

ARTICLE

Investigation of CYP3A induction by PF-05251749 in early clinical development: comparison of linear slope physiologically based pharmacokinetic prediction and biomarker response

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Abstract

PF-05251749 is a dual inhibitor of casein kinase 1 δ/ϵ under clinical development to treat disruption of circadian rhythm in Alzheimer's and Parkinson's diseases. In vitro, PF-05251749 (0.3–100 μM) induced CYP3A in cryopreserved human hepatocytes, demonstrating non-saturable, dose-dependent CYP3A mRNA increases, with induction slopes in the range 0.036–0.39 μM^{-1} . In a multiple-dose study (B8001002) in healthy participants, CYP3A activity was explored by measuring changes in 4 β -hydroxycholesterol/cholesterol ratio. Following repeated oral administration of PF-05251749, up to 400 mg q.d., no significant changes were observed in 4 β -hydroxycholesterol/cholesterol ratio; this ratio increased significantly (~1.5-fold) following administration of PF-05251749 at 750 mg q.d., suggesting potential CYP3A induction at this dose. Physiologically based pharmacokinetic (PBPK) models were developed to characterize the observed clinical pharmacokinetics (PK) of PF-05251749 at 400 and 750 mg q.d.; the PBPK induction model was calibrated using the in vitro linear fit induction slope, with rifampin as reference compound ($\text{Ind}_{\text{max}} = 8$, $\text{EC}_{50} = 0.32 \mu\text{M}$). Clinical trial simulation following co-administration of PF-05251749, 400 mg q.d. with oral midazolam 2 mg, predicted no significant drug interaction risk. PBPK model predicted weak drug interaction following co-administration of PF-05251749, 750 mg q.d. with midazolam 2 mg. In conclusion, good agreement was obtained between CYP3A drug interaction risk predicted using linear-slope PBPK model and exploratory biomarker trends. This agreement between two orthogonal approaches enabled assessment of drug interaction risks of PF-05251749 in early clinical development, in the absence of a clinical drug–drug interaction study.

Clinical trial number: NCT02691702.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Physiologically based pharmacokinetic (PBPK) predictions are commonly applied in clinical development to predict drug interaction risks associated with CYP3A inducers. Changes in 4 β -hydroxycholesterol levels are explored to assess the clinical relevance of CYP3A induction in humans.

WHAT QUESTION DID THIS STUDY ADDRESS?

PF-05251749 was identified as a potential CYP3A inducer in vitro. The objective of this analysis was to investigate clinical drug–drug interaction (DDI) risk associated with PF-05251749 during early clinical development, using PBPK and biomarker outcomes.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study exemplifies the assessment of drug interaction risks in early clinical development, using changes in 4 β -hydroxycholesterol/cholesterol ratio as preliminary evidence of CYP3A activity, leading to investigation using a PBPK model-informed approach. This PBPK model was developed using in vitro CYP3A mRNA induction linear-fit-slope. This investigation shows good concordance of linear-fit-slope-calibrated PBPK predictions with observed trends in hepatic CYP3A biomarker changes.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Measuring changes in 4 β -hydroxycholesterol/cholesterol ratio should be considered for potential CYP3A inducers during phase I multiple-dose studies. Assessment of DDI risks during early development should consider totality of data including in vitro, in vivo/biomarker, and PBPK approaches.

INTRODUCTION

In mammals, circadian processes are regulated by transcriptional feedback loops of clock proteins and transcription factors BMAL1, CLOCK, PER1/2, and CRY.¹ Mutations, dysregulations, as well as post-translational processes like phosphorylation of these clock components can result in alterations in sleep cycles and resting–active phases. In vitro, *PER2* gene has been suggested as a potential phosphorylation site by the casein kinase CK1 ϵ . Behavioral arrhythmic patterns generated by mutation of clock proteins or by disruption of light/dark cycles in mice were shown to be restored by daily treatment with a CK1 inhibitor, PF-0670462,² suggesting therapeutic avenues for dysregulated circadian patterns and sleep disorders in Alzheimer's and Parkinson's diseases.

PF-05251749 (Figure S1) was identified as a potent and selective small-molecule, brain-penetrant CK1 inhibitor. In non-clinical studies (internal data), PF-05251749 had oral bioavailability of 46–72% in rat and 44% in dog, and volume of distribution 1.28 L/kg in rat and 1.87 L/kg in dog. Incubation of unlabeled PF-05251749 in human liver microsomes using chemical inhibitors and recombinant human CYP isoforms suggested metabolism

by CYPs 1A2 (34%), 3A4/5 (26%), 2B6 (22%), and 2C19 (18%). In vitro, PF-05251749 was identified as an inducer of CYP3A in cryopreserved human hepatocytes. The clinical relevance of CYP3A inducers has been studied^{3,4} and may lead to clinical drug interactions resulting in (1) increased oral clearance,⁵ (2) increased concentrations of metabolite(s)⁶ leading to safety considerations,⁷ or (3) dose adjustments to minimize any loss of efficacy of concomitantly administered CYP3A substrates.^{8,9} Therefore, drug interaction risks due to CYP3A induction are assessed in drug development, to understand its impact on dose optimization while designing proof-of-concept studies. Designing drug interaction studies during early development could be challenging due to uncertainties associated with pharmacokinetics, knowledge of efficacious dose range, and safety monitoring. The primary objective of the current analysis was to evaluate the CYP3A drug interaction risks of PF-05251749 in early clinical development using in vitro, in vivo biomarker changes, and physiologically based pharmacokinetic (PBPK) outputs. PBPK model predictions were compared with trends in endogenous CYP3A biomarker, measured in the multiple-dose study of PF-05251749 in healthy human participants.

METHODS

PF-05251749 was synthesized at Pfizer (Groton, CT). All solvents and buffers were of HPLC grade and were procured from Sigma-Aldrich (St. Louis, MO). The clinical study was conducted in compliance with the ethical principles deriving from the Declaration of Helsinki and International Council on Harmonisation Good Clinical Practice Guidelines and the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002). Approval from a local institutional review board (IRB) (IntegReview IRB, Austin, TX) was obtained prior to the start of the study, in the interest of greater protection and safety for the study participants.

