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Effects of fulvestrant 250 mg in premenopausal women with oestrogen receptor-positive primary breast cancer

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ABSTRACT

Fulvestrant (Faslodex™) reduces markers of hormone sensitivity and proliferation in postmenopausal women. This Phase II double-blind, randomised, multicentre study compared the effects of a single 250 mg intramuscular dose of fulvestrant and placebo 14–21 days prior to surgery of curative intent on the oestrogen receptor (ER), progesterone receptor and Ki67 levels in 66 premenopausal women with ER-positive primary breast cancer. There were no statistically significant differences between fulvestrant and placebo with respect to any of the three markers analysed. The most common adverse events in both groups were nausea, headache and pyrexia. Fulvestrant 250 mg had no effects on markers of hormone-sensitivity and proliferation in premenopausal women with primary breast cancer when measured at 14–21 days after injection. These findings suggest that a higher fulvestrant dose may be required in this patient population. Further clinical trials are necessary to evaluate the efficacy of fulvestrant in premenopausal women.

1. Introduction

Inhibiting oestrogen production or reducing the binding of oestrogen to the oestrogen receptor (ER) are established rationales for the design of therapeutic agents to treat hormone-sensitive breast cancer.[1] Fulvestrant (Faslodex™) is an ER antagonist with no agonist effects,[2,3] which binds, blocks and degrades the ER. This results in reduced cellular levels of both the ER and progesterone receptor (PgR).[4,5] Although fulvestrant is indicated for the treatment of advanced disease in postmenopausal women, in the premenopausal setting, antioestrogens have been used alone and in combination with luteinising hormone-releasing hormone agonists (LHRHa's). Fulvestrant lacks cross-resistance with many other commonly used endocrine agents (*e.g.* tamoxifen and other selective ER modulators [SERMs]) and therefore may have the potential to provide an alternative additional treatment in the premenopausal setting.

The biological effects of fulvestrant have been evaluated in trials in postmenopausal women with primary breast cancer. In this patient population, fulvestrant (6 mg or 18 mg daily for 7 days or a single 50 mg, 125 mg or 250 mg dose) has been shown to reduce levels of markers of hormone sensitivity (ER and PgR) and proliferation (Ki67).[5,6] Furthermore, results from a recent study in postmenopausal women receiving fulvestrant as first-line therapy for advanced breast cancer

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showed ER remains downregulated at 6 months compared with the pre-treatment samples.[7]

Fulvestrant (250 mg/month) has been shown to be at least as effective as the third-generation, selective aromatase inhibitor anastrozole (1 mg/day) in the treatment of postmenopausal patients with advanced breast cancer who had progressed on prior antioestrogen therapy.[8,9] Recently, data from phase II studies and from a compassionate use programme have suggested fulvestrant may also be effective in postmenopausal women with advanced disease following progression on other endocrine treatments including aromatase inhibitors.[10–13] The effects of fulvestrant in premenopausal women with ER-positive primary breast cancer are yet to be investigated.

The primary objective of this study was to compare the effects of a single 250 mg intramuscular (i.m.) dose of fulvestrant with placebo on ER, PgR and Ki67 levels in premenopausal women with ER-positive primary breast cancer. The study also assessed the safety and tolerability of single-dose fulvestrant in premenopausal women.

2. Patients and methods

2.1. Study design and patients

This was a phase II double-blind, randomised, placebo-controlled, multicentre European study that aimed to recruit 80 women with breast cancer (40 per treatment arm). The study included premenopausal women <50 years of age, with histologically or cytologically confirmed primary ER-positive breast cancer (T1–T3). Patients were also required to be fit for surgery within 1 month of randomisation.

Patients experiencing any recent changes in the frequency of menses and/or menopausal symptoms were not permitted in this study, however, women who had undergone hysterectomy with preservation of at least one ovary were eligible if their follicle-stimulating hormone [FSH] and oestradiol levels were within the premenopausal range. Patients with metastatic disease, those who had previously received treatment with tamoxifen or another hormonal therapy for breast cancer (or had received any other treatment affecting sex hormone levels within 4 weeks of randomisation) or radiotherapy to the primary tumour, were excluded. Patients with a history of disease affecting steroid metabolism or with evidence of severe or uncontrolled systemic disease were also excluded. Written informed consent was obtained from each patient. The first patient was recruited on 15 January 2001 and the last patient completed the study on 21 January 2002.

