Effect of asoprisnil on uterine proliferation markers and endometrial expression of the tumour suppressor gene, PTEN

J. Wilkens¹, A.R.W. Williams², K. Chwalisz³, C. Han⁴, I.T. Cameron⁵, and H.O.D. Critchley¹,⁶

¹Division of Reproductive and Developmental Sciences, Centre for Reproductive Biology, University of Edinburgh, The Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK ²Department of Pathology, University of Edinburgh, Edinburgh EH16 4SA, UK ³Abbott Laboratories, Abbott Park, IL, USA ⁴Takeda Global Research & Development Center, Lake Forest, IL, USA ⁵Developmental Origins of Health and Disease Division (DoHaD), University of Southampton, Southampton SO16 6YD, UK ⁶Correspondence address. Tel: +44-131-242-6858; Fax: +44-131-242-6441; E-mail: hilary.critchley@ed.ac.uk

BACKGROUND: The selective progesterone receptor modulator asoprisnil suppresses uterine bleeding and decreases leiomyoma volume while maintaining follicular phase estrogen concentrations. For safety of potential clinical applications, any proliferative effect of asoprisnil on uterine tissues, particularly endometrium, needs to be established.

METHODS: In a double-blind, randomized, placebo-controlled study (continuation of previously published trial No. NCT00150644 (Williams et al., 2007 and Wilkens et al., 2008)), 33 patients with symptomatic uterine leiomyomata received placebo, 10 or 25 mg asoprisnil daily for 12 weeks before hysterectomy. Proliferation markers Ki-67 and anti-phospho-histone H3 (PH3) were immunolocalized in endometrium, myometrium and leiomyoma tissue. Endometrial PTEN (phosphatase and tensin homologue, a tumour suppressor gene) expression was also assessed by immunohistochemistry. PH3-positive glandular and stromal cells were counted per measured endometrial area. Endometrial Ki-67 expression was assessed using stereological methods. Stained myometrial and leiomyoma cells were counted per 10 fields (×250). PTEN immunostaining was quantified using a histoscore. Each asoprisnil group was compared with placebo (secretory phase) with significance at 0.05 level.

RESULTS: Endometrial epithelial proliferation and PTEN expression were not significantly different between placebo and asoprisnil groups. Decreased stromal Ki-67 expression (P < 0.05) suggested any effect of asoprisnil on endometrial proliferation to be inhibitory. Immunolocalization of PTEN expression was not different between treatment groups in any tissue compartments. Myometrial Ki-67 expression decreased following asoprisnil 25 mg (P < 0.05).

CONCLUSIONS: Asoprisnil does not induce proliferation of uterine tissues and does not suppress endometrial PTEN expression.

Key words: asoprisnil / uterine tissues / proliferation / phosphatase and tensin homologue

Introduction

Selective progesterone receptor modulators (SPRM) are a novel class of progesterone receptor ligands exhibiting mixed and/or partial agonist and antagonist activity. Asoprisnil is the first SPRM that has been clinically evaluated in patients with symptomatic uterine fibroids and endometriosis (Chwalisz et al., 2005b; Wilkens et al., 2008). Asoprisnil is a 11β-benzaldoxime-substituted steroidal SPRM that shows a high degree of uterine selectivity (Schubert et al., 2005).

Previously conducted clinical studies have shown asoprisnil to reversibly suppress uterine bleeding by primarily targeting the endometrium (Chwalisz et al., 2005a). Asoprisnil has further been demonstrated to reduce the volume of uterine fibroids in a dose-dependent manner (Chwalisz et al., 2005b). It has, therefore, potential for the medical management of symptomatic uterine fibroids, which commonly cause heavy menstrual bleeding as well as pressure-related symptoms.