In vitro CYP3A induction following incubation of PF-05251749 in cryopreserved human hepatocytes

CYP3A induction by PF-05251749 was examined in cryopreserved human hepatocytes from three different donors (HH1025, HC7-4, and FOS)¹⁰ by measuring changes in CYP3A mRNA and enzyme activity. Briefly, primary cultures were seeded (7.0×10^5 viable hepatocytes per ml) on collagen I-pre-coated plates with 0.25 ml per well for 48-well plates and 0.10 ml per well for 96-well plates. Dosing solutions containing solvent (0.1% DMSO) control, PF-05251749 (0.3, 1, 3, 10, 20, 30, 50, and 100 μ M), or prototypical CYP3A inducer rifampin (10 μ M) were changed every 24 h for 2 days (48 h total treatment). Cultures were maintained at 37°C under 5% CO₂ and 95% humidity. After 2 days of cell treatment, the media containing controls and test article were removed and the cells were rinsed three times with phosphate-buffered saline (PBS).¹¹ Following removal of the wash media, midazolam (30 μ M) in 0.1% DMSO was added to each well and the plate was returned to the incubator for 30 min. After incubation, 100 μ l of medium from each well was removed and analyzed for formation of 1-hydroxymidazolam using established liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods. Designated CYP enzyme and endogenous probe (glyceraldehyde-3-phosphate dehydrogenase) mRNA levels were quantified by real-time polymerase chain reaction (PCR) using TaqMan[®] assays, and relative quantitation of CYP3A4 mRNA was performed using the $\Delta\Delta$ CT method. A cytotoxicity assay using MTS cell proliferation reagent (Promega, Madison, WI) and visual inspection of cell cultures by microscopy showed no apparent reduction in hepatocyte viability after treatment with PF-05251749 at the tested concentrations.

Plasma PK following oral administration of repeated doses of PF-05251749 in healthy adult participants

The plasma PK following repeated doses of PF-05251749 were investigated in study B8001002 (NCT02691702). This was a randomized, double-blinded, sequential, placebo-controlled, multiple-dose trial in healthy male or female subjects of non-childbearing potential who, at the time of screening, were between the ages of 18 and 55 years inclusive. (This clinical study was designed in three Parts: Part A, Part B, and an optional Part C. CYP3A induction biomarker change was explored in Part A only; Part B and Part C are not described in this article.) Part A was designed in five cohorts, with 12 subjects/cohort randomized to PF-05251749/placebo/melatonin in a 4:1:1 ratio (melatonin was introduced in the study as a positive control for pharmacodynamic exploration and is included for completeness only). Subjects confirming eligibility were admitted to the clinical research unit on day –1. All doses were administered in an inpatient setting, to minimize risks to participants. Based on review of cumulative safety and PK of prior dose levels and protocol prespecified rules, subjects enrolled in subsequent cohorts of Part A were administered PF-05251749 doses of 100, 200, 400, and 750 mg q.d. in a sequential design. All doses in Part A were administered under fasted state, for 14 consecutive days. Blood samples (3 ml) to provide a minimum of 1 ml plasma for PK analysis were collected in K₂EDTA tubes on days 1 and 14 at pre-dose, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16, and 24 h post-dose and pre-dose on days 3, 4, 7, 8, and 10. After centrifugation, plasma samples were stored at approximately –20°C until analysis. Subjects were discharged on day 16 and planned for a follow-up on day 28, post last dose of drug product administration.

Bioanalysis of PF-05251749 in human plasma using LC–MS/MS

PF-05251749 samples were assayed using a validated high-performance liquid chromatography tandem mass spectrometric (HPLC–MS/MS) method. Samples (50.0 μ l) were extracted using liquid–liquid extraction (ethyl acetate) and extracts were evaporated to dryness followed by reconstitution in 500 μ l of acetonitrile:water (1:1 v/v). Reconstituted extracts were centrifuged, and 10 μ l of the supernatant was introduced for chromatographic separation on Phenomenex Kinetex C8, 2.6 μ m, 30 \times 2.1 mm, followed by precursor ion monitoring method (310.1 \rightarrow 211.0) in the positive ion mode of ionization. PF-06810368 (314.1 \rightarrow 211.3) was used

as the internal standard for this method. The between-day assay accuracy, expressed as percent relative error (%RE), for quality control (QC) concentrations, ranged from -4.7 to -1.1% . Calibration standard responses were linear over the range of 1.00 – 1000 ng/ml using weighted ($L/\text{concentration}^2$) linear least squares regression. Samples with concentrations above the upper limits of quantification were adequately diluted into calibration range. The lower limit of quantification (LLOQ) for PF-05251749 was 1.00 ng/ml. Clinical specimens with plasma PF-05251749 concentrations below the LLOQ were reported as below the LLOQ (<1.00 ng/ml).

Bioanalysis of plasma 4 β -hydroxycholesterol and cholesterol in healthy subjects

