The translational efficacy of a non-steroidal progesterone receptor antagonist, 4-[3-cyclopropyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy,-2,6-dimethylbenzonitrile (PF-02413873), on endometrial growth in macaque and human

David C. Howe, Natalie M. Mount, Kirsty Bess, Amy Brown, Peter Bungay, Karl R. Gibson, Tony Hawcock, Jacques Richard, Gareth Jones, Rosalind Walley, Alison McLeod, Coralie Apfeldorfer, Simeon Ramsey, Sarah Tweedy and Nick Pullen


Glaxosmithkline Research & Development, Gunnels Wood Road, Stevenage, Herts SG1 2NY (D.C.H.)

Sanofi-Aventis R&D, DSAR, 371 Avenue du Professeur Joseph Blayac, F-34184 Montpellier Cedex 04 France (J.R.)
Running Title: Translational efficacy of PF-02413873 in macaque and healthy females

Corresponding author:

Nick Pullen. Pfizer Global Research & Development, Ramsgate Road, Sandwich, Kent CT13 9NJ

Tel: +44 1304 644507

Email: dr.nick.pullen@gmail.com

Number of pages: 40

Number of tables: 6

Number of figures: 9

Number of references: 49

Number of words in the abstract: 214

Number of words in the introduction: 545

Number of words in the discussion: 1211

Abbreviations: PR, progesterone receptor; ZK-230211, 11β-(4-acetylphenyl)-17β-hydroxy-17α-(1,1,2,2,2-pentafluoroethyl)estra-4,9-dien-3-one; NHR, nuclear hormone receptor; GR, glucocorticoid receptor; PF-02367982, 2-[4-(4-cyano-phenoxy)-3,5-dicycloprenyl-1H-pyrazol-1-yl]-N-methylacetamide; PF-02413873, 4-[3-cycloprenyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy-2,6-dimethylbenzonitrile; RU-486, 11β-4-dimethylaminophenyl-17β-hydroxy-17α-propinyl-4,9-estradiene-3-one; PK, pharmacokinetics; AR, androgen receptor; MR, mineralocorticoid receptors; PDE, phosphodiesterase; BrdU, bromodeoxyuridine; LH lutenising hormone.

Recommended section assignment: Drug Discovery and Translational Medicine
Abstract

There is considerable ongoing investment in the research and development of selective progesterone receptor (PR) modulators for the treatment of gynaecological conditions such as endometriosis. Here we provide the first report on the clinical evaluation of a non-steroidal progesterone receptor antagonist 4-[3-cyclopropyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy,-2,6-dimethylbenzonitrile (PF-02413873) in healthy female subjects. In in vitro assays, PF-02413873 behaved as a selective and fully competitive PR antagonist, blocking progesterone binding and PR nuclear translocation. The pharmacological mode of action of PF-02413873 appears to differ from the founding member of the class of steroidal PR antagonists, RU-486 (mifepristone). Exposure-effect data from studies in the cynomolgus macaque, however, demonstrated that PF-02413873 reduced endometrial functionalis thickness to a comparable degree to RU-486 and this effect was accompanied by a decrease in proliferation rate (as measured by BrdU incorporation) for both RU-486 and high dose PF-02413873. These data were used to underwrite a clinical assessment of PF-02413873 in a randomised, double-blinded, 3rd party open, placebo controlled, dose escalation study in healthy female volunteers with dosing for 14 days. PF-02413873 blocked the follicular phase increase in endometrial thickness, the mid cycle LH surge and elevation in estradiol in a dose dependent fashion compared with placebo. This is the first report of translational efficacy data with a non-steroidal PR antagonist in cynomolgus macaque and human subjects.
Introduction

The physiological effects of progesterone are mediated by two nuclear receptor transcription factors, PR-A and PR-B, which are produced from a single gene and upon binding progesterone regulate the expression of specific gene networks in reproductive tissues. Both PR null mutation and selective disruption of the PR-A isoform in the mouse leads to a failure of ovulation due to disabled follicular rupture in response to gonadotrophin stimulation (Mulac-Jericevic and Conneely, 2005). Whilst there is still much to be delineated in the mechanisms by which PR controls the function of reproductive tissue and the hypothalamic-pituitary-ovary axis, as alterations in PR function also appear to contribute to pathological conditions such as endometriosis and cancer, there continues to be considerable interest in agents which modulate PR activity (Turgeon et al., 2004; Marx, 2006; Spitz, 2009; Kobayashi et al., 2010). As a class, steroidal progesterone receptor antagonists, such as RU-486/mifepristone, have been approved clinically for use in pregnancy termination and emergency contraception (Spitz, 2009). Studies with RU-486 and other steroidal PR antagonists, such as onapristone, ZK-137316 and ZK-230211, have revealed that these agents, as well as directly antagonizing progesterone function, can block ovarian function and also arrest the effect of estrogen on the endometrium in women and non-human primates (Wolf et al., 1989; Slayden and Brenner, 1994; Slayden et al., 2001a; Baird et al., 2003; Brenner and Slayden, 2005; Chabbert-Buffet et al., 2005; Narvekar et al., 2006; Slayden et al., 2006). The broader clinical utility of RU-486 as a new approach to the treatment of endometriosis, uterine fibroids and dysfunctional uterine bleeding, for instance, is limited due to antagonism at the glucocorticoid receptor (GR) (Heikinheimo et al., 1987) and effects on corticotropin secretion. Consequently, there has been considerable medicinal chemistry investment focussed on identifying alternative chemical equity which has greater selectivity for PR over GR as well as other nuclear hormone receptors (NHRs) (Attardi et al., 2002; Jones et al., 2005; Terefenko et al., 2005; Kern et al., 2007; Zhang et al., 2007a; Fensome et al., 2008; Zhang et al., 2008; Kern et al., 2009; Dack et al., 2010; Kern et al., 2010).
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. Once bound, ligands can induce different NHR conformations which, depending on cell type and context, can affect the specificity of the downstream signalling events (Dai et al., 2008; Kobayashi et al., 2010; Zhang et al., 2010) leading to complex expressions of pharmacology. The cornerstone of clinical translation is the development of a pre-clinical screen sequence which can be used to predict clinical outcome. We have previously reported on the in vitro pharmacological profile and PK/PD relationship of a novel potent non-steroidal PR antagonist, PF-02367982 (de Giorgio-Miller et al., 2008) in the rabbit and the macaque. The development of this molecule was curtailed before reaching clinical testing and was superseded by a related compound, 4-[3-cyclopropyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy,-2,6-dimethylbenzonitrile (PF-02413873). Here we describe the in vitro pharmacological characteristics of PF-02413873, comparing them with the RU-486, and report on the comparative in vivo effect of PF-02413873 and RU-486 in the cynomolgus macaque. These data were used to underwrite a clinical evaluation, the first reporting efficacy of a non-steroidal PR antagonist on inhibition of endometrial growth in healthy female subjects.
Materials and Methods

PF-02413873 is an orally active non-steroidal PR antagonist (4-[3-cyclopropyl-1-(mesylmethyl)-5-methyl-1H-pyrazol-4-yl]oxy-2,6-dimethylbenzonitrile, Figure 1). The synthesis of PF-02413873 has been described elsewhere (Bradley et al., 2009). RU-486/mifepristone was purchased from Sigma-Aldrich (St. Louis, MO). The in vitro functional pharmacological properties of PF-02413873 for progesterone receptor (PR), androgen (AR), glucocorticoid (GR) and mineralocorticoid (MR) nuclear hormone receptors was determined as described previously (de Giorgio-Miller et al., 2008). CEREP (Poitiers, France) was used for supplemental wide receptor-profiling assay data and additional binding assays as described. Pamgene (Hertogenbosch, The Netherlands) were contracted to provide PR-LxxLL peptide binding data.

