Progesterone Resistance and Targeting the Progesterone Receptors: A Therapeutic Approach to Endometriosis

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1. Introduction

Endometriosis is characterised by the benign growth of endometrial glands and stroma on the surface of peritoneal tissues and other organs. It is generally regarded as an aberrant estrogen-dependent growth condition, which presents with symptoms of chronic pelvic pain, bleeding and infertility. Steroidal progestogens are already widely used in the treatment of the condition, dienogest (Visanne) the most recent of which has gained EU approval for clinical use (McCormack, 2010). Progestogens appear to work by both directly inhibiting the functional effects of estrogen on endometrial cell proliferation, and also suppressing ovarian function, to induce anovulatory amenorrhoea. The efficacy of this class of agents in patients with endometriosis, however, is relatively modest and the tolerability (breakthrough bleeding and bloating) as well as concerns on the long term safety (risk of breast cancer and thromboembolism, effect on bone mineral density) has also limited their broader utility. Progesterone receptor antagonists (PRAs) have emerged in recent years as an alternative approach to treating the disease. This class of agents has contrasting effects on reproductive function compared with progestogens. This review will focus on what we know about the PRA mechanism of action from pre-clinical in vitro and in vivo evidence and how clinical data have shaped confidence in this class of agents as a new approach to treating endometriosis symptoms and disease progression[1].

2. Progesterone receptor structure & function

The steroid hormone, progesterone, is a key modulator of normal reproductive function, including ovulation, uterine and mammary gland development and the neurobehavioral expression associated with sexual responsiveness (Clarke & Sutherland, 1990; Lydon et al., 1995). Progesterone is absolutely essential for the maintenance of pregnancy, maintaining uterine quiescence by suppressing expression of genes that mediate increased myometrial

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[1] Zhu Y, Bond J & Thomas P (2003) Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. *Proc. Natl Acad. Sci.* USA 100(5):2237-2242.

contractility, such as the oxytocin receptor. The physiological effects of progesterone (P_4) are principally mediated by interaction with two specific intracellular receptors termed PR-A and PR-B. PR-A and PR-B are members of the nuclear receptor superfamily of transcription factors (Mangelsdorf et al., 1995; McKenna et al., 2009; O'Malley & Conneely, 1992; Tsai & O'Malley, 1994). Nuclear hormone receptors regulate gene transcription by discriminative binding to specific DNA sequences, so-called progesterone response elements (PREs) Specific interactions with co-activator and/or co-repressor proteins, induced by ligand binding, trigger interactions with the RNA polymerase complex (McKenna & O'Malley, 2001). PR-A and PR-B are expressed from a single gene as a result of transcription from two alternative promoters and translation initiation at two alternative initiation codons (Kraus et al., 1993). The human PR-A and PR-B are identical except for an additional N-terminal 164 amino acid sequence present in PR-B. Both isoforms have two activation function domains, AF-1 proximal to the DNA-binding domain, and a ligand-dependent AF-2 domain in the C terminus (Tetel et al., 1999). By virtue of the longer N-terminus, PR-B also has a unique AF-3 domain that may contribute to its differential trans-activation properties compared with PR-A (Tung et al., 2006).

Ligand binding (progesterone as well as other synthetic PR ligands such as progestogens (e.g. dienogest, tanaproget, medroxyprogesterone acetate), the progesterone receptor antagonists (PRAs, such as RU-486) and modulators (e.g. J-867)) triggers a conformational change that causes the dissociation of bound heat shock proteins, receptor phosphorylation, receptor dimerisation, nuclear translocation and DNA binding. Binding occurs at specific PRE sequences in promoters of progesterone response genes, and alters transcriptional activity, negatively or positively, depending on PRE sequence, the conformation of the ligand-bound complex and cell-specific context. In the presence of steroidal PRAs, such as RU-486, the complex becomes transcriptionally inactive due to recruitment of co-repressors such as NCoR1 (Wagner et al., 1998), whereas selective PR modulators, such as J-867, elicit a mixed agonist/antagonist response (Elger et al., 2000; Madauss et al., 2007).

Studies in mice with selective ablation of PR isoforms have revealed that PR-A is necessary for ovulation and modulates the anti-proliferative effects of progesterone in the uterus. In contrast PR-B knockout mice are fertile and sustain a normal pregnancy, but PR-B appears to be required for normal mammary gland development and function (Mulac-Jericevic & Conneely, 2005). In an attempt to understand the function of PR-B in the endometrium, one group has used PRAs and siRNA to knockdown gene expression in an immortalised human endometrial stromal cell line (Wu & Guo, 2006; Wu et al., 2008). Ablation of PR-B promoted cellular proliferation, by approximately 20% compared with control, supporting the notion that PR-B acts as a break on progesterone function. It is noteworthy that breast and endometrial malignancy is often accompanied by disruption of PR-A and PR-B expression or altered functional PR responses (Arnett-Mansfield et al., 2004; De Vivo et al., 2002; Kobayashi et al., 2010; McGowan et al., 2004), an observation which has triggered interest in the potential utility of PRAs for oncology (Fuhrmann et al., 2000; Poole et al., 2006; Tieszen et al., 2011; Wiehle et al., 2011). Recent evidence has also confirmed the existence of a functional third isoform, PR-C which lacks AF-2 and AF-3 domains and appears to act as a sink for progesterone and have a function in regulating the onset of parturition (Condon et al., 2006).

Beyond the genomic function of progesterone mediated by PR-A and PR-B, progesterone also appears to elicit non-genomic activity. Neurosteroidal function of progesterone and progesterone metabolites, such as allopregnelone (3α-hydroxy-5α-pregnan-20-one), augment GABAergic channel burst durations by increasing the opening frequency through positive allosteric modulation in the hypothalamus (Henderson, 2007). In the female rat, direct administration of allopregnelone in specific regions in the hypothalamus rapidly facilitates lordosis, suggesting a direct non-genomic effect on reproductive function. Outside of the predicted protection from catamenial exacerbation of epileptic seizures and premenstrual dysphoric disorder by neurosteroids (Biagini et al., 2010), the non-genomic effects of progesterone in reproductive function in the female human have been less well characterised. Several other reported progesterone receptors (mPR, PGRMC1 and CatSper, for instance) have also been touted to contribute to the non-genomic effects of progesterone (Dressing et al., 2011; Gellersen et al., 2009; Lishko et al., 2011; Zhu et al., 2003). Based on expression data and some functional characterisation both mPR and PGRMC1 may have a role in reproductive function and are speculated to regulate implantation and myometrial contractility. Whilst there are no evidence reported suggesting a contributing role for mPR or PGRMC1 in endometriosis, this is not the case for PR.