Sample analysis for measurements of 4 β -hydroxycholesterol and cholesterol were performed at Q Squared Solutions (Ithaca, NY). Briefly, blood samples (3 ml) were collected at pre-dose on days 1 and 14. The samples were collected in lithium heparin tubes and centrifuged at approximately 1200 g for approximately 15 min at 4°C . The plasma was transferred and equally divided into two screw-capped polypropylene tubes containing 5 μl butylated hydroxytoluene (BHT) in ethanol (22.69 mM or 5 mg/ml). Each tube was filled with approximately 0.5 ml of plasma and stored at -70°C within 1 hour of collection. The lithium heparin plasma containing BHT (50 $\mu\text{g}/\text{ml}$) was measured for 4 β -hydroxycholesterol and cholesterol using reverse-phase liquid chromatography with tandem mass spectrometric detection. For analysis of 4 β -hydroxycholesterol, 80 μl of sample was saponified via base hydrolysis followed by liquid–liquid extraction. Plasma samples were quantified by a standard curve prepared in 5% bovine serum albumin (BSA) in 10 mM PBS treated with 50 $\mu\text{g}/\text{ml}$ BHT. Sample extracts were analyzed by HPLC–MS/MS in the positive ion mode. The LLOQ was 3.00 ng/ml with an overall assay precision of $\leq 14.6\%$ and %RE from -9.9 to -3.9% . For the measurement of total cholesterol, 10 μl of human plasma were saponified followed by liquid–liquid extraction and subsequent derivatization with picolinic acid. Plasma samples were quantified by a standard curve prepared in 5% BSA in 10 mM PBS treated with 50 $\mu\text{g}/\text{mL}$ BHT. Sample extracts were analyzed by LC–MS/MS in the positive ion mode. The LLOQ was 500 $\mu\text{g}/\text{mL}$ with an overall assay precision of $\leq 6.4\%$ and %RE from -3.3 to -2.7% . Data were normalized by use of stable-labeled internal standards – [$^2\text{H}7$]-4 β -hydroxycholesterol and [$^2\text{H}7$]-cholesterol for 4 β -hydroxycholesterol and cholesterol, respectively.

Development of PBPK models of PF-05251749

The population-based PBPK simulator SimCYP® (version 18; Certara, NJ) was used to predict the interaction of PF-05251749 with midazolam. Base models of PF-05251749 at 400 and 750 mg q.d. were developed using drug-specific input parameters including molecular weight, passive permeability, pK_a , log P, free fraction in human plasma, and human blood-to-plasma partitioning. The in vitro Ralph Russ canine kidney (RRCK)-measured permeability of PF-05251749 was 31.6×10^{-6} cm/s. By applying a permeability scalar of 2.5 (with midazolam as the reference compound), SimCYP-predicted P_{eff} value of 10.0×10^{-4} cm/s were applied. To adequately characterize the observed time to maximum plasma drug concentration (T_{max}) of PF-05251749, a sensitivity analysis of first-order absorption rate constant (K_a) was performed. The observed plasma concentration–time profile was fitted to a two-compartment model, using a PBPK model platform, and estimates of apparent clearance (CL_{po}), steady-state volume of distribution (V_{ss}), volume of distribution of single adjusted compartment (V_{sac}), and inter-compartmental clearance (Q) were obtained.

Next, the base models of PF-05251749 were calibrated using CYP3A induction slopes estimated from each of the three lots of cryopreserved hepatocytes, normalized using rifampin as a reference calibrator (rifampin $\text{Ind}_{\text{max}} = 8$). Observed (linear fit) and calibrated induction slopes of PF-05251749 estimated for each of the three donor lots are summarized in Table S1. The most potent calibrated slope ($0.18 \mu\text{M}^{-1}$) was used to predict the interaction of PF-05251749 with the sensitive CYP3A substrate, midazolam.

CYP3A drug interaction trial simulations of PF-05251749

All clinical trial simulations in SimCYP were performed in a virtual population of healthy population (10 trials of 10 individuals age 20–50 years, 50% female, fasted conditions). The output sampling interval in the SimCYP simulation toolbox was set to 0.2 h in all simulations. The magnitude of the CYP3A induction of PF-05251749 in healthy adult participants was assessed by maximum plasma drug concentration (C_{max}) and area under the plasma drug concentration–time curve during dosing interval (AUC_{inf}) following single oral dose of midazolam (2 mg), administered alone or co-administered with PF-05251749 at doses of 400 or 750 mg q.d.

DATA ANALYSES

In vitro induction slope assessment

The potency of induction of PF-05251749 was determined in vitro by the incubation of PF-05251749 at 0.3–100 μM in cryopreserved human hepatocytes. The observed fold induction in CYP3A mRNA was fitted to a sigmoid (three- or four-parameter) model and a linear model, using Equations (1) and (2), respectively, in GraphPad Prism (La Jolla, CA),

$$\text{Fold induction} = 1 + \frac{(\text{Ind}_{\text{max}} - 1) \times C^\gamma}{C^\gamma + \text{EC}_{50}^\gamma} \quad (1)$$

$$\text{Fold induction} = 1 + \text{Slope} \times C \quad (2)$$

where C is the substrate concentration of PF-05251749, Ind_{max} is the maximum fold induction ($\text{Ind}_{\text{max}} = E_{\text{max}} + 1$), EC_{50} is the concentration of inducer that produced half E_{max} , and γ is the Hill coefficient. With the non-linear fit, the Hill coefficient with variable γ (four-parameter fit) was compared to a Hill coefficient with γ fixed at 1 (three-parameter fit) using the built-in extra sum-of-squares F test. The three-parameter fit was preferred unless the p value was <0.05 . In both cases, the baseline was set to 1 (vehicle induction). If neither fits converged or if the three-parameter fit was preferred, an additional comparison was made between the three-parameter and linear fit (with y -intercept fixed at 1). The linear fit was preferred unless the p value from the extra-sum-of-squares test was <0.05 .

Clinical PK of PF-05251749

Plasma PK parameters following oral administration of PF-05251749 were estimated from the observed plasma concentration–time data, using non-compartmental analyses, as per Pfizer internal SOPs and as data permitted. The PK parameters, maximum plasma drug concentration (C_{max}), area under the plasma drug concentration–time curve during dosing interval (AUC_{tau}), apparent oral clearance (CL/F), apparent volume of distribution (V_z/F), and plasma elimination half-life ($t_{1/2}$) were determined using non-compartmental analysis. Concentrations below the limit of quantitation were recorded as 0 ng/ml. The observed accumulation ratio R_{ac} , C_{max} and R_{ac} , AUC_{tau} were estimated as day 14 C_{max} /day 1 C_{max} and day 14 AUC_{tau} /day 1 AUC_{tau} , respectively. The observed dose-normalized C_{max} ($C_{\text{max}} [\text{dn}]$) and dose-normalized AUC_{tau} ($\text{AUC}_{\text{tau}} [\text{dn}]$) were estimated as $C_{\text{max}}/\text{dose}$ and $\text{AUC}_{\text{tau}}/\text{dose}$, respectively.

