Pharmacokinetics, pharmacological and anti-tumour effects of the specific anti-oestrogen ICI 182780 in women with advanced breast cancer

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Summary We have assessed the pharmacokinetics, pharmacological and anti-tumour effects of the specific steroidal anti-oestrogen ICI 182780 in 19 patients with advanced breast cancer resistant to tamoxifen. The agent was administered as a monthly depot intramuscular injection. Peak levels of ICI 182780 occurred a median of 8–9 days after dosing and then declined but were above the projected therapeutic threshold at day 28. C_{max} during the first month was 10.5 ng/ml^{-1} and during the sixth month was 12.6 ng ml^{-1}. The AUCs were 140.5 and 206.8 ng day ml^{-1} on the first and sixth month of dosing respectively, suggesting some drug accumulation. Luteinising hormone (LH) and follicle-stimulating hormone (FSH) levels rose after withdrawal of tamoxifen and then plateaued, suggesting no effect of ICI 182780 on the pituitary–hypothalamic axis. There were no significant changes in serum levels of prolactin, sex hormone-binding globulin (SHBG) or lipids. Side-effects were infrequent. Hot-flushes and sweats were not induced and there was no apparent effect of treatment upon the endometrium or vagina. Thirteen (69%) patients responded (seven had partial responses and six showed 'no change' responses) to ICI 182780, after progression on tamoxifen, for a median duration of 25 months. Thus ICI 182780, given by monthly depot injection, and at the drug levels described, is an active second-line anti-oestrogen without apparent negative effects on the liver, brain or genital tract and warrants further evaluation in patients with advanced breast cancer.

Keywords: ICI 182780; advanced breast cancer

Half of the patients with advanced breast cancer have tumours that either regress or remain stable when treated with tamoxifen. Despite initial response all such tumours eventually become resistant to this anti-oestrogen after a median duration of remission of about 18 months (Cole *et al.*, 1971; Patterson *et al.*, 1981). Although it acts as an oestrogen antagonist with respect to the tumour, tamoxifen is oestrogenic with respect to bone (Turken *et al.*, 1989), the liver (Bertelli *et al.*, 1988) and the endometrium (Fornander *et al.*, 1989). Potential causes of treatment failure may result from tamoxifen, or its metabolites (Osborne *et al.*, 1991) becoming oestrogenic with respect to the tumour (Howell *et al.*, 1990) or from tamoxifen becoming sequestered away from the oestrogen receptor (ER) and rendered inactive (Pavlick *et al.*, 1992).

A new class of specific anti-oestrogens has been developed that produce more complete suppression of the proliferative effects of oestrogen upon tumours. Substitution of a long side-chain at the 7 alpha position of the oestradiol molecule has produced compounds that appear more active as anti-oestrogens than the triphenylethylene derivatives such as tamoxifen (Wakeling and Bowler, 1987, 1988). The structure of the prototype specific anti-oestrogen, ICI 164384, is shown in Figure 1 together with that of ICI 182780, {7a-[9(4,4,5,5,pentafluoropentyl-sulphinyl)nonyl]oestra-1,3,5,(10)-triene-3,17β-diol} the compound selected for clinical evaluation because of its greater potency and affinity for the ER (Wakeling *et al.*, 1991).

Both compounds have been assessed using *in vitro* and animal models of human breast cancer and compared with non-steroidal, partial agonist anti-oestrogens, including tamoxifen, and also with oestrogen withdrawal. The specific anti-oestrogens have shown superiority over these alternative methods of oestrogen deprivation with respect to inhibition of cell proliferation and oestrogen-induced gene expression. ICI 164384 and ICI 182780 are up to two orders of magnitude more potent than tamoxifen as inhibitors of cell growth *in vitro*. (Wakeling and Bowler, 1987, 1988) and produce a more profound blockade of cell division in the G_1 phase of the cell cycle. The specific anti-oestrogens are more effective than tamoxifen in suppressing expression of oestrogen-induced genes, such as progesterone receptor (PgR), pS2 and cathepsin D (Nicholson *et al.*, 1994) by breast cancer cells. The specific anti-oestrogens have also been shown to produce a rapid reduction of intracellular ER levels, possibly as a result of inhibition of ER dimerisation and reduction of the ER half-life (Fawell *et al.*, 1990; Dauvois *et al.*, 1992). This latter effect is in contrast to that of tamoxifen, which has been shown to increase ER expression by breast cancer cells *in vitro* (Wakeling *et al.*, 1989).