3. Evidence of progesterone resistance in women with endometriosis

The uterus is composed of heterogeneous cell types which undergo synchronous waves of proliferation and differentiation in response to cyclical changes in estrogen (E_2) and progesterone levels. The spatiotemporal expression of PRs in epithelial, stromal and myometrial cellular compartments are under the control of estrogen, the primary endometrial mitogen. Progesterone appears to exert proliferative function or induce differentiation depending on the cell type; on epithelia, progesterone, acting via stromal PRs, inhibits estrogen-driven cell proliferation and on stroma progesterone appears to orchestrate a more complex pattern of proliferation and differentiation.

While the actions of progesterone are critical to the establishment and maintenance of pregnancy, approximately one third of women with endometriosis also present with infertility. For some women, the loss of tubal patency, the modification of the pelvis and the inflammation associated with condition appear to be causally related to the presentation of infertility. However, the characterization of the eutopic endometrium from women with endometriosis has also revealed many defects, including altered patterns of angiogenesis, dyssynchrony with the window of implantation as well as ultra structural abnormalities which may contribute to the infertility. Supporting this, isolated endometrial stromal fibroblasts from women with endometriosis do not appear to undergo a normal decidualisation response (Aghajanova et al., 2010), suggesting an impairment of the progesterone-mediated differentiation programme. Specific alterations in the expression molecular markers of endometrial receptivity have also been widely documented, especially the integrin $\alpha_v\beta_3$, certain steroid hormone receptors and HOXA10 gene expression, the latter of which has been identified from a genome wide association study of moderate to severe endometriosis as a potential candidate disease locus with proximity to the 7p15.2 SNP association (Painter et al., 2011).

Early studies also pointed to alteration in the normal pathways of estrogen metabolism in ectopic endometrial tissue; specifically expression of 17βHSD-2, the enzyme responsible for

the conversion of estradiol to estrone appears to be reduced compared with the eutopic compartment (Bulun et al., 2010; Zeitoun et al., 1998). As 17βHSD-2 is a progesterone response gene, one group has published evidence to suggest that this may be due to apparent reduction in PR levels and especially in PR-B in ectopic tissue (Attia et al., 2000; Wu et al., 2006). However, this signpost to progesterone resistance has had relatively little formal observational replication; indeed others have not been able to find evidence of alteration in the PR-A/PR-B ratio (Bergqvist & Ferno, 1993; Igarashi et al., 2005). Furthermore, decrease in PR-A mRNA and an increase in the PR-B to PR-A ratio and total PR protein levels have been detected in eutopic samples obtained from a murine endometriosis model (Lee et al., 2009). PR expression has also been found to be similarly unaltered in the eutopic endometrium of baboons with experimentally induced endometriosis compared with baseline (Fazleabas et al., 2003).

Microarray studies performed on isolated cells, eutopic/ectopic tissue biopsies and cells excised by laser capture microdissection from patients have been revealed several pathways of altered gene expression. For instance Kao et al (2002; 2003) collected biopsies from eutopic endometrium from normal women and women with endometriosis at days 8-10 after the mid-cycle LH surge and performed a microarray analysis to identify differentially expressed genes. Whilst a formal analysis of progesterone-response genes, whose expression was either elevated or suppressed in diseased versus normal tissue, was not undertaken, the expression of several progesterone response genes, including Dickkopf-1 and glycodelin, was suppressed in diseased tissues samples. The suppression of the Dickkopf-1 response has been supported by more recent studies of progesterone response in isolated human endometrial stromal fibroblasts from normal women and women with endometriosis (Aghajanova et al., 2011). In studies comparing ectopic and eutopic gene expression obtained from women with endometriosis one of the key observational fingerprints was an impairment in the normal progesterone response, especially the expression of PR, IHH, FOXO1A and Cyp26A1 amongst others (Burney et al., 2007). Relatively few genes have been qualified as progesterone responsive by testing with a PRA and this has limited larger data assignments in published microarray data. Even when microarray analysis was performed on RNA extracted from human endometrial explants cultured in the presence of E_2/P_4 and treated with RU-486, only a small population of gene expression were differentially regulated (Catalano et al., 2003). Of these, JAK1 and JNK1 appeared to be down regulated in the presence of RU-486. These observations are intriguing as JNK activity is unregulated in women with endometriosis (Uz et al., 2011) and in a *Scid* mouse experimental model of endometriosis, JNK inhibitor treatment reduces disease burden (Altan et al., 2008).

Taken together, while the data support the notion that there is an abnormal progesterone response in eutopic and ectopic endometrial compartments in women with endometriosis, it is not clear whether this is a direct effect, causally associated with infertility, or could be used for diagnostic purposes. One of the key gaps is that many of these genes have not been formally tested to be directly PR mediated, and therefore lack the qualification of alteration.

Another, somewhat controversial, observation linked with the molecular basis of progesterone resistance has been revealed from studying functional polymorphisms in the promotor region of PR. A putative functional polymorphism in the PR promoter (+331C/T; rs10895068), creates an additional TATA box that provides a unique transcriptional start site and favours increased production of PR-B relative to PR-A (De Vivo et al., 2002). Berchuck et

al. (2004) first suggested a reduced risk of endometriosis associated with the T allele of the +331 variant and because of increased production of PR-B, this variant was suggested to reduce the risk of endometriosis. However the original findings suggesting that PR-B is not expressed in ectopic lesions have not had broad replication and Treloar et al. (2005) found no association with endometriosis and this variant either in a large study which included more than 900 families. Another putative functional variant in the *PGR* gene is termed the PROGINS allele. Cells prepared from the eutopic endometria of women carrying the PROGINS allele appear to respond with greater proliferative capacity to estradiol and progesterone, supporting the contention that the PROGINS polymorphism enhances the endometriosis phenotype (D'Amora et al., 2009). However while several studies have suggested that the variant increases susceptibility to endometriosis (De Carvalho et al., 2007; Lattuada et al., 2004; Wieser et al., 2002), others have not found an association (Govindan et al., 2007; Treloar et al., 2005; van Kaam et al., 2007). Therefore taken together, there is only modest supporting evidence of altered progesterone receptor expression, and progesterone resistance in contributing to endometriosis susceptibility and disease symptoms. Notwithstanding this, the current clinical utility of progesterone receptor agonists and anticipated benefit of PRAs, outweighs the confidence in PR as a therapeutic target attained from a molecular understanding of the protein and condition.

