TITLE: Prediction of Oral Pharmacokinetics of cMet Kinase Inhibitors in Humans: Physiologically Based Pharmacokinetic Model versus Traditional One Compartment Model

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ABBREVIATIONS:

ADME, absorption, distribution, metabolism and excretion; AUC, area under the plasma concentration-time curve; AUMC, area under the first moment of the plasma concentration-time curve; BRW, brain weight; CL, clearance; CL\text{blood}, blood clearance; CL\text{hep}, hepatic clearance; CL\text{int}, in vitro intrinsic clearance; CL\text{int,vivo}, in vivo intrinsic clearance; CL\text{plasma}, plasma clearance; C\text{max}, maximum plasma concentration; CV, coefficients of variation; F\text{abs}, fraction absorbed; F\text{oral}, oral bioavailability; f_u\text{,blood}, unbound fraction in whole blood; f_u\text{,hepatocytes}, unbound fraction in hepatocytes; f_u\text{,microsomes}, unbound fraction in microsomes; f_u\text{,plasma}, unbound fraction in plasma; HPLC, high performance liquid chromatography; IVIVE, in vitro-in vivo extrapolation; k_a, absorption rate constant; K_p, tissue-to-plasma partition coefficient; LC-MS/MS, liquid-chromatography tandem mass spectrometry; MLP, maximum life span; PF02341066, (R)-3-[1-(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine; PF04217903, 2-[4-(3-quinolin-6-ylmethyl-3H-[1,2,3]triazolo[4,5-b]pyrazin-5-yl)-pyrazol-1-yl]-ethanol; PBPK, physiologically-based pharmacokinetics; PK, pharmacokinetics; R_{bp}, blood-to-plasma concentration ratio; t_{1/2}, apparent disposition half-life; t_{max}, the time at which C\text{max} occurred; V_{ss}, volume of distribution at steady-state; V_{ss,u}, unbound volume of distribution at steady-state; W, body weight.
ABSTRACT:

The objective of this study was to assess physiologically-based pharmacokinetic (PBPK) model for predicting plasma concentration-time profiles of orally available cMet kinase inhibitors, (R)-3-[1-(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine (PF02341066) and 2-[4-(3-quinolin-6-ylmethyl-3H-[1,2,3]triazolo[4,5-b]pyrazin-5-yl)-pyrazol-1-yl]-ethanol (PF04217903), in humans. The prediction accuracy of pharmacokinetics (PK) by the PBPK modeling was compared to traditional one-compartment PK model based on allometric scaling. The predicted clearance values from allometric scaling with the correction for the interspecies differences in protein binding were used as representative comparison, which showed more accurate PK prediction in humans than the other methods. Overall the PBPK modeling provided a better prediction of the area under the plasma concentration-time curves for both PF02341066 (1.2-fold error) and PF04217903 (1.3-fold error) compared to the one-compartment PK model (1.8- and 1.9-fold errors, respectively). More importantly, the simulated plasma concentration-time profiles of PF02341066 and PF04217903 by the PBPK modeling appeared to be consistent with the observed profiles showing multi-exponential declines, resulting in more accurate prediction on the apparent half-lives (t1/2): the observed and predicted t1/2 values were respective 10 and 12 h for PF02341066 and 6.6 and 6.3 h for PF04217903. The predicted t1/2 values by the one-compartment PK model were 17 h for PF02341066 and 1.9 h for PF04217903. Therefore, the PBPK modeling has the potential to be more useful and reliable for the PK prediction of PF02341066 and PF04217903 in humans compared the traditional one-compartment...
PK model. In summary, the present study has shown examples that PBPK model can be used to predict PK profiles in humans.
INTRODUCTION

Potential clinical drug candidates are routinely screened for their absorption, distribution, metabolism and excretion (ADME) properties in the drug discovery and development process. In order to improve and expedite clinical drug candidate selection, there has been an increased demand to predict pharmacokinetics (PK) in humans as early as possible. The development and application of reliable methods to predict PK in humans could help selecting the best candidates for clinical development. As the most therapeutic drugs are designed to be given orally to patients and the majority of these are intended to act systemically, the most important PK parameters related to dosing regimen are systemic clearance (CL), volume of distribution at steady-state (Vss) and fraction absorbed (Fabs). Among these parameters, CL plays a central role as it relates both the drug’s half-life (t1/2) and oral bioavailability (Foral) whereas Vss and Fabs are also required to fully characterize the dosing regimen such as dose size and frequency. Methods of predicting CL in humans include species scaling by allometry (Boxenbaum, 1984; Mordenti, 1986; Ings, 1990), in vitro to in vivo extrapolation (IVIVE) (Houston and Carlile, 1997; Obach et al, 1997; Rostami-Hodjegan and Tucker, 2007) and the combined use of in vivo animal and in vitro human data (Ubeaud et al, 1995, Lavé et al, 1999; Yamazaki et al, 2004). Allometric scaling is simply based on the similarity of anatomical, physiological and biochemical variables across species including humans. Although allometric scaling is an empirical approach, it has been widely used and provided reliable predictions for a number of highly metabolized and renally excreted drugs (Boxenbaum, 1984; Mordenti, 1986; Mahmood and Balian, 1996). Over the last 2 decades, the methods for extrapolating in vivo CL from in vitro data (i.e., IVIVE) have
been applied extensively with the increased availability of human liver samples (e.g., microsomes, hepatocytes, liver slices, etc.,) and have demonstrated prediction accuracy for metabolic CL of low to high hepatic extraction compounds (Houston and Carlile, 1997; Obach et al, 199; Obach, 1999; Riley et al., 2005; Shiran et al., 2006). In recent years, there has been a growing interest in physiologically-based pharmacokinetic (PBPK) model, which provides the disposition profiles in various species to be predicted from physico-biochemical properties of compounds with the species-specific physiological parameters (Jones et al, 2006; De Buck et al, 2007; Lavé et al, 2007; Nestorov, 2007). Advances in the prediction of $V_{ss}$ from in silico modeling (Poulin and Theil, 2002; Berezhkovskiy, 2004; Rodgers et al., 2005; Rodgers and Rowland 2006) have greatly extended the applicability of the PBPK model to compounds in the early stage of drug discovery to the late phase of drug development.

(R)-3-[1-(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine (PF02341066) and 2-[4-(3-quinolin-6-ylmethyl-3H-[1,2,3]triazolo[4,5-b]pyrazin-5-yl)-pyrazol-1-yl]-ethanol (PF04217903) (Fig. 1) were identified as orally available ATP-competitive cMet kinase inhibitors ($K_i$ 4-5 nM) (Zou et al., 2007, 2008). PF02341066 and PF04217903 potently inhibited in vitro cMet phosphorylation and signal transduction as well as cMet-dependent proliferation, migration, or invasion of human tumor cells ($IC_{50}$ 5-20 nM). PF02341066 also potently inhibited phosphorylation of nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) in Karpas299 and SU-DHL-1 ALCL cells as well as NPM-ALK–dependent cell proliferation or apoptosis ($IC_{50}$ 20-40 nM) (Christensen et al., 2007). PF02341066 and PF04217903 are currently being developed for the clinical treatment of cancer.
These kinase inhibitors were selected to investigate the PK prediction accuracy in humans using in vitro and in vivo data because of relatively different physicochemical and PK properties. PF02341066 is a strong basic compound having pKa values of 5.4 and 8.9 with logP of 4.3 whereas PF04217903 is a weak basic compound having pKa value of 4.7 with logP of 0.52. The solubility of PF02341066 in water (34 µg/mL) is approximately 50-fold higher than that of PF04217903 (0.6 µg/mL). PF02341066 showed moderate in vivo CL and relatively large Vss whereas PF04217903 showed relatively low CL and Vss. These differences positioned these inhibitors as interesting compounds to investigate the prediction accuracy of the oral PK parameters in humans. Moreover, the quantitative prediction of the plasma concentration-time profiles of clinical candidate drugs has become a matter of major interest to industry. The main reason for this focus is the fact that the plasma concentration-time profiles of clinical candidates are seen as the first surrogate markers of the pharmacological and toxicological effects. Therefore, we evaluated the prediction accuracy of the plasma concentration-time profiles of PF02341066 and PF04217903 in humans based on the PBPK modeling compared to the traditional one-compartment PK modeling.
Materials and Methods

Chemicals

PF02341066 (hydrochloride salt: chemical purity $>99\%$), PF04217903 (free base: chemical purity $>99\%$) and structurally-related in house compounds (internal standard for analysis) were synthesized by Pfizer Worldwide Research & Development (San Diego, CA). All other commercially available reagents and solvents were of either analytical or high performance liquid chromatography (HPLC) grade.