Statistical analyses of changes in plasma 4 β -hydroxycholesterol/cholesterol ratio following administration of repeated doses of PF-05251749

Plasma concentrations of cholesterol and 4 β -hydroxycholesterol, from individual subjects administered PF-05251749/placebo/melatonin, were measured using a validated bioanalytical method. The plasma 4 β -hydroxycholesterol/cholesterol was computed in each individual subject and summarized by treatment and by day. Fold change in 4 β -hydroxycholesterol/cholesterol pre-dose on day 14 relative to day 1 was determined for each individual subject. Statistical significance of the median fold change in 4 β -hydroxycholesterol/cholesterol relative to placebo was estimated in SAS version 9.4.

PBPK model evaluation and drug interaction trial simulations

PK models for PF-05251749 were developed at 400 and 750 mg q.d. using the parameter estimation function in SimCYP. Models of PF-05251749 were evaluated by goodness-of-fit between observed versus predicted time–concentration profiles on day 14. Observed/predicted ratios for geometric mean C_{max} and geometric mean AUC_{tau} and %predicted error were assessed for complete model evaluation. The mean, 5th and 95th percentiles of the population were predicted by SimCYP using default algorithms. In drug interaction simulation trials, the 90% CI geometric mean of plasma C_{max} and AUC_{inf} of midazolam 2 mg alone or co-administered with PF-05251749 at 400 or 750 mg q.d. were computed by a default statistical analyses algorithm in SimCYP.

RESULTS

In vitro CYP3A induction following incubation of PF-05251749 in cryopreserved human hepatocytes

Treatment of cryopreserved human hepatocytes with PF-05251749 (0.3–100 μM) resulted in concentration–dependent increases in CYP3A mRNA in all three lots. Concentration–dependent increases in CYP3A enzyme activity were observed in only 1/3 hepatocyte lots (HC7-4). Data are summarized in Figure 1. The induction parameters were estimated using sigmoid and linear fits; the sigmoid model was fitted with variable γ (four-parameter fit) or with γ fixed to 1 (three-parameter fit).

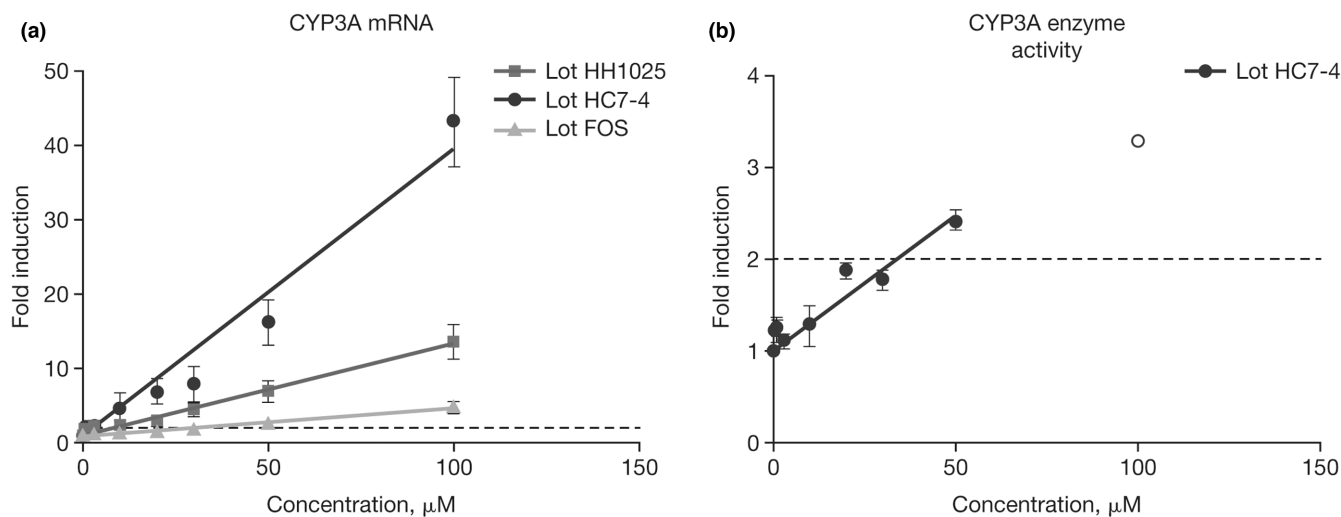


FIGURE 1 Summary of the fold induction of (a) CYP3A mRNA activity and (b) CYP3A enzyme activity in three different lots of cryopreserved human hepatocytes, treated with PF-05251749, at substrate concentrations of 0.3–100 μM. Fold induction in each hepatocyte lot was fit to a linear model. Dashed lines at 2-fold represent the threshold for induction.

Best fit of the fold-induction mRNA response in all three lots of hepatocytes and CYP3A activity in hepatocyte lot HC7-4 was obtained using a linear model (with y-intercept fixed at 1). A linear fit was preferred based on the p value of <0.05 using a sum-of-least-squares test. A summary of parameter estimates of the linear fit model is summarized in Table S2.