4. Discovery of small molecule modulators of PR function

The identification of drug-like, potent and selective PR antagonists has been challenging. As well as being highly lipophilic, the ligand binding sites between homologous NHRs are highly conserved and PR has the highest sequence homology with GR, AR, MR and ER (Figure 1). Early classes of anti-progestagens were poorly selective, yet some, such as gestrinone, still found clinical utility in the treatment of endometriosis (Cornillie et al., 1986; Coutinho, 1982). Furthermore, the ligand binding domains of PR-A and PR-B are identical and yet several in vitro and in vivo lines of evidence suggest that the effects of progesterone on transcriptional activation and repression by PR-A and PR-B are different (Conneely et al., 2001; Tung et al., 2006). To date, however, there are no agonist or antagonist agents that have been characterised with selectivity for PR-A over PR-B or vice versa.

The development of selective and safe steroidal PRAs has been challenging, both due to reported hepatoxicity as well as potential dose-limiting anti-glucocorticoid effects, due to lack of selectivity (Robertson et al., 1999). More recently, an additional concern has emerged following histological evaluations of subjects dosed for more than 3 months on steroidal PRAs. The endometrium of these individuals undergoes a characteristic cystic histological change which may be difficult to distinguish from endometrial hyperplasia without specialist evaluation (Ioffe et al., 2009; Mutter et al., 2008; Williams et al., 2007).

Mifepristone/RU-486, the founding member of the steroidal class of PRAs, was originally produced by Roussel-Uclaf and licensed for use for medical abortion and as an emergency contraceptive. The in vivo pharmacokinetic/pharmacodynamic profile of RU-486 is challenging to model as RU-486 generates a large number of pharmacologically active metabolites (Heikinheimo et al., 1987). Since its identification, >100 related analogues, principally by modifying the C-11 and/or C-17 positions of the steroid ring, have been synthesized and have shown all degrees of anti-progestagenic activity. The pharmacological profile of these examples range from pure antagonists, such as RU-486 to those with mixed antagonist/agonist activity, such as J-867 (Table 1).
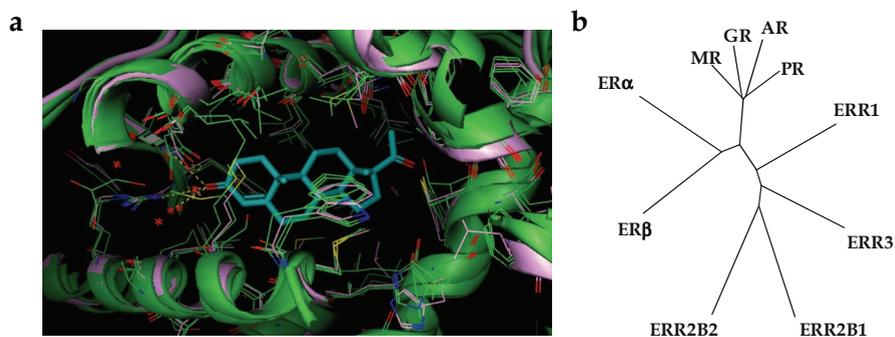
Fig. 1. (a) X-ray structure of progesterone (cyan) bound to the PR ligand binding pocket (pink) (Williams & Sigler, 1998) and overlaid with the x-ray co-ordinates of GR, MR and AR (in green) to illustrate the high degree of structural similarity between homologous NHRs (b) Phylogentic tree illustrating the sequence similarity of homologous NHRs with PR.

More recently, several alternative non-steroidal chemical PRA scaffolds have been published. These might have advantages over the steroidal templates due to simpler synthetic route, and potential for greater selectivity and metabolic stability compared with steroidal templates (Dack et al., 2010; Fensome et al., 2008; Terefenko et al., 2005; Zhang et al., 2002),. With a few exceptions, these classes of agents tend to mimic the steroid A ring ketone with a cyanoaryl group isostere, as is also seen with tanaproget, the non-steroidal progestogen (Fensome et al., 2005).

Steroidal PRAs have been characterised as facilitating PR dimerisation and nuclear translocation, but induce a conformation of the DNA-bound complex which recruits co-repressors to directly shutdown transcriptional PRE activity and other transcriptional promoters by trans-repression. The pharmacological profile of the non-steroidal compounds appears to be subtly different from steroidal examples (Howe et al., 2011; Zhang et al., 2007). In vitro pharmacological profiling of PRA-910 and PF-02413873 has indicated that at low concentrations the compounds inhibit the expression of progesterone-reporter genes, but at high concentrations, they induce agonism. For PF-02413873, the inhibition of progesterone-reporter gene expression appears to be due to blocking PR nuclear translocation and then at high concentrations PF-02413873 facilitates it, recruits co-activators and induces gene transcription.

Compound	Structure	Pharmacology data	In vivo observations	Reference
Non-steroidal PRAs				
PF-02367982		PR binding K_i=76 nM; functional IC_{50}=40.3 nM >3000x functional selectivity over GR, AR, MR	Dose dependent inhibition of arborisation of the immature rabbit and luteal phase endometrium of the intact macaque	(de Giorgio-Miller et al., 2008)

