DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



# Examination of the Human Cytochrome P4503A4 Induction Potential of PF-06282999, an Irreversible Myeloperoxidase Inactivator: Integration of Preclinical, *In Silico*, and Biomarker Methodologies in the Prediction of the Clinical Outcome

Jennifer Q. Dong, James R. Gosset, Odette A. Fahmi, Zhiwu Lin, Jeffrey R. Chabot, Steven G. Terra, Vu Le, Kristin Chidsey, Parya Nouri, Albert Kim, Leonard Buckbinder, and Amit S. Kalgutkar

*Clinical Pharmacology, Pfizer Inc., Cambridge, MA (J.Q.D.), Pharmacokinetics, Pharmacodynamics, and Metabolism, Pfizer Inc., Groton, CT (O.A.F., Z.L.) and Cambridge, MA (J.R.G., J.R.C., A.S.K.), Clinical Development, Pfizer Inc., Groton, CT (S.G.T.), Statistics, Pfizer Inc., (V.L.), Early Clinical Development, Pfizer Inc., Cambridge, MA (K.C., A.K.), Clinical Assay Group, Pfizer Inc., Groton, CT (P.N.), Cardiovascular and Metabolic Disease Research Unit, Pfizer Inc., Cambridge, MA (L.B.)*

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Running Heading**: CYP3A4 Induction Potential of PF-06282999

**Address correspondence to:** Amit S. Kalgutkar, Pharmacokinetics, Dynamics, and Metabolism-New Chemical Entities, Pfizer Worldwide Research and Development, 610 Main Street, Cambridge, MA 02139, USA. Tel: +(617)-551-3336. E-mail: amit.kalgutkar@pfizer.com

**Text Pages** (including references): 34

**Tables:** 7

**Figures:** 5

**References:** 51

**Abstract:** 245

**Introduction:** 639

**Discussion:** 1633

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Abbreviations used are:** CYP, cytochrome P450; mRNA, messenger RNA; DDI, Drug-drug interaction; PF-06282999, 2-(6-(5-chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamide; MPO, myeloperoxidase; FIH, first-in-human; PK, pharmacokinetics; MAD, multiple ascending dose; AUC, area under the plasma concentration time curve; PBPK, physiologic-based pharmacokinetic modeling; DMSO, dimethyl sulfoxide; TID, three times daily; BID, twice daily; LC–MS/MS, liquid chromatography tandem mass spectrometry; $C_{max}$, peak plasma concentration, $t_{max}$, time to reach $C_{max}$; $C_{min}$, minimum or trough concentration; $C_{av}$, average concentration; $t_{1/2}$, half–life; m/z, mass-to-charge; CI, confidence interval.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Abstract:**

The propensity for cytochrome P450 (CYP)3A4 induction by 2-(6-(5-chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamide (PF-06282999), an irreversible inactivator of myeloperoxidase, was examined in the present work. Studies using human hepatocytes revealed moderate increases in CYP3A4 messenger RNA (mRNA) and midazolam-1'-hydroxylase activity in a PF-06282999 dose-dependent fashion. At the highest tested concentration of 300 μM, PF-06282999 caused maximal induction in CYP3A4 mRNA and enzyme activity ranging from 56%–86% and 47%–72%, respectively, of rifampicin response across the three hepatocyte donor pools. In a clinical drug-drug interaction (DDI) study, mean midazolam $C_{max}$ and AUC following 14-day treatment with PF-06282999 decreased in a dose-dependent fashion with a maximum decrease in midazolam $AUC_{0\text{-}inf}$ and $C_{max}$ of ~57.2% and 41.1% observed at the 500 mg BID dose. The moderate impact on midazolam pharmacokinetics at the 500 mg BID dose of PF-06282999 was also reflected in statistically significant changes in plasma 4β-hydroxycholesterol/cholesterol and urinary 6β-hydroxycortisol/cortisol ratios. Changes in plasma and urinary CYP3A4 biomarkers did not reach statistical significance at the 125 mg TID dose of PF-06282999, despite a modest decrease in midazolam systemic exposure. Predicted DDI magnitude based on the in vitro induction parameters and simulated pharmacokinetics of perpetrator (PF-06282999) and victim (midazolam) using Simcyp population-based simulator were in reasonable agreement with the observed clinical data. As the magnitude of the 4β-hydroxycholesterol or 6β-hydroxycortisol ratio change was generally smaller than the magnitude of midazolam AUC change with PF-06282999, a pharmacokinetic interaction study with midazolam ultimately proved important for assessment of drug-drug interaction via CYP3A4 induction.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



## Introduction

The important role of CYP enzymes in drug metabolism is highlighted by the fact that the CYP1, 2, and 3 families combined are responsible for the biotransformation of most xenobiotics including 70–80% of all medicinal products in clinical use (Nelson, 2006; Guengerich, 2008; Zanger et al., 2008). Within the human CYP superfamily of drug-metabolizing enzymes, constitutively expressed CYP3A4 has the highest liver expression and plays a major role in the metabolism of ~55% of drugs from almost all therapeutic categories (Wienkers and Heath, 2005; Bu, 2006; Liu et al., 2007). DDIs caused by inhibition and/or induction of drug-metabolizing enzymes, particularly CYP3A4, can impact the efficacy and safety of co-administered drugs (Lin and Lu, 1998; Isoherranen et al., 2012). In vitro characterization of CYP inhibitory/induction potential of new investigational drug substances and interpretation of the generated results are critical steps in the drug development process, particularly in the design of relevant clinical DDI studies, and is also required for regulatory filing (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm292362.pdf; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf ).

2-(6-(5-Chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamide (PF-06282999, Figure 1) is an irreversible inactivator of the human myeloperoxidase (MPO) enzyme (Ruggeri et al., 2015) and is a clinical candidate for the treatment of acute coronary syndrome and related cardiovascular disorders. PF-06282999 has been evaluated in first-in-human (FIH) clinical studies for assessment of safety and tolerability (Dong et al., 2016). PF-06282999 is virtually resistant to metabolic turnover in human liver microsomes and human

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



hepatocytes, and is principally eliminated in unchanged form via renal excretion in healthy adult volunteers (Dong et al., 2016). Although PF-06282999 was devoid of inhibitory (reversible and/or time-dependent) effects against major human CYP enzymes including CYP3A4, subsequent studies in cryopreserved human hepatocytes revealed a propensity for CYP3A4 induction upon pretreatment with PF-06282999. Because the degree of MPO inhibition that leads to a clinical benefit in cardiovascular disease remain uncharacterized at the present time, there was much uncertainty around the clinically efficacious dose (and relevant systemic exposure) of PF-06282999 in relation to DDI potential with "victim" drugs (e.g., calcium channel blockers, lipid-lowering agents, etc.) that are subject to metabolism by CYP3A4 in a cardiovascular disease patient population (Niemi et al., 2003; Neuvonen et al., 2006).

Assessment of clinical DDIs arising from inhibition and/or induction of CYP3A4 by investigational drug substances usually relies on the use of midazolam as a victim drug and an exogenous marker of CYP3A4 metabolic activity (Sinz et al., 2008; Fahmi et al., 2009; Kirwan et al., 2010; Jones et al., 2016). Apart from pharmacokinetics (PK) interaction studies with probe CYP3A4 substrates, changes in plasma and urinary ratios of 4β-hydroxycholesterol to cholesterol and 6β-hydroxycortisol to cortisol, respectively, can be used as endogenous biomarkers of CYP3A4 activity (Bodin et al., 2001; Nowak et al., 2002; Galteau and Shamsa, 2003; Konishi et al., 2004; Diczfalusy et al., 2011; Marde Arrhen et al., 2013). The selective CYP3A4-mediated metabolic conversion of cholesterol to 4β-hydroxycholesterol and/or cortisol to 6β-hydroxycortisol, respectively, is utilized increasingly in clinical studies as non-invasive phenotypic markers of CYP3A4 induction, and are often implemented in phase 1 multiple ascending dose (MAD) clinical studies (Bjorkhem-Bergman et al., 2013; Dutreix et al., 2014). In our present work, a MAD study in healthy volunteers was conducted with PF-06282999 to

assess the safety, tolerability and PK upon repeated oral dosing, with a particular emphasis on the measurement of the endogenous plasma (4β-hydroxycholesterol/cholesterol) and urine (6β-hydroxycortisol/cortisol) biomarkers as surrogates for CYP3A4 induction. A DDI protocol was then integrated into the study design and included the co-administration of PF-06282999 with midazolam to estimate the magnitude of area under the plasma concentration time curve (AUC) change for midazolam as a result of CYP3A4 induction by PF-06282999. Finally, *in vitro* CYP3A4 induction data in human hepatocyte cultures and the available clinical PK data were used to rationalize the clinical observations via dynamic physiologically-based pharmacokinetic (PBPK) modeling using the Simcyp population-based simulator. The collective findings are summarized, herein.

## Materials and Methods

**Materials.** PF-06282999 (chemical purity > 99% by high-performance liquid chromatography and nuclear magnetic resonance) was synthesized at Pfizer Worldwide Research and Development (Groton, CT). Hepatocyte thawing, plating, and incubation media as well as the Torpedo antibiotic mix for the media were purchased from Bioreclamation IVT Celsis (Baltimore, MD) and CellzDirect (Pittsboro, NC). Rifampin, midazolam, 1'-hydroxymidazolam, Dulbecco's phosphate-buffered saline, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Cryopreserved human hepatocytes lot # HH1089 (Caucasian female, 57 years), HC7-4 (Caucasian male, 7 years), and FOS (Arabic male, 34 years) were purchased from In Vitro ADMET Laboratories (Columbia, MD), XenoTech (Lenexa, KS), and Bioreclamation IVT Celsis, (Baltimore, MD), respectively.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**CYP3A4 Induction in Human Hepatocytes**. Cryopreserved human hepatocytes were used to examine CYP3A4 induction potential of PF-06282999 using established protocols (Fahmi et al., 2008b). Briefly, primary cultures of human hepatocytes from three donors (HH1089, HC7-4, and FOS) were seeded in collagen I-precoated 24-well plates, and each well had a cell density of ~ 2 x $10^5$ viable cells. Hepatocyte cultures were treated for 3 consecutive days with medium containing solvent (0.1% DMSO) control, PF-0628999 (0.3, 2, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, and 300 μM), or prototypical CYP3A4 inducer rifampicin (0.01, 0.1, 0.5, 1, 5 and 10 μM). Following the incubation phase of the experiment, the medium containing test compounds or vehicle was removed and the cells were washed with phosphate buffered saline and fresh medium was added to all wells for a 30 minute wash. Following removal of the wash media, midazolam (30 μM) in 0.1% DMSO was added to each well and the plate was returned to the incubator for 30 minutes. After incubation, 200 μl of the medium from each well was removed and analyzed for 1-hydroxymidazolam formation using established liquid chromatography tandem mass spectrometry (LC-MS/MS) protocols (Walsky and Obach, 2004). Quantification of CYP3A4 mRNA was performed using the TaqMan® as previously described (Fahmi et al., 2008b).