Animals

All experiments with animals were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals, as well as with internal company policies and guidelines. Male Sprague-Dawley rats (Charles River Laboratories, Hollister, CA), weighing 0.25 to 0.28 kg, male beagle dogs (Pfizer dog colony, Kalamazoo, MI), weighing 7.9 to 12 kg, and male cynomolgus monkeys (Pfizer monkey colony, Kalamazoo, MI), weighing 2.4 to 4.1 kg, were used for the PK studies. Each animal was housed in stainless cages or metabolic cages (urine collection) under controlled conditions (20 - 26°C, 30-70% relative humidity and 12 h light/dark cycle).

In Vitro Incubation - Microsomes

Pooled liver microsomes of Sprague-Dawley rats, beagle dogs, cynomolgus monkeys and humans ($n = 5$, Caucasians) were purchased from BD Gentest (Woburn, MA). The incubation mixture consisted of liver microsomes (0.5 to 0.8 mg/mL), 125 mM MgCl$_2$ and PF02341066 or PF04217903 (1 µM) in 100 mM potassium phosphate, pH 7.4. The reaction was initiated by the addition of NADPH (final 1 mM)
and the mixture (0.2 mL) was incubated at 37°C for 0, 5, 10, 15, 30, 45 and 60 minutes. The reaction was terminated by the addition of cold acetonitrile (0.1 mL) containing the internal standard (1 μM) followed by vortexing for 1 minute on a SP Multi-tube Vortexer (Baxter, McGaw Park, IL). The sample was centrifuged (Allegra 6KR with GH-3.8A rotor) at 2000 × g for 10 minutes and the supernatant (0.1 mL) was mixed with an equal volume of water in an HPLC plate for liquid-chromatography tandem mass spectrometry (LC-MS/MS) analysis. All of the incubations were performed in triplicate.

**In Vitro Incubation - Hepatocytes**

Cryopreserved hepatocytes of Sprague-Dawley rats, beagle dogs, cynomolgus monkeys and humans (n = 5, Caucasians) were obtained from In Vitro Technologies (Baltimore, MD) and used according to the manufacturer’s instructions. Cell viability was routinely checked by the trypan blue (0.4% w/v) exclusion test and preparations in excess of 90% viable were used. The incubations with hepatocytes (final volume of 0.05 mL) were carried out at a cell density of 0.5 to 1 × 10⁶ cells/ml in Williams E Media supplemented with 10 mM HEPES buffer (pH 7.4) at 37°C up to 4 hours in a CO₂:O₂ (5:95) stationary incubator. Reaction was started by the addition of either PF02341066 or PF04217903 (1 μM) and terminated by the addition of methanol (0.2 mL) containing the internal standard (1 μM) at each time point (0, 0.25, 0.5, 1, 2, 3 and 4 hours). The sample was vortexed and centrifuged at 1900 × g (Beckman-Coulter GH3.8A rotor) for 10 minutes. The supernatant (0.1 mL) was diluted with equal amount of water and injected onto LC-MS/MS for analysis. All of the incubations were performed in triplicate.
In Vitro Plasma Protein Binding

In vitro plasma protein bindings of PF02341066 (0.5 to 20 μM) and PF04217903 (0.2 to 10 μM) were determined in Sprague-Dawley rats, beagle dogs, cynomolgus monkeys and humans (n = 3, Caucasians) using the equilibrium dialysis technique. The study was conducted in a 96-well Teflon® dialysis chamber (HTDialysis LLC, Gales Ferry, CT) using a semi-permeable membrane (Spectra/Por®4, Spectrum, Laguna Hills, CA) with a 12,000-14,000 Da molecular mass cut-off. The plate was covered with a top seal film to avoid evaporation and incubated at 37°C for 6 h. An appropriate volume of all samples was analyzed by LC-MS/MS. All of the incubations were performed in triplicate. Pilot experiments revealed that 1) PF02341066 and PF04217903 were stable in plasma and 2) protein binding reached equilibrium at 37°C within 6 h. The unbound fraction in plasma (fu, plasma) was calculated by the following equation:

\[ f_{u,\text{plasma}} = \frac{C_{\text{buffer}}}{C_{\text{plasma}}} \]

where \( C_{\text{buffer}} \) and \( C_{\text{plasma}} \) denote the drug concentrations in buffer and plasma, respectively, after the incubation.

In Vitro Non-Specific Binding

In vitro non-specific bindings of PF02341066 and PF04217903 were determined in liver microsomes using the equilibrium dialysis technique as described above. Dialysis mixtures contained liver microsomes (0.5 to 0.8 mg/mL), 125 mM MgCl₂ and PF02341066 or PF04217903 (1 μM) in 100 mM potassium phosphate, pH 7.4, in a final volume of 0.2 mL. All of the incubations were performed in triplicate. The unbound fraction in microsomes (fu, microsomes) was calculated by the following equation:

\[ f_{u,\text{microsomes}} = \frac{C_{\text{buffer}}}{C_{\text{microsomes}}} \]
where \( C_{\text{buffer}} \) and \( C_{\text{microsomes}} \) denote the drug concentrations in buffer and microsomes, respectively, after the incubation.

In vitro non-specific bindings of PF02341066 and PF04217903 in hepatocytes \( (f_{u,\text{hepatocytes}}) \) were calculated by the following equation (Kilford et al., 2008):

\[
f_{u,\text{hepatocytes}} = \frac{1}{1 + \left( \frac{R_{\text{hepatocytes}}}{R_{\text{microsomes}}} \right) \left( \frac{V_R}{P} \right) \left( 1 - \frac{f_{u,\text{microsomes}}}{f_{u,\text{microsomes}}} \right)}
\]

where \( R_{\text{hepatocytes}} \) is the cell to medium concentration ratio in hepatocytes, \( R_{\text{microsomes}} \) is the microsomal protein binding affinity, \( V_R \) is the cell to volume ratio in the hepatocytes and \( P \) is the microsomal protein concentration (mg/mL), respectively.

The \( R_{\text{hepatocytes}} \) over \( R_{\text{microsomes}} \) ratio of 125 and the \( V_R \) of 0.005 at the cell concentration of \( 10^6 \) cells/mL were used for the \( f_{u,\text{hepatocytes}} \) calculation (Kilford et al., 2008).