Patients received fulvestrant 250 mg (or matching placebo) as a single 5 ml, long-acting i.m. injection into the gluteus maximus and then underwent curative-intent surgery for their primary tumour 14–21 days later. The date of surgery was flexible within these limits in order to accommodate hospital surgical procedures.

2.2. Assessment of tumour markers

2.2.1. Biopsy and surgical specimens

A biopsy was taken from the primary tumour tissue for diagnostic purposes before the study treatment was administered, using a core-cut or tru-cut device with a 14-gauge needle. A sample of the tumour was also obtained at the time of surgery (between 14 and 21 days after administration of study treatment). These biopsy samples were fixed in formalin and embedded in paraffin wax blocks prior to histological examination. Assessment of the ER, PgR and Ki67 levels was performed at the Tenovus Cancer Research Institute, Cardiff, UK. For all the tumour marker analyses, matched pre- and post-breast cancer samples for each patient were run as paired samples in the same assay, and included a positive control breast cancer slide of known marker positivity for quality control purposes. Any areas of normal or benign breast tissue were excluded from the assessment of tumour immunostaining.

2.2.2. ER and PgR levels

Two different immunohistochemistry assays were used for the measurement of ER levels (using H222[14] and ID5 antibodies[15,16]) and two were also used for assessing PgR levels (using KD68[14] and PgR636[17] antibodies). This approach was taken to ensure maximal detection of steroid receptors in the premenopausal breast cancer material. The H222 and KD68 antibodies were a kind gift of Dr. Geoffrey L. Greene, at the Ben May Institute for Cancer Research, University of Chicago. The ID5 and PgR636 antibodies (DakoCytomation, Cambridgeshire, UK) are compatible with heat-mediated antigen retrieval and thus ensure restoration of maximal ER/PgR antigenicity and highly reproducible staining using an avidin-biotin complex procedure (immunopositivity appearing clearly as a brown nuclear signal). The assays were fully optimised and validated for routine use in clinical breast cancer material and regularly monitored to maintain high standards through the National External Quality Assurance Scheme for Immunhistochemistry. The H222 and KD68 assays were performed for consistency and to allow comparison with previous studies, where they had proved to be of acceptable sensitivity for use on clinical, paraffin-embedded breast cancer material. Pronase retrieval was used for the H222 assays and no retrieval was used with KD68, as described previously[5]. Both assays were fully optimised and validated for routine use in clinical breast cancer material.

For ER and PgR, tumour epithelial cell nuclear immunostaining was assessed in the breast cancer sections by the consensus agreement of two personnel. Examination of immunostaining was performed for both the pre- and post-surgical specimens for each patient. An overall examination of nuclear staining in each section was performed at an ocular magnification of ×10. Five fields of representatives of the staining across the tumour were then chosen at ×20, and each examined in detail at ×40 for tumour percentage nuclear positivity and nuclear staining intensity (aiming to encompass at least 2000 tumour cells/sample). A consensus value for percentage tumour epithelial cells staining for ER or PgR in each staining intensity category (*i.e.* negative, very weak +/−, weak +, moderate ++, and strong +++) was ascertained and recorded for each sample [5].

Both the ER and PgR levels were expressed as *H*-scores, where

$$H\text{-score} = ([0.5 \times \% +/-] + [1 \times \% +] + [2 \times \% ++] + [3 \times \% +++])$$

H-scores > 0 imply an ER-positive and PgR-positive state with a range between 0 and 300.

2.2.3. *Ki67 levels*

Ki67 is a nuclear antigen expressed by proliferating cells. Assessment of Ki67 labelling index in pre- and post-treatment surgical samples was via immunohistochemistry using a reference mouse monoclonal antibody to the Ki67 nuclear antigen (MIB1).[18,19] This antibody recognises proliferating cells at all stages of the cell cycle (late G1, S, M and G2 phases), but not those in G0 and is applicable for use in formalin-fixed, paraffin-embedded tissue. For this assay, only percentage nuclear immunopositivity was assessed – methodology was as for ER and PgR assays. The Ki67 level was defined as the percentage of cells stained, *i.e.* cells were either positive or negative for staining.