**In Vitro Data Analysis.** Data for mRNA and activity (normalized to DMSO vehicle control) were plotted versus the concentration of the PF-06282999 or rifampin. Individual fitting was carried out on each hepatocyte donor using GraphPad Prism (version 6), and was fit to either a four-parameter sigmoidal model (equation 1, fitting $E_{max}$, Hill slope $\gamma$, and $EC_{50}$, with baseline fixed at 1) or a three-parameter sigmoidal model (equation 2, fitting $E_{max}$ and $EC_{50}$, with Hill slope and baseline each fixed at 1). The four parameter model was preferred if adding the extra

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



parameter produced a fit with a $p$ value < 0.05 compared to the three parameter model by the built-in GraphPad extra sum-of-squares F test.

$$Fold\ Induction = 1 + \frac{(Emax-1)\cdot Conc^{\gamma}}{EC50^{\gamma}+Conc^{\gamma}} \quad \text{equation 1}$$

$$Fold\ Induction = 1 + \frac{(Emax-1)\cdot Conc}{EC50+Conc} \quad \text{equation 2}$$

**Clinical DDI Study Design.** The DDI potential of PF-06282999 was characterized in a clinical study (clinical trial identifier NCT01707082) comprising of healthy adult volunteers. This study was conducted according to the ethical principles of the Declaration of Helsinki and all protocols and informed consent documents were approved by the Ethics Committee for the study center. All subjects signed an approved written informed consent form before any study-related activities. There were two parts to this clinical study. Part A was a blinded, randomized, placebo-controlled, parallel group, escalating multiple oral dose study in healthy overweight adult subjects to assess the safety, tolerability and PK of PF-06282999 following 14 days of oral administration. A total of 48 male subjects and 2 female subjects (86% Caucasian, 4% Black and 10% Other) aged 22 to 55 years old, with a body weight range of 62.2-116.5 kg and a BMI range of 27.0–34.8 kg/m$^2$, were enrolled in 5 cohorts (10 per cohort, 8 active and 2 placebo). The doses tested were 10 mg three times daily (TID), 30 mg TID, 100 mg TID, 250 mg TID and 500 mg twice daily (BID), all administered with food. PF-06282999 was administered as an immediate-release tablet during both periods of the study. The safety and tolerability of PF 06282999 was assessed by adverse events, electrocardiograms, vital signs, and safety laboratory measurements. In addition to collection of safety and PK data, concentrations of plasma (4β-hydroxycholesterol/cholesterol) and urinary (6β-hydroxycortisol/cortisol) biomarkers associated with CYP3A4 induction were also assessed at the various PF-06282999 dose groups.

Part B of the study was an open label, fixed sequence DDI study in healthy adult volunteers evaluating the impact of PF-06282999 on the single dose PK of oral midazolam. A total of 18 male subjects (94% Caucasian and 6% Other) aged 20 to 48 years old, with a body weight range of 57.3–86.0 kg and a body mass index range of 19–27.3 $kg/m^2$, were enrolled in 2 cohorts (9 per cohort) in order to evaluate the effect of 2 dose levels of PF-06282999 on the PK of oral midazolam. The two dose levels of PF-06282999 (125 mg TID and 500 mg BID) were selected based on review of emerging data from Part A of the study, including the review of the available urinary and plasma biomarker data. This DDI part of the study was conducted in 2 periods. On day 1 of period 1 after an overnight fast, subjects received a single 7.5 mg tablet oral dose of midazolam followed by collection of blood samples for up to 16 hours post dose for evaluation of midazolam PK. On the next day, period 2 day 1 commenced when subjects received their initial dose of PF-06282999. All doses were provided at approximately the same time of day throughout the study for 13 days. All doses of PF-06282999 were administered following completion of a standard meal. On day 14 of period 2 after an overnight fast, subjects received another single 7.5 mg oral dose of midazolam. Two hours later, PF-06282999 was administered following completion of a standard meal (the only dose to be administered on day 14). Midazolam was administered at a similar time on day 1 period 1 and day 14 period 2.

**Evaluation of PF-06282999 PK and Endogenous Biomarkers of CYP3A4 Induction.** For part A, serial blood samples were collected prior to dosing and up to 48 hours after the first and the last dose for quantitation of circulating levels of PF-06282999. Sparse sampling was also performed on subsequent dosing days to confirm steady-state status of systemic exposure. For part B, only pre-dose blood samples were collected on selected days to confirm adequate exposures to PF-06282999. Blood samples were centrifuged to generate plasma and

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



concentrations of PF-06282999 were determined using a validated LC-MS/MS method (Dong et al., 2016). PK parameters were calculated using non-compartmental analysis (eNCA, a Pfizer internally validated system of non-compartmental analysis). The steady state plasma PK parameters area under the concentration-time curve over dosing interval ($AUC_{0-\tau}$), peak plasma concentration ($C_{max}$), time to peak concentration ($t_{max}$), minimum or trough concentration ($C_{min}$), and average concentration ($C_{av}$) over a dosing interval, half-life ($t_{1/2}$), and the observed accumulation ratio for $AUC_{0-\tau}$ and $C_{max}$ were summarized descriptively by dose.

Measurement of plasma (cholesterol and 4β-hydroxycholesterol) and urine (cortisol and 6β-hydroxycortisol) biomarkers was performed at SGS Cephac Europe (Saint Benoît Cedex, France). Blood samples were also collected in both part A and part B segments of the study for analysis of plasma cholesterol and 4β-hydroxycholesterol concentrations. Cholesterol levels were measured following chemical derivatization of plasma cholesterol to the corresponding dansylated derivative (Tang and Guengerich, 2010). Plasma samples were diluted (100-fold) with a solution of bovine serum albumin in phosphate buffered saline (40 g/l) containing 1% butyl-4-hydroxytoluene (22.69 mM)), followed by the addition of internal standard [$^2H_6$]-cholesterol (50 μg/ml) to ~ 100 μl portions of the diluted plasma samples. The samples were then treated with ethanolic sodium hydroxide solution (9 M) in water and incubated for 5-15 minutes at 37 °C followed by the addition of 200 μl of derivatization solution (dansyl chloride (150 mg) and dimethylaminopyridine (150 mg) in 15 ml of dichloromethane) in the presence of 2% diisopropylethylamine solution and heated to approximately 65 °C for 1 hour as previously described (Tang and Guengerich, 2010). The samples were centrifuged and the organic phase was evaporated to dryness, and the residue was reconstituted with 200 μl of mobile phase (5 mM ammonium acetate in acetonitrile) prior to LC-MS/MS analysis. Samples (5-10 μl) were

analyzed on a API-4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA) with a Phenomenex, Kinetex C18 column (2.6 μm, 150 x 2.1 mm, 100 Å) at 40-55 °C. For separation of dansyl cholesterol from endogenous plasma components, liquid chromatography was performed under isocratic conditions using a mobile phase comprising of 5 mM ammonium acetate in acetonitrile at a flow rate of 0.5 ml/min. Under these conditions, the retention time for dansyl cholesterol (and its stable-labeled internal standard) was 1.8 minutes. Dansylated derivatives of cholesterol and the internal standard [$^2H_6$]-cholesterol were detected using electrospray ionization (positive ion mode) in the multiple reaction monitoring mode, monitoring for mass-to-charge (m/z) transitions 620→252 (cholesterol) and 626→252 ([$^2H_6$]-cholesterol). The lower limit of quantification was 1.0 μg/ml with an overall assay precision of $\leq$8.0%, and % relative error from -8.26% to 2.07%.

For measurement of 4β-hydroxycholesterol plasma levels, plasma samples (200 μl) were diluted with methanol (20 μl) containing the internal standard ([$^2H_7$]-4β-hydroxycholesterol (1250 ng/ml). The samples were centrifuged (3000 x g) for 10 min and the supernatant was dried under a steady stream of nitrogen. The residue was reconstituted with hexane containing 0.5% isopropanol, loaded onto solid phase extraction cartridges (Biotage, Isolute Si 100 mg, 3cc), and eluted with 3 ml of hexane/isopropanol (70/30; v/v). The eluate was evaporated and reconstituted with HPLC mobile phase prior to analysis. The liquid chromatography method consisted of a 6 minute gradient with first two minutes at 20% solvent A (5 mM ammonium acetate in methanol/water (80/20; v/v)) and 80% solvent B (5 mM ammonium acetate in methanol) followed by gradual increase (1.5 minutes) to 100% solvent B. The flow rate was 0.3 ml/minute and the retention time for 4β-hydroxycholesterol and its stable-labeled internal standard were 3.0 and 2.9 minutes, respectively. 4β-Hydroxycholesterol and [$^2H_7$]-4β-

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



hydroxycholesterol were detected using electrospray ionization (positive ion mode) in the multiple reaction monitoring mode, monitoring for m/z transitions 402→385 (4β-hydroxycholesterol) and 409→392 ([$^2H_7$]- 4β-hydroxycholesterol). The lower limit of quantification was 3.0 ng/ml with overall assay precision of <5.0%, and % relative error from -5.20% to 4.59%.