The experiments outlined above were performed on cell lines but similar results were demonstrated when a short-acting, propylene glycol-based formulation of ICI 182780 was administered by daily intramuscular injection for 1 week before surgery to women with primary breast cancer. Compared with pretreatment tumour samples (obtained by Tru-cut biopsy), those obtained after treatment with the specific anti-oestrogen showed reduced proliferation (Ki67 expression) and reduced or absent expression of ER, PgR and pS2 (DeFriend *et al.*, 1994*b*; Nicholson *et al.*, 1994). Similar clinical experiments with tamoxifen produced no change in ER expression, slightly increased PgR expression and a reduction in labelling index (Howell *et al.*, 1988; Roberston *et al.*, 1991; Clarke *et al.*, 1993; Nicholson *et al.*, 1994).

The aims of the study reported here were to assess the long-term efficacy and toxicity of the specific anti-oestrogen ICI 182780 in patients with advanced breast cancer and to evaluate the pharmacokinetics of the long-acting formulation used. Since tamoxifen-resistant breast cancer cell lines have been shown to retain sensitivity to specific anti-oestrogens

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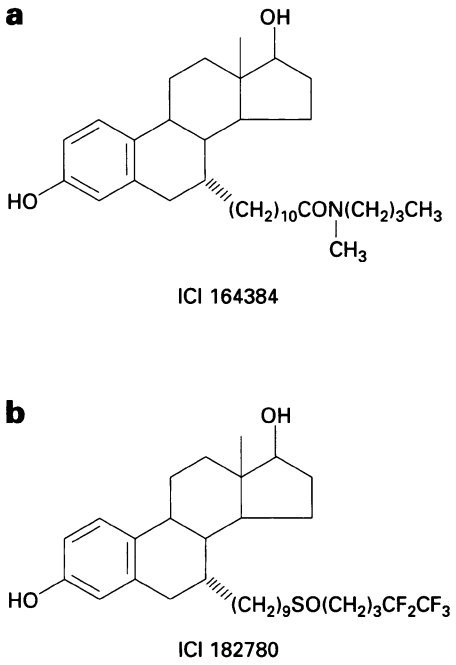

Figure 1 Comparison of the structures of ICI 164384 and ICI 182780.

when grown either *in vitro* (Lykkesfeld and Sorenson, 1992; Brunner *et al.*, 1993*a*,*b*; Lykkesfeld *et al.*, 1994) or as xenografts in nude mice *in vivo* (Gottardis *et al.*, 1989; Osborne *et al.*, 1991), the effects of ICI 182780 were evaluated in a group of post-menopausal patients with tamoxifen-resistant breast cancer. Since the partial agonist activity of tamoxifen on bone density and lipid levels has been reported to the beneficial in post-menopausal patients, the effects of ICI 182780 at other oestrogen target sites, including the hypothalamus/pituitary gland, the liver and the endometrium has also been assessed in this study.

We report that although some drug accumulation occurred at the dose level used in this study, administration of ICI 182780 was associated with a lower than expected incidence of side-effects (such as hot flushes and vaginal problems) together with a high response rate and long response duration in women previously treated with tamoxifen. A preliminary report of the early clinical result of this study has been published (Howell *et al.*, 1995).