2.2.4. *Plasma concentrations of fulvestrant, FSH, luteinising hormone (LH), oestradiol and progesterone*

Blood samples were taken at baseline (prior to administration of study treatment) and prior to surgery to determine plasma concentrations of fulvestrant, FSH, LH, oestradiol and progesterone. Steroid and pituitary hormone levels, together with the date of the last menses, were used to determine the stage (follicular or luteal) of the patient's menstrual cycle at baseline. Phase of the menstrual cycle was used as a covariate in the statistical analysis of the tumour marker data. This is because endogenous oestrogen levels are generally higher in the follicular phase than during the luteal phase, which may impact on fulvestrant's ability to elicit an effect on tumour/proliferation markers. Analyses of plasma fulvestrant concentrations were performed at the Drug Metabolism and Pharmacokinetics Department, AstraZeneca, Macclesfield, UK, and endocrinology assessments were conducted at Pivotal Laboratories (York, UK). Plasma concentrations were summarised using the mean, standard deviation (SD), and median change from baseline values.

2.3. *Tolerability assessments*

Adverse events were recorded throughout the study and follow-up period (*i.e.* until 8 weeks after the injection). Adverse events were categorised using the MedRA (Medical Dictionary of Regulatory Activities) dictionary and events summarised by the MedRA preferred term and system organ class. Safety data were summarised for all randomised patients by the treatment received, but were not formally analysed.

2.4. *Statistical analyses*

As this was a proof of concept study, the power required for statistical testing was set as 80%. The three primary endpoints (change in ER, PgR and Ki67 levels) were considered of equal importance and the study was powered to detect differences in these variables between the fulvestrant and placebo treatment groups. The number of patients required was estimated using data from a previous study.[5] In that study, the inter-patient SD for the ER, PgR and Ki67 levels were 0.517, 0.291 and 0.588 (log-transformed data), respectively. The differences between the least square means (Lsmeans) for fulvestrant and placebo were 0.61, 0.36 and 0.53 (ratio of geometric [G]Ls means), respectively. For the present study, it was anticipated that fulvestrant would decrease the ER, PgR and Ki67 levels and that placebo would have no effect. Assuming the above SDs, 30 patients per treatment group were calculated as being required to show a difference between treatments of 0.381 (ER), 0.215 (PgR) and 0.644 (Ki67) with 80% power using a 2-sided significance level of 5%.

Hence, a minimum of 30 patients per treatment group was required to complete the study. In order to account for withdrawals and non-compliance, a total of 80 patients (40 per treatment group), were to be recruited.

A per protocol analysis was used to evaluate the effect of fulvestrant 250 mg on the ER, PgR and Ki67 levels. The per protocol analysis excluded data from patients with significant protocol violations or deviations. Only those per protocol patients who had a baseline ER *H*-score > 0 were included in ER analyses and only those with a baseline PgR *H*-score > 0 were included in the PgR analyses. For the Ki67 analysis, all per protocol patients with a value for both baseline and surgery visits were included.

All three tumour markers were assessed using analysis of covariance (ANCOVA). Treatment group, centre, and phase of the menstrual cycle (follicular or luteal) were included in the model as class covariates and baseline tumour marker as a continuous covariate. A test for a treatment-by-centre interaction was performed at the 1% level and a test for both treatment-by-baseline tumour interaction and treatment-by-phase of menstrual cycle interaction together was performed at the 5% level, using an *F*-test. The difference in tumour marker levels between fulvestrant 250 mg and placebo treatment groups was presented in terms of the difference in the Lsmeans and the associated 95% confidence intervals (CI) and *P*-value. The *P*-values were obtained from Type III sum of squares for the variable of treatment group.

3. Results

3.1. *Patients*

Thirty-nine patients received fulvestrant 250 mg and 40 patients received placebo. Of these 79 patients, 13 were excluded from the per protocol population because of major protocol violations/deviations (eight in the fulvestrant group and five in the placebo group). Reasons for exclusion from the per protocol population included no histological/cytological confirmation of breast cancer (one patient), commencement of treatment affecting sex hormone status/disease response (one patient) and surgery not between 14 and 21 days after administration of study treatment (12 patients) [patients could have had more than one reason for exclusion]. Of these 12 patients, four were lost to follow-up, one patient was withdrawn because of protocol non-compliance and seven patients had surgery outside of the specified days (surgery occurred on day 13 or between days 22–27 following administration of study treatment in these patients).