**Blood-to-Plasma Concentration Ratio**

The blood-to-plasma concentration ratios of PF02341066 and PF04217903 were determined by incubating the compounds with whole blood from Sprague-Dawley rats, beagle dogs, cynomolgus monkeys and humans (Caucasian). PF02341066 or PF04217903 (final 1 \( \mu \)M), dissolved in methanol or acetonitrile:water (1:1, v/v), was added to whole blood and the spiked whole blood was incubated at 37°C for 1 hour. Aliquots of the spiked whole blood and the harvested plasma were transferred into a tube with acetonitrile:methanol (1:1, v/v) containing the internal standard (1 \( \mu \)M). An appropriate volume of all samples was analyzed by LC-MS/MS. All of the incubations were performed in triplicate. The blood-to-plasma concentration ratio \( (R_{bp}) \) and the unbound fraction in whole blood \( (f_{u,blood}) \) were calculated by the following equations:

\[ R_{bp} = \frac{C_{\text{blood}}}{C_{\text{plasma}}} \]
\[ f_{u,\text{blood}} = f_{u,\text{plasma}} / R_{up} \]

where \( C_{\text{blood}} \) and \( C_{\text{plasma}} \) denote the drug concentrations in whole blood and plasma, respectively, after the incubation.

**In Vitro Hepatic CL Calculation**

The in vitro intrinsic clearance (\( \text{CL}_{\text{int}} \)) was calculated from \( t_{1/2} \) of the parent drug disappearance, which was determined by the slope \( (k) \) of log-linear regression analysis from the concentration versus time profiles, i.e., \( t_{1/2} = -\ln(2)/k \). (Lavé et al., 1999).

Values of \( \text{CL}_{\text{int}} \) were scaled to in vivo units using scaling factors such as the hepatic microsomal protein concentrations (45 mg/g liver) or the hepatocellularity (135, 240, 120 and \( 120 \times 10^6 \) cells/g liver in rats, dogs, monkeys, and humans, respectively) and the liver weights (40, 32, 32, and 21 g/kg body weight, respectively) (Hosea et al, 2009). In vitro hepatic clearance (\( \text{CL}_{\text{hep}} \)) was then calculated using the equation for the well-stirred models, and hepatic extraction ratio (\( \text{ER} \)) was calculated from \( \text{CL}_{\text{hep}} \) divided by the hepatic blood flow \( (Q_b) \). Recently the use of validated scaling factors, 32 mg/g liver for human microsomal protein and \( 99 \times 10^6 \) cells/g liver for human hepatocellularity, have been recommended by Barter et al. (2007). The use of these scaling factors resulted in 15 to 30% lower \( \text{CL}_{\text{hep}} \) values of PF02341066 and PF04217903. For consistency with the previous reports (Obach et al, 1997; Obach 1999; Naritomi et al, 2003; Riley et al, 2005; Hosea et al, 2009), the scaling factors indicated above were used for this study.

In the well-stirred model (WS-I), \( \text{CL}_{\text{hep}} \) value was calculated using \( \text{CL}_{\text{int}}, f_{u,\text{blood}}, \) the unbound fraction in microsomes or hepatocytes \( (f_{u,\text{vitro}}) \) and \( Q_b \) (70, 40, 44, and 20 mL/min/kg, in rats, dogs, monkeys and humans, respectively) (Hosea et al, 2009):
Many investigators have suggested that the well-stirred model disregarding all bindings (i.e., $f_{u,blood}$ and $f_{u,vitro}$) provides a better estimate of in vivo CL (Obach, 1999; Riley et al., 2005; De Buck et al., 2007). Therefore, $CL_{hep}'$ was also calculated based on the modified well-stirred model (WS-II) using only $CL_{int}$ and $Q_b$ under the assumption that $f_{u,blood}$ and $f_{u,vitro}$ effectively nullify in the liver model:

$$CL_{hep}' = \frac{Q_b \cdot CL_{int}}{Q_b + f_{u,blood} \cdot CL_{int} / f_{u,vitro}}$$

**Preclinical PK Studies**

PF02341066 and PF04217903 were dissolved in saline and PEG400/saline (4:6, v/v), respectively, for the intravenous administration while they were suspended in 0.5% methylcellulose solution for the oral administration. All dose levels of PF02341066 and PF04217903 were expressed as free base equivalents. Male Sprague-Dawley rats were given intravenously PF02341066 (5 mg/kg) or PF04217903 (2.5 mg/kg) by bolus injection through a catheter implanted into the jugular vein; orally PF02341066 (25 mg/kg) or PF04217903 (10 mg/kg) by gavage. Male beagle dogs were given intravenously PF02341066 (5 mg/kg) or PF04217903 (2.5 mg/kg) by bolus injection through the jugular vein; orally PF02341066 (25 mg/kg) or PF04217903 (10 mg/kg) via gastric gavage. Male cynomolgus monkeys were given intravenously PF02341066 (5 mg/kg) by bolus injection via the saphenous jugular vein; orally PF02341066 (25 mg/kg) via gastric intubation. The number of animals dosed was 3 per group for PF02341066 and 2 per group for PF04217903. Blood samples were collected from all animals at pre-determined time points with K$_2$EDTA or K$_3$EDTA as the anticoagulant.
and were then centrifuged. The resulting plasma samples were stored at approximately -20°C until analysis. Urine samples were also collected from the intravenous dosing groups over 24 or 48 h post-dose with a minimal cage rinse and the samples were stored at approximately -20°C until analysis.

**PF02341066 and PF04217903 Analysis**

Concentrations of PF02341066 and PF04217903 in preclinical biological samples were quantitatively determined by LC-MS/MS analysis. The chromatography was performed with an Agilent HP1100 HPLC system (Palo Alto, CA) or Shimadzu LC-10AD HPLC system (Columbia, MD) using a reverse phase column (Agilent XDB-C18, 2.1 × 50 mm, 5 μm). Mass spectrometric analysis was performed on a Quattro Ultima triple-stage quadrupole mass spectrometer (Micromass, Beverly, MA) or an API 4000 triple-stage quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). The mobile phase consisted of A = 98% HPLC grade water, 2% acetonitrile and 0.1% formic acid, and B = 98% acetonitrile, 2% HPLC grade water and 0.1% formic acid at a flow rate of 0.4 to 0.6 mL/min. The gradient elution was programmed from B = 5% to 60% over 2.5 minutes for PF02341066 or B = 2% to 90% over 4 minutes for PF04217903. Sample analysis was performed in the positive ionization multiple reaction monitoring mode with unit resolution for the transitions of m/z 450→260 for PF02341066 and m/z 377→348 for its internal standard or m/z 373→142 for PF04217903 and m/z 305→160 for its internal standard. Total time for the analytical run was 4 to 5 min. All sample analyses were conducted according to the internal assay quality guidelines based on the workshop/conference report (Shah et al, 2000).
Clinical PK Studies

Plasma concentrations of PF02341066 and PF04217903 in the clinic were provided by Oncology Business Unite, Pfizer Inc (San Diego, CA). PF02341066 (100 mg) was orally administered once daily to 4 cancer patients, aged between 35 and 57 years old with body weights of 63 to 99 kg. Serial blood samples were collected over 24 hours after the first dose. A single oral dose of PF04217903 (30 mg) was administered to 6 healthy volunteers, aged between 23 and 50 years old with body weights of 73 to 98 kg. Serial blood samples were collected up to 96 hours post-dose. The plasma concentrations of PF02341066 and PF04217903 were quantitatively determined by validated LC-MS/MS methods based on preclinical assay. Clinical protocols were approved by the investigational review boards of participating institutions and all subjects gave written informed consent for participation in the clinical trials. Detailed clinical information of PF02341066 has been reported recently (Kwak et al., 2010) while clinical data of PF04217903 were previously reported by Tan et al (2009) and its detailed information will be published elsewhere (Tan W, Checchio TM, Grace N, Wang R and Wilner KD. manuscript in preparation).

Non-compartment PK Analysis

For the in vivo studies, plasma concentration-time data for each species were analyzed by model-independent methods. The area under the plasma concentration-time curve from time zero to the last time point ($t_{last}$) with a quantifiable concentration ($C_{last}$), $AUC_{0-last}$, was calculated using the linear trapezoidal rule. The area under the plasma concentration-time curve was extrapolated to infinity time ($AUC_{0-\infty}$) by the following equations:
AUC$_{0-\infty} = AUC_{0\text{-last}} + C_{\text{last}} / \lambda$

where $\lambda$ was the elimination rate constant determined by linear regression of the last two to four quantifiable data points in the log plasma concentration-time curves.