Twenty four-hour urine collections both before and after the 14-days oral administration of PF-06282999 were analyzed for the amount of urinary 6β-hydroxycortisol and cortisol. Urine samples (500 µl) diluted with 500 µl phosphate buffer (pH 8.0) were treated with 25 µl of the internal standard (30000/1000 ng/mL ([$^2H_3$]-6β-hydroxycortisol/[$^2H_4$]-cortisol in 50/50 methanol/water; v:v) and loaded onto preconditioned (with 0.5 ml methanol and 0.5 ml water) Oasis HLB cartridges and eluted with 300 µl of methanol. The samples were centrifuged (3500 x *g*, 1 minute at 5 °C), evaporated to dryness under a steady stream of nitrogen, and the residue was reconstituted with 0.3 ml of a methanol/water (50/50) mixture containing 0.1% formic acid. The samples (20 µl) were analyzed on an API-4000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA) with a Merck Chromolith RP-18e column (100 x 4.6 mm) at 30 °C. Analytes were eluted using a binary gradient comprising of water/acetonitrile (90/10; v/v) with 0.5% formic acid (mobile phase A) and water/acetonitrile (80/20; v/v) with 0.5 % formic acid (mobile phase B). Initial conditions (100% A) were held at 4 minutes at a flow rate of 2.0 ml/minute and then changed to 100% B over 7.5 minutes (flow rate = 3.0 ml/min) and held for 2 minutes. The flow rate was split into the mass spectrometer at a flow rate of 0.5 ml/minute. The retention times for 6β-hydroxycortisol and cortisol were at 3.0 minutes. The retention times of the internal standards were at 9.8 minutes. The mass spectrometer was operated in positive electrospray mode and the m/z transitions used were 363→121 (cortisol), 379→343 (6β-

hydroxycortisol), 367→121 ([$^2H_4$]-cortisol) and 382→346 ([$^2H_3$]-6β-hydroxycortisol). The lower limit of quantification of cortisol was 1.00 ng/ml with assay precision of ≤6.0%, and % relative error from -4.40% to 0.33%. The lower limit of quantification of 6β-hydroxycortisol was 10.0 ng/ml with assay precision of ≤5.0%, and % relative error from -0.89% to 6.56%.

The 4β-hydroxycholesterol/cholesterol ratio and 6β-hydroxycortisol/cortisol ratio were calculated for individual subjects at pre- and post-treatment. For part A, the fold induction was derived as the ratio of post-/pre-treatment 4β-hydroxycholesterol ratio or 6β-hydroxycortisol ratio for each of the PF-06282999 dose group or placebo. Pairwise comparison between each PF-06282999 dose group versus placebo was performed using *t*-test to examine the dose-dependent changes in the biomarkers of CYP3A4 induction. For Part B, 4β-hydroxycholesterol ratio and 6β-hydroxycortisol ratio data were log-transformed then back transformed to derive the fold induction with a 90% confidence interval (CI). P–values were from *t*-test and a 90% CI excluding 1 would indicate statistically significant change from baseline in the markers of CYP3A4 induction.

**Assessment of Clinical DDI with Midazolam.** In the DDI part of the study, serial blood samples were collected for midazolam PK analysis (16 hour PK profile) on day 1 of period 1 for midazolam given alone and day 14/period 2 for midazolam and PF-06282999 co-administration. Plasma samples were analyzed for midazolam concentrations at Covance Bioanalytical Services (Shanghai) using a validated, sensitive and specific LC-MS/MS method (Covance data on file). Calibration standard responses were linear over the range of 0.05–50 ng/ml, using a weighted ($1/concentration^2$) linear least squares regression. The lower limit of quantification for midazolam was 0.05 ng/ml. The overall assay precision was ≤7.9%, with % relative error of -

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



5.6% to 0.0%. PK parameters for midazolam were calculated using non-compartmental analysis (eNCA). Plasma midazolam PK parameters $C_{max}$, $AUC_{0\text{-}16hour}$, and $AUC_{0\text{-}inf}$ were summarized descriptively by dose and period. The interactive effect on $C_{max}$, $AUC_{0\text{-}16hour}$, and $AUC_{0\text{-}inf}$ of midazolam were determined by construction 90% CI's around the estimate difference between the test and reference treatments using a mixed-effects model based on the log transformed data. The mixed effect models with treatment as fixed effects and subject as a random effect was implemented using SAS Proc Mixed, with REML estimation method and Kenward-Roger degrees of freedom algorithm. Estimates of the adjusted mean differences (test/reference) and corresponding 90% CI's for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means (test/reference) and 90% CIs for the ratios. Midazolam alone was the reference treatment, while the midazolam co-administered with PF-06282999 was the test treatment.

**Simcyp Model.** PBPK modeling was used to simulate the effect of PF-06282999 on midazolam metabolism. Simulations were carried out using the Simcyp population-based absorption, distribution, metabolism, and elimination (ADME) simulator (Simcyp Ltd, Sheffield, UK; version 15.1). The PF-06282999 Simcyp perpetrator file was built using the clinical PK information to ensure the models reasonably recapitulated day 14 plasma-concentration time profiles reported in part A. Input parameters for PF-06282999 are shown in Table 1; data for absorption and renal clearance parameters of PF-06282999 at the dose used in the clinical study were obtained from previous clinical studies (Dong et al., 2016). For midazolam, the Simcyp default midazolam model was used. Individual in vitro CYP3A4 mRNA induction data ($EC_{50}$, $E_{max}$ and the Hill coefficient, $\gamma$) for PF-06282999 and rifampin from the three hepatocyte donors (Table 2) were integrated into the Simcyp induction calibrator to allow for correction of PF-

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



06282999 induction parameters in reference to the known inducer rifampin (Table 3). $E_{max}$ and $EC_{50}$ values were directly inputted as $Ind_{max}$ and $Ind_{C50}$; media binding was calculated to be negligible for PF-06282999 (Kalvass and Maurer, 2002).

Simulations were performed for 100 healthy subjects of North American Caucasian origin (i.e., 10 different subjects were chosen randomly for each of the 10 trial simulations); simulated subjects had an average body weight of 74 kg, were aged between 20 and 50 years, and included both genders. The $AUC_T$ of the test (co-administration of midazolam and PF-06282999) and that of the reference (midazolam alone) treatment were determined by Simcyp algorithms. Dose, dose interval, and duration of administration of PF-06282999 and midazolam were set according to the clinical protocol, such that PF-06282999 was administered 125 mg TID or 500 mg BID for 15 days with co-administration of midazolam (7.5 mg single dose) with the first dose of PF-06282999 on Day 14.

## Results

**In Vitro Induction Assessment.** The potential for induction of CYP3A4 mRNA and enzyme activity was examined in cryopreserved human hepatocytes treated with PF-06282999 at several concentrations (0.3-300 μM). PF-06282999 demonstrated moderate induction of CYP3A4 mRNA levels and midazolam-1'-hydroxylase activity in a dose-dependent fashion in all three donor hepatocytes (Figure 1). In contrast, treatment with positive control rifampicin led to a marked induction of CYP3A4 as measured from increases in mRNA and midazolam-1'-hydroxylase activity in cryopreserved human hepatocytes from the three donors. The estimated $EC_{50}$ and $E_{max}$ values for fold increases in CYP3A4 mRNA levels and enzyme activity in the three lots of human hepatocytes for PF-06282999 and rifampicin are shown in Table 2. A

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



cytotoxicity assay using MTS Cell Proliferation reagent (Promega, WI) and visual inspection showed no apparent reduction in hepatocyte viability after treatment with PF-06282999 at the tested concentrations.

**Safety and Clinical Pharmacokinetics.** PF-06282999 was safe and well tolerated after multiple oral doses up to 500 mg BID for 14 days in healthy adult volunteers. The corresponding $C_{max}$ of PF 06282999 was achieved with a median $t_{max}$ of 2.5-3.5 hours on day 1 and 2.0-3.0 hours on day 14 (Table 4). Steady state exposures were attained by day 2 with minimal to modest accumulation (<2-fold) of PF 06282999 in plasma observed after BID and TID dosing, which was consistent with $t_{1/2}$ values in the range of 4.4 to 7.3 hours in this study as well as an earlier single ascending dose study in healthy volunteers (Dong et al., 2016). Both $C_{max}$ and $AUC_{0-\tau}$ increased proportionally with increasing dose from 10 mg to 250 mg TID and 500 mg BID, with maximum $C_{max}$ and $AUC_{0-\tau}$ of 5.14 µg/ml and 35.6 h µg/ml respectively, achieved at 500 mg BID (Table 4). For the midazolam DDI part of the study (part B), mean $C_{min}$ PF-06282999 concentrations were 1.57 µg/ml on Day 7 and 1.90 µg/ml on Day 14 for 500 mg BID, and 1.11 µg/ml on Day 7 and 1.09 µg/ml on Day 14 for 125 mg TID, respectively. These $C_{min}$ values were comparable to those achieved in part A at similar doses (Table 4).

**Effects on Biomarkers of CYP3A4 Induction.** Because PF-06282999 induced CYP3A4 activity in human hepatocytes, endogenous clinical biomarkers of CYP3A4 activity were monitored in the MAD study with PF-06282999. Dose-dependent increases in both plasma 4β-hydroxycholesterol ratio and 24-hour urinary 6β–hydroxycortisol ratio were observed following daily administrations of PF-06282999 for 14 days, with an apparent induction effect starting at the 100 mg TID dose level (Part A of study) (Figure 2). Comparison between placebo and PF-06282999 dose groups was performed using the *t*-test. For 4β-hydroxycholesterol ratio,

significant differences from placebo were detected in the 250 mg TID and 500 mg BID dose groups ($p$=0.0044 and $p$=0.0003, respectively). For the 6β–hydroxycortisol ratio, significant differences from placebo were found in the 100 mg TID, 250 mg TID and 500 mg BID dose groups ($p$=0.0206, $p$=0.0029 and $p$=0.0018, respectively).

**Midazolam DDI Study.** The observed changes in the biomarkers of CYP3A4 induction during part A of the study formed the basis of dose selection for the DDI portion of the study (part B), i.e., to confirm the extent of CYP3A4 induction following 125 mg TID and 500 mg BID PF-06282999 administrations for 15 days using oral midazolam as a probe. Median midazolam plasma concentrations over the 16 hours sampling interval were lower in the presence of multiple PF-06282999 administrations than when midazolam was administered alone. Midazolam was rapidly absorbed following a single 7.5 mg oral dose administered either alone or with multiple-dose PF 06282999 (500 mg BID or 125 mg TID), with similar median $t_{max}$ of 0.5 to 1 hour. Mean terminal $t_{1/2}$ for midazolam was similar when administered alone and with multiple-dose PF 06282999 ranging from 3.3 to 4.6 hours. Following the co-administration of midazolam and PF-06282999, midazolam AUC and $C_{max}$ values were lower than those when midazolam was administered alone (Table 5). Results of the statistical comparison indicate that multiple-dose administration of PF-06282999 significantly decreased both peak and total midazolam exposures in a dose-dependent manner (Table 5). Following co-administration of 500 mg BID PF-06282999, the mean (90% CI) midazolam $AUC_{0\text{-inf}}$ and $C_{max}$ were decreased by 57.2% (52.1%, 61.7%) and 41.1% (24.6%, 54.06%), respectively, compared to when midazolam was administered alone. The exposures were decreased by 34.0% (26.2%, 41.0%) for $AUC_{0\text{-inf}}$ and 30.8% (11.4%, 46.0%) for $C_{max}$, respectively, for midazolam with PF-06282999 125 mg TID.