Plasma PK following oral administration of repeated doses of PF-05251749 in healthy adult participants

In Part A, a total of 61 participants were assigned to PF-05251749/placebo/melatonin (Table S3); 51/61 participants completed the study. Ten participants (2/10 receiving placebo, 1/11 receiving melatonin, 1/8 receiving PF-05251749 50 mg, 3/8 receiving 400 mg, and 3/8 receiving 750 mg) discontinued from the study (Table S4). PF-05251749 was administered at doses of 50–750 mg q.d. for 14 days. The day 14 median plasma concentration–time profile is summarized in Figure S2. Day 1 and steady-state plasma PK parameters of PF-05251749, estimated by non-compartmental analyses, are summarized in Table S5. PF-05251749 was rapidly absorbed, followed by biphasic decline at all dose levels. Steady-state generally appeared to have been reached by day 3, based on trough-level concentrations (Figure S3). Based on dose-normalized plasma PK parameters, C_{max} and AUC_{tau} increased dose-proportionally up to 200 mg q.d. Less than dose-proportional increases in C_{max} (but not AUC_{tau}) were observed following administration of 400 and 750 mg q.d. The steady-state terminal $t_{1/2}$ ranged from approximately

9 to 11 hours. On day 14, geometric mean accumulation ratio (R_{ac}) for C_{max} and AUC_{tau} ranged from 1.0 to 1.3. At steady-state, $<0.2\%$ of the administered dose was recovered as unchanged PF-05251749 in urine across all doses tested (internal data).

Statistical analyses of plasma 4β-hydroxycholesterol/cholesterol ratios

The changes in 4β-hydroxycholesterol/cholesterol following administration of PF-05251749/placebo/melatonin q.d. for 14 days is summarized in Figure 2 and Table 1. Significant interindividual variability was observed at baseline and in post-dose levels of cholesterol and 4β-hydroxycholesterol in all treatment groups. The fold change on day 14 relative to day 1 was similar following administration of placebo or PF-05251749 50–400 mg q.d. As observed in Table 1, administration of PF-05251749 at 750 mg q.d. led to a ~1.55-fold increase in the ratio of 4β-hydroxycholesterol/cholesterol on day 14 as compared to that on day 1 and achieved statistical significance relative to placebo.

Development of PBPK models of PF-05251749

The steady-state plasma PK of PF-05251749, 400 and 750 mg q.d. observed in healthy adult participants (B8001002, Part A) was characterized in SimCYP using a two-step approach. In the first step, base models were qualified using drug-specific in vitro input parameters

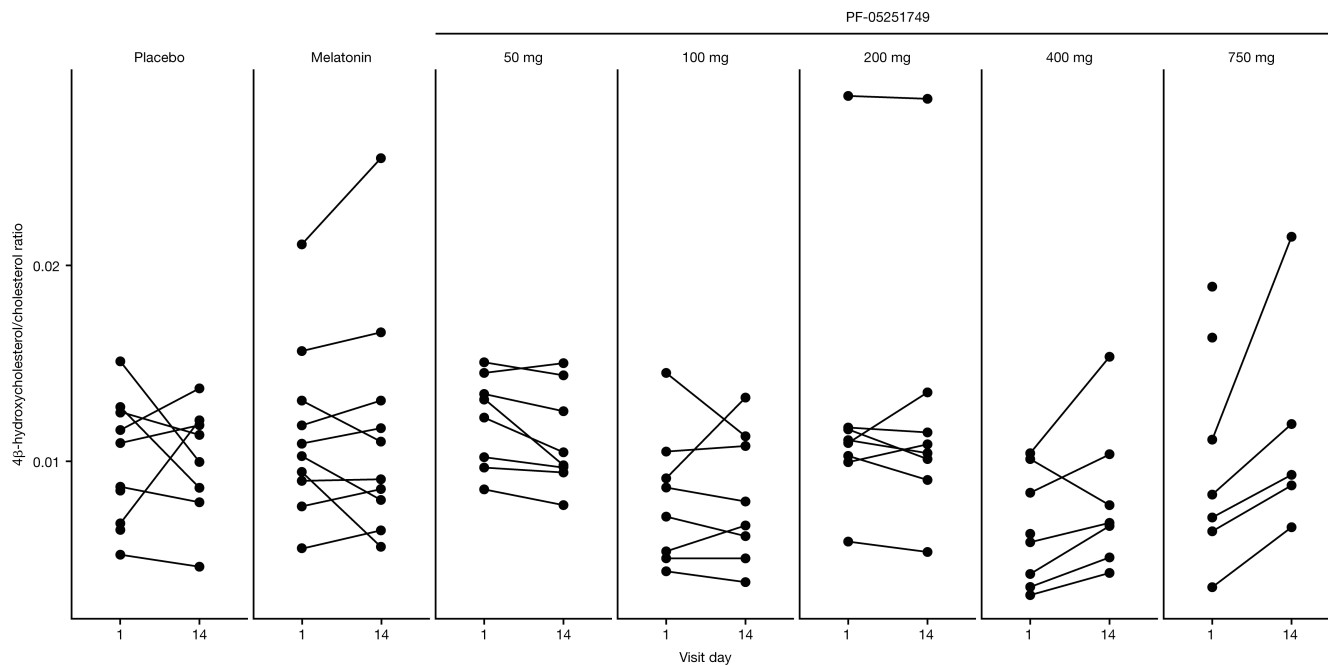


FIGURE 2 Individual plasma 4 β -hydroxycholesterol/cholesterol ratio at pre-dose on day 1 and day 14, following administration of PF-05251749/placebo at 50–750 mg and melatonin q.d. for 14 days. In the PF-05251749 400 mg group, 1/8 subjects discontinued on day 3 due to unwillingness to participate and 2/8 subjects discontinued due to loss of follow-up with last dosing occurring on day 14. In the PF-05251749 750 mg dose group, 2/8 subjects discontinued as they were no longer willing to participate in the study and 1/8 subjects were withdrawn due to misconduct.

| Treatment, q.d. | LSM ratio, baseline adjusted fold change | 90% CI | P value vs. placebo |
|-----------------|------------------------------------------|----------------|---------------------|
| Placebo | 1.019 | (0.910, 1.130) | N/A |
| 50 mg | 0.947 | (0.833, inf) | 0.5175 |
| 100 mg | 0.999 | (0.887, inf) | 0.5001 |
| 200 mg | 1.018 | (0.903, inf) | 0.5000 |
| 400 mg | 1.245 | (1.120, inf) | 0.1534 |
| 750 mg | 1.549 | (1.401, inf) | 0.0012 |

TABLE 1 Baseline-adjusted LSM fold change in median plasma 4 β -hydroxycholesterol/cholesterol ratio on day 14 relative to day 1, following administration of PF-05251749/placebo at 50–750 mg q.d. for 14 days

Note: P values for fold change compared to placebo.