Asoprisnil and other structurally related SPRMs demonstrated endometrial antiproliferative effects in non-human primates (Chwalisz et al., 2005b; Schubert et al., 2005). In cynomolgus monkeys, asoprisnil induced profound endometrial atrophy in the presence of early luteal phase estrogen concentrations (Brenner et al., 2005). This effect was accompanied by a decrease in the proliferation markers Ki-67 and
anti-phospho-histone H3 (PH3). Similar endometrial antiproliferative effects were described in the macaque endometrium with mifepris-
tone (Slayden and Brenner, 1994) and other progesterone receptor
antagonists (antiprogestogens) including ZK 137 316 (Slayden et al.,
1998). The exact mechanism of the endometrial antiproliferative
effects of SPRMs and antiprogestogens remains poorly understood
in spite of extensive studies conducted in non-human primates
(Brenner and Slayden, 2005).

We previously described the effects of asoprisnil on the mor-
phology of the endometrium, myometrium and leiomyomata in
patients with symptomatic uterine leiomyomata treated with asoprisnil
for 3 months prior to hysterectomy (Williams et al., 2007). The results
of this study showed that asoprisnil suppressed endometrial prolifer-
ation, as evidenced by a low mitotic index in endometrial glands and
stroma, which is consistent with the studies conducted in non-human
primates. However, differences in endometrial morphology in
response to asoprisnil treatment were noted in humans compared
with macaques. Although this study also revealed a decrease in endo-
metrial thickness, unique endometrial morphology was observed in
women treated with asoprisnil for 3 months. This was characterized
by weakly secretory endometrial glands with scarce or absent
mitotic activity, variable stromal changes ranging from stromal
compaction to focal pre-deciyal reaction and thickening of the
walls of spiral arterioles. These unusual morphological appearances
were further referred to as 'non-physiologic secretory effects' or
'endometrial SPRM effects' (Williams et al., 2007).

The clinical and histological effects of asoprisnil described above
have been demonstrated in the presence of follicular phase estrogen
concentrations (Wilkens et al., 2008). Concern has previously been
expressed that administration of a compound with progesterone
antagonistic activity may leave the endometrium at risk of hyperplastic
or even malignant changes due to exposure to unopposed estrogen.
Previous studies have reported features of endometrial hyperplasia
following administration of the progesterone antagonist mifepristone
(Eisinger et al., 2003). However, hyperplasia was not observed with
use of low doses of 2 or 5 mg mifepristone, which after administration
to 90 women for 120 days resulted in suppression of endometrial pro-
iferation marker expression (Ki-67 and mitotic index) (Baird et al.,
2003). No study or case report has described complex hyperplasia
or cytological atypia. No endometrial hyperplasia or evidence of unop-
posed estrogen effects have been described with asoprisnil after treat-
ment for up to 3 months (Chwalisz et al., 2005a, 2007).

Endometrial proliferation can be assessed in full thickness endo-
metrial biopsies by means of Ki-67 and PH3 immunohistochemistry.
The nuclear antigen Ki-67 can be detected in all phases of the replicat-
ing cell (G1, S, G2 and M) representing the growth fraction of a cell
population (Gerdes et al., 1984; Endl and Gerdes, 2000). More
recently, Ki-67 has been described to play a role in ribosomal RNA
synthesis as well as mitosis and may therefore also be expressed in
non-proliferating cells (Bullwinkel et al., 2006). The Ki-67 index is
less specific than the mitosis-specific marker PH3, which is only
expressed during the actual phase of mitosis (M) (Brenner et al.,
2003). When proliferation of normal endometrium in different
phases of the menstrual cycle is assessed using various proliferation
markers, they all show a high proliferation index during the prolifera-
tive phase with a significant decrease in the secretory phase. There is a
high correlation between the direct mitotic count and the PH3 count,
while the Ki-67 index shows the same trend but is less specific
(Brenner et al., 2003).