Mean age, age distribution and weight were similar in the two groups. At the start of the treatment, similar number of patients were found to be in the follicular and luteal phases of the menstrual cycle in the fulvestrant 250 mg group, while

Table 1 – Patient characteristics at baseline (per protocol population)

	Treatment group	
	Fulvestrant 250 mg ($n = 31$)	Placebo ($n = 35$)
Mean age, years (range)	44.2 (35–49)	43.3 (25–49)
Age distribution, n (%)		
<35 years	0 (0)	3 (8.6)
35–44 years	15 (48.4)	14 (40.0)
⩾45 years	16 (51.6)	18 (51.4)
Mean weight, kg (range)	66.1 (48.0–91.2)	64.3 (40.8–88.0)
Race, n (%)		
White	31 (100.0)	35 (100.0)
Phase of menstrual cycle, n (%)[a]		
Follicular	15 (48.4)	21 (60.0)
Luteal	16 (51.6)	14 (40.0)

a Assigned at baseline (prior to administration of study treatment) by the study team physician using baseline FSH, LH, oestradiol and progesterone values and date of onset of last menses.

in the placebo group slightly more patients were in the follicular phase than in the luteal phase (Table 1).

3.2. *Endocrine values and tumour markers*

Results of the analyses comparing the effects of fulvestrant 250 mg and placebo on ER (using H222 and ID5 assays), PgR (using KD68 and PgR636 assays), and Ki67 levels are shown in Tables 2 and 3. There were no statistically significant differences between fulvestrant 250 mg and placebo with respect to any of the three tumour markers analysed. A reduction in PgR levels was observed in the fulvestrant group using the KD68 assay, but this was not statistically significant at the 5% level. No reduction in PgR levels was observed using the PgR636 assay. An informal post-hoc analysis of treatment effect by the centre showed similar results to the overall analysis. As there were only 11 patients in the Ki67 per protocol analysis (fulvestrant $n = 5$, placebo $n = 6$) who had either missing or zero ER or PgR *H*-scores at baseline, excluding these patients would make no substantial difference to the results.

A total of 22 patients (fulvestrant $n = 10$; placebo $n = 12$) had a baseline ER *H*-score ⩾80. In this subset of patients, who might be considered as most likely to respond to endocrine therapy, there were no apparent differences between the fulvestrant and placebo groups with respect to any of the three markers and as such, reflect the findings in the overall population (Table 4).

Mean plasma concentrations of fulvestrant were similar in the follicular and luteal phases (4.57 ng/ml and 3.84 ng/ml, respectively) and the overall mean (SD) for both phases combined was 4.21 (1.28) ng/ml. There was no clear evidence of a trend between the magnitude of change in each tumour marker and the plasma fulvestrant concentration on the day of surgery or the oestradiol/plasma fulvestrant concentration. However, there was a large difference in the change from baseline in oestradiol concentration for patients starting treatment during the luteal phase in the fulvestrant 250 mg group (median change 450.0 pmol/L), compared with placebo (median change 42.0 pmol/ml) (Table 5). This difference was not related to the development of adverse events or clinical symptoms and is of unknown clinical significance. Differences between fulvestrant 250 mg and placebo groups for FSH, LH and progesterone were small.

3.3. *Tolerability*

Overall, the incidence and type of adverse events were similar between groups. One patient experienced a serious adverse event (fulvestrant 250 mg group), a relapse of pre-existing pancreatitis and cholecystitis. Excluding the adverse events related to surgery, the most common events in the fulvestrant 250 mg group were nausea (five patients, 12.8%) and headache (three patients, 7.7%) and in the placebo group were nausea

Table 2 – Primary analyses of the effect of fulvestrant on tumour markers (per protocol population) using the H222 assay for ER and the KD68 assay for PgR