Table 1. Pharmacological properties of key non-steroidal and steroidal PRAs [1] In these assays, the activity of RU-486 was PR binding IC_{50}=9 nM; T47D IC_{50}=7.6 nM; GR binding IC_{50}=10 nM; IC_{50}=5.9 nM; AR binding IC_{50}=45 nM; [2] In these assays, the activity of RU-486 was PR binding IC_{50}=0.028 nM; GR binding IC_{50}=2.2 nM; AR binding IC_{50}=10 nM
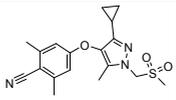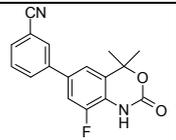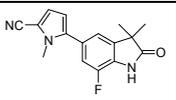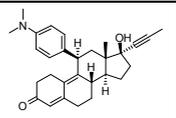
PF-02413873		PR binding K_i=2.6 nM; functional K_i=9.7 nM; pK_B= 8.0 [Agonist format: ~25% activation at 10 µM] AR binding IC_{50}=2100 nM; functional K_i=1130 nM MR functional K_i=307 nM GR binding K_i=410 nM; functional K_i=2710 nM	Dose dependent inhibition in functionalis thickness & BrdU incorporation of follicular phase macaque endometrium; maximum effects at 10 mg/kg (p.o, *b.i.d*)	(Howe et al., 2011)
PRA-910		PR binding Kd=4.4 nM; functional 14 nM [Agonist format: ~60-70% activation at 0.1 µM AR binding IC_{50}=1292 nM GR binding IC_{50}=1756 nM MR binding IC_{50}=2369 nM ER binding IC_{50} >10000 nM	Dose dependent inhibition of the P_4-induced rat decidual response (mean ED_{50} =0.3 mg/kg) with no evidence of agonism at 10 mg/kg. 5 mg/kg reduced BrdU incorporation in the E_2/OVX macaque, but no effect on endometrial thickness c.f. control	(Zhang et al., 2002; 2007)
WAY-255348		PR binding IC_{50}=5 nM; functional IC_{50}=5 nM AR functional IC_{50}=196 nM MR functional IC_{50}=3700 nM >No significant activity at GR, ER	Inhibition of P_4-induced rat decidual response (ED_{50} = 0.3 mg/kg). Dose dependent inhibition of ovulation in the macaque. All the animals treated at 10 mg/kg had thin-atrophied endometria	(Fensome et al., 2008)
Steroidal PRAs				
RU-486 / mifepristone		PR binding K_i=0.5 nM; T47D IC_{50}=0.2 nM AR functional C_{50}=20 nM GR binding K_i=1.4 nM; Functional IC_{50}=3 nM MR functional IC_{50}=3 nM	Dose dependent induction of menses in intact and E_2/P_4 artificially cycled macaque. Inhibition of ovulation and endometrial proliferation. ~75% reduction in peritoneal disease volume in a surgical model of endometriosis in the macaque	(de Giorgio-Miller et al., 2008; Grow et al., 1996; Slayden & Brenner, 1994; Slayden et al., 2001; Wolf et al., 1989)

Table 1. (continuation) Pharmacological properties of key non-steroidal and steroidal PRAs[1] In these assays, the activity of RU-486 was PR binding IC_{50}=9 nM; T47D IC_{50}=7.6 nM; GR binding IC_{50}=10 nM; IC_{50}=5.9 nM; AR binding IC_{50}=45 nM; [2] In these assays, the activity of RU-486 was PR binding IC_{50}=0.028 nM; GR binding IC_{50}=2.2 nM; AR binding IC_{50}=10 nM
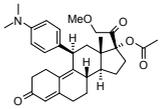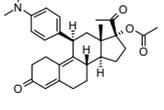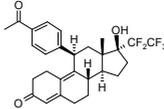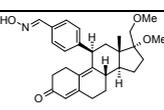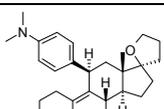
CDB-4124 / proellex		PR binding IC_{50}=19 nM; T47D functional IC_{50}=11 nM[1] GR binding IC_{50}=17 nM IC_{50}=130 nM AR binding IC_{50}=288 nM	Dose dependent suppressed cell proliferation and tumour latency in a N-methyl-N-nitrosourea-induced mammary carcinogenesis rat model	(Attardi et al., 2002; Brenner et al., 2010; Wiehle et al., 2011)
CDB-2914 / ulipristal		PR binding IC_{50}=7 nM; T47D functional IC_{50}=7 nM GR binding IC_{50}=18 nM; functional IC_{50}=73.8 nM AR binding IC_{50} = 65 nM[1]	Intrauterine delivery of CDB-2914 suppressed endometrial growth and menstruation in artificially cycled Rhesus macaques	(Attardi et al., 2002; Brenner et al., 2010; Gainer & Ulmann, 2003)
ZK-230211 / linaprostone		PR binding IC_{50} = 0.0036 nM GR binding IC_{50} = 16 nM AR binding IC_{50} = 54 nM[2]	Dose dependent interruption of pregnancy in the rat and reduction in tumour mass in a DMBA-induced rat mammary tumour model. Dose dependent inhibition of endometrial growth in the macaque	(Afhüppe et al., 2010; Fuhrmann et al., 2000; Slayden et al., 2001)
J-867 / asoprisnil		PR functional IC_{50}=0.2 nM AR functional IC_{50}=6.1 nM GR functional IC_{50}=85 nM MR functional IC_{50}=1.6 µM ER functional IC_{50}= 1.9 µM	Partial agonist properties in McPhail's assay. Marginal labour-inducing activity during mid-pregnancy and ineffective in inducing preterm parturition in the guinea pig. Abolition of menstrual cyclicity and induction of endometrial atrophy in the macaque	(DeManno et al., 2003; Elger et al., 2000; Fensome et al., 2008)
ORG-31710		PR relative binding affinity equivalent to RU-486. 30 fold lower GR relative binding affinity c.f. RU-486	ORG-31710 administered with desogestrel reduced the incidence of unscheduled vaginal bleeding c.f. desogestrel alone in the macaque	(Kloosterboer et al., 1994; 2000; Verbost et al., 2005)

Table 1. (continuation) Pharmacological properties of key non-steroidal and steroidal PRAs [1] In these assays, the activity of RU-486 was PR binding IC_{50}=9 nM; T47D IC_{50}=7.6 nM; GR binding IC_{50}=10 nM; IC_{50}=5.9 nM; AR binding IC_{50}=45 nM; [2] In these assays, the activity of RU-486 was PR binding IC_{50}=0.028 nM; GR binding IC_{50}=2.2 nM; AR binding IC_{50}=10 nM
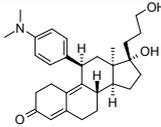
ZK-98299 / Onapristone		PR relative binding affinity equivalent to RU-486	Inhibition of arborisation of the immature rabbit. Reduction in cell proliferation of cells in ectopic lesion in a rat endometriosis model. Inhibition of ovulation and endometrial growth in monkeys	(Elger et al., 2000; Gopalkrishnan et al., 2003; Ishwad et al., 1993; Stoeckemann et al., 1995)
ZK-137316		PR relative binding affinity equivalent to RU-486	Inhibition of arborisation of the immature rabbit. Reduction in cell proliferation of cells in ectopic lesion in a rat endometriosis model. Inhibition of ovulation and endometrial growth in macaques.	(Borman et al., 2003; Slayden et al., 1998; 2001; Slayden & Brenner, 2003; Stoeckemann et al., 1995; Zelinski-Wooten et al., 1998)

Table 1. (continuation) Pharmacological properties of key non-steroidal and steroidal PRAs [1]
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5. Pre-clinical effects of PR antagonists in pre-clinical models

The study of the effects of PRAs in pre-clinical models has shed light on the site of and mechanism of action of PRAs in normal reproductive physiology and disease. The classical models for quantifying PRA activity are the modified assay in juvenile rabbits according to McPhail (McPhail, 1933), the induction of luteolysis in the guinea pig (Elger et al., 2000) and the inhibition of decidualisation in the rat. For the purposes of this review, I have focussed on the pre-clinical models which have been used to support a role for PR in the pathogenesis and treatment of endometriosis, principally focussing on studies in the rodent and macaque. Many data have been acceded from studies in the normal animal, but several experimental models of endometriosis have been also developed in normal as well as immune compromised rodents and non-human primates (D'Hooghe et al., 2009; Grummer, 2006; Laschke & Menger, 2007). In these models, cystic ectopic endometrial lesions develop following the transplantation of syngeneic or human uterine endometrial tissue under the control of ovarian estradiol. Measuring temporal changes in the size of these lesions and their proliferative capacity as well other aspects of the disease presentation is a powerful pre-clinical yardstick for testing the efficacy of experimental drugs.