Abbreviations: CI, confidence interval; inf, infinity; LSM, least squares mean; N/A, not assessed; q.d., once daily.

and using first-order absorption, minimal PBPK, two-compartmental elimination model. The observed T_{\max} of PF-05251749 was characterized using a sensitivity analysis of K_a (Figure S4). All input parameters for base model qualification are summarized in Table 2.

The PBPK models of PF-05251749 in healthy human participants were qualified by superposition of day 14 observed and SimCYP-predicted plasma concentration–time profiles. The predicted (95% CI) C_{\max} , T_{\max} , and AUC_{τ} of PF-05251749 agreed well with observed clinical data (Figure 3 and Table 3). In the second step, base models of PF-05251749 at 400 and 750 mg q.d. were calibrated to develop corresponding induction models. These models were developed in SimCYP version 18 using in vitro mRNA induction linear-fit-slope and with rifampin as the reference inducer

(rifampin $\text{Ind}_{\max} = 8$). The calibrated slopes of CYP3A mRNA induction in each of the three hepatocyte lots are summarized in Table S1. The final PBPK induction models of PF-05251749 were developed with the most potent calibrated slope ($0.18 \mu\text{M}^{-1}$) from donor HC7-4 (Table 2).

CYP3A drug interaction trial simulations of PF-05251749

In a virtual healthy adult population in SimCYP, a single oral dose of midazolam 2 mg was co-administered at steady-state of PF-05251749 at 400 or 750 mg q.d. Following co-administration of single oral dose of midazolam with PF-05251749, 400 mg q.d., no significant

TABLE 2 Input parameters of PF-05251749 for SimCYP® model qualification

| Parameters | Value | Source |
|-----------------------------------------------|-------------------|-----------------------------------------------------------------------|
| Physicochemical properties | | |
| Molecular weight (g/mol) | 309 | |
| log P | 1.18 | In silico |
| Compound type | Neutral | |
| $f_{u,p}$ | 0.231 | Experimental data |
| Blood/plasma ratio | 0.7 | Experimental data |
| Absorption | | |
| Absorption type | First-order | |
| F_a | 1 | Assumed |
| K_a (1/h) | 2 | Sensitivity analyses |
| Q_{gut} (L/h) | 18.3 | Predicted by MDCK |
| MDCK permeability P_{app} (10^{-6} cm/s) | 30.4 | Measured RRCK value |
| Permeability scalar | 2.5 | Using midazolam RRCK value (24.5×10^{-6} cm/s) as reference |
| $F_{u,gut}$ | 1 | Assumed |
| Distribution | | |
| Model | Minimal PBPK | |
| V_{ss} (L/kg) | 3.1 | 400 mg/750 mg, fitting |
| V_{SAC} (L/kg) | 1.6/1.3 | 400 mg/750 mg, fitting |
| Q (L/h) | 5.8/11 | 400 mg/750 mg, fitting |
| Elimination | | |
| | In vivo clearance | |
| CL_{po} (L/h) | 29.3/22.5 | 400 mg/750 mg, clinical data |
| Interaction | | |
| Induction slope (1/ μ M) | 0.18 | mRNA, calibrated with rifampin |

Abbreviations: CL_{po} , clearance observed following oral administration; F_a , fraction absorbed; $F_{u,gut}$, fraction unbound in gut; $F_{u,p}$, fraction unbound in plasma; h, hour; K_a , first-order absorption rate constant; MDCK, Madin Darby canine kidney; Q , intercompartmental clearance; Q_{gut} , composite of enterocyte permeability and blood flow across the gut, assuming well-stirred model; PBPK, physiologically based pharmacokinetic; RRCK, Ralph Russ canine kidney; V_{SAC} , volume of distribution for single adjusting compartment; V_{ss} , apparent volume of distribution at steady state.

changes in midazolam exposures were predicted. In a similar trial design, when PF-05251749 750 mg q.d. was co-administered with a single oral dose of midazolam 2 mg, geometric mean $C_{max,GMR}$ and $AUC_{inf,GMR}$ of midazolam were predicted to be 0.71 (90% CI 0.70–0.73) and 0.66 (90% CI 0.64–0.68), respectively. Results of the trial simulations are summarized in Figure S5 and Table 4.

DISCUSSION

In vitro, PF-05251749 was identified as an inducer of CYP3A (Figure 1) in three different donor lots of cryopreserved human hepatocytes¹² based on increases in CYP3A mRNA and enzyme activity. The slope of induction of CYP3A mRNA and enzyme activity was estimated using a linear fit model (Table S2). PF-05251749 was not an inhibitor of CYP isozymes. Many antiepileptic

and antiretroviral therapies that are CYP3A inducers increase the rate of synthesis of 4 β -hydroxycholesterol from endogenous cholesterol, due to increased activity of hepatic CYP3A.^{13–15} These observations have been consistently replicated following multiple doses of rifampin (20–600 mg q.d.), a strong CYP3A inducer.^{16,17} Increases in 4 β -hydroxycholesterol have been observed as early as 1-week post-rifampin administration and up to the end of treatment (2 weeks), followed by a decrease in 4 β -hydroxycholesterol levels after rifampin dosing was discontinued.¹⁶ As PF-05251749 was identified to be a potential CYP3A inducer in vitro, changes in cholesterol and 4 β -hydroxycholesterol levels, following repeated oral administration of PF-05251749 in B8001002 (Part A), were explored as biomarkers of hepatic CYP3A activity. While 4 β -hydroxycholesterol has been shown to be a promising marker of CYP3A activity, significant interindividual variability has been