PTEN (phosphatase and tensin homologue) expression in endo-
metrial tissue can be studied as a marker of early carcinogenesis.
PTEN is a tumour suppressor gene product, which has been described
as a gatekeeper for initiation of carcinogenesis in the endometrium
(Mutter, 2001; Daikoku et al., 2008). Loss of PTEN function has been
demonstrated to occur as an early event in endometrial carcino-
genesis and has therefore been suggested as a biomarker for prema-
lignant disease even in histologically normal endometrium (Mutter,
et al., 2000, 2001). The PTEN tumour suppressor gene is inactivated
in up to 83% of endometrioid endometrial adenocarcinomas, the most
common form of endometrial cancer, which has also been associated
with the risk factor of exposure to unopposed estrogen (Mutter,
2001). It has been shown that exogenous hormones can alter PTEN
expression. Progesterone has been shown to play an important role
in eliminating PTEN-deficient endometrial cells when administered
via a progesterin-impregnated intrauterine device (Orbo et al., 2006)
and systemically (Zheng et al., 2004). A compound with partial
progesterone antagonist activity may raise the concern of an unfavour-
able effect on PTEN expression and therefore on the potential to
influence the predisposition to latent endometrial precancerous
lesions.

The objective of this study, which is a continuation of the above-
mentioned study (Williams et al., 2007), was to investigate in detail
the effects of asoprisnil on proliferation of human endometrial, myo-
metrial and leiomyoma tissues and on endometrial PTEN expression.

Materials and Methods

Study design
This was a Phase II multi-centre, double-blind, randomized, placebo-
controlled study of asoprisnil administered for 12 weeks. The study
group was composed of 33 premenopausal women from four centres
(Edinburgh, Southampton, Glasgow, Liverpool) in good general health
screening for entry into the study. The study was performed according
to ethical principles of the Declaration of Helsinki (1989 revision).
The Institutional Review Board (Multicentre Research Ethics Committee)
approved the protocol. All subjects voluntarily signed a full informed
consent form.

Subjects were randomly assigned to one of three parallel treatment
groups in a 1:1:1 ratio to receive daily doses of asoprisnil 10, 25 mg or
placebo. Asoprisnil or placebo capsules were supplied in blister cards
of identical appearance packaged in sealed kits. The drug was self adminis-
tered as a single oral dose taken once daily. Treatment was initiated no
later than the fifth day of the subject’s menstrual cycle and continued
for at least 12 weeks. Hysterectomy was performed within 24 h after
the final dose of drug. Blood specimens for determination of estradiol (E2) and progesterone were collected within 24 h prior to hysterectomy. Compliance was monitored by the subjects returning all used and unused study medication blister cards.

**Sample collection**

After removal of the uterus in the operating theatre, the unfixed specimen was placed on ice and taken without delay to the local pathology laboratory. The specimen was oriented and a probe inserted through the external os of the cervix to define the position of the cavity. The uterus was opened using a long-bladed knife along the plane of the probe. The opened specimen was then placed in an adequate volume of 10% buffered formaldehyde and allowed to fix overnight. For large specimens, parallel parasagittal slices 2 cm in thickness were made to permit adequate fixation. The following day, the pathologist sampled the specimen for routine diagnostic reporting and took additional blocks for study assessment. Study blocks included full thickness endometrium with underlying myometrium. Leiomyomata were also sampled. All study samples were processed by routine methods for paraaffin wax, and 5 μm sections prepared. Endometrial assessment was carried out by microscopic examination of haematoxylin–eosin stained sections. Phase of cycle was assessed using the updated conventional descriptive Noyes criteria of the normal menstrual cycle as described in Blaustein’s Pathology of the Female Genital Tract (Mutter and Ferenczy, 2002).

**Immunohistochemistry**

Five-micrometre paraffin sections were de-waxed in Histoclear (National Diagnostics, Atlanta, GA, USA) for 10 min before rehydration in descending grades of alcohol. The slides were washed with 0.01 M phosphate-buffered saline (PBS; pH 7.4; Sigma) and pressure-cooked in 0.01 M sodium citrate (pH 6) for 5 min at setting 2/high (Tefal, Clipso, Nottingham, UK) for antigen retrieval. The tissue sections were cooled for 20 min and then washed again in PBS before blocking endogenous peroxidase activity by immersion in 3% hydrogen peroxide for 10 min at room temperature. After washing in PBS, the subsequent protocol differed for the two proliferation markers Ki-67 and PH3.