Patients and methods

Patients

Nineteen patients with advanced breast cancer resistant to tamoxifen were treated with ICI 182780. The study was approved by the ethics committees of each clinical centre. Patients were eligible for the study if they were post-menopausal and age less than 81 years, with histologically verified breast cancer. Patients were included if they had been treated with tamoxifen as an adjuvant to surgery for more than 2 years and then relapsed, or if they had been treated with tamoxifen for advanced disease, had a complete or partial remission or disease stabilisation ('no change') for at least 6 months, and subsequently progressed while taking tamoxifen. Patients were excluded if they had serious intercurrent disease, a WHO performance status of greater than 2, and a life expectancy of less than 3 months or had received previous cytotoxic chemotherapy for advanced breast cancer. The characteristics of the patients studied are summarised in Table I. One patient had adjuvant therapy for only 9 months and progressed and was thus a protocol violation, but is included in the analysis. She had progressive disease when treated with ICI 182780.

Study design

After giving informed consent, all patients participating in the study underwent baseline staging investigations before commencing treatment with ICI 182780 including X-rays, liver ultrasound or computerised tomography (CT) scan and isotope bone scan. ICI 182780 was administered as a long-acting formulation contained in a castor oil-based vehicle by monthly i.m. injection (5 ml) into the buttock. For appraisal of drug safety, the first four patients received escalating doses of ICI 182780, starting with 100 mg in the first month and increasing to 250 mg i.m. from the second month onwards, following confirmation of lack of local or systemic drug toxicity at the 100 mg dose. Patients 5–19 received 250 mg $month^{-1}$ i.m. from the outset. Treatment with ICI 182780 was continued until objective tumour progression occurred. Patients were seen at intervals of 3–7 days during the first month after commencing treatment with ICI 182780 in order to monitor local and systemic drug tolerability and to collect blood samples for pharmacokinetic studies. Thereafter, patients were reviewed at monthly intervals in order to evaluate objective tumour response to ICI 182780 and to further monitor local and systemic drug tolerability. Blood samples were taken before commencing treatment with ICI 182780 and at monthly intervals thereafter for measurement of full blood count, clinical biochemistry and serum hormone, SHBG and lipid levels. Tumour response to therapy was evaluated according to UICC criteria (Hayward *et al.*, 1977). To qualify for the 'no change' category, tumour growth had to stabilise for more than 6 months (Howell *et al.*, 1988; Robertson *et al.*, 1989). Body weight was recorded at each monthly review in the majority of patients.

Serum estimations

The concentration of ICI 182780 in serum samples was determined by radioimmunoassay (RIA), using antibodies raised in sheep to ICI 182780 coupled at the 17-position to thyroglobulin and tritiated ICI 182780. The procedure was applied after solid base clean up of a diethyl ether/hexane extract of serum. The study limit of quantification was 0.68 ng ml^{-1}. The RIA procedure is believed to be specific for ICI 182780, since comparative analysis of plasma samples from preclinical studies by RIA and high-performance liquid chromatography (HPLC) showed a good correlation for ICI 182780 concentrations. Further, ICI 182780 metabolites present in these samples were not detected by the RIA. Gonadotrophins follicle-stimulating hormone and lutenising hormone (FSH and LH) and SHBG were measured by RIA in the Regional Radioimmunoassay Laboratory of the University Hospital of South Manchester. Prolactin was measured by immunoradiometric assay using reagents supplied by Netria. Total cholesterol levels were determined enzymatically using a commercially available reagent (Diamed, Switzerland). Triglyceride levels were determined by the glyceryl phosphate oxidase–peroxidase–antiperoxidase method using a commercially available kit (Boehringer Mannheim, Germany). High-density lipoprotein (HDL) cholesterol levels were measured after pretreatment of the serum samples with buffered magnesium phosphotungstate, which selectively precipitates low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol and chylomicrons, leaving HDL cholesterol in the supernatant. Serum levels of LDL cholesterol were calculated using the Friedewald equation (Friedewald *et al.*, 1972).

Endometrial assessment

Endometrial thickness was measured in transverse section by transabdominal ultrasound using a Siemens Sonoline SL2 with a 3.5 MHz sector probe. Baseline and repeat scans at 3–6 monthly intervals were performed in five patients. Endometrial histology was reviewed on one patient who had a hysterectomy for uterine prolapse after 18 months on ICI 182780.