PR Nuclear Translocation Assay

U2OS cells recombinantly expressing PR with a prolink tag and the nuclear restricted “enzyme acceptor” were purchased from DiscoveRx and maintained in phenol red free Minimal Eagle’s Medium (MEM) supplemented with 10% (v/v) heat inactivated foetal calf serum (FCS), 2 mM L-glutaMAX, 600 µg/mL Geneticin and 250 µg/mL Hygromycin B. Cells were seeded in 384 well plates (10,000 cells, 20 µL/well) in phenol red free MEM supplemented with 1% (v/v) charcoal/dextran stripped FCS and 2 mM L-GlutaMAX and incubated overnight at 37°C. Diluent (5 µL; phosphate buffered saline supplemented with 0.025% pluronic acid and 2.4% DMSO) or serial dilutions of RU-486 (1 pM to 10 nM), or PF-02413873 (1 nM to 10 µM) were added to the cells alone or followed by serial dilutions of progesterone (5 µL; 1.58 pM to 158 nM) in diluent. Cells were incubated at 37°C for 3 h to enable nuclear translocation; after which DiscoveRxTM PathHunterTM reagents (15µL) were added according to manufacturer’s instructions 60 min prior to reading on a luminescence counter (Perkin Elmer EnVison).

Analysis of Plasma Samples for PF-02413873 and RU-486
Plasma concentrations of RU-486 and PF-02413873 were determined in 50 μL aliquots of thawed macaque plasma using tert-butyl methylether extraction followed by high-performance liquid chromatography and on-line tandem mass spectrophotometric analysis. Plasma protein binding of PF-02413873 was determined by equilibrium dialysis, and unbound plasma concentrations were then calculated using values for “free fraction” of 0.046 and 0.031 for cynomolgus macaque and human respectively. The free fraction value for RU-486 was 0.028 for cynomolgus macaque (de Giorgio-Miller et al., 2008).

Human plasma samples were analyzed for PF-02413873 concentrations at GVK Biosciences Ltd (Hyderabad, India) using a validated analytical specific LC-MS/MS assay. Specimens were stored at approximately -20°C until analysis and assayed within the 154 days of established stability data generated during validation. Calibration standard responses were linear over the range of 1 - 5 ng/mL and the lower limit of quantification for PF-02413873 was 3 ng/mL. Assay precision, expressed as the between-day CV (%) of the mean estimated concentrations of QC samples was 6.2% for low (3 ng/mL), medium (249 ng/mL) and high (400 ng/mL) concentrations.

**Evaluating the Effects of PF-02413873 on the Naturally Cycling Cynomolgus Macaque Endometrium**

The in-life phase of the study was performed at Covance (Münster, Germany) in cynomolgus macaques (weight range 3.7-5.7 kg and aged 5-6 years old) previously used to assess the effects of PF-02367982 (de Giorgio-Miller et al., 2008). The study was approved by local and Covance ethics review boards and conducted in accordance with local animal husbandry procedures and legislation. A chronic dosing study was supported with a preliminary single dose oral pharmacokinetics study from which doses for the pivotal efficacy study were extrapolated. For this, 20 sexually mature female cynomolgus macaques underwent daily menstrual cycle inspection by morning examination of external genitalia and vaginal smears. Menstrual bleeding was checked by inserting a cotton bud into the vagina. Animals were allowed to complete an observation cycle prior to any surgical intervention or drug administration. All animals completed a normal menstrual cycle within 35 days. The first day of the next observation cycle was deemed the first day of menstruation and the first day of dosing.
Animals were dosed vehicle (2% (w/v) hydroxyprolyl cellulose, 0.1% (v/v) Tween-80 and 0.1% (w/v) sodium lauryl sulphate in water), PF-02413873 (2.5 and 10 mg/kg b.i.d) and RU-486 (20 mg/kg q.d) in a vehicle suspension by oral gavage (n=5 per dose group). The doses were selected after a pilot single dose (3 mg/kg) PK study and by projection from a previous study with RU-486 and the related non-steroidal progesterone receptor antagonist PF-02367982 (de Giorgio-Miller et al., 2008) with the objective of targeting 0.5 and 2 nmol.h/mL PF-02413873 exposures. Animals were dosed for 10 days and 1 h prior to euthanasia, approximately 4 h after the final drug dose, the animals were treated with bromodeoxyuridine (BrdU, 100 mg/animal i.v.). Drug exposure levels were determined by pharmacokinetic sampling on days 1, 5 and 10 of dosing.

**Histomorphometric analysis**

Multiple sections of vagina, cervix, and uterus were embedded in paraffin before sectioning at 4 μm and staining with haematoxylin and eosin using the Leica 5030 auto-stainer. A representative section of endometrium which had the full thickness of endometrium and where possible longitudinal sections of endometrial glands, was chosen for evaluation. Sections were assessed for degree of reduction in endometrial thickness relative to negative control. A virtual 0.63x image was used to measure the endometrial thickness. All measurements were performed using Image Pro-Plus (Media Cybernetics). The border of endometrium and myometrium was manually delineated using the measurements line drawing tool within Image Pro-Plus. Between 6 and 15 representative distances (from luminal epithelial surface to the endometrial/myometrial border) were captured per sample. Whenever possible, all measurements followed the gland direction. The basalis and functionalis zones were manually delineated using the measurements line drawing tool within Image Pro-Plus and representative thickness measurements were drawn along glands. Between 6 and 10 representative measurements were taken for each sample.