**Ki-67 immunohistochemistry**

Slides were incubated in non-immune horse serum (NHS; Vector Laboratories Inc, UK) in PBS for 20 min at room temperature in order to block non-specific binding of the primary antibody. The primary antibody Ki-67 Novocastra NCL-Ki-67-MM1 (Novocastra, Newcastle-upon-Tyne, UK; 1:100 dilution in NHS/PBS) was added and the slides incubated for 30 min at 37°C. For the negative controls, the primary antibody was replaced with non-immune mouse immunoglobulin (IgG1 antibody at a matched antibody concentration to the Ki-67 antibody (1:1000). Subsequently, the sections were washed in PBS with added Tween 20 (PBST) before incubating in biotinylated horse anti-mouse antibody (Vector Laboratories) for 30 min at room temperature. Following another wash in PBST, an avidin–biotin–peroxidase complex (ABC-HRP; Vectorstain Laboratories) was applied for 30 min at room temperature before the final wash with PBST.

**PH3 immunohistochemistry**

At room temperature, sections were incubated in avidin (Vector Laboratories) for 15 min, rinsed in PBS and then incubated in biotin (Vector Laboratories) for a further 15 min. To block non-specific binding of the primary antibody, slides were incubated in non-immune goat serum (NGS; Autogen Bioclear Cat# 7) in PBS with 5% bovine serum albumin (BSA; Sigma Cat# A-7888) for 20 min at room temperature. The primary antibody PH3 (Cat# 06–570, Upstate Biotechnology, Buckingham, UK; 1:1000 dilution in NGS/PBS/BSA) was added and the slides incubated overnight at room temperature. For the negative controls, the primary antibody was replaced with non-immune rabbit antibody IgG at a matched antibody concentration to H3 (1:1000). Sections were washed in PBST before incubating in anti-rabbit enVision kit (EnVision+ System-HRP, DAKO Cytomation) for 30 min at room temperature and washing again with PBST.

After the final wash with PBST, the protocols for both proliferation markers Ki-67 and PH3 were then followed by the addition of the chromagen 3,3’-diaminobenzidine (DAKO). The reaction was stopped with distilled water when nuclear staining was detected by inspection under the microscope. Harris’s haematoxylin was used for counterstaining. The sections were then dehydrated and finally mounted with Pertex (Cellpath plc, Hemel Hempstead, UK).

**PTEN immunohistochemistry**

For PTEN immunohistochemistry, the paraffin sections were de-waxed, rehydrated and pressure-cooked as described earlier. After cooling down for 20 min, the sections were transferred to the Bond-X immunostaining machine and processed using the Bond Refine Polymer Detection kit (Cat. No DS9800 Vision Biosystems BondTM, Newcastle-upon-Tyne, UK). The primary antibody was PTEN (NCL-PTEN, Novocastra; 1:600 dilution in Bond Antibody diluent, Vision Biosystems) and IgG from the same species and at the same concentration was used as the negative control (Mouse IgG, kappa, Sigma). Sections were dehydrated and mounted as described earlier.

**Scoring**

In myometrium and leiomyoma tissue, Ki-67 and PH3 immunostaining was assessed by randomly selecting 10 fields at ×250 magnification and counting all stained cells. To quantify the amount of staining in endometrium, stereological methods were applied and varied for assessment of Ki-67 and PH3, respectively. For assessment of PTEN immunostaining in endometrium, a histoscore was applied by two independent observers as previously described (Aasmundstad et al., 1992). A separate histoscore was applied to surface epithelium, glandular epithelium, stroma, perivascular cells and endothelium, respectively.