**Comparison of CYP3A4 Induction Biomarkers.** In the midazolam DDI part of the study, the CYP3A4 induction after 2 weeks of daily 125 mg TID or 500 mg BID of PF-06282999 treatment were also assessed by measuring plasma 4β-hydroxycholesterol/cholesterol ratio and urinary 6β–hydroxycortisol/cortisol ratio. There appeared to be comparable levels of baseline CYP3A4 enzyme activities (based on plasma 4β-hydroxycholesterol/cholesterol ratio, urinary 6β-hydroxycortisol/cortisol ratio and midazolam pharmacokinetics) before treatment with PF-06282999 within the two dose cohorts (Figure 3). Statistical analysis comparing the after PF-06282999 treatment values with baseline values revealed significant changes in the 4β-hydroxycholesterol/cholesterol ratio and 6β-hydroxycortisol/cortisol ratio following 500 mg PF-06282999 BID dosing regimen (Table 6). This was associated with moderate impact on midazolam pharmacokinetics (ratio to baseline of 0.44 for midazolam $AUC_{0\text{-}16hours}$ and 0.59 for midazolam $C_{max}$, respectively, Table 6). Dosing of PF-06282999 at 125 mg TID did not demonstrate significant changes in the 4β-hydroxycholesterol/cholesterol ratio or 6β-hydroxycortisol/cortisol ratio, albeit a modest impact on midazolam exposure was observed (Table 6). The magnitude of fold induction was the largest for the midazolam AUC changes, followed by the 6β-hydroxycortisol/cortisol ratio, and with plasma 4β-hydroxycholesterol/cholesterol ratio showing the minimal change (Table 6). Whereas both 4β-hydroxycholesterol/cholesterol ratio and 6β-hydroxycortisol/cortisol ratio performed similarly in detecting CYP3A4 induction, the 6β-hydroxycortisol/cortisol ratio in urine showed more inter-individual variability (Figure 3).

**Simcyp Simulation.** Simcyp was used to simulate the effect of co-administration of PF-06282999 on midazolam PK. Model simulations for PF-06282999 successfully captured the day 14 plasma-concentration time profiles from the part A (Figure 4). Incorporation of the most

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



sensitive estimate of rifampin calibrated mRNA CYP3A4 induction parameters for PF-06282999 (donor FOS) reasonably characterized the magnitude of CYP3A4 enzyme induction on the PK of midazolam (Figure 5). The Simcyp model predicted an AUCR of 0.62 (90% CI: 0.59-0.65), for midazolam with 500 mg BID PF-06282999, compared to the observed interaction (AUCR = 0.43) in part B (Table 7). At the lower dose of 125mg TID PF-06282999 the model predicted an AUCR 0.91 (90% CI: 0.90-0.92) for midazolam compared to the observed interaction (AUCR = 0.66) (Table 7).

**Discussion**

PF-06282999 is devoid of reversible and/or time-dependent inhibitory effects against major human CYP enzymes including CYP3A4, which largely mitigates the likelihood of clinical DDI's arising from CYP inhibition by PF-06282999 (Dong et al., 2016). Concentration-dependent increases in CYP3A4 mRNA and midazolam-1'-hydroxylase activity, however, were noted in human hepatocytes treated with PF-06282999 indicating that PF-06282999 is a CYP3A4 inducer in vitro. At the highest tested concentration of 300 μM, PF-06282999 caused maximal induction in CYP3A4 mRNA and enzyme activity ranging from 56% to 86% and 47% to 72%, respectively, of the positive control rifampicin response in the three individual hepatocyte lots. Consistent with previous reports (Fahmi et al., 2008a; 2008b), significant variability in the $EC_{50}$ (43–105 μM) and $E_{max}$ (12.7–24.5) values for induction of CYP3A4 mRNA was noted amongst the 3 different lots of human hepatocytes. Resistance of PF-06282999 towards metabolic turnover in human hepatocytes (Ruggeri et al., 2015; Dong et al., 2016) suggests that CYP3A4 induction is solely mediated by the parent compound with little to no contribution of metabolites. Clinical PK and safety studies in healthy adult volunteers revealed that PF-06282999 was safe and well tolerated after administration of multiple oral doses

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



(10 mg TID-500 mg BID) for 14 days. Systemic exposures as reflected by the $C_{max}$ and $AUC_{0-\tau}$ increased in a dose–proportional manner; maximum $C_{max}$ and $AUC_{0-\tau}$ values of 5.14 μg/ml and 35.6 h μg/ml respectively, were achieved at the top 500 mg BID dose group. The corresponding unbound $C_{max}$ (5.94 μM, PF-06282999 molecular weight = 325 and human plasma unbound fraction = 0.376) and $C_{avg}$ (1.71 μM) concentrations of PF-06282999 at the 500 mg BID dose were approximately 2.2–7.8–fold in excess over the $IC_{50}$ value (0.76 μM) for inhibition of MPO activity in human whole blood (Ruggeri et al., 2015), and this dose was potentially considered to be a therapeutic dose to test proof-of-pharmacology in phase 2 efficacy studies.

Because PF-06282999 induced CYP3A4 activity in human hepatocytes, measurement of plasma 4β-hydroxycholesterol/cholesterol and urinary 6β-hydroxycortisol/cortisol ratios was incorporated in the MAD study. Daily administration of PF-06282999 for 14 days led to dose-dependent increases in both plasma 4β-hydroxycholesterol/cholesterol ratio and 24-hour urinary 6β–hydroxycortisol/cortisol ratios. The urinary biomarker 6β–hydroxycortisol/cortisol ratio was more variable than the plasma 4β-hydroxycholesterol/cholesterol ratio, although in the present scenario, the 6β–hydroxycortisol/cortisol ratio appeared to be more sensitive in detecting mean fold induction upon repeated dosing with PF-06282999. High inter- and intrasubject variabilities in urinary 6β–hydroxycortisol/cortisol ratio, previously noted across several clinical studies in healthy volunteers, have been attributed to interindividual variability in CYP3A4 expression in human liver and/or variability in cortisol secretion into the circulation that is influenced by several factors such as stress, infections and circadian rhythm (Forrester et al., 1992; Galteau and Shamsa, 2003; Dutreix et al., 2014). Consistent with the dose-dependent increases in endogenous CYP3A4 biomarker ratios in the MAD study, mean midazolam $C_{max}$ and AUC following 14-day treatment with PF-06282999 decreased in a dose-dependent fashion with a

maximum decrease in midazolam $AUC_{0\text{-}inf}$ and $C_{max}$ of 57.2% and 41.1% observed at the 500 mg BID PF-06282999 dose group. The moderate impact on midazolam PK at the 500 mg BID dose of PF-06282999 was also reflected in statistically significant changes in plasma 4β-hydroxycholesterol/cholesterol and urinary 6β-hydroxycortisol/cortisol ratio. Interestingly, changes in plasma and urinary CYP3A4 biomarkers did not reach statistical significance at the 125 mg TID dose of PF-06282999, despite a modest impact on midazolam systemic exposure.

In retrospect, both plasma and urinary biomarkers performed relatively well in the MAD study as early indicators of the CYP3A4 induction potential of PF-06282999. However, the magnitude of CYP3A4 induction determined by oral midazolam systemic exposure was larger than the fold induction ascertained from plasma and urinary 4β-hydroxycholesterol/cholesterol and 6β-hydroxycortisol/cortisol ratios, respectively. Similar findings have also been recently reported in a clinical 14-day rifampin/midazolam DDI study (Björkhem-Bergman et al., 2013). Across several rifampin dose groups (10, 20 and 100 mg), the median fold induction from baseline was 2.0, 2.6, and 4.0 for the estimated midazolam clearance; 1.7, 2.9, and 3.1 for the 6β-hydroxycortisol/cortisol ratio; and 1.3, 1.6, and 2.5 for the 4β-cholesterol/cholesterol ratio. It is possible that the plasma 4β-hydroxycholesterol/cholesterol ratio is not at steady state in the 2-week MAD/DDI studies because of the slow turnover of 4β-hydroxycholesterol ($t_{1/2}$ = 17 days), and therefore does not fully reflect the induced CYP3A4 levels. In contrast, the 6β-hydroxycortisol/cortisol ratio reaches steady state relatively faster because of the shorter $t_{1/2}$ (~ 1 hour) of cortisol and its 6β-hydroxyl metabolite, with little delay or lag time for changes in CYP3A4 activity in vivo (Weitzman et al., 1971; Dutreix et al., 2014). In addition, oral midazolam pharmacokinetics takes into account intestinal and hepatic metabolism by CYP3A4. Therefore, the greater fold induction of midazolam AUC changes may be due to induction of

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



both intestinal and hepatic CYP3A4 activity by the perpetrator agents (e.g., PF-06282999 and/or rifampin). As such, simcyp simulation of the steady state pharmacokinetics of PF-06282999 at the 125 mg TID and 500 mg BID dose groups using the calibrated induction data predict approximately similar increases in active CYP3A4 enzyme of ~1.1- and 1.5-fold respectively, further strengthening the hypothesis around DDI arising from induction of both intestinal and liver CYP3A4 activity. However, in accordance with previous literature reports (Dresser et al., 2003; Imai et al., 2008; Davis et al., 2012), lack of significant changes in the terminal $t_{1/2}$ for midazolam in the 14-day DDI study with both dose groups of PF-06282999 suggests that PF-06282999 mainly induces intestinal CYP3A4. Additional clinical studies between PF-06282999 and midazolam (e.g., measurement of intravenous and oral AUC and/or clearance of midazolam in the presence of PF-06282999) will be required in order to delineate DDIs arising from CYP3A4 induction in the gut wall or the liver or both. Overall, the clinical studies conducted to investigate the potential of PF-06282999 as DDI perpetrator confirmed that this compound was a moderate inducer of CYP3A4 according to the Food and Drug Administration USA classification system (http://www.fda/gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf)

Simcyp, a population-based ADME simulator, has been successfully utilized by various laboratories to quantitatively predict metabolism-based DDIs (Almond et al., 2016; Xu et al., 2011; Fahmi et al., 2009). Relying on the in vitro CYP3A4 induction data, the Simcyp simulator can predict the extent of in vivo induction in a virtual population using a concentration-dependent dynamic induction model, as was recently demonstrated with rifampin, carbamazepine, and phenobarbital (Xu et al., 2011). Consequently, a retrospective analysis of

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



the clinical PF-06282999/midazolam DDI data was performed using Simcyp. The predicted steady-state PK of PF-06282999 (125 mg TID and 500 mg BID) and the PK ($C_{max}$ and AUC) of midazolam after a single 7.5 mg dose correlated reasonably well with the data obtained from our clinical study. Using PF-06282999 and midazolam kinetics and the in vitro induction parameters ($EC_{50}$ and $E_{max}$ for the three individual hepatocyte lots) for CYP3A4 mRNA increase, the induction DDIs were simulated. The predicted magnitudes of DDIs were shown to be in reasonable agreement particularly at the high PF-06282999 dose (500 mg BID). PF-06282999 exhibited dose- and dosing duration-dependent effects on the magnitude of midazolam victim DDI. Consistent with the conclusions noted in previous studies (Almond et al. 2016), Simcyp could be a useful tool for prospectively predicting clinical DDI risks for drug candidates that induce CYP3A4 enzyme levels.