5.1 Rodent

Mouse knockout studies have elegantly described differences in the function of PR-A and PR-B. Both PR null mutation (PRKO) and selective disruption of the PR-A isoform (PRAKO)

in the mouse leads to a failure of ovulation due to disabled follicular rupture in response to gonadotrophin stimulation (Lydon et al., 1995; Mulac-Jericevic & Conneely, 2005). The histological characterisation of uteri from PRKO mice confirmed extensive epithelial hyperplasia (Lydon et al., 1995). In contrast, the stromal compartment was distinctly oedematous and infiltrated with neutrophils and macrophages. While these data strongly support the notion of PR in suppressing ER function in the uterus, the cystic dilation, epithelial hyperplasia and associated inflammation are also histological hallmarks of endometriosis, especially those characterised in rodent disease models (Bruner et al., 1997; Grummer, 2006; Hull et al., 2003; Vernon & Wilson, 1985). The endometrial epithelial hyperplasia observed in the uteri of PRAKO mice was similar to that observed for PRKO mice suggesting that PR-B is unable to compensate for the loss of PR-A (Mulac-Jericevic et al., 2000). In contrast, ovarian and uterine response to E_2/P_4 appear to be normal in PR-B knockout mice, whereas mammary lobuloalveolar development was markedly reduced due to decreased ductal and alveolar epithelial cell proliferation (Mulac-Jericevic et al., 2003). Taken together these findings demonstrate the extremely important role PR plays in regulating ovarian function and spatiotemporal cell growth in different tissue compartments in response to E_2/P_4 in the mouse.

PRKO mice have been used to explore the role of PR in the development and growth of ectopic lesions in a syngenic mouse model of endometriosis (Fang et al., 2004). In this study, the volumes of PRKO lesions collected from animals treated with E_2 were approximately 20% larger than those from corresponding wild-type animals. Additionally, the effects of P_4 on PRKO lesions were ablated compared with those from wild-type animals, underscoring the important role that PR plays in regulating E_2-dependent cell proliferation in the rodent.

Whilst the evaluation of gene ablation on eutopic and ectopic endometrial cell growth has been revealing, the studies of pharmacological modulation contrast these observations to a certain extent as both progestogens and PRAs reduce ectopic endometrial cell proliferation and disease burden in pre-clinical rodent models of endometriosis (Bruner-Tran et al., 2006; Chwalisz et al., 1998; Katayama et al., 2010; Katsuki et al., 1998; Stoeckemann et al., 1995). An explanation of this phenomenon compared with the phenotype of PRKO animals has been revealed by studies with PRAs in the non-human primate.

5.2 Macaque

Given the evolutionary and physiological proximity of the macaque menstrual cycle with the human, many groups have evaluated the role of PR and the effects of PRAs on the macaque endometrium. Most data revealing the effect of PRAs on the endometrium have come from studies evaluating the effects of steroidal PRAs. When administered acutely after the mid-cycle LH surge, or during the progesterone phase in artificially cycled animals, PRAs impair the effects of progesterone on endometrial arborisation and induce an early menstruation. In the intact macaque, animals undergo an anovulatory amenorrhoea under the influence of continuous steroidal PRA exposure (Brenner et al., 2010; Slayden et al., 2001). In these animals, the endometrium is characterised by decreased wet weight, thickness and mitotic activity. The endometrium undergoes a characteristic atrophy and compaction of the stroma, glandular apoptosis as well as degeneration of the endometrial spiral arterioles. These effects are characteristically anti-estrogenic in nature, and yet the effects occur in the presence of mid-follicular levels of E_2, levels that should be sufficient to

facilitate endometrial growth. Studies in ovariectomised (OVX) and E_2-supplemented macaques, have been perhaps even more revealing with respect to the mechanism driving this effect, especially the direct as well as indirect effects of PRAs on the hypothalamic-pituitary-gonadal axis and endometrium. Firstly, RU-486 has been shown to suppress the estrogen-induced LH surge in the OVX macaque (Wolf et al., 1989), underwriting a role for PR in regulating the hypothalamic-pituitary axis in higher species as suggested by early studies in knockout mice (Conneely et al., 2001). Furthermore, as RU-486 does not appear to blunt GnRH-induced LH secretion in the macaque (Heikinheimo et al., 1995), this has suggested that PRAs directly block ovarian folliculogenesis. In intact macaques with PRA doses that are too low to block ovulation or in the OVX/E_2 macaque, steroidal PRAs that include can also directly suppress the effects of estrogen on the endometrium by inhibiting cell proliferation and thickness suggesting that PR regulates reproductive function at multiple points (Brenner et al., 2010; DeManno et al., 2003; Hodgen et al., 1994; Ishwad et al., 1993; Slayden et al., 1998; 2001; 2006; Wolf et al., 1989; Zelinski-Wooten et al., 1998).