	Baseline mean ± SD (range)	Surgery mean ± SD (range)	Mean ± SD change from baseline (range)	Lsmean	Treatment effect[a] (95% CI)	P-value
ER level (H222)[b]						
Fulvestrant 250 mg ($n = 29$)	57.8 ± 46.6 (1–180)	40.4 ± 26.2 (0–95)	−17.4 ± 48.2 (−138 to 53)	49.5	5 (−9 to 20)	0.48
Placebo ($n = 31$)	64.2 ± 41.5 (1–138)	39.6 ± 39.0 (0–120)	−24.6 ± 48.1 (−118 to 88)	44.3		
PgR level (KD68)[c]						
Fulvestrant 250 mg ($n = 26$)	81.0 ± 54.8 (1–190)	73.5 ± 57.7 (0–170)	−7.5 ± 6.4 (−168 to 105)	64.7	−23 (−49 to 3)	0.08
Placebo ($n = 26$)	76.7 ± 57.2 (1–170)	95.6 ± 58.4 (0–180)	18.9 ± 45.1 (−65 to 109)	87.8		
Ki67 level[d]						
Fulvestrant 250 mg ($n = 30$)	23.6 ± 21.5 (0–80)	20.7 ± 16.7 (0–70)	−2.8 ± 22.5 (−65 to 48)	24.0	0 (−7 to 7)	0.97
Placebo ($n = 32$)	29.4 ± 20.7 (1–80)	22.2 ± 22.8 (1–80)	−7.1 ± 18.0 (−55 to 30)	23.8		

ER, oestrogen receptor; PgR, progesterone receptor; Lsmean, least square mean.
a Difference in Lsmean.
b Includes patients with ER-positive tumours who had an ER *H*-score > 0 at surgery.
c Includes patients with PgR-positive tumours who had a PgR *H*-score > 0 at surgery.
d Includes all per-protocol patients with a Ki76 value at baseline and surgery, expressed as a percentage of cells staining.

Table 3 – Supportive analyses of the effect of fulvestrant on tumour markers (per protocol population) using the 1D5 assay for ER and the 636 assay for PgR

	Baseline mean ± SD (range)	Surgery mean ± SD (range)	Change from baseline mean ± SD (range)	Lsmean	Treatment effect[a] (95% CI)	P-value
ER level (ID5)[b]						
Fulvestrant 250 mg (*n* = 30)	135.6 ± 62.3 (1–250)	92.0 ± 49.1 (4–179)	−43.6 ± 71.1 (−246 to 59)	116.0	−3.7 (−30 to 22)	0.77
Placebo (*n* = 30)	164.3 ± 54.1 (8–240)	106.7 ± 69.0 (0–210)	−57.6 ± 80.1 (−180 to 183)	119.7		
PgR level (636)[c]						
Fulvestrant 250 mg (*n* = 27)	134.8 ± 63.9 (7–230)	115.1 ± 62.0 (6–220)	−197.0 ± 70.7 (−204 to 140)	115.7	−13.9 (−41 to 13)	0.31
Placebo (*n* = 27)	138.5 ± 70.6 (1–210)	131 ± 65.7 (3–210)	−7.5 ± 4.6 (−110 to 120)	129.6		

ER, oestrogen receptor; PgR, progesterone receptor; Lsmean, least square mean.
a Difference in Lsmean.
b Includes patients with ER-positive tumours who had an ER *H*-score > 0 at surgery.
c Includes patients with PgR-positive tumours who had a PgR *H*-score > 0 at surgery.

Table 4 – The effect of fulvestrant on tumour markers (patients with an ER level ⩾ 80 at baseline) using the H222 assay for ER and the KD68 assay for PgR

	Baseline mean ± SD (range)	Surgery mean ± SD (range)	Mean ± SD change from baseline mean (range)
ER level (H222)[a]			
Fulvestrant 250 mg (*n* = 10)	112.8 ± 29.7 (85–180)	43.8 ± 32.6 (3–95)	−69.0 ± 40.2 (−138 to 0)
Placebo (*n* = 12)	107.5 ± 0.18.7 (85–138)	45.8 ± 44.2 (3–110)	−61.7 ± 47.7 (−118 to 20)
PgR level (KD68)[b]			
Fulvestrant 250 mg (*n* = 10)	99.7 ± 62.4 (12–190)	73.2 ± 52.5 (0–140)	−26.5 ± 75.4 (−168 to 80)
Placebo (*n* = 12)	80.7 ± 56.4 (1–170)	117.5 ± 45.2 (0–170)	36.8 ± 49.5 (−30 to 109)
Ki67 level[c]			
Fulvestrant 250 mg (*n* = 10)	35.5 ± 21.8 (15–80)	13.4 ± 6.8 (2–25)	−22.1 ± 22.5 (−65 to 5)
Placebo (*n* = 12)	27.4 ± 18.4 (7–60)	13.7 ± 18.4 (1–53)	−13.7 ± 16.8 (−48 to 15)