Induction of expression of drug metabolizing enzymes (and hepatobiliary transport proteins) by small molecule xenobiotics, including clinical drugs, often occurs through activation of nuclear receptors, resulting in transcriptional upregulation of the receptor's target genes. Nuclear receptors commonly involved in regulation of CYP enzymes include the aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR). Activation of AhR results in upregulation of the CYP1A genes, while activation of PXR and/or CAR results in upregulation of CYP3A, CYP2B and CYP2C genes (Raucy et al., 2002; Raucy, 2003; Hewitt et al., 2007; Tirona and Kim, 2005; Sahi et al., 2009). Induction of CYP3A4 expression by xenobiotics (including drugs) primarily occurs via PXR activation, although there is overlap with CAR both in terms of ligand activators and target genes (Moore et al., 2000; Wilson and Kliewer, 2002; Wang et al., 2003; Wang et al., 2004; Faucette et al., 2006; Faucette et al., 2007). Induction of CYP3A4 expression by PF-06282999, which ultimately manifested in

a clinical DDI with midazolam, has been clearly demonstrated in this work; however, the propensity for DDI's resulting from the potential induction of CYP2B6, CYP2C9, and/or CYP2C19 enzymes by PF-06282999 remain to be examined. As such, PF-06282999 (2-300 μM) does not induce CYP1A2–mediated ethoxyresorufin-*O*-deethylase activity in human hepatocytes, suggesting that PF-06282999 does not activate AhR (Pfizer data on file). In vitro studies using PXR and CAR reporter assays (Luo et al., 2002) are currently in progress to ascertain whether the biochemical basis of CYP3A4 induction by PF-06282999 arise through activation of PXR and/or CAR. The knowledge gained from these ongoing studies can be potentially utilized to design the next generation of selective MPO inactivators that are devoid of the CYP3A4 induction liability in the clinic. Likewise, the clinical development plan for PF-06282999 has been modified to include additional in vitro studies in human hepatocytes to examine induction of CYP2C9 and/or CYP2C19 mRNA and activity levels, information that can be potentially used in designing appropriate clinical DDI studies with relevant isozyme-selective substrates of the CYP2C family.

Our studies clearly demonstrate the informative value of endogenous biomarker measurement in FIH studies to examine CYP3A4 induction in vivo; However, as the magnitude of the 4β-hydroxycholesterol or 6β-hydroxycortisol ratio change was generally smaller than the magnitude of an oral midazolam AUC change with PF-06282999, an oral midazolam DDI study was ultimately needed to gauge DDI via CYP3A4 induction, and is likely to be the case for weak to moderate CYP3A4 inducers or inducers that have a significant intestinal CYP3A4 induction component.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



## Authorship Contributions

*Participated in research design:* Jennifer Dong, Amit Kalgutkar, Odette Fahmi, Steven Terra, Vu Le, Kristin Chidsey, Albert Kim, and Leonard Buckbinder

*Conducted in vitro experiments:* Zhiwu Lin and Parya Nouri

*Contributed new reagents or analytic tools:* Parya Nouri

*Performed data analysis:* James Gosset, Zhiwu Lin, Odette Fahmi, Jeffery Chabot, Jennifer Dong, and Vu Le

*Wrote or contributed to the writing of the manuscript:* Amit Kalgutkar, Jennifer Dong, James Gosset, Jeffery Chabot, Steven Terra, and Odette Fahmi

## Declaration of interest

All authors are employees of, and/or hold stock in Pfizer Inc. when this work was carried out.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



## References

Almond LM, Mukadam S, Gardner I, Okialda K, Wong S, Hatley O, Tay S, Rowland-Yeo K, Jamei M, Rostami-Hodjegan A, and Kenny JR (2016) Prediction of drug-drug interactions arising from CYP3A induction using a physiologically based dynamic model. *Drug Metab Dispos* **44:**821–832.

Björkhem-Bergman L, Bäckström T, Nylén H, Rönquist-Nii Y, Bredberg E, Andersson TB, Bertilsson L, and Diczfalusy U (2013) Comparison of endogenous 4beta-hydroxycholesterol with midazolam as markers for CYP3A4 induction by rifampicin. *Drug Metab Dispos* **41:**1488–1493.

Bodin K, Bretillon L, Aden Y, Bertilsson L, Broomé U, Einarsson C, and Diczfalusy U (2001) Antiepileptic drugs increase plasma levels of 4beta-hydroxycholesterol in humans: evidence for involvement of cytochrome p450 3A4. *J Biol Chem* **276:**38685–38689.

Bu HZ (2006) A literature review of enzyme kinetic parameters for CYP3A4-mediated metabolic reactions of 113 drugs in human liver microsomes: structure-kinetics relationship assessment. *Curr Drug Metab* **7:**231–249.

Davis J, Langdon G, Layton G, Chong CL, Ndongo MN, and Vourvahis M (2012) The effect of lersivirine, a next-generation NNRTI, on the pharmacokinetics of midazolam and oral contraceptives in healthy subjects. *Eur J Clin Pharmacol* **68:**1567–1572.

Diczfalusy U, Kanebratt KP, Bredberg E, Andersson TB, Böttiger Y, and Bertilsson L (2009) 4beta-hydroxycholesterol as an endogenous marker for CYP3A4/5 activity. Stability and half-life of elimination after induction with rifampicin. *Br J Clin Pharmacol* **67:**38–43.

Diczfalusy U, Nylen H, Elander P, and Bertilsson L (2011) 4beta-Hydroxycholesterol, an endogenous marker of CYP3A4/5 activity in humans. *Br J Clin Pharmacol* **71:**183–189.

Dong JQ, Varma MV, Wolford A, Ryder T, Di L, Feng B, Terra SG, Sagawa K, and Kalgutkar AS (2016) Pharmacokinetics and Disposition of the Thiouracil Derivative PF-06282999, an Orally Bioavailable, Irreversible Inactivator of Myeloperoxidase Enzyme, Across Animals and Humans. *Drug Metab Dispos* **44:**209–219.

Dresser GK, Schwarz UI, Wilkinson GR, and Kim RB (2003) Coordinate induction of both cytochrome P4503A and MDR1 by St John's wort in healthy subjects. *Clin Pharmacol Ther* **73:**41–50.

Dutreix C, Lorenzo S, and Wang Y (2014) Comparison of two endogenous biomarkers of CYP3A4 activity in a drug-drug interaction study between midostaurin and rifampicin. *Eur J Clin Pharmacol* **70:**915–920.

Fahmi OA, Boldt S, Kish M, Obach RS, and Tremaine LM (2008a) Prediction of drug-drug interactions from in vitro induction data: application of the relative induction score approach using cryopreserved human hepatocytes. *Drug Metab Dispos* **36:**1971–1974.

Fahmi OA, Maurer TS, Kish M, Cardenas E, Boldt S, and Nettleton D (2008b) A combined model for predicting CYP3A4 clinical net drug-drug interaction based on CYP3A4 inhibition, inactivation, and induction determined in vitro. *Drug Metab Dispos* **36:**1698–1708.

Fahmi OA, Hurst S, Plowchalk D, Cook J, Guo F, Youdim K, Dickins M, Phipps A, Darekar A, Hyland R, and Obach RS (2009) Comparison of different algorithms for predicting clinical

drug-drug interactions, based on the use of CYP3A4 in vitro data: predictions of compounds as precipitants of interaction. *Drug Metab Dispos* **37:**1658–1666.

Forrester LM, Henderson CJ, Glancey MJ, Back DJ, Park BK, Ball SE, Kitteringham NR, McLaren AW, Miles JS, and Skett P (1992) Relative expression of cytochrome P450 isozymes in human liver and association with the metabolism of drugs and xenobiotics. *Biochem J* **281:**359-368.

Faucette SR, Sueyoshi T, Smith CM, Negishi M, LeCluyse EL, and Wang H (2006) Differential regulation of hepatic CYP2B6 and CYP3A4 genes by constitutive androstane receptor but not pregnane X receptor. *J Pharmacol Exp Ther* **317:**1200–1209.

Faucette SR, Zhang TC, Moore R, Sueyoshi T, Omiecinski CJ, LeCluyse EL, Negishi M, and Wang H (2007) Relative activation of human pregnane X receptor versus constitutive androstane receptor defines distinct classes of CYP2B6 and CYP3A4 inducers. *J Pharmacol Exp Ther* **320:**72–80.

Galteau MM and Shamsa F (2003) Urinary 6beta-hydroxycortisol: a validated test for evaluating drug induction or drug inhibition mediated through CYP3A in humans and in animals. *Eur J Clin Pharmacol* **59:**713–733.

Guengerich FP (2008) Cytochrome p450 and chemical toxicology. *Chem Res Toxicol* **21:**70–83.

Hewitt NJ, Lecluyse EL, and Ferguson SS (2007) Induction of hepatic cytochrome P450 enzymes: methods, mechanisms, recommendations, and in vitro-in vivo correlations. *Xenobiotica* **37:**1196–1224.

Imai H, Kotegawa T, Tsutsumi K, Morimoto T, Eshima N, Nakano S, and Ohashik K (2008) The recovery time-course of CYP3A after induction by St John's wort administration. *Br J Clin Pharmacol* **65:**701–707.

Isoherranen N, Lutz JD, Chung SP, Hachad H, Levy RH, and Ragueneau-Majlessi I (2012) Importance of multi-p450 inhibition in drug-drug interactions: evaluation of incidence, inhibition magnitude, and prediction from in vitro data. *Chem Res Toxicol* **25:**2285–2300.

Jones BC, Rollison H, Johansson S, Kanebratt KP, Lambert C, Vishwanathan K, and Andersson TB (2016) Managing the risk of CYP3A induction in drug development: a strategic approach. *Drug Metab Dispos* DOI:10.1124/dmd.116.072025.