Table I Patient characteristics, tumour receptor status and response to ICI 182780

No.	Age at entry	Time to relapse	Duration of adjuvant tamoxifen	Duration of tamoxifen for adv	Response to tamoxifen	Sites of disease	ER[a]	PR[a]	Response to 182780	Duration (months)
1 (ERD)	51	48	48	–	–	Bone	0	0	PD	<2
2 (PF)	75	–	–	8	NC	Breast Nodes	99	99	NC	29
3 (SAS)	49	74	68	–	–	Lung Pleura	0	22	PR	12
4 (AS)	53	45	45	–	–	Bone	16	0	PD	<2
5 (AR)	68	20	20	–	–	Bone Pleura	74	<5	PR	8
6 (FC)	58	77	77	–	–	Nodes	73	6	PR	3
7 (SC)	61	–	–	8	PR	Bone Breast	ND	ND	PD	<2
8 (LH)	55	48	–	19	PR	Breast Node	70	95	PR	25
9 (NT)	70	42	9	–	–	Local Bone	95	80	PD	<2
10 (MC)	64	201	–	8	NC	Local	100	100	PR	33+
11 (FWT)	70	271	–	7	NC	Nodes Bone Breast	60	<5	PD	<2
12 (MEU)	51	77	74	–	–	Bone	99	97	NC	33+
13 (KG)	62	–	–	12	NC	Nodes Bone	90	60	PD	<2
14 (IN)	78	120	–	24	PR	Bone	100	0	PR	32+
15 (CA)	48	61	61	–	–	Bone	ND	ND	NC	
16 (AC)	64	68	67	–	–	Nodes Breast	30	<5	PR	25
17 (LM)	67	52	–	34	NC	Breast Bone	70	30	NC	9
18 (JKJ)	65	80	–	48	NC	Bone	(1828)[b]	(1)	NC	30+
19 (MB)	64	23	23	–	–	Bone	29	29	NC	30+

adv, advanced disease; PD, progressive disease; NC, no change; PR, partial response; ND, not done. [a]% cells positive, immunoassay. [b]Biochemical assay (mol l^{-1}).

Statistical analysis

All statistical analyses were performed on an Apple Macintosh personal computer, using the StatView SE software programme (Abacus Concepts, Berkeley, CA, USA). Pharmacokinectic data were analysed using parametric statistics. Data relating to body weight, serum gonadotrophin, SHBG and lipid levels were analysed using non-parametric statistics. The null hypothesis was rejected at a probability level of $P \leqslant 0.05$.

Results

Pharmacokinetics

Serum concentrations of ICI 182780 were measured during the first month of treatment in 15 patients who started treatment at the 250 mg dose level and in 11 patients who remained on treatment with ICI 182780 during the sixth month. In the majority of patients, the measured C_{max} was reached 8 or 9 days after the start of the drug administration. However, samples were not available between day 2 and day 8. The profile was quite flat between days 2 and 8, supported by preclinical data in dogs where the C_{max} was seen on day 1 or 2. Following both the 100 mg and 250 mg doses, continuous release of drug from the ICI 182780 slow release formulation was shown throughout the one month dosing interval. The profiles of the serum concentration of ICI 182780 are shown in Figure 2. Comparison of data after the first and sixth monthly 250 mg doses of ICI 182780 showed that the mean exposure to the drug increased slightly after multiple dosing. Mean C_{max} (which occurred on day 7) increased from 10.5 ng ml^{-1} to 12.8 ng ml^{-1}, accompanied by increases in mean end-of-month concentrations from 3.1 ng ml^{-1} to 5.6 ng ml^{-1} and AUC values from 140.5 ng day ml^{-1} to 206.8 ng day ml^{-1} for the first and sixth months respectively in the 11 patients studied. Multiple dosing produced a 1.2-fold increase in C_{max} and a 1.5-fold increase in AUC, indicating a degree of accumulation at the 250 mg dose level. This greater exposure was not associated with any increased side-effects or irritancy (see below). There was no significant difference in the median C_{max} and AUC between responders and non-responders to treatment (Table II). After 6 months of treatment there was no significant difference between C_{max} and AUC for patients who had a partial reponse (PR) compared with those with a no change (NC) response.