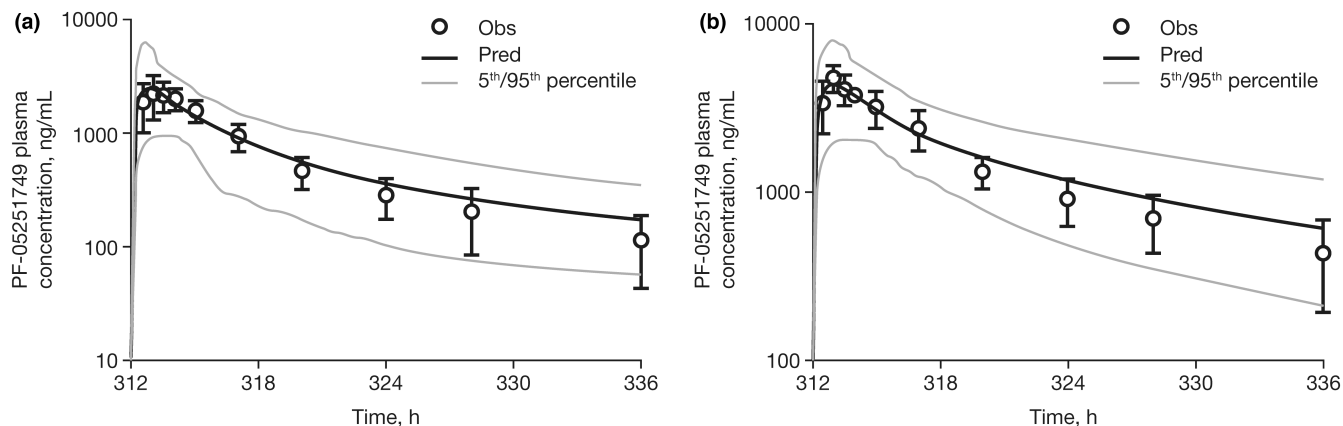


FIGURE 3 Characterization of the mean plasma concentration–time profile of PF-05251749 (a) 400 mg q.d. and (b) 750 mg q.d. Open circles represent the day-14-observed mean plasma concentration–time profile of PF-05251749; the dark lines represent the SimCYP®-predicted population mean concentration–time profile; the light lines represent the SimCYP-predicted 5th and 95th percentile concentration–time profile.

TABLE 3 Summary of the day-14 geometric mean (%CV) observed and SimCYP®-predicted steady-state plasma pharmacokinetic parameters C_{max} and AUC_{tau} , and median T_{max} , following administration of PF-05251749 at 400 and 750 mg q.d. in virtual healthy population ($n = 100$)

| PF-05251749 | C_{max} , ng/ml | | | AUC_{tau} , ng h/ml | | | T_{max} , h | | |
|-------------|-------------------|-----------|----------|-----------------------|-------------|----------|---------------|------|----------|
| | Pred | Obs | Pred/Obs | Pred | Obs | Pred/Obs | Pred | Obs | Pred/Obs |
| 400 mg q.d. | 2351 (64) | 2613 (28) | 0.90 | 14,672 (30) | 13,640 (28) | 1.08 | 1.17 | 1.00 | 1.17 |
| 750 mg q.d. | 4094 (46) | 4726 (19) | 0.87 | 35,833 (30) | 33,290 (17) | 1.08 | 1.28 | 1.00 | 1.17 |

Note: The summary statistics presented in this table are geometric mean (geometric %CV) for C_{max} and AUC_{tau} and median for T_{max} .

Abbreviations: AUC_{tau} , area under the plasma drug concentration–time curve over the dosing interval, tau; C_{max} , maximum plasma drug concentration; CV, coefficient of variance; h, hour; Obs, observed; Pred, predicted; q.d., once daily; T_{max} , time to maximum plasma drug concentration.

TABLE 4 SimCYP®-predicted geometric mean pharmacokinetic (PK) parameters and ratio of PK parameters of midazolam, following co-administration of a single oral dose of midazolam 2 mg with or without PF-05251749 at 400 or 750 mg q.d. in virtual healthy population ($n = 100$)

| PF-05251749 dose | C_{max} , ng/ml | AUC_{inf} , ng h/ml | C_{max} , ng/ml | AUC_{inf} , ng h/ml | $C_{max,GMR}$ | $AUC_{inf,GMR}$ |
|------------------|-------------------------------------------|-----------------------|----------------------------------------------|-----------------------|-------------------|-------------------|
| | Reference (midazolam alone), mean (range) | | Test (midazolam + PF-05251749), mean (range) | | Mean (90% CI) | Mean (90% CI) |
| 400 mg q.d. | 8.4 (7.59, 9.29) | 25.1 (22.4, 28.1) | 7.19, (6.50, 7.96) | 20.7 (18.5, 23.2) | 0.86 (0.85, 0.87) | 0.83 (0.81, 0.84) |
| 750 mg q.d. | 8.4 (7.59, 9.29) | 25.1 (22.4, 28.1) | 5.98 (5.39, 6.62) | 16.5 (14.8, 18.5) | 0.71 (0.70, 0.73) | 0.66 (0.64, 0.68) |

Note: Data are geometric mean (range) for PK parameters and geometric mean ratio (90% CI) for $C_{max,GMR}$ and $AUC_{inf,GMR}$. AUC_{inf} , area under the plasma drug concentration–time curve from zero to infinity; $AUC_{inf,GMR}$, ratio of the AUC_{inf} of midazolam co-administered with PF-05251749 to AUC_{inf} of midazolam administered alone; CI, confidence interval; C_{max} , maximum plasma drug concentration; $C_{max,GMR}$, ratio of the C_{max} of midazolam co-administered with PF-05251749 to C_{max} of midazolam administered alone; q.d., once daily.

observed in this marker (associated with its precursor cholesterol and attributed to diet/exercise, age, lifestyle, disease status/co-medications, etc.).¹⁸ Phase I trials are not statistically powered to account for variability associated with exploratory biomarkers; in addition, uncertainty in 4 β -hydroxycholesterol levels may exist due to its prolonged terminal elimination half-life of ~17 days¹⁶ whereas duration of dosing in first-in-human studies

in healthy subjects is typically 10–14 days. Due to these uncertainties associated with measurements of absolute levels of 4 β -hydroxycholesterol, changes in the ratio of 4 β -hydroxycholesterol/cholesterol have been used as a marker of hepatic CYP3A activity.¹⁹ Thus, changes in 4 β -hydroxycholesterol/cholesterol ratio at trough levels on day 14 relative to baseline were evaluated in B8001002.; no significant changes were observed up to 400 mg q.d.