Kalvass JC and Maurer T (2002) Influence of nonspecific brain and plasma binding on CNS exposure: implications for rational drug discovery. *Biopharm Drug Dispos* **23:**327–338.

Kirwan C, MacPhee I, and Philips B (2010) Using drug probes to monitor hepatic drug metabolism in critically ill patients: midazolam, a flawed but useful tool for clinical investigation of CYP3A activity? *Expert Opin Drug Metab Toxicol* **6:**761–771.

Konishi H, Tanaka K, Minouchi T, and Yamaji A (2004) Urinary 6beta-hydroxycortisol/17-hydroxycorticosteroids ratio as a measure of hepatic CYP3A4 capacity after enzyme induction. *Ann Clin Biochem* **41:**335–337.

Lin JH and Lu AY (1998) Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* **35:**361–390.

Liu YT, Hao HP, Liu CX, Wang GJ, and Xie HG (2007) Drugs as CYP3A probes, inducers, and inhibitors. *Drug Metab Rev* **39:**699–721.

Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, Hamilton G, Rizzo C, Jolley S, Gilbert D, Downey A, Mudra D, Graham R, Carroll K, Xie J, Madan A, Parkinson A, Christ D, Selling B, LeCluyse E, and Gan LS (2002) CYP3A4 induction by drugs: correlation between a pregnane X receptor gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab Dispos* **30:**795–804.

Mårde Arrhén Y, Nylén H, Lövgren-Sandblom A, Kanebratt KP, Wide K, and Diczfalusy U (2013) A comparison of 4beta-hydroxycholesterol : cholesterol and 6beta-hydroxycortisol : cortisol as markers of CYP3A4 induction. *Br J Clin Pharmacol* **75:**1536–1540.

Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, Goodwin B, Liddle C, Blanchard SG, Wilson TM, Collins JL, and Kliewer SA (2000) Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. *J Biol Chem* **275:**15122–15127.

Nelson DR (2006) Cytochrome P450 nomenclature, 2004. *Methods in molecular biology* **320:**1–10.

Neuvonen PJ, Niemi M, and Backman JT (2006) Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* **80:**565–581.

Niemi M, Backman JT, Fromm MF, Neuvonen PJ, and Kivisto KT (2003) Pharmacokinetic interactions with rifampicin : clinical relevance. *Clin Pharmacokinet* **42:**819–850.

Nowak SN, Edwards DJ, Clarke A, Anderson GD, and Jaber LA (2002) Pioglitazone: effect on CYP3A4 activity. *J Clin Pharmacol* **42:**1299–1302.

Raucy JL, Mueller L, Duan K, Allen SW, Strom S, and Lasker JM (2002) Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. *J Pharmacol Exp Ther* **302:**475 482.

Raucy JL (2003) Regulation of CYP3A4 expression in human hepatocytes by pharmaceuticals and natural products. *Drug Metab Dipos* **31:**533–539.

Ripp SL, Mills JB, Fahmi OA, Trevena KA, Liras JL, Maurer TS, and de Morais SM (2006) Use of immortalized human hepatocytes to predict the magnitude of clinical drug-drug interactions caused by CYP3A4 induction. *Drug Metab Dispos* **34:**1742–1748.

Ruggeri RB, Buckbinder L, Bagley SW, Carpino PA, Conn EL, Dowling MS, Fernando DP, Jiao W, Kung DW, Orr ST, Qi Y, Rocke BN, Smith A, Warmus JS, Zhang Y, Bowles D, Widlicka DW, Eng H, Ryder T, Sharma R, Wolford A, Okerberg C, Walters K, Maurer TS, Zhang Y, Bonin PD, Spath SN, Xing G, Hepworth D, Ahn K, and Kalgutkar AS (2015) Discovery of 2-(6-(5-chloro-2-methoxyphenyl)-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamide (PF-2999): A highly selective mechanism-based myeloperoxidase inhibitor for the treatment of cardiovascular diseases. *J Med Chem* **58:**8513–8528.

Sahi J, Shord SS, Lindley C, Ferguson S, and LeCluyse EL (2009) Regulation of cytochrome P450 2C9 expression in primary cultures of human hepatocytes. *J Biochem Mol Toxicol* **23:**43–58.

Sinz M, Wallace G, and Sahi J (2008) Current industrial practices in assessing CYP450 enzyme induction: preclinical and clinical. *AAPS J* **10:**391–400.

Tang Z and Guengerich FP (2010) Dansylation of unactivated alcohols for improved mass spectral sensitivity and application to analysis of cytochrome P450 oxidation products in tissue extracts. *Anal Chem* **82:**7706–7712.

Tirona RG and Kim RB (2005) Nuclear receptors and drug disposition gene regulation. *J Pharm Sci* **94:**1169–1186.

Walsky RL and Obach RS (2004) Validated assays for human cytochrome P450 activities. *Drug Metab Dispos* **32:**647–660.

Wang H, Faucette S., Sueyoshi T., Moore R., Ferguson S., Negishi M, and LeCluyse EL (2003) A novel distal enhancer module regulated by pregnane X receptor/constitutive androstane receptor is essential for the maximal induction of CYP2B6 gene expression. *J Biol Chem* **278:**14146–14152.

Wang H, Faucette S, Moore R, Sueyoshi T, Negishi M, and LeCluyse E (2004) Human constitutive androstane receptor mediates induction of CYP2B6 gene expression by phenytoin. *J Biol Chem* **279:**29295–29301.

Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, and Hellman L (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab* **33:**14-22.

Wide K, Larsson H, Bertilsson L, and Diczfalusy U (2008) Time course of the increase in 4beta-hydroxycholesterol concentration during carbamazepine treatment of paediatric patients with epilepsy. *Br J Clin Pharmacol* **65:**708–715.

Wienkers LC and Heath TG (2005) Predicting in vivo drug interactions from in vitro drug discovery data. *Nat Rev Drug Discov* **4:**825–833.

Wilson TM and Kliewer SA (2002) PXR, CAR and drug metabolism. *Nat Rev Drug Discov* **1:**259–266.

Xu Y, Zhou Y, Hayashi M, Shou M, and Skiles GL (2011) Simulation of clinical drug-drug interactions from hepatocyte CYP3A4 induction data and its potential utility in trial designs. *Drug Metab Dispos* **39:**1139–1148.

Zanger UM, Turpeinen M, Klein K, and Schwab M (2008) Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. *Anal Bioanal Chem* **392:**1093–1108.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



## Figure Legends

**Figure 1.** Fold induction in mRNA expression (A) and enzyme activity (B) of CYP3A4 (A) in cultured human hepatocyte cultures treated with PF-06282999 (0.3, 2, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300 μM) from three human donors ( HH1089, HC7-4, FOS). For purposes of comparison, increases in CYP3A mRNA and enzyme activity with rifampicin (10 μM) are also depicted. The chemical structure of PF-06282999 is also shown.

**Figure 2.** Dose-dependent fold-induction of urinary and plasma biomarkers of CYP3A4 induction, 4β-hydroxycholesterol/cholesterol ratio (4β-OHcholesterol Ratio) and 6β-hydroxycortisol/cortisol ratio (6β-OHcortisol Ratio), following daily administrations of placebo or PF-06282999 for 14 days.

The numbers at the top designate the median fold induction in each treatment group. Pairwise comparison was performed between each treatment group versus placebo using t-test.

**Figure 3.** Comparison of three different markers for CYP3A4 activities: 4β-hydroxycholesterol/cholesterol ratio (4β-OHcholesterol Ratio), 6β-hydroxycortisol/cortisol ratio (4β-OHcortisol Ratio), midazolam $AUC_{0\text{-}16h}$, and midazolam $C_{max}$ before and after 14 days of PF-06282999 administrations with graphical illustration of the treatment effect.

**Figure 4.** Simcyp-simulated versus observed plasma profile of PF-06282999 following 500 mg BID oral administration for 14 days.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Figure 5**. Simcyp-simulated versus observed plasma profiles following a single oral dose of 7.5 mg midazolam without and with 14 days co-administration of PF-06282999 500 mg BID (upper panel) and 125 mg TID (lower panel).

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



## Tables

TABLE 1

*PF-06282999 Simcyp model input parameters*

| **Input Parameters** | | | |
|---|---|---|---|
| Mol. wt (g/mol) | 325.77 | $F_a$ | 1.0 |
| cLog P | 0.49 | $k_a$ ($h^{-1}$) | 0.3 |
| $pK_a$ (Monoprotic base) | 7.82 | $Vd_{ss}$ (L/kg) | 0.45 |
| Blood/plasma ($R_{bp}$) | 0.94 | | |
| $f_{u,plasma}$ | 0.376 | $CL_{po}$ (L/hr) | 14 |
| $f_{u,gut}$ | equal to $f_{u,plasma}$ | $CL_{renal}$ (L/h) | 7.8 |
| $Q_{gut}$ (l /hour) | 5.0 | | |
| **CYP3A4** | | | |
| $k_{deg,CYP3A4}$ ($hour^{-1}$) | 0.0289 | | |

cLogP–calculated partition coefficient (Dong et al., 2016), $pK_a$–acid dissociation constant (Dong et al., 2016), $R_{bp}$–blood to plasma ratio (Dong et al., 2016), $f_{u,plasma}$–unbound fraction in plasma (Dong et al., 2016), $f_{u,gut}$–unbound fraction in gut, $Q_{gut}$–gut blood flow, $k_{deg,CYP3A4}$–degradation rate constant for CYP3A4, $F_a$–fraction absorbed , $k_a$–absorption rate constant (Dong et al., 2016), $Vd_{ss}$–steady-state volume of distribution (Dong et al., 2016), $CL_{po}$–oral clearance (Dong et al., 2016), $CL_{renal}$–renal clearance (Dong et al., 2016).