Plasma clearance (CL$_{\text{plasma}}$), blood clearance (CL$_{\text{blood}}$), the apparent disposition $t_{1/2}$ and $V_{ss}$ were calculated by the respective following equations:

$$\text{CL}_{\text{plasma}} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}}$$

$$\text{CL}_{\text{blood}} = \frac{\text{CL}_{\text{plasma}}}{R_{\text{bp}}}$$

$$t_{1/2} = \frac{\ln(2)}{\lambda}$$

$$V_{ss} = \frac{\text{CL}_{\text{plasma}} \cdot \text{AUMC}_{0-\infty}}{\text{AUC}_{0-\infty}}$$

where AUMC$_{0-\infty}$ was the area under the first moment of the plasma concentration-time curve from time zero to infinity:

$$\text{AUMC}_{0-\infty} = \text{AUMC}_{0\text{-last}} + \frac{C_{\text{last}}}{\lambda^2} + \frac{C_{\text{last}} \cdot t_{\text{last}}}{\lambda}$$

In vivo hepatic extraction ratio (ER$_{\text{vivo}}$) was calculated from CL$_{\text{blood}}$ divided by the $Q_b$ values. The oral bioavailability (F$_{\text{oral}}$) was calculated from the following equation:

$$F_{\text{oral}} = \left(\frac{\text{AUC}_{\text{po}}}{\text{AUC}_{\text{iv}}}\right) \times \left(\frac{\text{Dose}_{\text{iv}}}{\text{Dose}_{\text{po}}}\right)$$

where AUC$_{\text{iv}}$ and AUC$_{\text{po}}$ are AUC estimates after the intravenous and oral administration, respectively, and Dose$_{\text{iv}}$ and Dose$_{\text{po}}$ are the administered dose for the intravenous and oral administration, respectively.

**Allometric Scaling Approach**

Allometric scaling assumes that PK parameters (Y) such as CL$_{\text{blood}}$ and $V_{ss}$ are correlated with their corresponding mean body weights ($W$, kg) by the allometric equation (Boxenbaum, 1984; Mordenti, 1986):

$$Y = a \cdot W^b$$
where \( a \) and \( b \) are allometric coefficient and exponent, respectively.

The values of the allometric coefficients \( (a) \) and exponent \( (b) \) were estimated by linear least square regression of the log transformed allometric equation 
\[
\log Y = \log a + b \log W
\]
To improve the predictive performance of allometric scaling for \( \text{CL}_{\text{blood}} \), Mahmood and Balian (1996) evaluated three different allometric scaling methods: 1) simple allometry \( (\text{CL}_{\text{blood}} = a \cdot W^b) \), 2) product of \( \text{CL}_{\text{blood}} \) and maximum life span potential (MLP) versus \( W \) \( (\text{CL}_{\text{blood}} \times \text{MLP} = a \cdot W^b) \), and 3) product of \( \text{CL}_{\text{blood}} \) and brain weights (BRW) versus \( W \) \( (\text{CL}_{\text{blood}} \times \text{BRW} = a \cdot W^b) \). They proposed the selection of one of these methods based upon the exponents of simple allometric scaling (the rule of exponent): 1) if the exponent of the simple allometry lies between 0.55 to 0.70, simple allometry will predict \( \text{CL}_{\text{blood}} \) more accurately than \( \text{CL}_{\text{blood}} \times \text{MLP} \) or \( \text{CL}_{\text{blood}} \times \text{BRW} \), 2) if the exponent of the simple allometry lies between 0.71 to 1.0, the \( \text{CL}_{\text{blood}} \times \text{MLP} \) approach will predict \( \text{CL}_{\text{blood}} \) better than simple allometry or \( \text{CL}_{\text{blood}} \times \text{BRW} \), and 3) if the exponent of the simple allometry is \( \geq 1.0 \), the \( \text{CL}_{\text{blood}} \times \text{BRW} \) approach is suitable to predict \( \text{CL}_{\text{blood}} \) in humans compared to the other two methods. Allometric scaling methods with the rule of exponent were also evaluated in the present study. Furthermore, as protein binding differences are known to affect PK comparisons across species and PK predictability in humans, the in vivo intrinsic clearance (\( \text{CL}_{\text{int,vivo}} \)) calculated from \( \text{CL}_{\text{blood}} \) by the well-stirred model equation was also used for the allometric scaling. Collectively four different allometric scaling methods for CL prediction were performed:

1) ALS-CL: \( \text{CL}_{\text{blood}} \) versus \( W \) (i.e., \( \text{CL}_{\text{blood}} = a \cdot W^b \))

2) ALS-MLP: product of \( \text{CL}_{\text{blood}} \) and MLP versus \( W \) (i.e., \( \text{CL}_{\text{blood}} \times \text{MLP} = a \cdot W^b \))

3) ALS-BRW: product of \( \text{CL}_{\text{blood}} \) and BRW versus \( W \) (i.e., \( \text{CL}_{\text{blood}} \times \text{BRW} = a \cdot W^b \))
Likewise, two difference allometric scaling methods were performed to predict

\( V_{ss} \) in humans:

1) ALS-\( V_{ss} \): \( V_{ss} \) versus \( W \) (i.e., \( V_{ss} = a \cdot W^b \))

2) ALS-\( V_{ss,u} \): unbound volume of distribution at steady-state (\( V_{ss,u} = V_{ss} / f_{u,plasma} \)) versus \( W \) (i.e., \( V_{ss,u} = a \cdot W^b \))

To predict plasma concentration-time profiles of PF02341066 and PF04217903 in humans based on allometric scaling, a standard one-compartment PK model with a first-order absorption rate was utilized with NONMEM version V (University of California at San Francisco, San Francisco CA). As inputs of the compartment PK model, the following 2 different sets of the predicted \( CL_{plasma} \) and \( V_{ss} \) from allometric scaling were used:

1) ALS-total: \( CL_{plasma} \) from ALS-CL and \( V_{ss} \) from ALS-\( V_{ss} \)

2) ALS-free: \( CL_{plasma} \) from ALS-CL\(_{int}\) and \( V_{ss} \) from ALS-\( V_{ss,u} \)

The absorption rate constants (\( k_a \)) of both the compounds were estimated in all preclinical species by the compartment PK analysis with NONMEM and the mean \( k_a \) values (0.30 h\(^{-1}\) for PF2341066 and 0.69 h\(^{-1}\) for PF04217903) in preclinical species tested were used as inputs of the one-compartment PK model: the estimates of \( k_a = 0.24, 0.38 \) and 0.28 h\(^{-1}\) in rats, dogs and monkeys, respectively, for PF02341066 and 0.76 and 0.61 h\(^{-1}\) in rats and dogs, respectively, for PF04217903.

**PBPK Modeling**

The GastroPlus 5.3 PBPK model and its built-in mass balance differential equations were used for all simulations in preclinical species and humans (Simulations
Plus Inc., Lancaster, CA). Briefly, the PBPK model was composed of 14 tissue compartments, including lung, spleen, liver, gut, adipose tissue, muscle, heart, brain, kidney, skin, testes, bone and rest of the body, which were linked by the venous and arterial blood circulation. It was assumed that drug distributes instantaneously and homogenously within each tissue compartment, and uptake of drug within each tissue compartment was limited by the blood flow (perfusion rate-limited uptake). All physiological parameters for tissue volume and blood flows data are summarized in Supplemental Data, Table 1. To predict the rate and extent of oral absorption of PF02341066 and PF04217903, the Advanced Compartment Absorption and Transit model (ACAT) within GastroPlus was used (Agoram et al., 2001). The ACAT model is a semi-physiologically-based transit model consisting of nine compartments corresponding to different segments of the gastro-intestinal tracts. The generic LogD model was used for the simulation of oral absorption of PF02341066 and PF04217903 in all species tested.

