistically using ANCOVA according to treatment received with terms included in the model for treatment, center, and the baseline tumor marker value. Patients in the per-protocol population who were ER-negative were excluded from the analysis of ER, Ki67, and AI, and patients who were PgR-negative were excluded from the analysis of PgR. In addition, any patients in the per-protocol population with a missing value for a tumor tissue marker were also excluded from the analysis for that particular marker. The ANCOVA allowed an overall assessment of differences between each dose of ICI 182,780 and tamoxifen and each dose of ICI 182,780 and placebo. A test for overall treatment

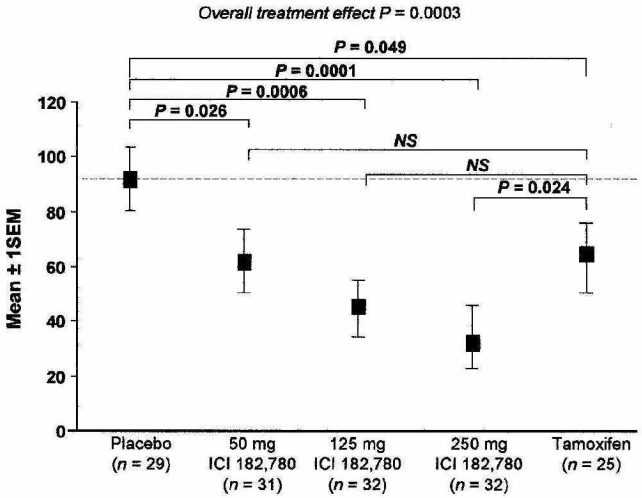

Fig. 3. Posttreatment mean ER H-scores after a single i.m. injection of 50 mg, 125 mg, or 250 mg of ICI 182,780 or Tamoxifen 20 mg once daily p.o. or tamoxifen placebo.

Table 3 Summary of results for ER H-score

	Placebo	ICI 182,780 50 mg	ICI 182,780 125 mg	ICI 182,780 250 mg	Tamoxifen
n	29	31	32	32	25
Pretreatment mean H-score	125	136	124	113	123
Percentage change (posttreatment)	−13	−39	−50	−59	−36
Overall treatment effect	P = 0.0003				
Treatment difference vs. placebo (95% CI)	NA[a]	−30 (−57, −4) P = 0.0255	−47 (−74, −21) P = 0.0006	−60 (−86, −34) P = 0.0001	−29 (−57, −0.2) P = 0.0485
Treatment difference vs. tamoxifen (95% CI)	29 (0.2, 57) P = 0.0485	−2 (−29, 26) P = 0.8955	−19 (−46, 9) P = 0.1833	−32 (−59, −4) P = 0.0239	NA

[a] NA, not applicable.

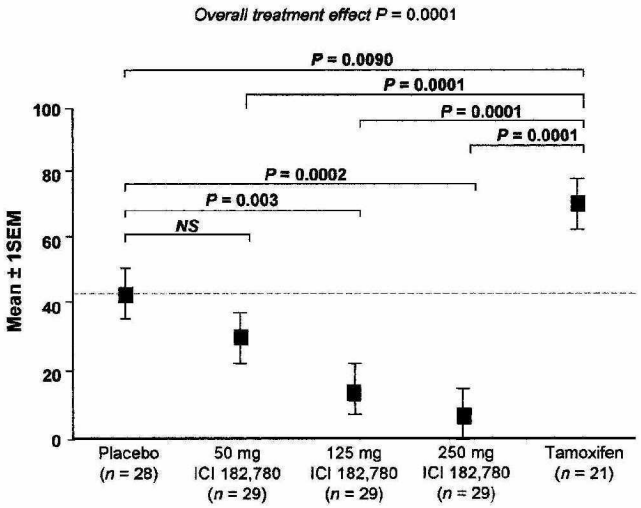

Fig. 4. Posttreatment mean PgR H-scores after a single i.m. injection of 50 mg, 125 mg, or 250 mg of ICI 182,780 or tamoxifen 20 mg once daily p.o. or tamoxifen placebo.