While many studies have been commonly undertaken by oral or systemic administration of PRAs, principally RU-486, some studies have also been undertaken by local, intrauterine administration, such as those with CDB-2914 and ZK-230211(Brenner et al., 2010; Nayak et al., 2007). In each case, the intrauterine administration resulted in the characteristic inhibition of normal menstrual bleeding, atrophy of endometrial spiral arterioles and functionalis thickness, consistent with observations from systemic administration (Figure 2). Unfortunately neither of these studies were supported with a confirmation of drug exposure, comparing the local versus systemic exposure to demonstrate that the effects were mediated by a local site of action and not an indirect effect. Nonetheless the data support others which suggest that PRAs can work locally to block estrogen effects on endometrial growth in the macaque.
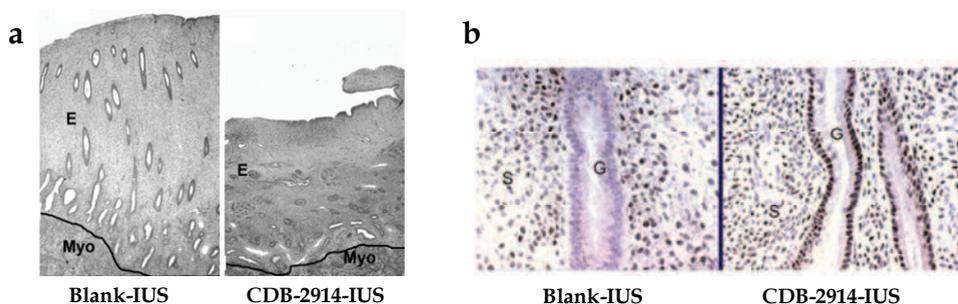

Fig. 2. (a) Induction of endometrial atrophy by CDB-2914-intrauterine system (IUS) versus blank-IUS (taken from (Brenner et al., 2010) with permission); E, endometrium; Myo, myometrium (original magnification ×25). (b) AR staining on endometrial tissue samples taken from macaques treated with CDB-2914-IUS or blank-IUS (original magnification ×340).

The mechanism of attenuation of estradiol effects on the endometrium is not well understood, and although steroidal PRAs appear to block cell proliferation in various *in vitro* cell-based systems, the concentrations needed for this effect are considerably greater than those which elicit the effect *in vivo* (Freeburg et al., 2009a; Goyeneche et al., 2007; Murphy et al., 2000; Ohara et al., 2007; Wu & Guo, 2006). One clue to a potential mechanism

has emerged from observations of elevated endometrial androgen receptor (AR) expression (Narvekar et al., 2004; Slayden & Brenner, 2003) following PRA administration and the known effects of AR modulators (e.g. danazol) on endometrium (Rose et al., 1988).

These observations have been functionally evaluated further (Slayden & Brenner, 2003) in which OVX/E_2 macaques were continuously treated with ZK-137316 or together with the AR antagonist, flutamide, for 28 days. Flutamide reversed the inhibitory effects of ZK-137316 on the E_2/OVX endometrium, restoring levels of endometrial proliferation and thickness to control levels (Table 2).

	E_2 alone	E_2/ZK-137316 (0.1 mg/kg i.m.)	E_2/ZK-137316 (0.1 mg/kg i.m.) + Flutamide (2 mg/kg s.c.)
Weight (mg)	360 ± 32	64 ± 10[b]	265 ± 92
Thickness (mm)	3.3 ± 0.4	1.1 ± 0.3[b]	2.2 ± 0.6
Stromal compaction[c]	45.5 ± 3.4	142.3 ± 63.7[b]	54.0 ± 4.6
Mitotic index[d]	6.3 ± 0.6	0.3 ±0.3[b]	5.2 ± 3.8

Table 2. Morphometric assessment of androgen receptor blockade of ZK-137316 effects on OVX/E_2 macaques[a] (Adapted from (Slayden & Brenner, 2003)) [a]All values represent mean ± SE; [b]p<0.05 compared with values in the same row; [c]stromal cells/10,000 µm²; [d]mitotic cells/1000 epithelial cells

That flutamide did not appear to inhibit the PR activity of ZK-137316 (i.e. ZK-137316-induced menstruation in E_2/P_4 artificially cycled animals in the presence of flutamide), suggested that the endometrial anti-proliferative effects of steroidal PRAs like ZK-137316 are mediated by a mechanism involving AR. However, despite this extremely important observation, the seminal Slayden publication has not been followed up further. For instance, it is not clear what ligand is driving the AR effect, as testosterone levels do not appear to be altered in ZK-137316 treated animals, or how the signal is transduced through AR; if it is genomic or non-genomic. If AR is inducing a genomic effect, what are the transcripts that are altered and confer the inhibitory effect on PRA? Other important and, as yet, unaddressed questions also include whether these effects are only manifested only by the steroidal class of PRAs, but the observation that RU-486 can elevate endometrial AR expression in women goes some way to understanding the translational significance of the macaque findings (Narvekar et al., 2004).

Non-steroidal classes of PRAs have also been studied in a similar way in the macaque. Of the novel class of cyanophenoxypyrazoles, PF-02367982 dose-dependently inhibited the progesterone-mediated aborisation of the endometrium and delayed menses induction when dosed for 20 days from the start of the menstrual cycle. PF-02367982 also increased AR protein expression in a similar manner to that observed by RU-486 and the non-steroidal PRA, WAY-255348 (de Giorgio-Miller et al., 2008; Fensome et al., 2008). These data are consistent with other non-steroidal PRAs that have been assessed, such as WAY-255348 (Fensome et al., 2008). More recently, PF-02413873 a more potent PRA than PF-02367982 (Table 1) has been shown to reduce endometrial cell proliferation and thickness in intact macaques dosed for 10 days from the start of menstruation (Howe et al., 2011). In this study, however, AR expression was not appreciably altered with PF-02413873 treatment compared with RU-486. While this may be, in part, due to the timepoint for the comparison and

assessment, PF-02413873 also appears to have a different pharmacological profile from RU-486 and other steroidal PRAs.

While these interesting observations are important in the context of normal endometrial physiology, few studies have been undertaken in macaques with endometriosis to build translational understanding to disease. Menstruating primates, such as the baboon and the macaque, develop spontaneous endometriosis and ectopic lesions that are histologically identical to the human disease (D'Hooghe et al., 2009). For many researchers, the proximity of this model to the human condition has made this the model of choice for the assessment of interventional agents the endometriosis. Spontaneous disease is acquired with a similar time course as experienced by the human female, developing slowly over a period of years and is not easily diagnosed without laparoscopy. Consequently, researchers have used intraperitoneal inoculation of autologous menstrual or endometrial tissue to develop an experimental model of endometriosis that is similar to that observed in women.