For the PBPK modeling in preclinical species, the observed in vivo CL_{blood} values obtained from a single intravenous administration of PF02341066 and PF04217903 were used as the CL_{hep} inputs on the PBPK modeling. As CL_{hep} inputs on the PBPK modeling in humans, CL_{hep} values predicted by the IVIVE using WS-II and the allometric scaling by ALS-CL_{int} were used as representative PBPK modeling. Renal clearances of both PF02341066 and PF04217903 in humans were set to be null for the PBPK modeling based on preclinical data (≤1% of the administered dose as parent drug in urine for both the compounds after the intravenous dose). This assumption was further supported by clinical data (≤2% of the dose in urine for both the compounds after the oral dose). Tissue-to-plasma partition coefficients (K_P) for each tissue compartment were predicted
from physicochemical parameters of each compound using the following equation developed by Poulin and Theil (2002) and corrected by Berezhkovskiy (2004).

**Assessment of Prediction Accuracy**

The accuracy of prediction was assessed from the prediction error (difference between predicted and observed values) for each PK parameter:

\[
\text{Fold error} = \frac{\text{PRED}}{\text{OBS}}, \text{ if PRED} > \text{OBS}
\]

\[
\text{Fold error} = \frac{\text{OBS}}{\text{PRED}}, \text{ if OBS} > \text{PRED}
\]

where PRED and OBS denote the predicted and observed values, respectively.
RESULTS

In Vivo Non-compartment PK in Preclinical Species and Humans

After a single intravenous administration of PF02341066 at the dose of 5 mg/kg to preclinical species, CL\textsubscript{plasma} values were estimated to be 29, 9.0 and 34 mL/min/kg in rats, dogs and monkeys (Table 1), respectively, demonstrating that PF02341066 was a moderate to high clearance compound in preclinical species. Estimate of V\textsubscript{ss} in preclinical species was 13 L/kg, suggesting extensive distribution into tissues. The estimated apparent t\textsubscript{1/2} values of PF02341066 were 5.5 to 17 hours. After a single oral administration of PF02341066 at the dose of 25 mg/kg to preclinical species, PF02341066 was moderately absorbed with C\textsubscript{max} values of 0.24 to 0.62 µg/mL at 4 to 6 hours post-dose. The estimated oral t\textsubscript{1/2} values (7.0 to 12 hours) were comparable to those for the intravenous administration (5.5 to 17 hours). F\textsubscript{oral} was estimated to be 42 to 65% across species. After oral administration of PF02341066 at the dose of 100 mg to patients, PF02341066 was moderately absorbed with a mean C\textsubscript{max} of 0.061 µg/mL at 2.5 hours post-dose (Table 1). Thereafter PF02341066 declined multi-exponentially with an apparent mean t\textsubscript{1/2} of 10 hours over the dosing interval of 24 hours.

After a single intravenous administration of PF04217903 at the dose of 2.5 mg/kg in preclinical species, CL\textsubscript{plasma} values were estimated to be 8.2 and 12 mL/min/kg in rats and dogs (Table 1), respectively, demonstrating that PF04217903 was a low to moderate clearance compound in preclinical species. Estimates of V\textsubscript{ss} in rats and dogs were 1.6 and 3.7 L/kg, respectively, suggesting moderate distribution into tissues. The estimated apparent t\textsubscript{1/2} values were 3.7 and 5.2 hours in rats and dogs, respectively. After a single oral administration of PF04217903 at the dose of 10 mg/kg to preclinical species,
PF04217903 was rapidly absorbed in rats and dogs with C_max values of 2.4 to 2.7 µg/mL at 0.8 to 2 hours post-dose. The estimated oral t_1/2 values (3.6 to 5.1 hours) were comparable to those for the intravenous administration (3.7 to 5.2 hours). F_oral was estimated to be approximately 70% and 100% in rats and dogs, respectively. After a single oral administration of PF04217903 at the dose of 30 mg to healthy volunteers, PF04217903 was rapidly absorbed with a mean C_max of 0.24 µg/mL at 1.2 hours post-dose (Table 1). Thereafter PF04217903 declined multi-exponentially with an apparent mean t_1/2 of 6.6 hours.

**In Vitro-In Vivo Extrapolation**

In vitro binding and blood partition data of PF02341066 and PF04217903 are summarized in Table 2. PF02341066 showed moderate to high in vitro bindings (f_u,blood = 0.051 to 0.092; f_u,vitro = 0.091 to 0.151) while PF04217903 showed low to moderate in vitro bindings (f_u,blood = 0.183 to 0.423; f_u,vitro = 0.780 to 0.843). The estimated R_bp values of PF02341066 and PF04217903 were approximately unity across species (0.850 to 1.37 and 0.806 to 0.980, respectively).

The estimated in vitro CL_hep values of PF02341066 and PF04217903 in liver microsomes and hepatocytes are summarized in Table 3. The in vitro CL_hep values of PF02341066 were 2 to 3-fold higher in microsomes than hepatocytes across species including humans. The CL_hep values of PF02341066 calculated from WS-I were slightly lower (<2-fold) than those values by WS-II since the f_u,blood values were approximately 2-fold lower than f_u,vitro values. When the in vitro CL_hep values of PF02341066 were compared to the in vivo CL_blood in preclinical species, the estimated CL_hep values in microsomes by WS-I and WS-II were reasonably consistent in rats and monkeys.
whereas those in dogs were >2-fold higher than the in vivo CL_{blood} value. The estimated CL_{hep} values of PF02341066 in hepatocytes by WS-I and WS-II were 2 to 4-fold lower than the in vivo CL_{blood} in rats and 1.3 to 2.5-fold lower than the CL_{blood} in monkeys. In contrast, the estimated CL_{hep} values in dog hepatocytes by WS-I and WS-II were reasonably consistent (<2-fold) with the CL_{blood} value. Overall the IVIVE for PF02341066 using both WS-I and WS-II provided reasonable predictions in rat microsomes, dog hepatocytes and monkey microsomes compared to rat hepatocytes, dog microsomes and monkey hepatocytes, respectively.

In contrast to PF02341066, the f_{u,blood} values of PF04217903 were 3 to 4-fold lower than f_{u,vitro} values (Table 2). As a result, the CL_{hep} values from WS-I were 1.5 to 4-fold lower than those from WS-II while the estimated CL_{hep} values were comparable between microsomes and hepatocytes. The estimated CL_{hep} values of PF04217903 in rat microsomes and hepatocytes by WS-I were reasonably consistent (<2-fold) with in vivo CL_{blood} whereas those in rat microsomes and hepatocytes by WS-II were 2 to 3-fold higher than in vivo CL_{blood}. In dog microsomes and hepatocytes, the estimated CL_{hep} values by WS-II were more consistent (<1.2-fold) to the in vivo CL_{blood} than those by WS-I. Overall a better IVIVE of PF04217903 was observed in rats using WS-I whereas WS-II provided a better IVIVE in dogs than WS-I, disregarding whether microsomes or hepatocytes were used. Collectively there was no consistent IVIVE method for PF02341066 and PF04217903 in preclinical species to provide a better projection of in vivo CL_{blood} from one in vitro system (i.e., microsomes versus hepatocytes) and/or one mathematical model (i.e., WS-I versus WS-II).
Allometric Scaling for CL and $V_{ss}$

To predict $C_{L\text{blood}}$ of PF02341066 and PF04217903 in humans, 4 different allometric scaling approaches were performed, i.e., ALS-CL, ALS-MLP, ALS-BRW and ALS-CL$_{int}$. Allometric parameters of PF02341066 and PF04217903 are summarized in Table 4. Predicted $C_{L\text{blood}}$ of PF02341066 by ALS-CL was 8.0 mL/min/kg whereas that by ALS-CL$_{int}$ was 14 mL/min/kg. The difference in the predicted $C_{L\text{blood}}$ values between ALS-CL and ALS-CL$_{int}$ largely resulted from the difference in $f_{u,\text{blood}}$ across species. Predicted $C_{L\text{blood}}$ values of PF02341066 in humans by ALS-MLP and ALS-BRW were 3.9 and 2.6 mL/min/kg, respectively. When the rule of exponent (Mahmood and Balian, 1996) was applied, the predicted $C_{L\text{blood}}$ was 3.9 mL/min/kg from ALS-MLP based upon the allometric exponent of 0.729 on ALS-CL.