Administration of repeated doses of PF-05251749 at 750 mg q.d. resulted in statistically significant increase in 4 β -hydroxycholesterol/cholesterol, relative to placebo (Table 1). While this increase suggested the potential for PF-05251749 to be a CYP3A inducer in humans, the magnitude of changes in 4 β -hydroxycholesterol/cholesterol as a suitable surrogate to predict magnitude of CYP3A drug interaction has not been validated to date. A semi-mechanistic PK/PD model to assess fold induction of CYP3A by characterizing concentration effects of enasidenib on changes in 4 β -hydroxycholesterol/cholesterol has been developed.²⁰ This model could potentially be applied to quantitate induction risks of a compound, if sufficient time-course data to characterize the effect of concentration or dose of the inducer on rate of change of 4 β -hydroxycholesterol/cholesterol are available. However, this model has limited utility, as the rate of change of 4 β -hydroxycholesterol/cholesterol has not been correlated with the magnitude of changes in midazolam AUC, when co-administered with enasidenib. In B8001002, cholesterol and 4 β -hydroxycholesterol were sparsely sampled as exploratory biomarkers; furthermore, biomarker changes were observed only at 750 mg q.d.; thereby no exposure-response assessment of CYP3A markers was undertaken. Therefore, the observed change in 4 β -hydroxycholesterol/cholesterol following administration of PF-05251749 750 mg q.d. was not used as a surrogate of magnitude of clinical drug interaction with midazolam. These changes were used as preliminary evidence of PF-05251749 as a potential inducer of CYP3A and investigation of drug interaction risks were undertaken using PBPK model.

The performance of PBPK models to predict metabolic drug interactions has demonstrated good concordance with the observed magnitude of clinical drug–drug interaction (DDI) outcomes and increased regulatory acceptance to de-risk metabolic DDIs in lieu of a clinical study.^{21,22} However, there are caveats in applying a PBPK model alone during early clinical development; mass balance/ADME (absorption, distribution, metabolism, and excretion) data are typically unavailable during early clinical stages, limiting the development of a mechanistic PBPK model and leading to uncertainties in model qualification. Limited understanding of an efficacious dose range of the investigational drug also introduces significant uncertainty in selecting doses for model-based drug interaction assessments, particularly in case of dose- or non-stationary PK. Amidst such uncertainties, a PBPK model could be used as a fit-for-purpose tool and may need to be supplemented with cumulative understanding of available non-clinical and clinical data. Based on observed increases in hepatic CYP3A activity markers following repeated dosing of PF-05251749, CYP3A DDI risk

assessment was undertaken using SimCYP. As nonlinear PK was observed following repeated doses >200 mg q.d., the base PBPK models at 400 and 750 mg q.d. were developed using dose-dependent PK estimates from B8001002; base models were qualified using the observed steady-state plasma concentration–time profile of PF-05251749.^{23,24} The induction model of PF-05251749 was developed in SimCYP⁷ by calibrating the base models with the most potent in vitro induction linear-fit slope (Table S1).

In virtual healthy populations, based on SimCYP predicted changes in midazolam exposures (Table 4), PF-05251749 750 mg q.d. was predicted to be a weak inducer of CYP3A. This PBPK prediction was in line with statistically significant changes observed in 4 β -hydroxycholesterol/cholesterol following PF-05251749 750 mg q.d. (Table 1). A similar drug interaction assessment of PF-05251749 was conducted at the dose of 400 mg q.d. Using the SimCYP-calibrated induction model, a single oral dose of midazolam 2 mg, co-administered with PF-05251749 400 mg q.d., predicted no significant drug interactions; this PBPK prediction was in agreement with no significant changes observed in 4 β -hydroxycholesterol/cholesterol at the 400 mg q.d. dose of PF-05251749.

Thus, the PBPK model predictions at potentially (weak) inducing, as well as non-inducing, doses of PF-05251749 were in line with the observed biomarker trends.²⁵ While the predictive performance of PBPK for CYP3A inducers has shown good concordance with the observed magnitude of clinical drug interactions,²² a limitation of this study is that the current PBPK based prospective assessment cannot be validated due to the absence of a midazolam clinical drug interaction study. Furthermore, no quantitative relationship between magnitude of change in 4 β -hydroxycholesterol/cholesterol ratio and magnitude of midazolam DDI has been reported to date.

In conclusion, this study investigated CYP3A drug interaction risk of PF-05251749, using PBPK models calibrated with linear-fit-induction slope. The PBPK model predictions were in good agreement with observed trends of the CYP3A markers 4 β -hydroxycholesterol/cholesterol ratio. This analysis exemplifies assessment of CYP3A inductions risks during early clinical development guided by biomarker changes and PBPK model outcomes.

AUTHOR CONTRIBUTIONS

A.S.-B., J.L., P.N., T.C.G., and F.G. wrote the manuscript. A.S.-B., J.L., N.J., Z.L., T.C.G., and F.G. designed the research. A.S.-B., J.L., N.J., and F.G. performed the research. A.S.-B., T.C.G., and F.G. analyzed the data.

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CONFLICT OF INTEREST

All authors are present or past employees and shareholders of Pfizer Inc.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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