ER, oestrogen receptor; PgR, progesterone receptor; SD, standard deviation.
a Includes patients with ER-positive tumours who had an ER *H*-score > 0 at surgery.
b Includes patients with PgR-positive tumours who had a PgR *H*-score > 0 at surgery.
c Includes all per-protocol patients with a Ki67 value at baseline and surgery, expressed as a percentage of cells staining.

Table 5 – Median change from baseline in FSH, LH, oestradiol and progesterone (all patients who received study treatment)

	Endocrine parameter			
	FSH (IU/L)	LH (IU/L)	Oestradiol (pmol/L)	Progesterone (nmol/L)
Fulvestrant 250 mg (*n* = 37)				
Follicular phase[a]	−1.50	−1.28	92.0	4.90
Luteal phase[a]	1.20	1.56	450.0	−17.15
Combined	−0.50	−0.30	194.0	0.00
Placebo (*n* = 36)				
Follicular phase[a]	−5.60	−2.80	114.0	14.95
Luteal phase[a]	3.00	2.45	42.0	−14.30
Combined	−1.05	−1.20	69.5	1.55

FSH, follicle-stimulating hormone; LH, luteinising hormone.
a Assigned at baseline (prior to administration of study treatment) by the Study team physician using baseline FSH, LH, oestradiol and progesterone values and date of onset of last menses.

Table 6 – Overview of adverse events experienced by >1 patient, excluding events related to surgery (all patients who received treatment)

Organ class and MedRA term	Number of patients[a] (%)	
	Fulvestrant 250 mg (*n* = 39)	Placebo (*n* = 40)
Total number of patients with adverse events	11 (28.2)	10 (25.0)
Gastrointestinal disorders, Nausea	5 (12.8)	2 (5.0)
General disorders, Pyrexia	0 (0)	2 (5.0)
Musculoskeletal and connective tissue disorders, Back pain	1 (2.6)	1 (2.5)
Nervous system disorders		
Headache	3 (7.7)	1 (2.5)
Dizziness	1 (2.6)	1 (2.5)

a Patients may have experienced >1 type of adverse event.

(two patients, 5.0%) and pyrexia (two patients, 5.0%) (Table 6). Both fulvestrant 250 mg and placebo were well tolerated and no patients experienced an adverse event as a result of changes in biochemical, haematologic or hormone levels.

4. Discussion

This trial investigated the effect of a single fulvestrant 250 mg i.m. dose on hormone receptors and an anti-proliferation marker in premenopausal women with ER-positive primary breast cancer. At the time of surgery, within 14–21 days after treatment administration, no significant differences in ER, PgR and Ki67 levels in breast tumour tissue were observed between patients who received fulvestrant and those who received placebo. Fulvestrant 250 mg did not downregulate cellular levels of ER and PgR but was well tolerated in this patient population. There are several possible reasons for these results, which are explored below.

Previously, in a phase I/II trial in postmenopausal women with primary breast cancer, a significant decrease in expression of ER and PgR was observed after treatment with seven daily doses of fulvestrant (6 mg or 18 mg).[6] Reduced tumour cell proliferation, indicated by reduced expression of Ki67 and the oestrogen-regulated protein pS2, was also observed.[6] In a subsequent study, postmenopausal women with primary breast cancer received either a single i.m. dose of fulvestrant 50 mg, 125 mg or 250 mg, or continuous oral daily tamoxifen, or matching placebo for 14–21 days prior to surgery of curative intent.[5] In this trial, dose-dependent reductions in ER levels and Ki67 labelling were observed at all fulvestrant doses.[5] Fulvestrant also produced significant reductions in PgR levels. In contrast, tamoxifen produced a significant increase in PgR, a finding attributed to its partial agonist properties and confirming that fulvestrant has a different mode of action to that of tamoxifen.