The $C_{L\text{blood}}$ values of PF04217903 in humans were predicted to be 22 and 7.6 mL/min/kg by ALS-CL and ALS-CL$_{int}$, respectively. Thus the predicted $C_{L\text{blood}}$ by ALS-CL was approximately 3-fold higher than that by ALS-CL$_{int}$ due to the difference in $f_{u,\text{blood}}$ across species. Predicted $C_{L\text{blood}}$ values of PF04217903 in humans by ALS-MLP and ALS-BRW were 7.8 and 4.3 mL/min/kg, respectively. When the rule of exponent was applied, the predicted $C_{L\text{blood}}$ was 4.3 mL/min/kg from ALS-BRW since the allometric exponent was 1.17 on ALS-CL.

To predict $V_{ss}$ of PF02341066 and PF04217903 in humans, 2 different allometric scaling approaches were performed, i.e., ALS-$V_{ss}$ and ALS-$V_{ss,u}$. Allometric parameters of PF02341066 and PF04217903 by ALS-$V_{ss}$ and ALS-$V_{ss,u}$ are summarized in Table 4. The $V_{ss}$ values of PF02341066 in humans were predicted to be 13 and 25 L/kg by ALS-$V_{ss}$ and ALS-$V_{ss,u}$, respectively. The difference in the predicted $V_{ss}$ values largely
resulted from the difference in $f_{u,\text{blood}}$ across species. The allometric exponents and correlation coefficients were close to unity on the both methods. The predicted $V_{ss}$ values of PF04217903 in humans by ALS-$V_{ss}$ and ALS-$V_{ss,u}$ were 6.3 and 2.2 L/kg respectively, with the allometric exponents of 1.25 and 1.09, respectively. Likewise, the difference in the predicted $V_{ss}$ values largely resulted from the difference in $f_{u,\text{blood}}$ across species.

**Prediction of Plasma Concentration-time Profiles of PF02341066 and PF04217903 in Humans based on Allometric Scaling Approach**

Plasma concentration-time profiles of PF02341066 and PF04217903 in humans were predicted by a one-compartment PK model using the 2 different sets of predicted $\text{CL}_{\text{plasma}}$ and $V_{ss}$ values from the allometric scaling methods, i.e., ALS-total and ALS-free (Fig. 2). The mean $k_a$ values of PF02341066 and PF04217903 (0.30 and 0.69 h$^{-1}$, respectively) estimated from preclinical species were used for the one-compartment modeling. The simulated PK parameters of PF02341066 and PF04217903 are summarized in Table 5 with the prediction accuracy, i.e., the fold error of the observed versus model-simulated PK parameters.

The predicted AUC value of PF02341066 by ALS-total was approximately 4-fold higher than the observed value although the $C_{\text{max}}$ value was predicted well with only 1.1-fold error. The over-prediction of AUC value was largely due to the over-prediction of $t_{1/2}$ (1.8-fold). The predicted AUC value with 4-fold error does not appear to fall in an acceptable range of the current industry criteria, i.e., 2-fold (Ward and Smith, 2004; Jones et al., 2006; Beaumont and Smith, 2009). In contrast, the fold errors for the AUC, $C_{\text{max}}$ and $t_{1/2}$ based on ALS-free were 1.8, 1.8 and 1.6, respectively. These predicted PK
parameters might be therefore within the acceptable range (<2-fold). However the predicted one-compartment plasma concentration-time profile of PF02341066 appeared to be considerably different from the observed profile, which showed multi-exponential elimination (Fig. 2A).

For the one-compartment PK modeling of PF04217903 in humans, the predicted $C_{\text{max}}$, AUC and $t_{1/2}$ values from ALS-total were under-predicted by approximately 4.0-, 1.8- and 2.0-fold, respectively (Table 5) whereas plasma concentration-time profiles were reasonably predicted after 6 h post-dose (Fig. 2B). In contrast, the predicted $C_{\text{max}}$ and AUC values from ALS-free were over-predicted by 1.3- and 1.9-fold, respectively, whereas the predicted $t_{1/2}$ value was significantly under-predicted by 3.4-fold.

**PBPK Modeling of PF02341066 and PF04217903 in Preclinical Species and Humans**

The predicted $K_p$ values and the resulting $V_{ss}$ values of PF02341066 and PF04217903 in rats, dogs and humans are summarized in Supplemental Data, Table 2. PF02341066 showed large $K_p$ values in adipose tissues (42 to 63 in rats, dogs and humans) compared to the other tissues, which appeared to be largely due to its high lipophilicity. The $K_p$ values of PF04217903 were relatively consistent across all tissues. The predicted $V_{ss}$ values of PF02341066 in rats (13 L/kg) and dogs (14 L/kg) were consistent with the observed values (13 L/kg) in rats and dogs. The $V_{ss}$ value of PF02341066 in humans was predicted to be 19 L/kg. Likewise, the predicted $V_{ss}$ values of PF04217903 in rats (2.4 L/kg) and dogs (3.1 L/kg) were reasonably consistent with the observed values in rats (1.6 L/kg) and dogs (3.7 L/kg). The predicted $V_{ss}$ of PF04217903 in humans was 2.9 L/kg. Thus the mathematically predicted $V_{ss}$ values of both
PF02341066 and PF04217903 were relatively consistent with the predicted values from allometric scaling (Table 4).

The observed and PBPK model-simulated plasma concentration-time profiles of PF02341066 and PF04217903 in preclinical species are graphically presented in Fig. 3 and Fig. 4, respectively. The $F_{\text{abs}}$ values of PF02341066 and PF04217903 estimated by the ACAT model were >90% in rats and dogs. Overall the PBPK modeling reasonably matched the plasma concentration-time profiles of both PF02341066 and PF04217903 in rats and dogs after single intravenous and oral administrations although there was a little discrepancy in the late phases (i.e., after 4 to 6 h post-dose) of the intravenous plasma concentration-time profiles of PF04217903 in rats and dogs. As tabulated in Supplemental Data, Table 3, the majority of PK parameters (i.e., $C_{\text{max}}$, $t_{\text{max}}$, AUC and $t_{1/2}$) calculated from the model-simulated plasma concentration-time profiles were within 2-fold of the observed values for both PF02341066 and PF04217903.