**Immunohistochemical assessment of BrdU and androgen receptor (AR) expression**

The paraffin blocks containing the optimal endometrial morphology were chosen and stained for BrdU and AR using a Ventana XT. A primary rat anti-BrdU antibody (1/100) was purchased from
Abcam (ab6326) and developed with a rabbit anti-rat biotin (1/200) from Vector Labs BA-4001. The DabMap kit used was manufactured by Ventana following the protocol number 136. Sections were stained for the androgen receptor using a rabbit anti-human AR (1/100) primary antibody from Santa Cruz (sc-816) and donkey anti-rabbit biotin (1/200) secondary from Jackson (711-065-152). Slides were dehydrated through graded alcohols into xylene and coverslipped using a Leica CV5030. All slides were cleaned, and digital images produced using a Nanozoomer digital slide scanner (Hamamatsu) at 20x magnification. BrdU positive nuclei were counted manually in a blinded fashion and the proportion of positive to negative nuclei recorded for between 5 and 9 fields (equivalent to approximately 300-700 nuclei). For AR expression, Image Pro-Plus was used to identify total areas of positive and negative stained nuclei per field of view. The optimum red-green-blue threshold level was chosen per stain and remained constant for each sample. The mean intensity of positive and negative nuclei was also recorded per sample. This was used to calculate the available (dynamic) range of intensities for each sample. A corrected intensity (the average intensity of positive area) was calculated, and from this, an Androgen Receptor Index was determined (= corrected intensity * positive area / total area), where corrected intensity is the average intensity of positive area (on increasing scale of 0 to 100) determined by the following:

\[
\frac{((255-\text{mean brown staining})-(255-\text{mean blue staining}))}{(\text{dynamic range}/100), \text{ where dynamic range} = 210-(255-\text{mean blue staining})}
\]

Nuclei area was determined from the pixel size of the region of interest, multiplying by the relevant objective magnification.

All statistical analysis was performed using GraphPad Prism 5. Statistical significance \((p \leq 0.05)\) was determined using two-way ANOVA with a Bonferroni adjustment for multiple comparisons.

Clinical Evaluation of PF-02413873 Given Chronically to Women of Child-bearing Potential
The study was managed by Pfizer Global Research and Development (the sponsor) and conducted by investigators contracted by and under the direction of the sponsor. The final protocol, its amendments, and informed consent documentation were reviewed and approved by the Institutional Review Board and/or Independent Ethics Committee at each of the investigational centres participating in the study. This study was conducted in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice Guidelines. In addition, all local regulatory requirements were followed; in particular, those affording greater protection to the safety of study participants. The study was conducted at Pfizer Clinical Research Units in Brussels, Belgium, and New Haven, US and the Vince and Associates Clinical Research Centre, Overland Park, Kansas. Medical clinical monitoring was conducted by the sponsor or its designated representatives. Study drug was packaged, labelled, and shipped by the study sponsor. Written informed consent was obtained from all participants in this study prior to screen. This was a double-blind, third party open, randomized, placebo-controlled, parallel group, oral dose escalation study in healthy women of child bearing potential, aged 18-35 with a regular menstrual cycle, at least 1 functioning ovary and a normal endometrium. Subjects had to be willing to have their cycle synchronised with the combined pill for at least 2 weeks (and up to 8 weeks) until 7 days before randomization and, if non-abstinent, have a male partner who was willing to use adequate contraception (Figure 2). Subjects were excluded if they had an abnormal papanicolaou smear, chlamydia, or gonorrhoea, or if on screening transvaginal ultrasound any of the following were detected: uterine fibroids >3 cm in diameter which distorted the uterine cavity, uterine polyp, hydrosalpinx or tubo-ovarian mass, benign or malignant ovarian mass (including functional ovarian cysts >4 cm).

Doses were selected on the basis of a previous preliminary single dose safety and toleration dose escalation study. A sample size of 10 subjects per cohort (4:1 active:placebo) for 4 cohorts was planned to allow detection of a difference of 5 mm in endometrial thickness with >80% power at a two-sided significance level of 5%. The calculation was based on assumptions that endometrial thickness is normally distributed, the between subject standard deviation estimated to be 3.2 mm
based on published data (Premkumar et al., 2007) and that the data will be pooled across the cohorts for the statistical analysis. Cohorts were run sequentially and within each cohort subjects were randomized to treatment. Each dose cohort was intended to be dosed for 14 days (hereafter referred to as the treatment period) and then observed for a further 14 days (hereafter referred to as the observation period). Initially for each cohort, 10 subjects were randomised to treatment and subjects withdrawing for reasons unrelated to safety and toleration were replaced. The dose cohorts were as follows:

Cohort 1: 8 subjects received 20 mg PF-02413873 and 3 subjects received placebo.

Cohort 2: 9 subjects received 100 mg PF-02413873 and 2 subjects received placebo.

Cohort 3: 8 subjects received 500 mg PF-02413873 and 2 subjects received placebo. Dosing was stopped on Day 11 due to the occurrence of rash-related AEs in 4 subjects on active treatment; however, subjects continued in the study and other procedures were completed as planned.

Cohort 4: 8 subjects received 250 mg PF-02413873 and 2 subjects received placebo.

4 subjects (1 on placebo, 1 on 100 mg, 1 on 250 mg and 1 on 500 mg PF-02413873 doses) were discontinued from the study and as such had incomplete data. Endometrial thickness and all other data were collected for all other subjects

PF-02413873 or placebo was administered as an extemporaneous preparation with a standard meal. The safety/tolerability and steady state pharmacokinetic data of PF-02413873 (up to 24 h after the last dose) were reviewed after each cohort. Each dose escalation was based on safety (including determination of potential prolongation of corrected QT interval), tolerability and pharmacokinetic data from the previous dose.

Blood samples were collected for confirmation of PF-02413873 exposure and the determination of plasma hormone concentrations during the treatment and observation periods (Days 0 to 24). The clinical laboratory sample analyses were performed at the local laboratories. All pharmacokinetic
samples were sent to SGS Cephac Europe (France) for urine analysis and GVK Biosciences Ltd (Hyderabad, India) for plasma analysis.

Real-time ultrasonography was conducted with a 5.0 to 7.5 MHz vaginal transducer pre-dose (usually Day -1) and on Day 13 (notional). Briefly, the vaginal probe was covered with a coupling gel and inserted into a condom, which was coated with gel and inserted into the vaginal fornix, with the subject in the lithotomy position. During the ultrasound examination particular attention was paid to the uterus and ovaries. After obtaining a proper longitudinal view of the uterus, the uterus was observed for a few minutes during which the endometrium was continuously focused upon, and any wave-like contractions in the sub-endometrial layer observed. A still image of the uterus was captured between contractions and the endometrium was arbitrarily divided into 3 roughly equal portions for the purposes of measurement: an upper third of the endometrial stripe at the uterine fundus, middle third between upper and lower portions, and a lower third that was adjacent to the cervix. In each of the 3 regions of the uterus, the following measurements (to the nearest 0.1 mm) were obtained in the relaxed phase between any sub-endometrial or myometrial contractions:

- Antero-posterior thickness of any pool of intrauterine fluid (Measurement A).

- The endometrial thickness (obtained by placing electronic calipers at the anterior and posterior uterine walls at the margins of the basal layers of the endometrium delineated by the highly echogenic interface between endometrium and inner myometrium) (Measurement B). The true endometrial thickness was measured as B-A.