**Ki-67**

Inspection of the endometrial tissue after immunostaining for Ki-67 gave the impression of scanty staining in the majority of sections. In order to quantify the level of immunoreactivity, stereological methods were used as previously described (Mahood et al., 2005). The program used was Image-Pro plus 4.5.1 with Stereology-Pro 5.0 plug-in software (Media Cybernetics UK, Wokingham, Berkshire, UK) in combination with an Olympus BH-2 microscope fitted with a Prior automatic stage (Prior Scientific Instruments Ltd, Cambridge, UK). With the aid of the software, random fields were selected for counting and grids were placed over the fields at ×250 magnification. All 432 intersections of a grid were defined as points, and all points falling over tissue were counted as one of the following categories: (a) unstained epithelial cell, (b) stained epithelial cell, (c) unstained stromal cell, (d) stained stromal cell and (e) lumen (i.e. the empty space within glands or vessels). The proportion of tissue occupied by each of these categories was expressed as a percentage of total points counted.

The number of fields counted was dependent on obtaining a percentage SE value of <10%. For all but four tissue sections, 10 fields (i.e. 4320 points) were sufficient to obtain a percentage SE of <10% for the categories of unstained cells and lumen. For three of the remaining tissue sections, 50 fields (i.e. 21 600 points) and in one case 20 fields...
istical analysis. The remaining eight placebo-treated subjects had been undergone hysterectomy in the proliferative phase of their menstrual cycle and were excluded from the statistical analysis. Two placebo-treated subjects had undergone hysterectomy in the proliferative phase of the menstrual cycle. There was satisfactory drug compliance in all groups. Two placebo-treated groups had a well-matched distribution for age, weight and height (Table I). Ten subjects had been treated with placebo, 12 had received 10 mg asoprisnil and 11 had received 25 mg asoprisnil for an average of 95 days. There was no statistically significant difference between treatment groups in Ki-67 expression in endometrial glandular epithelium (Fig. 1B). The median of counted stained endometrial cells following immunostaining for PH3 was generally very low in epithelium and stroma of both asoprisnil- and secretory phase placebo-treated subjects (<3/mm²). There was no statistically significant difference between treatment groups (Fig. 2). The PH3 data were normalized to the total area of endometrium. The data were also assessed in relation to only epithelial or stromal cells, and those results were comparable and did not add to the results as presented.

### Results

A total of 33 patients were included in the study. Placebo and asoprisnil groups had a well-matched distribution for age, weight and height (Table I). Ten subjects had been treated with placebo, 12 had received 10 mg asoprisnil and 11 had received 25 mg asoprisnil for an average of 95 days. There was satisfactory drug compliance in all groups. Two placebo-treated subjects had undergone hysterectomy in the proliferative phase of their menstrual cycle and were excluded from the statistical analysis. The remaining eight placebo-treated subjects had been in the secretory phase of their cycle and constituted the subgroup for comparison to the asoprisnil-treated groups.

Proliferation marker expression was low in all asoprisnil-treated subjects as well as placebo-treated subjects in the secretory phase in all uterine tissues. There was high proliferation marker expression in the endometrium of the two placebo-treated subjects in the proliferative phase of the menstrual cycle.

### Expression of Ki-67 and PH3 in endometrium

Assessment of proliferation marker expression in endometrial stroma is suggestive of a suppressive effect of asoprisnil (Fig. 1A). Following the method of quantifying Ki-67 immunostaining as described earlier, the median of grid points occupied by stained cells in endometrial epithelium and stroma was <2% in specimens from both asoprisnil- and placebo-treated subjects. There was a statistically significant and dose-dependent decrease in Ki-67 expression in endometrial stroma (Fig. 1A) compared with the placebo-treated subjects in the secretory phase of their menstrual cycle. There was no statistically significant difference between treatment groups in Ki-67 expression in endometrial glandular epithelium (Fig. 1B). The median of counted stained endometrial cells following immunostaining for PH3 was generally very low in epithelium and stroma of both asoprisnil- and secretory phase placebo-treated subjects (<3/mm²). There was no statistically significant difference between treatment groups (Fig. 2). The PH3 data were normalized to the total area of endometrium. The data were also assessed in relation to only epithelial or stromal cells, and those results were comparable and did not add to the results as presented.