As CL inputs for the PBPK modeling in humans, the CL$_{\text{hep}}$ values from the IVIVE method, WS-II, and the allometric scaling, ALS-CL$_{\text{int}}$, were used as representative PBPK modeling. That is, the CL$_{\text{hep}}$ values of PF02341066 used for the PBPK modeling were 9.3 and 14 mL/min/kg, respectively, while those of PF04217903 were 3.6 and 7.6 mL/min/kg, respectively. The observed and PBPK model-simulated plasma concentration-time profiles of PF02341066 and PF04217903 in humans are graphically presented in Fig. 5. The $F_{\text{abs}}$ values of both PF02341066 and PF04217903 estimated by the ACAT model were >90% in humans. The PK parameters calculated from the model-simulated plasma concentration-time profiles of PF02341066 and PF04217903 are summarized in Table 5 with the prediction accuracy.
Using the predicted CL\textsubscript{blood} value from WS-II, the C\textsubscript{max} and AUC values of PF02341066 were over-predicted (2.6- and 1.9-fold, respectively) whereas the predicted t\textsubscript{1/2} (12 h) was comparable to the observed value (10 h). Using the predicted CL\textsubscript{blood} from ALS-CL\textsubscript{int}, the C\textsubscript{max} and AUC values of PF02341066 were reasonably consistent (<1.3-fold) with the observed values. The predicted t\textsubscript{1/2} (12 h) was also comparable to the observed value. Overall the predicted plasma concentration-time profiles of PF02341066 by the PBPK modeling were reasonably matched with the observed profiles (Fig. 5A).

Regarding the PBPK modeling of PF04217903 using the predicted CL\textsubscript{blood} value from WS-II, the C\textsubscript{max} value of PF04217903 was slightly under-predicted by 1.4-fold while the AUC value was over-predicted by 2.2-fold. The predicted t\textsubscript{1/2} (11 h) was 1.7-fold longer than the observed value (6.6 h). Using the predicted CL\textsubscript{blood} from ALS-CL\textsubscript{int}, the C\textsubscript{max} value was under-predicted by 2.3-fold while AUC value was relatively consistent (1.3-fold error). The predicted t\textsubscript{1/2} (6.3 h) was also consistent with the observed value. Thus the PBPK modeling of PF04217903 tends to under-predict C\textsubscript{max} values by approximately 2-fold whereas AUC and t\textsubscript{1/2} values were within an acceptable range of 2-fold. Overall the predicted plasma concentration-time profiles of PF04217903 by the PBPK modeling using the predicted CL\textsubscript{blood} from ALS-CL\textsubscript{int} were reasonably matched with the observed profiles (Fig. 5B).
DISCUSSION

The PBPK modeling is a mechanistic approach which allows the disposition of drug candidates to be predicted in different species including humans (Jones et al, 2006; De Buck et al, 2007; Lavé et al, 2007; Nestorov, 2007). The modeling is generally achieved by using physiological parameters (e.g., organ volume, blood flow, etc.) and drug-dependent parameter (e.g., permeability, Kp, CL, etc.). The recently developed mechanistic tissue composition-based equations could accurately predict Vss based on the physicochemical properties of each compound (Poulin and Theil, 2002; Berezhkovskiy, 2004; Rodgers et al., 2005; Rodgers and Rowland, 2006). These equations have greatly extended the applicability of the PBPK modeling to PK prediction (Jones et al, 2006; De Buck et al, 2007; Lavé et al, 2007; Nestorov, 2007). In contrast, CL as one of the most important inputs on the PBPK modeling is predicted by a variety of methods such as IVIVE, ALS, etc. Therefore the PBPK modeling has to rely on at least one of CL prediction methods. In general, animal data are used to verify which IVIVE method would be used for CL prediction in humans. However, it is often difficult to choose the best IVIVE method based on animal data. A number of investigators have reported hepatocytes to be a superior system for CL prediction, with microsomes also providing reasonable data (Houston and Carlile, 1997, Obach, 1999; Naritomi et al., 2003). Both microsomes and hepatocytes were employed in the present study to investigate IVIVE approach because oxidative biotransformation was considered to be the major clearance pathway since 1) PF02341066 and PF04217903 were mainly metabolized to oxidative metabolites across species with little qualitative species-differences in metabolite profiles (in house data) and 2) the contributions of urinary and
biliary excretions of PF02341066 (<1% and 15% of the dose as parent drug, respectively) and PF04217903 (<1% and 5% of the dose, respectively) to systemic clearance were minimal in preclinical species. The decision of whether to incorporate $f_{u,\text{blood}}$ and $f_{u,\text{vitr}}$ into IVVIE method appears to remain controversial (Obach, 1999; Riley et al., 2005; De Buck et al., 2007). The inclusion of both the unbound fractions (i.e., WS-I) has been generally suggested whereas several investigators reported that disregarding all bindings (i.e., WS-II) could provide the better CL predictions for some compound classes (Obach et al., 1997; Obach, 1999; De Buck et al., 2007). Since PF02341066 showed similar values of $f_{u,\text{blood}}$ and $f_{u,\text{vitr}}$, the $CL_{\text{hep}}$ values calculated by WS-I and WS-II were relatively comparable (Table 3). Overall the estimated $CL_{\text{hep}}$ values from rat and monkey microsomes were more consistent with the $CL_{\text{blood}}$ compared to respective hepatocytes, whereas the $CL_{\text{hep}}$ values from dog hepatocytes were more consistent with the $CL_{\text{blood}}$ than its microsomes. PF04217903 showed lower $f_{u,\text{blood}}$ compared to $f_{u,\text{vitr}}$, thus the calculated $CL_{\text{hep}}$ values by WS-I were 1.5 to 4-fold lower than those by WS-II. Disregarding whether microsomes or hepatocytes were used, the $CL_{\text{hep}}$ values of PF04217903 calculated from WS-I were more consistent with the $CL_{\text{blood}}$ than those from WS-II in rats whereas the opposite trend was observed in dogs, where the $CL_{\text{hep}}$ values from WS-II were more consistent with the $CL_{\text{blood}}$ in dogs than those from WS-I. Thus there was no consistent IVIVE method to provide a better projection of in vivo $CL_{\text{blood}}$ for each PF02341066 and PF04217903 from one in vitro system and/or one mathematical model. Since the best use of an IVIVE method depends upon knowing which in vitro metabolism system, which mathematical model and which animal species are the most
representatives in humans, it represents CL prediction of PF02341066 and PF04217903
in humans by the IVIVE methods is highly challenging.

While the IVIVE approach has a physiological basis with a theoretical
mathematic modeling compared to the empirical allometric scaling, studies comparing
the CL prediction by IVIVE and ALS have been minimal with a few controversial reports
(Obach et al., 1997; Mahmood, 2002; Shiran et al., 2006; Hosea et al., 2009). Therefore
there does not appear to be a clear answer which method is more reliable for PK
prediction in humans. Concerning ALS, Mahmood and Balian, (1996) proposed a rule of
exponents, which recommended judicious use of either MLP or BRW depending upon
ALS exponent. When the rule of exponent was applied to PF02341066, the predicted
CL_{blood} was 3.9 mL/min/kg by ALS-MLP (Table 4). The prediction accuracy with this
method was worse than those with ALS-CL and ALS-CL_{int}. Simple CL extrapolation
from monkeys to humans based on the difference in Q_b was reported by Jolivette and
Ward (2005). Predicted CL_{blood} by this method was 11 mL/min/kg, which was relatively
consistent with the predicted values from ALS-CL_{int} and IVIVE in microsomes. For
PF04217903, we could not evaluate an exponent of the ALS-CL because of a lack of the
3rd species. It has been reported that each species has a different influence on allometric
scaling (Lavé et al., 1995; Tang and Mayersohn, 2005). A species close to the center of
the regression, e.g., monkeys between rats and dogs, has less influence on the prediction
because its data point lies close to the center of the regression. On the other hand, the
importance of monkey data for CL prediction was reported (Jolivette and Ward, 2005;
Goteti et al., 2008). Currently it’s not clear how monkey data make an impact on CL
prediction of PF04217903. Several investigators (Obach et al., 1997; Chiou et al., 1998;
Hosea et al, 2009) reported that ALS including inter-species difference in $f_{u,blood}$ was more reliable than simple ALS. The current study result appears to be consistent with these reports: ALS-CL\textsubscript{int} provided the most accurate CL inputs of the PBPK modeling for both PF02341066 and PF04217903.