Effects on hormones and lipids

The serum levels of FSH, LH, prolactin and SHBG, before and during treatment with ICI 182780, are shown in Figure 3. The median levels of FSH and LH before starting treatment with ICI 182780 were below the normal range for post-menopausal women, whereas the median SHBG level was above the normal range, both possibly related to the

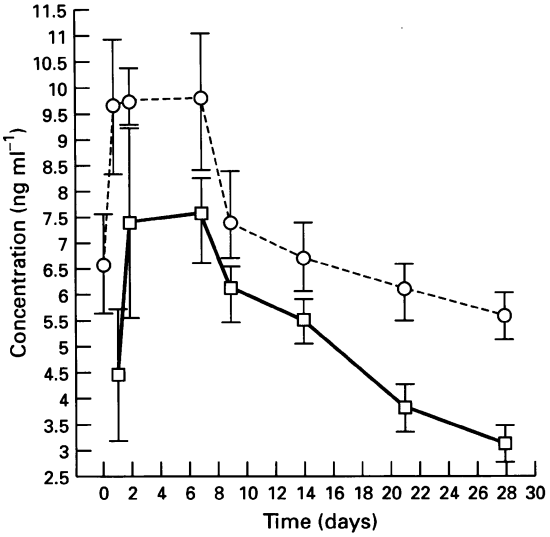

Figure 2 Mean serum concentrations of ICI 182780 during the first and sixth months of treatment. ——, Profile at entry; - - -, profile month 6.

agonist activity of previous treatment with tamoxifen. During the first 3 months of administration of ICI 182780, there was significant increases in the serum concentration of FSH (median pre- and post-treatment values 26 and 52 IU l^{-1} respectively; $P<0.05$, Wilcoxon's matched-pairs signed-rank test) and LH (median pre- and post-treatment values 26 and 42 IU l^{-1} respectively; $P<0.005$). Thereafter, no further significant overall changes occurred in serum gonadotrophin levels but wide variation between individual patients were observed, reflected in the broad interquartile ranges seen in Figure 3. Serum SHBG levels showed an overall trend to decrease following treatment with the specific anti-oestrogen, falling from a median level of 100 mmol l^{-1} pretreatment to 55 mmol l^{-1} after 8 months of treatment (P=NS. Figure 3c). This overall reduction appeared to result predominantly from four patients who continued tamoxifen up to the time of starting ICI 182780. The remaining patients, including 11 others on tamoxifen and four who had stopped tamoxifen some time before entry, showed very little change in serum SHBG levels during treatment. Serum prolactin levels remained within the normal range, and did not change significantly throughout the treatment period. There were no significant changes in serum levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride (Figure 3) for the 12 patients treated in the South Manchester Breast Unit during treatment with ICI 182780.

Side-effects

No serious drug-related adverse events occurred in any of the 19 patients treated with ICI 182780. Minor systemic adverse events were reported by two patients and comprised a transient bloodstained vaginal discharge and a subjective feeling of living in a 'dream-like state' (similar to one she had while taking tamoxifen) in one patient and alteration of body odour (noticed by her husband for a 1 month period), possibly associated with increased hair greasiness, in the other. Administration of the pure anti-oestrogen was not associated with any alteration in the frequency of night sweats or hot flushes, if already present, and none were initiated. None of the patients reported vaginal dryness or altered libido despite direct questioning at each monthly out-patient attendence. The long-acting formulation of ICI 182780 used in this study appeared well tolerated locally at the site of injection despite the relatively large volume (5 ml) administered. One patient developed bruising over the buttock and a second developed tenderness at the injection site following drug administration on one occasion each, and a third patient had local erythema at the injection site on one occasion. No clinically significant changes in full blood count or unexpected changes in the biochemical profile occurred in any of the patients participating in the study.