- The longitudinal, transverse and antero-posterior length of the uterus.

Endometrial thickness was measured on a midline sagittal image of the uterus and was a summation of the antero-posterior width of both the anterior and posterior endometrial layers, exclusive of possible intracavitary content. For each region, true endometrial thickness was analyzed using analysis of covariance with baseline (Day-1) as a covariate. From the analysis of covariance, the difference in treatment means and the 95% confidence interval (C.I.) for the difference were presented.
The daily estradiol and LH plasma concentrations in individual subjects dosed with PF-02413873 or placebo were used to confirm ovulation. The presence of an LH peak (defined as >1.5x baseline LH) after a rise in estradiol to >200 pg/mL (visual inspection by expert reviewer) was taken to signify ovulation. Where there was no LH peak present, the value recorded on the mean peak day was used, where mean peak day was the mean day of LH peak for those subjects with a peak.
Results

In Vitro Pharmacological Profile of PF-02413873

PF-02413873 was identified from a drug discovery campaign for agents which could block PR signalling function. PF-02413873 was assessed for its ability to block an in vitro native progesterone response in a human T47D mammary carcinoma cell based functional reporter gene assay (de Giorgio-Miller et al., 2008) and block binding to PR. PF-02413873 blocked radioligand binding to PR in a CEREP MCF-7 cytosol binding assay with a Ki value of 2.6 nM (Table 1). PF-02413873 showed potent PR antagonist activity with a derived Ki value of 9.7 nM (95% C.I. 7.3 -13.0 nM, n = 9; Table 1) in the T47D native functional assay. While, PF-02413873 did not appear to elicit a PR agonist response at concentrations below 3 μM, at concentrations higher than this PF-02413873 appeared to induce a partial PR agonist response, an effect which has been observed with other non-steroidal PR-As (Zhang et al., 2007b). To explore this further, Schild experiments were conducted with Lew and Angus non linear regression analysis (Lew and Angus, 1995) where dextral displacement of a progesterone concentration response curve was observed for both the steroidal anti-progestin RU-486 and PF-02413873 (Figure 3) with pKᵦ values of 10.5 (95% C.I. 10.4 - 10.5; n=3) and 8.0 (95% C.I 7.9 - 8.0; n=3) respectively. In contrast to RU-486, at high doses of PF-02413873 there was an apparent increase in the basal response, indicative of the agonism of PF-02413873 observed at high concentrations in the absence of progesterone. Similar data were generated in a recombinant β-lactamase reporter cell-line where the effects PF-02413873 on the progesterone-induced response on PR are coupled to a mouse mammary tumour virus promoter (de Giorgio-Miller et al., 2008), suggesting the observed pharmacological response was not due to the T47D cell line (data not shown). When the effect of RU-486 and PF-02413873 was assessed in an enzyme complementation assay which measures PR nuclear translocation, RU-486 induced nuclear translocation consistent with its functional antagonist potency (Figure 4A) and did not alter the progesterone concentration response (Figure 4B). In contrast, PF-02413873 did not induce nuclear translocation at antagonist concentrations, but appeared to block progesterone induced nuclear translocation (Figure 4C). Only at high concentrations (>3 μM), was there an apparent facilitation of
nuclear translocation (Figure 4A), consistent with the observed agonism that PF-02413873 appears to induce in other systems (Figure 3).

We explored the nature of the PF-02413873 interaction in more detail by evaluating the interaction between a recombinantly expressed glutathione-S-transferase-tagged PR ligand binding domain and suite of immobilised peptides covering the LxxLL motifs of all the major NHR co-activators/co-repressors in response to increasing concentrations of progesterone, PF-02413873 and RU-486. Representative examples of the ligand-induced interactions between PR and LxxLL peptides from NCoA-1 and NCoR-1 are depicted in Figure 5. In the system, progesterone induced interactions between PR and the co-activator NCoA-1 peptide and reduced interactions with a peptide raised against the LxxLL motif on the co-repressor NCoR-1 peptide in a concentration-dependent manner. This profile was broadly mimicked in the PF-02413873 concentration effect response, although both the magnitude and potency of the effect were considerably reduced compared with progesterone (Figure 5). In contrast, RU-486 facilitated PR recruitment to the NCoR-1 peptide and reduced PR affinity for the NCoA-1 peptide in a concentration-dependent fashion.

PF-02413873 selectivity was evaluated in recombinant functional reporter assays expressing the homologous nuclear hormone receptors, GR, AR and MR as well as against a panel of broad receptors, enzymes and ion channels available at CEREP (Table 1). In these systems, PF-02413873 demonstrated greater than 30 fold selectivity for PR.

**In vivo Effect of PF-02413873 in Naturally Cycling Cynomolgus Macaques**

PF-02413873 Doses of PF-02413873 (2.5 and 10 mg/kg; p.o b.i.d) and RU-486 (20 mg/kg; p.o q.d) were selected based on previous experience (de Giorgio-Miller et al., 2008) and pilot PK data (Table 2). The effects of PF-02413873 and RU-486 on estrogen induced endometrial growth were confirmed by histology on samples of uterus obtained from cynomolgus macaques dosed with vehicle, RU-486 (20 mg/kg qd) and PF-02413873 (2.5 and 10 mg/kg bid) for 10 days from the start of the menstrual cycle. Both RU-486 and PF-02413873 induced a statistically significant reduction in endometrial thickness compared with vehicle control animals (Figure 6 and 7). The cynomolgus and human PR
primary amino acid sequence are highly homologous (data not shown) and the PF-02413873 exposures achieved with <10x the multiple of the human Kᵢ value (Table 2). The mean percentage change in endometrial thickness in animals dosed with PF-02413873 (2.5 and 10 mg/kg p.o. bid) or the steroidal progesterone receptor antagonist RU-486 (20 mg/kg p.o. qd) was -43%, -56% and -34% respectively compared with a vehicle control (Figure 7). The effect on endometrial thickness appeared to be due to specific changes in the thickness of the functionalis compartment, as there were no observed statistically significant changes in basalis thickness. To correlate the change in endometrial thickness observed in cynomolgus macaques treated with RU-486 and PF-02413873 with changes in proliferation rate, samples of endometrium were stained for BrdU accumulation. Positive nuclei in luminal epithelium as well as functionalis glandular epithelium and stromal cell compartments were counted for each group. RU-486 induced a specific statistically significant reduction in BrdU accumulation in the functionalis stromal compartment by 88% compared with the vehicle. With the caveat of the single time point of evaluation, the effects of PF-02413873 were in contrast to the effects of RU-486 and only the highest dose of PF-02413873 tested (10 mg/kg bid) induced a statistically significant reduction in BrdU incorporation of 43% compared with the vehicle control group (Figure 7B).