effect was undertaken. If this was significant at the 5% level, then the following pairwise comparisons were made: ICI 182,780 50 mg versus placebo; ICI 182,780 125 mg versus placebo; ICI 182,780 250 mg versus placebo and ICI 182,780 50 mg versus tamoxifen; ICI 182,780 125 mg versus tamoxifen; and ICI 182,780 250 mg versus tamoxifen. A supplementary comparison of tamoxifen versus placebo was also undertaken. For ER and PgR, the comparisons are presented as treatment differences with 95% confidence intervals. The mean change from baseline was also calculated for each treatment group and expressed as a percentage of the baseline mean value. For both Ki67LI and AI, the data showed evidence of nonnormality, so all values were log- (base e) transformed for the ANCOVA analysis, and the comparisons are, therefore, presented as treatment ratios with 95% confidence intervals. In addition, the median change from baseline was calculated for each treatment group and expressed as a percentage of the baseline median value. Plots of means ± 1 SE by treatment group for each end point are also presented.

RESULTS

Patient Characteristics. A total of 201 postmenopausal women (mean age, 67.6 years; range: 48–86 years) were randomized into the trial, and 190 completed the trial. One patient did not take any trial treatment, and 10 patients withdrew from the trial. The withdrawal rates were similar for the ICI 182,780 groups (1/treatment group) but four patients withdrew from the tamoxifen treatment group and three from the tamoxifen placebo group. Of those patients in the per-protocol population, 155 were ER-positive. Groups were well balanced with respect to age, disease stage, and tumor grade at surgery. The ER and PgR status of the tumors at study entry are given in Table 1. The demographic characteristics of the ER-positive per-protocol patients in the five treatment groups are summarized in Table 2.

ER Expression. Treatment of ER-positive tumors with ICI 182,780 resulted in a marked reduction of nuclear ER content that could easily be seen under the light microscope (Fig. 2). This was confirmed by statistical analysis of the ER H-score, which showed a significant overall treatment effect (P = 0.0003). The posttreatment mean ER H-scores are shown in Fig. 3, and the summary of results are shown in Table 3. ICI 182,780 produced a dose-dependent reduction in the ER H-scores, and all doses significantly reduced the ER H-score compared with placebo. The reduction in ER H-scores seen at the lower doses of ICI 182,780 (50 mg and 125 mg) were not statistically significantly different from those caused by tamoxifen, although the comparison between the 250-mg dose of ICI 182,780 and tamoxifen did reach significance (P = 0.0239).

PgR Expression. Analysis of the PgR H-scores showed a significant overall treatment effect (P = 0.0001). Posttreatment mean PgR H-scores are shown in Fig. 4, and the summary of results is shown in Table 4. There was a dose-dependent reduction in PgR H-score with ICI 182,780, with the 125 mg and 250 mg doses of ICI 182,780 producing significantly greater reductions in PgR H-score than placebo. Tamoxifen caused a significant increase in PgR H-score compared with placebo; consequently, each dose of ICI 182,780 resulted in a PgR H-score that was significantly lower than that of tamoxifen.

Ki67LI. Analysis of the Ki67LI showed a significant overall treatment effect (P = 0.0029). The posttreatment mean Ki67LIs are shown in Fig. 5 and the summary of results are shown in Table 5. ICI 182,780

Table 4 Summary of results for PgR H-score

	Placebo	ICI 182,780 50 mg	ICI 182,780 125 mg	ICI 182,780 250 mg	Tamoxifen
n	28	29	29	29	21
Pretreatment mean H-score	30	47	28	33	49
Percentage change (posttreatment)	+43	−12	−52	−67	+63
Overall treatment effect	P = 0.0001				
Treatment difference vs. placebo (95% CI)	NA[a]	−14 (−32, 5) P = 0.1455	−28 (−46, −10) P = 0.0030	−35 (−53, −17) P = 0.0002	27 (7, 47) P = 0.0090
Treatment difference vs. tamoxifen (95% CI)	−27 (−47, −7) P = 0.0090	−40 (−60, −21) P = 0.0001	−55 (−75, −34) P = 0.0001	−62 (−82, −42) P = 0.0001	NA

[a] NA, not applicable.

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