### Expression of Ki-67 and PH3 in myometrium and leiomyomata

There also appeared to be a suppressive effect of asoprisnil on myometrial proliferation. There was very low PH3 expression in both asoprisnil- and secretory phase placebo-treated groups (median of less than 10 stained cells per 10 fields at ×250 magnification), and the differences were not statistically significant. There was however a dose-dependent decrease in Ki-67 expression in both asoprisnil-treated groups compared with the secretory phase placebo-treated subjects, but only the difference between asoprisnil 25 mg and secretory phase placebo reached statistical significance (Table II).

#### Table I Demographic data for patients with symptomatic uterine leiomyomata given placebo or asoprisnil daily for 12 weeks before hysterectomy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
<th>Placebo (n = 10)</th>
<th>Asoprisnil 10 mg (n = 12)</th>
<th>Asoprisnil 25 mg (n = 11)</th>
<th>All subjects (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>41.8 (3.6)</td>
<td>45.1 (3.5)</td>
<td>44.6 (6.0)</td>
<td>43.9 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>37–48</td>
<td>39–50</td>
<td>35–52</td>
<td>35–52</td>
<td></td>
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<tr>
<td>Weight (kg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>73.4 (11.7)</td>
<td>73.8 (17.7)</td>
<td>75.9 (11.8)</td>
<td>74.4 (13.8)</td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>54–89</td>
<td>45–105</td>
<td>60–96</td>
<td>45–105</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>165.3 (6.4)</td>
<td>164.3 (4.7)</td>
<td>165.6 (7.3)</td>
<td>165.1 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>158–177</td>
<td>156–172</td>
<td>157–178</td>
<td>156–178</td>
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</tr>
</tbody>
</table>
Assessment of Ki-67 and PH3 expression in leiomyoma tissue revealed a similar trend. There was very low PH3 expression in tissue sections from asoprisnil- and placebo-treated subjects, and the differences between groups were not statistically significant. There was an apparent reduction in Ki-67 expression in both asoprisnil-treated groups, but the differences did not reach statistical significance (Table III).

Endometrial expression of PTEN

There was no difference in immunohistochemical detection of PTEN expression between treatment groups in any of the tissue compartments assessed. PTEN immunohistochemistry resulted in a nuclear staining pattern with similar intensity in glandular epithelium and stroma (Fig. 3). The staining intensity was strongest in the surface epithelium (median histoscores of 125–180) and only weak in endometrial and perivascular cells (median histoscores of 25–32.5 for perivascular cells and 30–45 for endothelium). In asoprisnil and placebo groups, there were inter-individual variations independent of the treatment received with some subjects showing very weak and others quite strong staining. However, there was no significant difference between treatment groups (Fig. 4).

Blood concentrations of E2 and progesterone

E2 and progesterone levels were measured within 24 h prior to hysterectomy (Table IV). The mean E2 value was lower in the 25-mg asoprisnil group compared with both placebo and 10-mg asoprisnil groups. Mean progesterone values were substantially lower in both asoprisnil-treated groups compared with placebo. Although variability across the groups was high, the median progesterone values revealed the same pattern.

Discussion

This study shows that asoprisnil administered daily for 12 weeks exerts an inhibitory effect on endometrial proliferation and proliferation of myometrium and leiomyomata.
Within the placebo group in this study, endometrial glands and stroma showed high proliferation marker expression in subjects who had undergone hysterectomy in the proliferative phase of their menstrual cycle compared with low expression in subjects in the secretory phase, as previously reported (Brenner et al., 2006). Proliferation marker expression following 12 weeks of treatment with asoprisnil was low in most uterine tissues and comparable to the low level of proliferation observed in the placebo-treated subjects in the secretory phase. There was no difference in PH3 expression between treatment groups in endometrium, myometrium or leiomyoma tissue, a likely reflection of the very scanty immunostaining. Ki-67 expression in endometrial epithelium was not significantly different in asoprisnil-treated subjects compared with placebo-treated subjects in the secretory phase. Asoprisnil had a dose-dependent inhibitory effect on proliferation in endometrial stroma as indicated by decreased Ki-67 expression. There was no evidence of altered endometrial PTEN expression by administration of asoprisnil. Dose-dependent suppression of proliferation was demonstrated similarly in myometrium but was only significant with the higher dose of asoprisnil. Proliferation of leiomyoma tissue appeared to be inhibited by asoprisnil even though the differences did not reach statistical significance.