The effects of both RU-486 and PF-02413873 on the anticipated increase in endometrial AR expression (Slayden et al., 2001b; Brenner et al., 2002; Brenner et al., 2003; Slayden and Brenner, 2004) were similarly determined by semi-quantitative immunohistochemistry. AR positive nuclei in the functionalis stroma were counted for each group. RU-486 induced an approximate two-fold increase in AR expression compared with vehicle. In contrast, despite similar effects of PF-02413873 on endometrial thickness as RU-486, PF-02413873 was without apparent effect on endometrial AR expression compared with the vehicle control for all doses tested (Table 3).

Effect of PF-02413873 on Endometrial Thickness in Women

Using a similar methodology to that employed in the macaque, the effects of PF-02413873 on endometrial growth during the early proliferative phase were examined in healthy women of child
bearing potential. This was a double-blind, third party open, randomized, placebo-controlled, parallel
group study evaluating escalating multiple doses of PF-02413873 (20, 100, 250, 500 mg) or placebo
given once a day for 14 days. Dosing in the 500 mg cohort was stopped after the Day 10 dosing due
to the occurrence of rash in 4 subjects on active treatment; however, subjects continued in the study
and other procedures were completed as planned. A single incidence of muculopapular rash was also
reported in the 250 mg dose cohort.

Plasma concentrations of PF-02413873 were quantifiable after all administered doses (Table 4).
Following single and multiple-dose oral administration, maximum concentrations of PF-02413873
were achieved between 3 and 5 hours on both Days 1 and 14. The mean $C_{\text{max}}$ of the highest dose
tested was approximately $10 \times$ the human $K_i$ value (Table 4). The estimated $t_\frac{1}{2}$ ranged between 34
and 48 hours across the dose range and plasma concentration-time profiles exhibited at least a bi-
exponential decline over time (data not shown). PF-02413873 showed a slightly less than dose-
proportional increase of $C_{\text{max}}$, both after single and multiple dosing. For a 5-fold increase in dose (20
- 100 mg) there was a 4-fold increase in $C_{\text{max}}$ and for a 25-fold increase in dose (20 - 500 mg) there
was a 15-fold increase in $C_{\text{max}}$. PF-02413873 accumulation was less than 2-fold with $qd$ oral dosing
(Table 4).

The main efficacy endpoints were a change in endometrial thickness compared with the placebo
control group measured by transvaginal ultrasound and change in ovarian hormone and LH responses
over the period of dosing. Three different zones of the endometrium were assessed by ultrasound. At
the highest dose tested PF-02413873 induced a reduction in the thickness of the uterine fundus and
middle third regions (Figure 8 and Table 5) and these differences were statistically significant at the
5% level. This effect was more marked for the uterine fundus data where the difference was 5.4 mm
(95% C.I, 2.5, 8.4). The differences in endometrial thickness observed at the lower doses of PF-
02413873 (20 mg, 100 mg and 250 mg) were smaller as compared with placebo (Table 5). PF-
02413873 had no inhibitory effect on the lower-third region compared with subjects receiving placebo
(Figure 8C).
The effects of PF-02413873 on plasma LH and estradiol levels were determined by sampling during the dosing period and washout phase for all PF-02413873 dosed subjects and compared with placebo. The data were compiled and centred around the mid cycle LH surge (Figure 9). The peak plasma concentrations of LH and FSH (data not shown) were lower in subjects treated with PF-02413873 doses when compared with placebo (Figure 9A). This reduction in peak plasma LH concentrations was most marked in the PF-02413873 highest dose (500 mg) group compared with placebo (Figure 9B). Peak plasma estradiol concentrations (Figure 9C) followed a similar pattern as the plasma LH profiles, a clear suppression in the individuals treated with the highest dose of PF-02413873.

Ovulation was defined as the presence of an LH peak (defined as >1.5x increase over baseline LH) after a rise in estradiol to >200 pg/mL. Inhibition of ovulation response rate is summarized in Table 6. There was an apparent dose dependent inhibition of ovulation. Inhibition of ovulation was highest (85%) for the PF-02413873 500 mg dose and persisted after cessation of dosing (Table 6). Both suppression of endometrial growth and ovarian function were achieved with PF-02413873 plasma exposures which were reasonable multiples of the primary pharmacology (Table 4).
Discussion

The role of progesterone as a pregnancy hormone only has evolved in recent years. Studies in knockout mice and by pharmacological modulation in non-human primates have revealed a direct role for the progesterone receptor in ovarian function and endometrial growth (Conneely et al., 2001; Slayden et al., 2001a; Brenner et al., 2010), suppressing the proliferative effects of estrogen on the endometrium (Wolf et al., 1989; Hodgen et al., 1994; Slayden and Brenner, 2004; Slayden et al., 2006; Brenner et al., 2010).

As a consequence, there has been considerable interest in the class of PR antagonists, typified by agents such as RU-486, as alternative contraceptives and for the treatment of gynaecological conditions such as endometriosis and uterine fibroids (Spitz, 2009). The development of selective and safe steroidal PR antagonists has been challenging, both due to reported hepatoxicity as well as potential dose-limiting anti-glucocorticoid effects. More recently, histological evaluations of subjects dosed for more than 3 months on steroidal PR antagonists appear to induce a characteristic cystic histological change in the endometrium which may be difficult to distinguish from endometrial hyperplasia without specialist evaluation (Williams et al., 2007; Mutter et al., 2008; Ioffe et al., 2009).

Building on our previous experience (de Giorgio-Miller et al., 2008), we have identified and characterised a novel and selective non-steroidal PR antagonist, PF-02413873.

PF-02413873 exhibited potent PR antagonism in human in vitro functional and binding assays and greater than 30-fold selectivity over closely related members of the nuclear hormone receptor family as well as other enzymes, receptors and ion-channels (Table 1). We characterised the nature of the PF-02413873 interaction with PR in more detail by Schild analysis, assessment on nuclear translocation and LxxLL peptide binding compared with RU-486. RU-486 behaved in these assays as anticipated from previous literature reports, facilitating PR translocation and antagonising PR function though recruitment of co-repressors (Bocquel et al., 1993; Madauss et al., 2007; Afhüppe et al., 2010). In contrast, PF-02413873 blocked PR nuclear translocation at concentrations which blocked PR.
function in the T47D functional assay. At suprapharmacological concentrations, however, it induced a nuclear translocation and partial agonist activation of the receptor; observations which were matched with a recruitment of co-activator at these concentrations. When taken together, these data suggest that PF-02413873 has mixed antagonist-agonist pharmacology. At concentrations which block PR function, PF-02413873 behaves as a neutral antagonist, inhibiting progesterone binding and nuclear translocation in a competitive manner. At suprapharmacological concentrations, PF-02413873 may induce a conformational change in the PR complex to facilitate nuclear translocation and partial agonism. These pharmacological properties of PF-02413873 as well as other non-steroidal examples we have profiled (data not shown) appear to contrast the class of steroidal anti-progestins exemplified by RU-486.