The lack of reductions in ER levels in the present study suggests that the 250 mg fulvestrant dose may be insufficient to provide antioestrogen activity in the premenopausal setting. This is likely to be related to the logarithmically higher oestradiol levels present in premenopausal women that may outcompete fulvestrant for binding the ER. The results of a recent study comparing the biological effects of a single dose of fulvestrant 750 mg with tamoxifen 20 mg/day for 14–16 days prior to surgery in premenopausal patients with ER-positive breast cancer are in support of this hypothesis.[20,21] Both fulvestrant and tamoxifen significantly reduced ER levels although the fall in ER was significantly greater in the fulvestrant group. Both agents also significantly reduced proliferation and fulvestrant but not tamoxifen which significantly reduced PgR levels.[21] These data suggest that fulvestrant 750 mg rather than 250 mg may be the clinically effective dose in premenopausal patients. However, until results from studies directly assessing the clinical activity of fulvestrant 750 mg in premenopausal patients are available, the optimum dose in this setting is yet to be determined. In line with the findings of the present study, fulvestrant 50, 125 and 250 mg (every 4 weeks for a total of 12 weeks) did not significantly reduce fibroid volume, endometrial thickness or change endpoints such as endometrial histology or vaginal bleeding in premenopausal patients awaiting hysterectomy for uterine fibroid growth.[22] In an earlier study of fulvestrant in premenopausal women with benign gynaecological disease, an i.m. fulvestrant dose of 12 mg/day (short-acting formulation) for 7 days prior to surgery reduced endometrial Ki67 levels but did not markedly reduce ER or PgR levels, although significantly lower ER levels were observed in the myometrial cells of the treated group.[23] Interestingly, similar to observations in the present study, a significant increase in plasma oestradiol ($P < 0.05$) was noted in the fulvestrant group on days 5, 6 and 7 of treatment compared with the control group. In the present study, the increase in oestradiol levels during fulvestrant treatment was only noted in patients who were in the luteal phase at baseline – currently the mechanism for this effect is unknown. Despite the continued oestradiol stimulation noted in the study by Thomas *et al.*, there was no significant endometrial growth in the fulvestrant group.[24] The lack of oestrogen agonist activity on the endometrium with fulvestrant treatment and the ability to abrogate oestrogen action was subsequently confirmed in a study in healthy volunteers.[25]

Overall, there are few published clinical trial data on the biological effects of endocrine therapy specifically in premenopausal patients with breast cancer. Prior to the recently published preoperative study mentioned above,[20,21] there were no available data on the biological effects of tamoxifen in premenopausal breast cancer patients. However, a study had investigated the effects of tamoxifen (5, 10 or 20 mg/day for 50 days) on ER, PgR and Ki67 levels in normal breast tissue from premenopausal patients with fibroadenoma. Similar to the data from Young *et al.*, all doses of tamoxifen were found to significantly reduce levels of ER and Ki67 compared with placebo. In contrast, tamoxifen treatment was also found to significantly reduce PgR levels in this study.[26]

Fulvestrant 250 mg/month is approved in the USA and elsewhere for the treatment of postmenopausal women with advanced, ER-positive breast cancer who have progressed or relapsed following prior antioestrogen therapy. In contrast to results observed in a similar study in the postmenopausal setting, our study showed that a single dose of fulvestrant 250 mg had no significant effects on markers of hormone-sensitivity and proliferation in premenopausal women with primary breast cancer. The clinical significance of these findings is not known, however, the fulvestrant dose used may not accurately reflect the clinical activity of this agent and higher doses may be required in premenopausal patients. Nonetheless, a lack of biological effect at two weeks may not necessarily reflect a lack of clinical effect of fulvestrant 250 mg in premenopausal women. Current and future clinical trials, some of which use loading or high-dose fulvestrant regimens, will help determine the efficacy of fulvestrant in this setting and the postmenopausal setting.

Conflict of interest statement

The following authors have other potential financial conflicts of interest: J.F.R. Robertson has performed contract work and received speaking honoraria from AstraZeneca. R.I. Nicholson and J.M.W. Gee have a joint research grant from AstraZeneca. J. Armstrong is an employee of AstraZeneca. The remaining authors have no conflicting interests to declare.

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