Serial endometrial ultrasound examinations were per-

Table II Results of C_{max} and AUC during months 1 and 6 according to response categories. There were no significant differences in drug kinetics between responders and non-responders

		On entry		*At month 6*	
Response	*Patient*	C_{max} (ng ml^{-1})	*AUC* (ng day ml^{-1})	C_{max} (ng ml^{-1})	*AUC* (ng day ml^{-1})
Progressive disease	1	4.4[a]	53.1[a]		
	4	1.6[a]	25.1[a]		
	7	5.5	105.7		
	9	9.7	138.2		
	11	29.9	289.3		
	13	5.6	36.7	15.8	243.7
	Median	5.6	79.4		
No change	2	1.8[a]	23.2[a]	7.5	135.8
	12	7.2	143.6	12.2	179.3
	15	9.0	107.8	15.8	201.7
	17	9.5	125.5	14.9	297.6
	18	10.3	183.2	10.2	156.1
	19	11.0	137.8	17.2	308.0
	Median	9.3	131.6	12.8	190.9
Partial response	3	2.9[a]	56.2[a]	9.9	139.5
	5	17.4	188.4	17.6	203.0
	6	7.7	118.3		
	8	5.9	118.7	10.0	175.2
	10	14.8	206.6	9.1	191.7
	14	9.1	134.6	13.5	266.0
	16	4.4	72.8	12.0	190.8
	Median	7.7	118.7	11.0	191.0

[a] Patients 1–4 received 100 mg dose at entry and 250 mg dose from month 2 onwards.

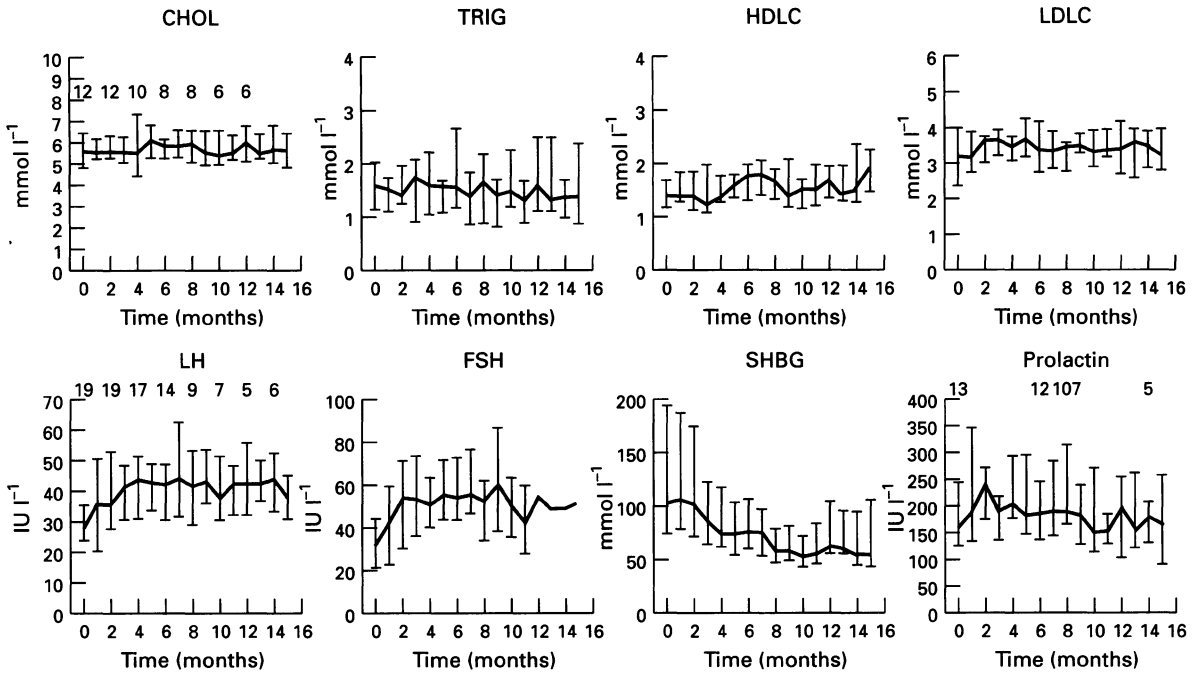

Figure 3 Median and interquartile ranges of lipids and hormones during treatment with ICI 182780. Numbers above the curves refer to the numbers of patients tested. Twelve patients were tested for the four lipids (CHOL, cholesterol; TRIG, triglyceride; HDLC, high-density lipoprotein; LDLC, low-density lipoprotein), 19 for the LH, FSH and SHBG and 13 for prolactin. Numbers decline because patients go off study after progression.