The endometrial antiproliferative effect of asoprisnil demonstrated in this study is consistent with previous clinical trial reports. Proliferation marker expression was suppressed or unchanged compared with the secretory menstrual cycle phase. In a previous trial, various doses of asoprisnil (5 mg once daily to 50 mg twice daily) were administered to healthy premenopausal women for 28 days. In endometrial biopsies collected following treatment (with a Pipelle® endometrial sampler), there was no evidence of endometrial hyperplasia or other appearances suggestive of an unopposed estrogen effect. Importantly, there was a common finding of a unique endometrial appearance, which has since been classified as ‘non-physiologic secretory effect’ and which has not been previously described with any hormonally active agent (Chwalisz et al., 2005a). This appearance, now referred to as ‘SPRM endometrial effect’, seems to reflect the mixed progesterone agonistic/antagonistic properties of asoprisnil in the human endometrium.

In a further trial with administration of 5, 10 or 25 mg asoprisnil to women with uterine leiomyomata for 12 weeks, Pipelle® endometrial biopsies were also obtained following treatment. Histological assessment revealed similar findings. There was no case of endometrial hyperplasia or cytological atypia. A majority of endometrial samples following asoprisnil treatment showed distinct changes consistent with the ‘SPRM endometrial effect’ (Chwalisz et al., 2007). In a 39-week toxicity study with asoprisnil in adult cynomolgus monkeys, the endometrium appeared atrophic with suppressed gland proliferation and stromal compaction. Some glands appeared dilated and cystic in the absence of any hyperplastic features (DeManno et al., 2003). However, this study neither showed secretory changes in endometrial glands nor thickening of the wall of spiral arterioles.

Studies in non-human primate models have been crucial in the discovery and development of SPRMs and have provided valuable insight into their possible mechanisms of action (Chwalisz et al., 2006). Endometria from monkeys as well as humans have shown a distinct morphological response following exposure to SPRMs pointing to an antiproliferative effect (Wolf et al., 1989). However, comparisons between monkeys and humans have also indicated some important differences highlighting the need for caution when extrapolating results from non-human primate studies to the human. This may be due to differences in the steroid receptor pharmacology of the monkey and human endometrium (Chwalisz et al., 2006). It is possible that the balance between agonist and antagonist effect of asoprisnil is in favour of the agonist side in humans. This could result from species differences in metabolic end-products or from species-specific cellular environments creating a different balance of coactivator and corepressor expressions (Chwalisz et al., 2008). The findings of secretory

| Table II | Proliferation marker expression in myometrium by immunohistochemistry |
|----------|-----------------------------|----------------|------------------|-----------------------------|
|          | Placebo/secretory            | Asoprisnil 10 mg | Asoprisnil 25 mg |
| Ki-67: count of stained cells per 10 fields at ×250 | 80.0 | 31.5 | 0.558 | 19.0 | 0.024$ |
| PH3: count of stained cells per 10 fields at ×250 | 8.0 | 3.5 | 0.099 | 5.0 | 0.582 |

Each asoprisnil group is compared with placebo using Wilcoxon’s rank sum test; $ denotes statistical significance at 0.05 level using Hochberg’s multiple comparison procedure. PH3, anti-phospho-histone H3.

| Table III | Proliferation marker expression in leiomyoma by immunohistochemistry |
|----------|-----------------------------|----------------|------------------|-----------------------------|
|          | Placebo/secretory            | Asoprisnil 10 mg | Asoprisnil 25 mg |
| Ki-67: count of stained cells per 10 fields at ×250 | 237.0 | 66.0 | 0.078 | 86.0 | 0.088 |
| PH3: count of stained cells per 10 fields at ×250 | 5.5 | 3.0 | 0.355 | 4.0 | 0.707 |