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



TABLE 2

*$EC_{50}$, $E_{max}$, and γ (Hill slope) (± standard error (SE)[a]) for induction of CYP3A4 mRNA and midazolam-1'-hydroxylase activity by PF-06282999 and Rifampin*

| PF-06282999 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | CYP3A4 mRNA[b] | | | CYP3A4 Activity[b] | | | |
| Donor | $EC_{50}$ ± SE (μM) | $E_{max}$ ± SE | γ ± SE[d] | $EC_{50}$ ± SE (μM) | $E_{max}$ ± SE | γ ± SE[d] | *n*[c] |
| HH1089 | 105 ± 11 | 24.5 ± 1.27 | 1.25 ± 0.07 | 92.1 ± 25.6 | 3.76 ± 0.34 | 1.0 | 3 |
| HC7-4 | 56 ± 4.5 | 27.1 ± 1.28 | 2.20 ± 0.31 | 62.3 ± 6.2 | 3.70 ± 0.14 | 1.6 ± 0.2 | 3 |
| FOS | 43 ± 2.9 | 12.7 ± 0.45 | 1.99 ± 0.24 | 75.5 ± 11.5 | 4.64 ± 0.23 | 1.0 | 3 |

| Rifampin | | | | | | | |
|---|---|---|---|---|---|---|---|
| | CYP3A4 mRNA[b] | | | CYP3A4 Activity[b] | | | |
| Donor | $EC_{50}$ Geo. Mean (μM) | $EC_{50}$ Range[e] (μM) | $E_{max}$ ± SE | $EC_{50}$ Geo. Mean (μM) | $EC_{50}$ Range (μM) | $E_{max}$ ± SE | *n*[c] |
| HH1089 | 0.60 | 0.53 – 0.68 | 45.2 ± 16.7 | 0.57 | 0.30 – 1.09 | 6.1 ± 0.9 | 2 |
| HC7-4 | 1.0 | 0.82 – 1.22 | 53.6 ± 10.4 | 0.57 | 0.28 – 1.13 | 11.1 ± 1.8 | 4 |
| FOS | 1.16 | 0.54 – 2.47 | 37.1 ± 10.2 | 0.36 | 0.22 – 0.60 | 5.0 ± 1.2 | 3 |

[a]SE for PF-06282999 parameters reflects fit parameter uncertainty; SE for rifampin parameters determined by variability in fit parameters obtained for each independent run. [b] fold induction in CYP3A4 mRNA or enzyme activity (midazolam hydroxylation) expressed as pmol/min/$10^6$ cells. [c]n = number of replicates (for PF-06282999) or number of independent runs (for

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



rifampin). [d] where tabulated $\gamma \neq 1.0$, CYP3A4 mRNA and activity data was fit using a four-parameter sigmoidal model; otherwise data was fit using a three-parameter sigmoidal model. [e] $EC_{50}$ range for rifampin reflects ± 1 geometric standard error.

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



TABLE 3

*Simcyp calibrated in vitro CYP3A4 induction parameters for PF-06282999*

| | PF-06282999 | | |
|---|---|---|---|
| | CYP3A4 mRNA | | |
| Donor | $Ind_{max}$ (μM) | $Ind_{C50}$ (μM) | γ |
| HH1089 | 8.98 | 56.0 | 1.25 |
| HC7-4 | 8.44 | 17.9 | 2.20 |
| FOS | 5.86 | 12.0 | 1.99 |

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



TABLE 4

*Summary of PF-06282999 pharmacokinetic parameter following daily PF-06282999 administrations for 14 days*

| Parameter | 10 mg TID | 30 mg TID | 100 mg TID | 250 mg TID | 500 mg BID |
|---|---|---|---|---|---|
| *N* | 8 | 7 | 8 | 8 | 8 |
| $AUC_{0-\tau}$, hours µg/ml | | | | | |
| Geometric mean | 1.05 | 3.37 | 8.26 | 19.7 | 35.6 |
| % CV | 23 | 19 | 10 | 13 | 6 |
| $C_{max}$ , µg/ml | | | | | |
| Geometric mean | 0.198 | 0.604 | 1.52 | 3.54 | 5.14 |
| % CV | 22 | 18 | 17 | 15 | 5 |
| $t_{max}$, hours | | | | | |
| Median | 2.02 | 2.00 | 3.00 | 2.00 | 3.01 |
| Range | 2.00-3.00 | 1.00-3.98 | 2.00-4.02 | 2.00-4.00 | 3.00-4.00 |
| $C_{av}$, µg/ml | | | | | |
| Geometric mean | 0.131 | 0.422 | 1.03 | 2.47 | 2.97 |
| % CV | 23 | 19 | 10 | 13 | 6 |
| $C_{min}$, µg/ml | | | | | |
| Geometric mean | 0.0714 | 0.231 | 0.584 | 1.36 | 1.15 |
| % CV | 29 | 27 | 11 | 22 | 15 |
| $t_{1/2}$, hours | | | | | |
| Arithmetic mean | 4.43 | 4.90 | 4.75 | 7.35 | 6.08 |
| SD | 0.88 | 0.62 | 0.20 | 3.21 | 1.14 |
| $R_{ac,AUC}$ | | | | | |
| Geometric mean | 1.62 | 1.77 | 1.46 | 1.52 | 1.15 |
| % CV | 9 | 18 | 12 | 11 | 5 |
| $R_{ac,Cmax}$ | | | | | |
| Geometric mean | 1.62 | 1.56 | 1.33 | 1.36 | 1.07 |
| % CV | 11 | 16 | 8 | 15 | 7 |

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



$AUC_{0-\tau}$ area under the plasma concentration-time curve over a dosing interval τ, BID twice daily, $C_{av}$ average concentration, $C_{max}$ peak concentration, $C_{min}$ trough concentration, CV coefficient of variation, $R_{ac,AUC}$ accumulation ratio over 14 days for AUC, $R_{ac,Cmax}$ accumulation ratio over 14 days for $C_{max}$, SD standard deviation, $t_{1/2}$ half-life, $t_{max}$ time to peak concentration, TID three times daily, τ the dosing interval is 12 hours for BID dosing and 8 hours for TID dosing.

TABLE 5

*Statistical summary of pharmacokinetic parameters after an oral dose of 7.5 mg midazolam without and with co-administration of PF-06282999*

| Midazolam parameter | Interaction with 125 mg TID PF-06282999[a] | | | Interaction with 500 mg BID PF-06282999[a] | | |
|---|---|---|---|---|---|---|
| | With PF-06282999 (Test) | Without PF-06282999 (Reference) | Ratio of Test/Reference (90% CI)[b] | With PF-06282999 (Test) | Without PF-06282999 (Reference) | Ratio of Test/Reference (90% CI)[b] |
| $AUC_{0\text{-}inf}$, hour ng/ml | | | | | | |
| Adjusted geometric mean | 72.3 | 110 | 66.0 (59.0, 73.8) | 45.4 | 106 | 42.9 (38.3, 47.9) |
| $AUC_{0\text{-}16hours}$, hour ng/ml | | | | | | |
| Adjusted geometric mean | 70.0 | 104 | 67.4 (60.5, 75.2) | 44.6 | 102 | 43.7 (39.2, 48.7) |
| $C_{max}$, ng/ml | | | | | | |
| Adjusted geometric mean | 29.8 | 43.1 | 69.2 (54.0, 88.6) | 22.1 | 37.5 | 58.9 (46.0, 75.4) |

$AUC_{0\text{-}inf}$ area under the plasma concentration-time curve from zero to infinity, $AUC_{0\text{-}16hours}$ area under the plasma concentration-time curve from zero to 16 hours, BID twice daily, $C_{max}$ peak concentration, CI confidence interval, TID three times daily.

[a] n= 9 subjects per PF-06282999 dose.

[b] The ratios (and 90% CI's) are expressed as percentages.

TABLE 6

*Comparison of three different markers for CYP3A4 activities: 4β-hydroxycholesterol/cholesterol ratio (4β-OHcholesterol Ratio), 6β-hydroxycortisol/cortisol ratio (6β-OHcortisol Ratio), midazolam $AUC_{0\text{-}16hours}$, and midazolam $C_{max}$ before and after 14 days of PF-06282999 administrations with statistical analysis of the treatment effect*

| Markers for CYP3A4 | 125 mg BID PF-06282999[a] | | 500 mg BID PF-06282999[a] | |
|---|---|---|---|---|
| | Ratio to baseline[b] (90% CI) | p-value[c] | Ratio to baseline[b] (90% CI) | p-value[c] |
| 4β-OHcholesterol Ratio | 1.03 (0.95, 1.12) | 0.4657 | 1.14 (1.06, 1.24) | 0.0145 |
| 6β-OHcortisol Ratio | 1.10 (0.88, 1.39) | 0.4497 | 1.53 (1.35, 1.73) | 0.0002 |
| Midazolam $AUC_{0\text{-}16hours}$ | 0.67 (0.63, 0.73) | <0.0001 | 0.44 (0.38, 0.51) | <0.0001 |
| Midazolam $C_{max}$ | 0.69 (0.55, 0.87) | 0.0158 | 0.59 (0.44, 0.79) | 0.0107 |

[a] n= 9 subjects per PF-06282999 dose.
[b] Values have been back-transformed from the log scale.
[c] p-values from *t*-test.

TABLE 7

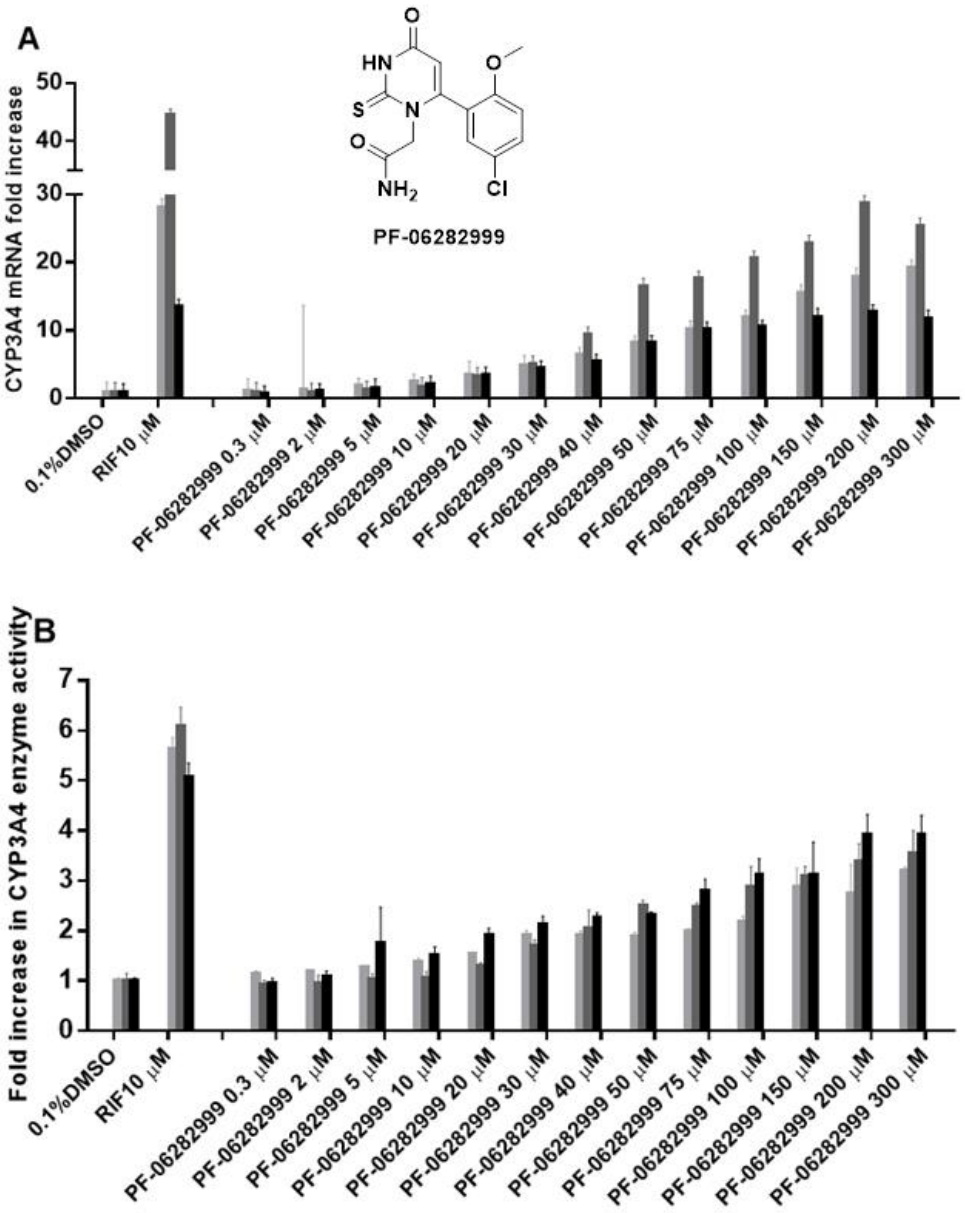
*Summary of predicted vs observed DDI using Simcyp*