We sought to determine whether the action of PF-02413873 could antagonise PR in vivo by studying the effects in pregnant rats and rabbits (data not shown) as well as on endometrial thickness in the intact macaque. In pregnant rats and rabbits, PF-02413873 induced the dose dependent and complete resorption of foetuses (data not shown), supporting its credentials as a PR antagonist. When macaques were dosed with vehicle, RU-486 or PF-02413873 from the first day of menstruation, both RU-486 and PF-02413873 induced a statistically significant reduction in the thickness of the endometrial functionalis layer compared with vehicle control animals. In contrast, the basalis thickness was unaffected, an observation which is consistent with other literature reports (Wolf et al., 1989; Slayden and Brenner, 1994; Slayden et al., 1998; Greb et al., 1999; Brenner et al., 2010). The effects on endometrial thickness were also coupled to a reduction in proliferation rate at the highest PF-02413873 dose tested compared with the vehicle control group. Whilst this may, in part, be due to the doses of PF-02413873 only achieving a modest multiple of Ki compared with that achieved the RU-486 (Table 2), it can not be ruled out that PF-02413873 achieved its effects on the endometrium by a different mechanism to RU-486. To this latter point, one of the anticipated pharmacological consequences of PR antagonism is an upregulation of AR expression in the endometrium, especially when assessed during luteal phase sampling (Slayden et al., 2001b; Brenner et al., 2002; Brenner et al., 2003; Narvekar et al., 2004; Slayden and Brenner, 2004; Heikinheimo et al., 2007; de Giorgio-
Miller et al., 2008). The consequences of PR blockade on AR expression during follicular phase dosing were different for RU-486 and PF-02413873 treated animals as only RU-486 had a statistically significant effect on AR expression compared with control animals. This observation when coupled with the data captured in Figures 4 and 5 suggest that the mode of action of PF-02413873 and RU-486 in the macaque appear to be different.

We explored the pharmacological effects of PF-02413873 by undertaking a clinical evaluation in healthy female subjects. PF-02413873 had been previously assessed in a preliminary single dose escalation to determine PK, safety and toleration (Bungay et al., 2011). PF-02413873 was considered safe and well tolerated up to the maximal dose tested (3 g) and behaved with a terminal half-life of approximately 40 h (Bungay et al., 2011). These data were used to underwrite a 14 day multiple dose double blind, third party open, randomised and placebo controlled study to determine the effects of PF-02413873 on endometrial growth and ovarian function. During this multiple dose study, idiosyncratic maculopapular rash was observed in both the 250 (n=1) and 500 mg (n=4) cohorts, which resulted in early curtailment of dosing in the 500 mg dose cohort. The rash resolved on cessation of dosing. The mechanism driving the rash is still under investigation and, whilst the possibility that this is a PR-mediated effect can not be excluded, as there are reports of rash with progestins, its incidence as well as that reported for steroidal PR antagonists is low. Fortunately, there were sufficient data collected from all dose cohorts to determine the effect of PF-02413873 on endometrial thickness and plasma LH/estradiol levels (Figure 8 and 9). In a manner consistent with the data generated in the macaque, PF-02413873 induced a dose dependent and statistically significant reduction in endometrial thickness at the highest PF-02413873 dose tested. The PF-02413873 plasma exposures at this dose were similar to those which reduced endometrial thickness in the macaque (compare Table 2 and 4). Additionally, on the basis of the plasma LH and estradiol profiles as well as the apparent breakthrough in the normal endocrine profile during the post-dosing observation phase (Figure 9), the menstrual cycle appeared to be blocked for the duration of exposure with the 500 mg dose. Taken together these data strongly underwrite the translational confidence in the screen sequence leading to the identification of PF-02413873.
In conclusion, we have pharmacologically characterized a novel, selective non-steroidal PR antagonist, PF-02413873. We have demonstrated that PF-02413873 suppresses endometrial growth in the macaque and human and established some preliminary evidence to suggest that the mechanism by which it achieves this appears to be different compared with conventional steroidal PR antagonists. Whether by retaining unligated PR in the cytosol or some other mechanism, the inhibition of PR function by PF-02413873 deserves further experimental investigation as this mode of action may give rise to an entirely different profile of effect on the endometrium than that characterised by the class of steroidal PR antagonists (Mutter et al., 2008; Ioffe et al., 2009).
Acknowledgements

We thank Baerbel Wittke for clinical pharmacology analysis support at Pfizer Global Research & Development, Dr. A. Fuchs at Covance for expert guidance and managing cynomolgus macaque studies and Dr Herco van Liere (Pamgene) for characterising the nature of the ligand induced PR/LxxLL peptide interaction. We are extremely grateful to the volunteers who participated in the clinical study.

Author contribution


Contributed new reagents or analytic tools: K.R.G.


Wrote or contributed to the writing of the manuscript: K.R.G., R.W., N.M.M, T.H., N.P., P.B., J.R., D.C.H.

Other: Operational oversight (S.T)
References


Footnote

This work was supported by Pfizer Global Research & Development.
Figure Legends

Figure 1: Chemical structure of PF-02413873

Figure 2: Design of multiple dose assessment of PF-02413873 in female subjects

Figure 3: Progesterone concentration response curves in the T47D functional assay to increasing concentrations of (A) RU-486 and (B) PF-02413873. All data are duplicate points for a representative from one experiment (RU-486, n=4; PF-02413873, n=3) +/- SD. A concentration response curve to PF-02413873 in the absence of progesterone is also shown as an inset to B. Non-linear regression to determine the $pK_B$ value for PF-02413873 did not use the progesterone EC$_{50}$ in the presence of 2.53 µM PF-02413873 due to the apparent agonist properties of PF-02413873 at this concentration. Two models of non linear regression were applied and compared. These are analogous to Schild analysis fixing the slope to one to model simple competitive antagonism and a second model with a free fitting slope. The simple competitive model was not appropriate for either RU-486 or PF-02413873 as the slope varies significantly from unity; however the $pK_B$ predicted correlated with the $K_i$ value. Geometric mean $pK_B$ figures quoted in the text were generated using the most appropriate model. In the case of RU-486, the determined $pK_B$ is an underestimate of the true $pK_B$ and in the case of PF-02413873 it is an overestimate of the true $pK_B$ value.

Figure 4: Concentration response curves for progesterone, RU-486 and PF-02413873 (A) and progesterone in the presence of increasing concentrations of RU-486 (B) or PF-02413873 (C) in the PR nuclear translocation assay. All data are duplicate points for a representative from one experiment (n>4). The mean EC$_{50}$ values for progesterone, RU-486 and PF-02413873 are described in the table inset.