The only study evaluating the effect of a steroidal PRA, RU-486, in a non-human primate model of endometriosis was reported by Grow *et al* (1996). This study was undertaken in a surgical induction model of endometriosis and disease allowed to develop prior to dosing. A baseline measure of burden (peritoneal lesion area) was undertaken and then macaques were treated with either RU-486, leuprolide or vehicle for one year. Both RU-486 and leuprolide induced an anovulatory amenorrhoea and reduced peritoneal disease levels to a similar levels, $\geq 75\%$, compared with the vehicle control group. The authors additionally evaluated the effect of RU-486 and leuprolide on bone mineral density as revealed by dual x-ray absorptiometry. Consistent with the post-menopausal levels of E_2 achieved, leuprolide induced a 0.035 g/cm^2 reduction in bone mass compared with +0.1 g/cm^2 for vehicle control and +0.25 g/cm^2 for the RU-486 treated animals. These data support earlier observations that steroidal PRAs are able to suppress endometrial cell growth whilst maintaining bone-sparing mid-follicular levels of E_2 (Heikinheimo et al., 1995).

6. Clinical evaluation in healthy women and women with endometriosis

RU-486 was originally developed for emergency contraception, however early observations with lower doses than those used clinically, indicated that when given acutely during the luteal phase, RU-486 would facilitate the onset of menstruation by the upregulation of endometrial prostaglandins and given chronically, RU-486 would delay menses (Hapangama et al., 2002; Shoupe et al., 1987). The effects of RU-486 on the ovarian cycle and endometrium appear to be dose dependent, that is low doses interfere with estrogen function and disrupt endometrial growth (Croxatto et al., 1993; Narvekar et al., 2004), but higher doses additionally suppress follicular development by impairing gonadotrophin secretion (Gemzell-Danielsson et al., 1996; Liu et al., 1987; Spitz et al., 1993; 1994). These observations strike a resounding chord with those data acceded in the macaque described earlier. The potential value of PRAs as alternative contraceptives to current combined or progestin-only pills have been long recognised and evaluated in a number of different dosing and delivery strategies (Baird et al., 2003; Brown et al., 2002; Chabbert-Buffet et al., 2007; Heikinheimo et al., 2007; Lakha et al., 2007; Nayak et al., 2007). Whilst no pregnancies were reported after 200 months in women who received 2-5 mg RU-486 daily (Brown et al., 2002), lower doses appeared to be less effective (Croxatto et al., 1998). Similar observations on the suppression of ovulation and the normal menstrual cycle have also been made with

other PR-As such as a onapristone, J-876 as well as CDB-2914 (Chabbert-Buffet et al., 2007; Chwalisz et al., 2005a; Katkam et al., 1995; Stratton et al., 2000). The utility of PRAs as a new class of oral contraceptives has still not been fully exploited and this feature alone is anticipated to have potential benefit in endometriosis patients by reducing cyclical menstrual pain.

Intrauterine delivery (IUD) of progestogens (levonorgestrel/Mirena) is an effective way of administering durable contraceptive exposures of drug and to bypass systemic side effects. IUD studies with PRAs in the macaque have also been followed up with a single human study. The study compared levonorgestrel with an IUD releasing ZK-230211. The dose of ZK-230211 was selected based on an equivalent IUD dose of ZK-230211 that suppressed ovulation and menstruation in the macaque (Heikinheimo et al., 2007). In contrast to the data acceded in the macaque, however, the ZK-230211 IUD did not appreciably alter bleeding patterns suggesting that either the local drug exposure was insufficient or that there are translational differences between the macaque and human.

Increasingly, however, the endometrial effects of PRAs have been subject to concern due observed histological changes in the endometrium with chronic exposure. In the past, endometrial hyperplasia has been reported as a safety concern with chronic use of RU-486 (Newfield et al., 2001). Recent, detailed histological analyses of endometrial biopsies from patients exposed to steroidal PRA for more than 3 months have indicated that these agents produce a slightly thickened endometrium with cystically dilated endometrial glands (Ioffe et al., 2009; Mutter et al., 2008; Williams et al., 2007). The appearance of glandular epithelium appears to change with dose and exposure duration, from inactive/non-mitotic to non-physiologic combinations of features usually seen separately in normal proliferative and secretory endometrium. These alterations do not appear to be limited to the glands only, as thick-walled vessels most commonly seen in endometrial polyps, become more widely distributed throughout the endometrium. Whether these effects on the endometrium are mediated by the unopposed effects of persistent follicular phase levels of estradiol, the pharmacological class or some non-specific effect of PRAs on the endometrium is not clear, but this appears to be a common feature of all steroidal PRAs assessed so far. Individuals on prolonged exposure to asoprisnil/J-867 were at a higher risk of developing endometrial changes sufficient to raise concern with regulatory authorities indicates that more research is needed to understand the phenomenon of PRA associated endometrial changes and whether this might be in part mitigated by an alternative dosing regimen from continuous dosing (Baird et al., 2003).

The first evidence that PRAs such as RU-486 would have a potential benefit in women with endometriosis was published by Kettel and co-workers (1991; 1994; 1996; 1998). The incentive for these early investigational studies was the clinical observation that RU-486 could block follicular maturation and ovulation when given early in the menstrual cycle, disrupt endometrial integrity when administered in the luteal phase and induce an anovulatory amenorrhoea when administered continuously. However, even earlier studies than those conducted with RU-486, indicated the potential utility of treating women with endometriosis with an anti-progestin (Coutinho, 1982). In this small open-label study, 20 patients with endometriosis were subjected to 6 months of continuous treatment with gestrinone (5 mg/twice weekly), a reportedly mixed antagonist with anti-progestogenic activity. All subjects became amenorrhoeic and had reportedly dramatic

improvements in dyspareunia symptoms and fertility outcomes. Whilst no visualisation of the change in the disease burden was made in this study, this assessment was followed up by others (Cornillie et al., 1986) and incorporated as part of the validation work performed by Kettel and co-workers with RU-486. In these studies, doses of RU-486 were carefully selected to avoid the known anti-glucocorticoid effects. Treatment of women with endometriosis with a daily dose of 5-100 mg for 3-6 months resulted in durable inhibition of the normal menstrual cycle (although not optimal for the 5 mg dose) and a suppression in ovarian hormone levels consistent with a block on folliculogenesis. Endometriosis-associated pain scores and American Fertility Scores determined by laparoscopic examination also decreased from baseline (Kettel et al., 1994; 1996; 1998; Murphy et al., 1995). These preliminary studies were not able to rule out the possibility that the visible changes in disease burden were secondary to the absence of ovarian hormone cyclicity; indeed this is still not known. From a safety perspective, there was no suppression of cortisol levels, indicative of anti-glucocorticoid effects. Serum estradiol concentrations were also maintained at a mid-follicular level which preserved femur and lumbar spinal bone mineral density (Kettel et al., 1996). These bone safety data are consistent with those reported in the cynomolgus macaque and contrast clinical observations of GnRH receptor agonists (Grow et al., 1996).