| Perpetrator drug | Victim drug | Predicted DDI AUCR Geometric Mean (90% CI) | Observed DDI AUCR |
|---|---|---|---|
| PF-6282999 500 mg BID | Midazolam 7.5 mg | 0.62 (0.59-0.65) | 0.43 (0.38, 0.48) |
| PF-6282999 125 mg TID | | 0.91 (0.90–0.92) | 0.66 (0.59, 0.74) |

DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



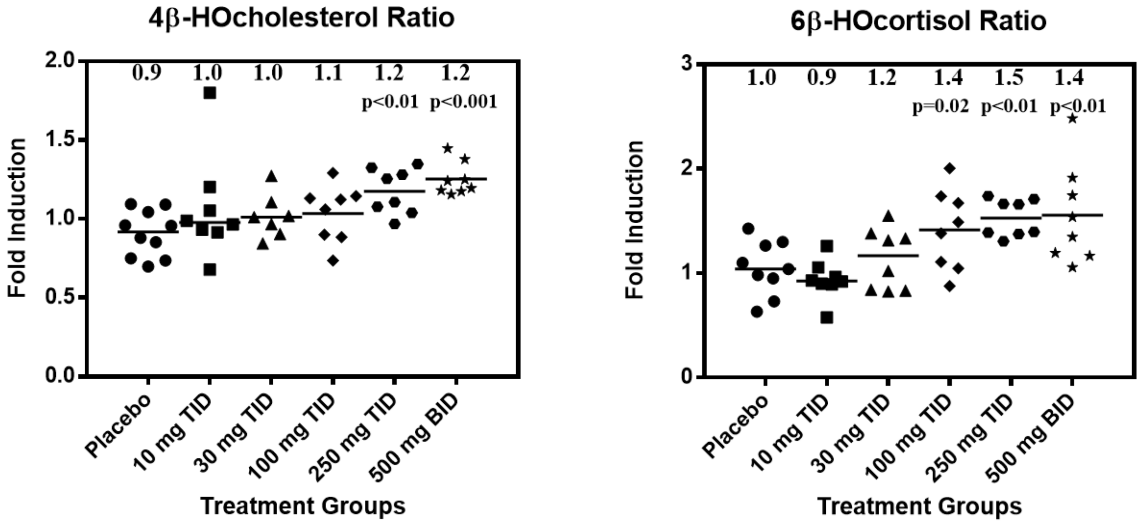
**Figure 1**



DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



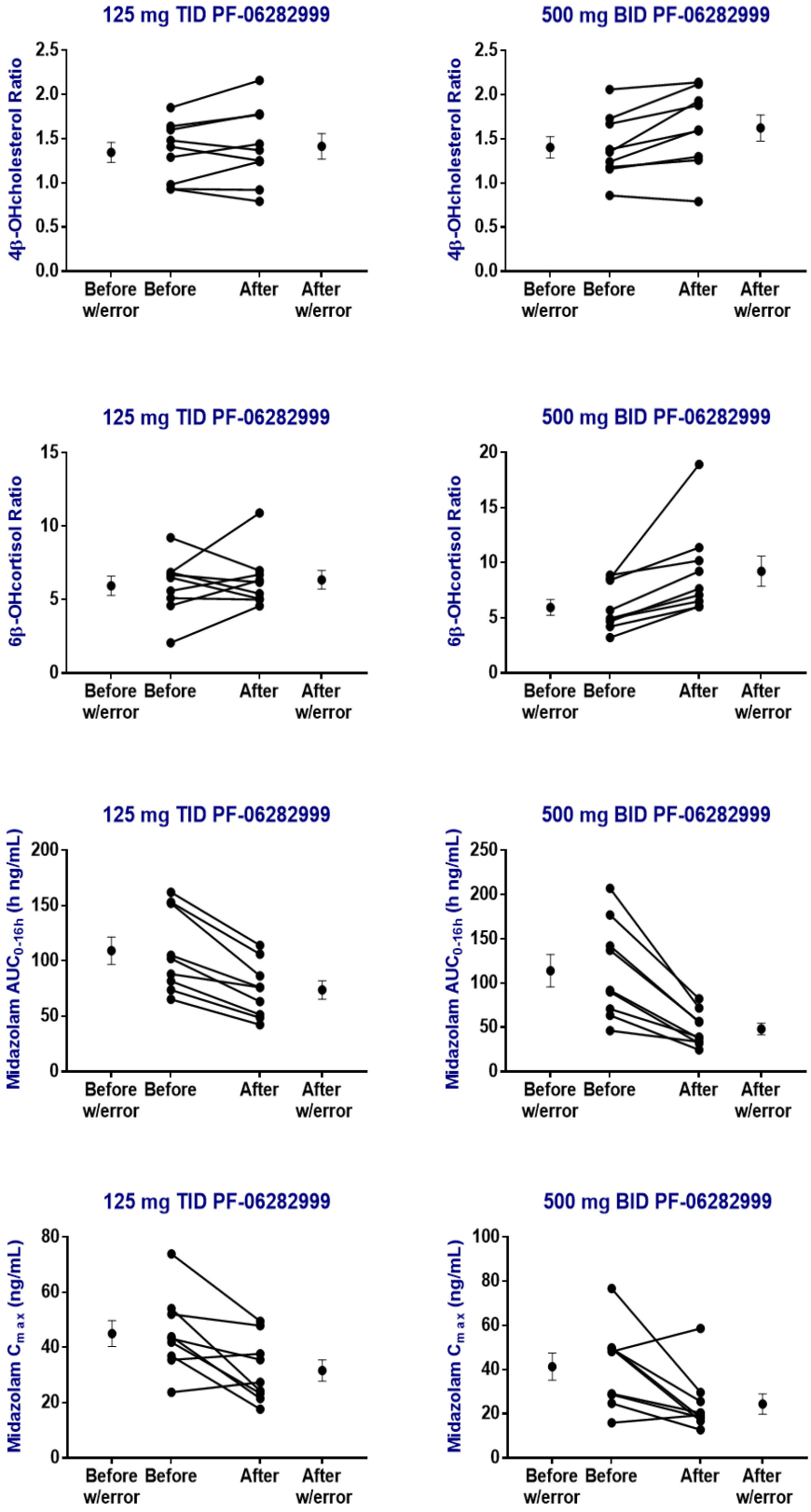
**Figure 2**



DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



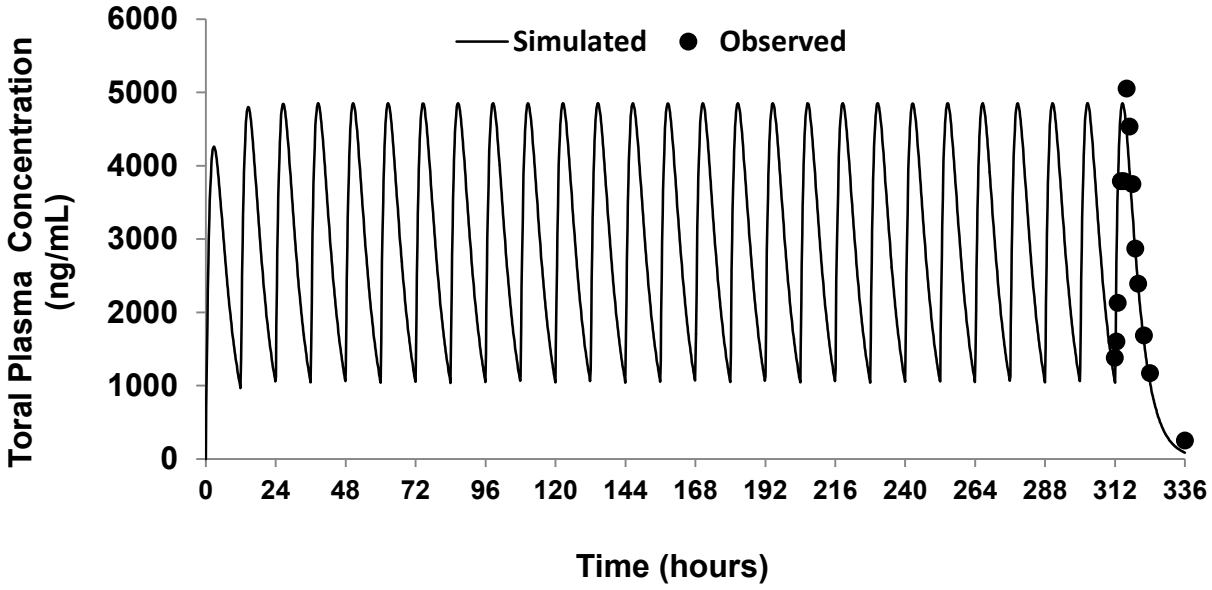
**Figure 3**



DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Figure 4**



DMD Fast Forward. Published on March 2, 2017 as DOI: 10.1124/dmd.116.074476
This article has not been copyedited and formatted. The final version may differ from this version.



**Figure 5**