Each asoprisnil group is compared with placebo using Wilcoxon’s rank sum test.
endometrial gland changes and formation of thick-walled endometrial spiral arteries following treatment with asoprisnil were unique to the human endometrium and had not previously been observed in the monkeys. Suppression of proliferation marker expression due to asoprisnil exposure was common to both human and non-human endometria. Endometrial Ki-67 and PH3 expression was suppressed in cynomolgus monkeys treated with 10, 30 or 90 mg/kg asoprisnil for 90 days. The suppression was significant when compared with samples from the placebo group in the proliferative cycle phase (Brenner et al., 2005). There was no significant difference between asoprisnil-treated monkeys and monkeys in the secretory cycle phase corresponding to the findings in this study. There was no differentiation into separate tissue compartments (stroma and epithelium) in the monkey studies, while this study showed that in the human Ki-67 expression particularly in endometrial stroma is significantly suppressed after 3 months asoprisnil treatment.

The exact mechanism of action of the endometrial antiproliferative effect of SPRMs has still not been fully elucidated. It has been suggested that the endometrial effects of asoprisnil are mediated by the endometrial vasculature and in particular the spiral arteries (Chwalisz et al., 2000), which may be targeted via the perivascular cells (Chwalisz et al., 2006). This hypothesis appears to be supported by the striking effect of asoprisnil on the formation of thick-walled spiral arterioles in humans. However, the absence of similar morphological vascular changes in non-human primate models suggests there may be another pathway, and more recently the role of the endometrial androgen receptor (AR) has been emphasized as a potential mechanism of the endometrial antiproliferative effect (Brenner et al., 2002; Brenner and Slayden, 2005). Androgens are known to inhibit estrogenic effects in the primate endometrium, and AR has been shown to be up-regulated by treatment with various progesterone antagonists and SPRMs in monkeys and mifepristone in humans (Brenner et al., 2002, 2005). Moreover, it was possible to suppress the endometrial antiproliferative effect induced by progesterone antagonists by adding the AR antagonist flutamide (Slayden and Brenner, 2003). These findings imply that a functional AR is required for SPRMs to exert their antiproliferative effect on the endometrium.

Previous in vitro studies have suggested asoprisnil to have a cell type-specific antiproliferative effect on uterine leiomyoma cells compared with normal myometrial cells (Chen et al., 2006). A direct effect of asoprisnil on leiomyoma cells may result in suppressed growth and therefore reduced fibroid volume as previously reported (Chwalisz et al., 2007). The concomitant suppression of myometrial proliferation observed in this study suggests there may be an additional mechanism of action, for example, an impact on uterine artery blood flow. Both

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**Figure 3** PTEN expression in endometrial stroma (A) and glandular epithelium (B) following administration of asoprisnil. PTEN expression is quantified by applying a histoscore (0–300). Each asoprisnil group is compared with placebo (secretory phase) using Wilcoxon’s rank sum test. None of the differences between an asoprisnil group and placebo are statistically significant. PTEN, phosphatase and tensin homologue.

**Figure 4** PTEN immunolocalization in endometrium; scale bar 100 μm. Placebo group in secretory cycle phase; inset—negative control (A). Treatment group asoprisnil 25 mg (B).
asoprisnil (Wilkens et al., 2008) and mifepristone (Reinsch et al., 1994) have been shown to reduce uterine artery blood flow.

This study has demonstrated that endometrial PTEN expression is not altered by administration of asoprisnil. This finding, together with the low endometrial Ki-67 and PH3 expression after 3 months treatment, supports the conclusion that the effect of asoprisnil on endometrium is antiproliferative and does not promote carcinogenesis. It justifies the assumption that the feature of cystically dilated glands occasionally found in asoprisnil-treated endometria is not associated with hyperplasia and is not pre-malignant.

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**References**


<table>
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<th>Table IV Blood E2 and progesterone concentrations within 24 h of hysterectomy</th>
<th>Treatment group</th>
<th>Placebo (n = 10)</th>
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<th>Asoprisnil 25 mg (n = 11)</th>
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<td>Estradiol (ng/dl), mean ± SD</td>
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