Asoprisnil has also been compared with placebo for treatment of pain in laparoscopically diagnosed endometriosis in a randomized, controlled trial. Whilst the results of this study were reported as an abstract only, a significant decrease in daily pain scores with all doses of asoprisnil (5, 10 or 25 mg) compared with placebo was noted (Chwalisz et al., 2005b). Intriguing as these data are, there has been a compelling lack of replication studies from double blinded, randomised and controlled trials in women with endometriosis, using empirical and objective outcome measures approved by regulatory bodies. This lack of evidence may be in part due to the level of investment needed for such an old drug as RU-486, or concerns over the safety of continuously administered RU-486 or PRAs like it. CDB-4124 is currently in development for the treatment of endometriosis and uterine fibroids and data are anticipated on its clinical efficacy/safety profile.

In contrast to the relatively large wealth or reported data with steroidal PRAs, there is only a single study evaluating the effects of a non-steroidal PRA, PF-02413873, in healthy female subjects (Howe et al., 2011). When orally dosed to healthy female volunteers, daily from the first day of the menstrual cycle, PF-02413873 blocked the mid-cycle LH surge and endometrial growth (Figure 3).

Whilst PF-02413873 development for endometriosis was curtailed due to a high incidence of idiosyncratic maculopapular rash, PF-02413873 proves the principle that a non-steroidal PRA can similarly block the effects of follicular hormones on endometrial growth as steroidal PRAs. Further data are needed to determine whether the histological changes encountered by the class of steroidal PRAs endometrial are similarly manifested by the non-steroidal PRA class.

Given the anti-proliferative effects observed in vitro and in vivo for PRAs (Freeburg et al., 2009b; Goyeneche et al., 2007; Ohara et al., 2007; Poole et al., 2006; Tieszen et al., 2011), the broader utility of this class in treating other benign and malignant growth conditions has not gone unnoticed (Chwalisz et al., 2007; Robertson et al., 1999; Rocereto et al., 2000; Wilkens et al., 2008). In the closely related condition of uterine fibroids, small studies have

demonstrated a reduction in myoma volume and uterine bleeding with asoprisnil and RU-486 (Chabbert-Buffet et al., 2005; DeManno et al., 2003; Fiscella et al., 2006). Larger studies have been completed for asoprisnil. In one randomized, controlled trial, 129 women with at least 1 fibroid greater than 3 cm in diameter or a uterine volume twice the normal (>200 cm^3) were treated for up to 3 months with asoprisnil (5, 10, or 25 mg) or placebo (Chwalisz et al., 2007). Significant reduction in uterine fibroid volume was noted by week 4 and persisted through the end of the study in a dose-dependent fashion.
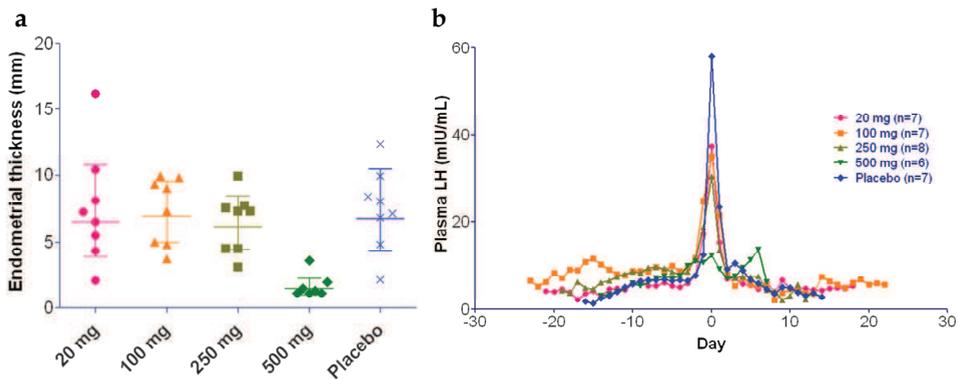

Fig. 3. Effect of escalating multiple dose of PF-02413873 on endometrial thickness (mm) (a) and the mid cycle LH surge (mIU/mL) (b) in healthy women compared with placebo (Howe et al., 2011)

7. Conclusion

There are compelling pre-clinical and clinical evidence to suggest that as well as directly antagonising the effect of progesterone, PRAs also functionally antagonise the effects of estrogen on the endometrium. This coupled with the suppression of ovarian folliculogenesis induces anovulatory amenorrhoea. Evaluation of the PR axis in animal models of endometriosis has suggested that PRAs can suppress the growth of ectopic endometriotic lesions. The mechanism driving this effect is still not clear, but it sufficient to maintain ovarian activity and estradiol levels adequately to protect bone as well as other potential post-menopausal symptoms more commonly encountered with ovarian suppression. In women with endometriosis, the data available from small clinical evaluations, strongly suggest that PRA treatment reduces disease symptoms, whilst maintaining normal levels of bone mineral density. Further clinical evaluation in larger randomised, placebo controlled and blinded studies are warranted, both to underscore the clinical benefit as well as understand the safety of the mechanism compared with existing standard of care therapy (endometrial, cardiovascular and bone safety, in particular). The medicinal chemistry challenge in designing potent, selective and safe PRAs is not inconsiderable, especially given the large number of examples whose clinical development have been curtailed (e.g. onapristone, PF-02413873, asoprisnil). However, the clinical evidence observed so far provides strong confidence that the class could have utility as a chronic treatment for endometriosis as well as a range of other gynaecological indications and malignant conditions.

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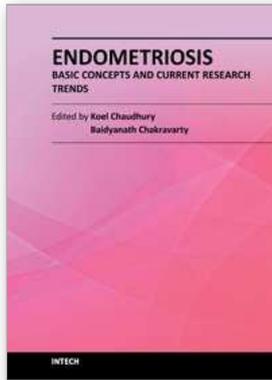
Endometriosis - Basic Concepts and Current Research Trends

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This book provides an insight into the emerging trends in pathogenesis, diagnosis and management of endometriosis. Key features of the book include overviews of endometriosis; endometrial angiogenesis, stem cells involvement, immunological and hormonal aspects related to the disease pathogenesis; recent research reports on infertility, endometrial receptivity, ovarian cancer and altered gene expression associated with endometriosis; various predictive markers, and imaging modalities including MRI and ultrasound for efficient diagnosis; as well as current non-hormonal and hormonal treatment strategies This book is expected to be a valuable resource for clinicians, scientists and students who would like to have an improved understanding of endometriosis and also appreciate recent research trends associated with this disease.

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