Figure 5: Concentration response curves for PR binding to (A) NCoA-1 (633_620_643) and (B) NCoR-1 (2263_2251_2273) peptides. All data are duplicate points for a representative from one experiment (n=2). The numbers in parentheses refer to the position of the LxxLL motif in the Swiss-Prot amino acid sequence, position of the first amino acid of the peptide sequence, position of the last amino acid of the peptide in the Swiss-Prot sequence.
Figure 6: Representative hematoxylin and eosin stained sections of uterus from cynomologus macaques treated with (A) vehicle, (B) RU-486 (20 mg/kg, p.o., q.d) (C) PF-02413873 (2.5 mg/kg p.o., b.i.d) and (D) PF-02413873 (10 mg/kg p.o., b.i.d).

Figure 7: Mean % changes in (A) endometrial thickness and (B) BrdU incorporation from a cynomologus macaques treated with RU-486 (20 mg/kg, p.o., q.d) and PF-02413873 (2.5 and 10 mg/kg p.o., b.i.d) compared with baseline vehicle-treated animals. Data are depicted as means ± SEM for n=5 animals for (A) total (black), functionalis (medium grey) and basalis (light grey) endometrial thickness and (B) luminal (black), functionalis (medium grey) and stromal (light grey) compartments per treatment group. There was a statistically significant effect of RU-486 and PF-02413873 on endometrial thickness and BrdU incorporation at a p < 0.001 (**) and 0.05 (*) level respectively compared with the vehicle control group samples.

Figure 8: Transvaginal ultrasound determined endometrial thickness measures in the (A) fundus, (B) middle and (C) lower third of the uterus in women treated with increasing oral doses of PF-02413873 and placebo. Data are presented as geometric means and a 95% confidence range. There was a statistically significant effect of PF-02413873 (500 mg) on endometrial thickness and at a p < 0.05 (*) level compared with the placebo control group for measures of thickness determined in the uterine fundus and middle third.

Figure 9: (A) Mean plasma LH concentrations in female subjects treated with placebo and increasing doses of PF-02413873. (B) Individual subject plasma LH responses to PF-02413873 (500 mg) compared with the mean placebo response. (C) Mean plasma estradiol concentrations in female subjects treated with placebo and increasing concentrations of PF-02413873. Data are
positioned around the mid cycle LH surge, indicated as Day 0. Data obtained prior to the mid cycle surge are indicated as minus (-) and vice versa. The data are presented as geometric means and without error bars for clarity. Individual LH responses (indicated as the corresponding blinded subject IDs) in the 500 mg dose group in (A) are shown in (B) and the corresponding estradiol levels in all dose groups shown in (C). The study groups sizes for analysis were as follows: 20 mg (n=7), 100 mg (n=7), 250 mg (n=8), 500 mg (n=6) for the PF-02413873 cohorts and placebo (n=7).
Table 1. PF-02413873 in vitro Pharmacological Profile. All values were obtained from assays using native or recombinant human receptors. Data are indicated as mean $K_i$ or IC$_{50}$ value (or pKB), 95% confidence interval range, and the number of repeat experiments.

<table>
<thead>
<tr>
<th>Assay Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro functional</strong></td>
<td></td>
</tr>
<tr>
<td>T47D-PR-AP</td>
<td>Antagonist format: $K_i = 9.7$ nM (95% C.I. 7.3 – 13.0 nM; n=9)</td>
</tr>
<tr>
<td></td>
<td>$pK_B = 8.0$ (95% C.I 7.9 - 8.0; n=3)</td>
</tr>
<tr>
<td></td>
<td>Agonist format: ~25% activation at 10 μM (95% C.I. 19-32%; n =4)</td>
</tr>
<tr>
<td>AR-βlac reporter</td>
<td>Antagonist format: $K_i=1130$ nM (95% C.I. 340 - 3800 nM; n=5)</td>
</tr>
<tr>
<td></td>
<td>Agonist format: No evidence of agonism at 10 μM</td>
</tr>
<tr>
<td>MR-βlac reporter</td>
<td>Antagonist format: $K_i= 307$ nM (95% C.I. 261 – 362 nM, n=16)</td>
</tr>
<tr>
<td></td>
<td>Agonist format: No evidence of agonism at 10 μM</td>
</tr>
<tr>
<td>GR-βlac reporter</td>
<td>Antagonist format: $K_i=2710$ nM (95% C.I. 1950 – 3770 nM; n=6)</td>
</tr>
<tr>
<td></td>
<td>Agonist format: No evidence of agonism at 10 μM</td>
</tr>
<tr>
<td><strong>In vitro binding</strong></td>
<td></td>
</tr>
<tr>
<td>MCF-7 cytosol PR</td>
<td>$K_i = 2.6$ nM</td>
</tr>
<tr>
<td>Full length human AR</td>
<td>IC$_{50} = 2100$ nM</td>
</tr>
<tr>
<td>IM-9 cell cytosol GR</td>
<td>$K_i = 410$ nM</td>
</tr>
<tr>
<td>Phosphodiesterases:</td>
<td></td>
</tr>
<tr>
<td>PDE1A</td>
<td>IC$_{50} = 6870$ nM</td>
</tr>
<tr>
<td>PDE3A</td>
<td>IC$_{50} = 1180$ nM</td>
</tr>
<tr>
<td>PDE3B</td>
<td>IC$_{50} = 1750$ nM</td>
</tr>
<tr>
<td>Compound</td>
<td>IC50 Values</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>PDE5</td>
<td>IC50 = 7540 nM</td>
</tr>
<tr>
<td>PDE10</td>
<td>IC50 = 1540 nM</td>
</tr>
<tr>
<td>[All other PDE’s tested showed IC50 = &gt; 10 μM]</td>
<td></td>
</tr>
<tr>
<td>CEREP panel (n=73 different enzymes, receptors, ion channels)</td>
<td></td>
</tr>
<tr>
<td>Rat Na+ site 2 channel (CEREP cat no. 862a)</td>
<td>Ki = 2200 nM</td>
</tr>
<tr>
<td>All others tested IC50 &gt; 10 μM</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. PF-02413873 and RU-486 Exposure Data from Single and Multiple Dose Studies in the Cynomolgus Macaque. Mean exposure data for both single and multiple dose studies with PF-02413873; multiple dose data shown are from day 5 of the dosing schedule.