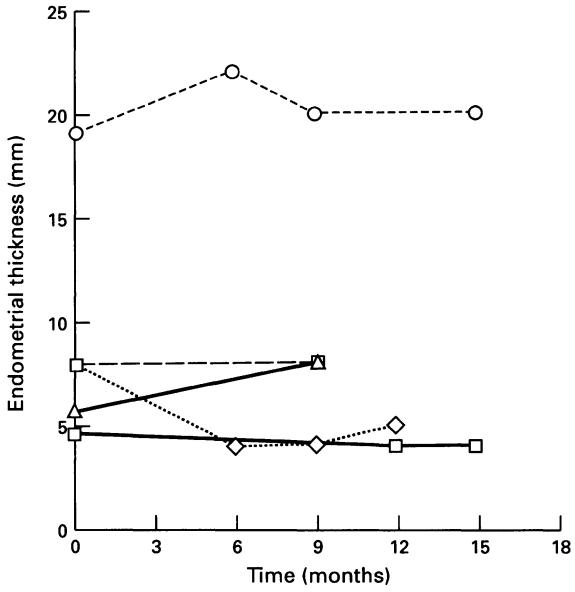

Figure 4 Serial ultrasound estimations of endometrial thickness in five patients. Thickened endometrium compared with normal post-menopausal women was thought to be due to treatment with tamoxifen. No significant change occurred up to 15 months of treatment with ICI 182780.

Table III Response rate and durations of response to ICI 182780 in relation to duration of previous treatment with tamoxifen

Response ICI 182780	*Number (%)*	*Durations (months)*
Partial	7 (37)	25+ (PR19)[a], 33+ (NC8), 32+ (PR24) 25+ (A67), 12 (A74), 8 (A20), 3 (A77)
No change	6 (32)	29+ (NC8), 33+ (A74), 23+ (A61), 30+ (NC48), 30+ (A23), 9 (NC34)
Progression	6 (31)	All patients progressed in <8 weeks (A48, A45, NC7, PR8, A9, NC12)

[a]Letters in brackets indicate response to tamoxifen when given for advanced disease (PR, partial remission; NC, no change; PD, progressive disease) or if given as an adjuvant therapy (A). The numbers in the brackets indicate duration of treatment with tamoxifen.

formed in five responding patients. Endometrial thickness was greater than the expected <2 mm usually found in post-menopausal women, in all patients. The thickness of the endometrium remained unchanged in all patients during treatment with ICI 182780 (Figure 4). Endometrial histology was reviewed on one patient who had a hysterectomy. This was reported as showing an atrophic post-menopausal pattern with cystic change. The glands were lined by flattened and cuboidal epithelium. There was no mitotic activity, epithelial ectoplasia or polyp formation. There were no significant changes in body weight during treatment with ICI 182870. Mean body weight (kg ± s.d.) was 63.8 ± 14.0 (n = 13) at the beginning of treatment, 64.9 ± 15.8 (n = 11) after 6 months, 64.5 ± 17.2 (n = 9) after 10 months and 64.2 ± 18.3 (n = 9) after 16 months of treatment with ICI 182780.

Response

All 19 patients are evaluable for response to ICI 182780 (Table III). Six patients were unresponsive *de novo* and showed objective evidence of disease progression within 2 months of commencing treatment. The remaining 13 patients (69%) all responded to treatment with the specific anti-oestrogen for a median duration of 25 months. Seven patients (37%) showed PRs for 33+, 32+, 25+, 25, 12, 8 and 3 months, and six patients (32%) showed NC responses for 33+, 30+, 30+, 29, 23 and 9 months. Thus five patients are still in remission and continuing treatment with ICI 182780 after 30–33 months. Responses have been observed in six of the nine women who progressed while receiving tamoxifen as

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