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$</th>
<th>$C_{\text{min}}$</th>
<th>AUC$_{0-24}$</th>
<th>$C_{\text{av}}$</th>
<th>CL/F</th>
<th>Vz/F</th>
<th>t$_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mL unbound nM (multiple of $K_i$)</td>
<td>ng/mL unbound nM (multiple of $K_i$)</td>
<td>ng.h/mL unbound nM (multiple of $K_i$)</td>
<td>mL/min/kg</td>
<td>L/kg</td>
<td>h</td>
<td></td>
</tr>
<tr>
<td>PF-02413873 (3 mg/kg; single)</td>
<td>162</td>
<td>20.7 (2.1x)</td>
<td>4.2</td>
<td>0.5 (0.05x)</td>
<td>1240</td>
<td>6.7 (0.7x)</td>
<td>41</td>
</tr>
<tr>
<td>PF-02413873 (2.5 mg/kg; b.i.d)</td>
<td>197</td>
<td>25.2 (2.5x)</td>
<td>14.8</td>
<td>1.9 (0.2x)</td>
<td>2295</td>
<td>12.4 (1.2x)</td>
<td>ND</td>
</tr>
<tr>
<td>PF-02413873 (10 mg/kg; b.i.d)</td>
<td>765</td>
<td>97.9 (9.8x)</td>
<td>185</td>
<td>23.6 (2.4x)</td>
<td>10320</td>
<td>55.0 (5.5x)</td>
<td>ND</td>
</tr>
<tr>
<td>RU-486 (20 mg/kg q.d.)</td>
<td>37.9</td>
<td>2.9 (30x)</td>
<td>11.1</td>
<td>0.85 (9.0x)</td>
<td>720</td>
<td>2.3 (23x)</td>
<td>ND</td>
</tr>
</tbody>
</table>

N.D Not determined
Table 3. Mean Androgen Receptor Index ± S.D in Functionalis Stromal Nuclei in RU-486 and PF-02413873 Treated Cynomolgus Macaques c.f. Vehicle Control

<table>
<thead>
<tr>
<th>Group</th>
<th>AR indexa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>14.6 ± 2.7</td>
</tr>
<tr>
<td>RU-486 (20 mg/kg qd)</td>
<td>26.6 ± 4.8 (p&lt;0.01)</td>
</tr>
<tr>
<td>PF-02413873 (2.5 mg/kg bid)</td>
<td>12.9 ± 10.1</td>
</tr>
<tr>
<td>PF-02413873 (10 mg/kg bid)</td>
<td>12.9 ± 1.5</td>
</tr>
</tbody>
</table>

a arithmetic mean
Table 4. Mean exposure data in women treated with PF-02413873

<table>
<thead>
<tr>
<th>Dose</th>
<th>C$_{\text{max}}$(^a) (ng/mL)</th>
<th>AUC$_{\text{tau}}$(^a) (ng.h/mL)</th>
<th>$t\frac{1}{2}$ (h)</th>
<th>C$_{\text{av}}$ (multiple of $K_i$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mL unbound nM (multiple of $K_i$)</td>
<td></td>
<td></td>
<td>unbound nM (multiple of $K_i$)</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg</td>
<td>79</td>
<td>6.8 (0.7x)</td>
<td>651</td>
<td></td>
</tr>
<tr>
<td>100 mg</td>
<td>283</td>
<td>24.4 (2.5x)</td>
<td>2705</td>
<td></td>
</tr>
<tr>
<td>250 mg</td>
<td>722</td>
<td>62.1 (6.4x)</td>
<td>7563</td>
<td></td>
</tr>
<tr>
<td>500 mg</td>
<td>1150</td>
<td>99.2 (10.2x)</td>
<td>11427</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg</td>
<td>111</td>
<td>9.6 (1x)</td>
<td>1218</td>
<td>4.4 (0.5x)</td>
</tr>
<tr>
<td>100 mg</td>
<td>401</td>
<td>34.6 (3.6x)</td>
<td>4462</td>
<td>16 (1.6x)</td>
</tr>
<tr>
<td>250 mg</td>
<td>888</td>
<td>76.4 (7.9x)</td>
<td>10544</td>
<td>37.9 (3.8x)</td>
</tr>
<tr>
<td>500 mg</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
<td>N.D</td>
</tr>
</tbody>
</table>

\(^a\)geometric mean; N.D not determined
Table 5. Adjusted Mean Changes in Ultrasound Determined Endometrial Thickness (in mm) in PF-02413873 Treated Women Compared with Placebo Controls

<table>
<thead>
<tr>
<th>Dose</th>
<th>Uterine Fundus</th>
<th>Middle Third Between Upper and Lower Portions</th>
<th>Lower Third Adjacent to Cervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg</td>
<td>-0.7 (95% C.I., -3.7, 2.3)</td>
<td>-0.9 (95% C.I., -3.8, 2.0)</td>
<td>0.4 (95% C.I., -1.0, 1.8)</td>
</tr>
<tr>
<td>100 mg</td>
<td>0.2 (95% C.I., -2.7, 3.1)</td>
<td>0.3 (95% C.I., -2.3, 2.8)</td>
<td>2.2 (95% C.I., 0.8, 3.5)</td>
</tr>
<tr>
<td>250 mg</td>
<td>-1.1 (95% C.I., -3.9, 1.8)</td>
<td>-1.7 (95% C.I., -4.3, 0.8)</td>
<td>1.5 (95% C.I., 0.1, 2.8)</td>
</tr>
<tr>
<td>500 mg</td>
<td>-5.4 (95% C.I., -8.4, -2.5)</td>
<td>-3.7 (95% C.I., -6.4, -1.1)</td>
<td>-0.7 (95% C.I., -2.1, 0.7)</td>
</tr>
</tbody>
</table>

*p<0.05
Table 6. Inhibition of Ovulation Response Rate

<table>
<thead>
<tr>
<th>Dose</th>
<th>n/N</th>
<th>Response rate (%)</th>
<th>n/N</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg</td>
<td>4/8</td>
<td>50</td>
<td>1/8</td>
<td>13</td>
</tr>
<tr>
<td>100 mg</td>
<td>3/8</td>
<td>38</td>
<td>1.8</td>
<td>13</td>
</tr>
<tr>
<td>250 mg</td>
<td>6/8</td>
<td>75</td>
<td>1/8</td>
<td>13</td>
</tr>
<tr>
<td>500 mg</td>
<td>6/7</td>
<td>86</td>
<td>6/7</td>
<td>86</td>
</tr>
<tr>
<td>Placebo</td>
<td>2/8</td>
<td>25</td>
<td>1/8</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 1

PF-02413873
**Figure 4**

<table>
<thead>
<tr>
<th>EC$_{50}$ value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>1.7 nM (95% C.I. 1.3–2.1)</td>
</tr>
<tr>
<td>RU-486</td>
<td>0.31 nM (95% C.I. 0.09–1.05)</td>
</tr>
<tr>
<td>PF-02413873</td>
<td>2018 nM (95% C.I. 1239–3286)</td>
</tr>
</tbody>
</table>
Figure 5

A

B
Figure 7

A

Mean % reduction in thickness c.f. control

RU-486 (20 mg/kg qd)  PF-02413873  PF-02413873
(2.5 mg/kg bid)  (10 mg/kg bid)

**  **  **

B

Mean % reduction in BrdU incorporation c.f. control

RU-486 (20 mg/kg qd)  PF-02413873  PF-02413873
(2.5 mg/kg bid)  (10 mg/kg bid)

*
Figure 8

A) Uterine Fundus

B) Middle Third

C) Lower Third

Endometrial Thickness (mm)

20 100 250 500 Placebo

PF-02413873 (mg)