The allometric exponents for $V_{ss}$ and $V_{ss,u}$ of PF02341066 and PF04217903 were close to unity (Table 4), suggesting that the $V_{ss}$ values of these compounds were linearly related to their body weights in preclinical species. As both PF02341066 and PF04217903 showed approximately 2-fold difference in $f_{u,blood}$ across species including humans (Table 2), the inclusion of protein binding correction in ALS for $V_{ss}$ resulted in 2 to 3-fold differences in the predicted $V_{ss}$ values for both compounds (Table 4). These differences largely affected the plasma concentration-time profile simulation. The predicted AUC values of PF02341066 by ALS-total were approximately 4-fold higher than the observed values. The simulated AUC and $C_{max}$ values of PF04217903 by ALS-total were 2 to 4-fold lower than the observed values. In contrast, the simulated AUC and $C_{max}$ values of PF02341066 and PF04217903 by ALS-free were within 2-fold of the observed values, demonstrating that the overall prediction accuracy by ALS could be better for both compounds when the species-difference in $f_{u,blood}$ was corrected. When the simulated plasma concentration-time profiles were compared between the PBPK modeling with CL from ALS-CL\textsubscript{int} versus the one-compartment PK modeling based on ALS-free, the PBPK modeling provided better AUC prediction for both PF02341066 (1.2- vs 1.8-fold error) and PF04217903 (1.3- vs 1.9-fold error) (Table 5). More importantly, the simulated plasma concentration-time profiles of PF02341066 and PF04217903 by the PBPK modeling appeared to be relatively consistent with the
observed profiles showing multi-exponential declines (Fig. 5), resulting in comparable apparent \( t_{1/2} \) values for both the compounds: the observed and predicted \( t_{1/2} \) values were respective 10 and 12 h for PF02341066 and 6.6 and 6.3 h for PF04217903. The predicted \( t_{1/2} \) values by the one-compartment PK model were 17 h for PF02341066 and 1.9 h for PF04217903. Therefore, the PBPK modeling has the potential to be more useful and reliable for the PK prediction of PF02341066 and PF04217903 compared to the traditional one-compartment PK modeling.

In summary, the present study has shown examples that PBPK modeling can be used to predict PK profiles in humans. Since the blood flows and tissue volumes are drug-independent components of the PBPK modeling, the success of in vivo PK prediction appears to largely depend upon the quality of the prediction accuracy of in vivo CL, which most likely varies from compound to compound. This will be key to a successful application of PBPK modeling. As presented in this paper, ADME scientists generally generate large amounts of preclinical in vitro and in vivo data for clinical drug candidates to predict PK parameters, especially CL and \( V_{ss} \), in humans. However ADME scientists are facing the dilemma of how to use the preclinical information in an appropriate manner to prospectively predict in vivo PK in humans (Beaumont and Smith, 2009). Furthermore, prediction accuracies of PK parameters such as CL, \( V_{ss} \) and \( F_{oral} \) for most of oral drugs are actually never evaluated because of a lack of intravenous PK data in humans, which is another dilemma ADME scientists are facing. For this gap, ADME scientists have started focusing on the prediction of plasma concentration-time profiles of clinical drug candidates by the PBPK modeling. The prediction of plasma concentration-time profiles is also in line with the current focus on translational
pharmacology, where the plasma concentration-time profiles of clinical candidates are seen as the first surrogate markers of pharmacological effects.
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Authorship Contribution

Participated in research design: Yamazaki and Koudriakova

Conduct experiments: Skaptason, Romero and Vekich

Performed data analysis: Yamazaki, Skaptason and Romero

Wrote or contribute to the writing of the manuscript: Yamazaki, Skaptason, Vekich, Jones and Koudriakova

Others: Tan and Wilner provided clinical PK data
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Footnotes

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Legends for Figures

Fig. 1. Chemical Structures of PF02341066 [(R)-3-[(2,6-Dichloro-3-fluoro-phenyl)ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine] and PF04217903 [2-[4-(3-quinolin-6-ylmethyl-3H-[1,2,3]triazolo[4,5-b]pyrazin-5-yl)-pyrazol-1-yl]-ethanol].

Fig. 2. Observed and one-compartment pharmacokinetic model-simulated plasma concentration-time profiles of PF02341066 (A) and PF04217903 (B) in humans after oral administration. Observed plasma concentration-time profiles (OBS) were obtained from 4 patients after oral administration of PF02341066 at 100 mg or from 6 healthy volunteers after oral administration of PF04217903 at 30 mg. Model-simulated concentrations were predicted by the one-compartment pharmacokinetic model using the 2 different sets of the predicted CL<sub>plasma</sub> and V<sub>ss</sub> from the allometric scaling (ALS), i.e., ALS-total (---) and ALS-free (−−)
Fig. 3. Observed and physiologically based pharmacokinetic model-simulated plasma concentration-time profiles of PF02341066 in rats (A and B) and dogs (C and D) after a single intravenous (A and C) or oral (B and D) administration.

Observed plasma concentration-time profiles were obtained from rats and dogs after a single intravenous and oral administration of PF02341066 at 5 and 25 mg/kg, respectively (n= 3 per group). OBS, observed plasma concentration-time profiles; PBPK, physiologically based pharmacokinetic model-simulated plasma concentration-time profiles.

Fig. 4. Observed and physiologically based pharmacokinetic model-simulated plasma concentration-time profiles of PF04217903 in rats (A and B) and dogs (C and D) after a single intravenous (A and C) or oral (B and D) administration.

Observed plasma concentration-time profiles were obtained from rats and dogs after a single intravenous and oral administration of PF04217903 at 2.5 and 10 mg/kg, respectively (n= 2 per group). OBS, observed plasma concentration-time profiles; PBPK, physiologically based pharmacokinetic model-simulated plasma concentration-time profiles.
Fig. 5. Observed and physiologically based pharmacokinetic model-simulated plasma concentration-time profiles of PF02341066 (A) and PF04217903 (B) in humans after oral administration. Observed plasma concentration-time profiles (OBS) were obtained from 4 patients after oral administration of PF02341066 at 100 mg or from 6 healthy volunteers after oral administration of PF04217903 at 30 mg. Model-simulated concentrations were predicted by the physiologically based pharmacokinetic modeling (PBPK) using the 2 different predicted CL_{hep} values from the IVIVE-WS-II (---) and ALS-CL_{int} (—).
### Table 1

**Pharmacokinetic parameters of PF02341066 and PF04217903 in preclinical species and humans after intravenous or oral administration**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Dosing route</th>
<th>Dose (mg/kg)</th>
<th>$CL_{plasma}$ (mL/min/kg)</th>
<th>$V_{ss}$ (L/kg)</th>
<th>$C_{max}$ (µg/mL)</th>
<th>$t_{max}$ (h)</th>
<th>$AUC_{0-\infty}$ (µg·h/mL)</th>
<th>$t_{1/2}$ (h)</th>
<th>$F_{oral}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF02341066</td>
<td>Rat</td>
<td>IV</td>
<td>5</td>
<td>29 ± 8</td>
<td>13 ± 4</td>
<td>−</td>
<td>−</td>
<td>3.0 ± 0.9</td>
<td>7.7 ± 1.8</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td></td>
<td>25</td>
<td>−</td>
<td>−</td>
<td>0.53 ± 0.10</td>
<td>4.7 ± 1.2</td>
<td>5.6 ± 0.8</td>
<td>7.0 ± 0.4</td>
<td>63</td>
</tr>
<tr>
<td>PF02341066</td>
<td>Dog</td>
<td>IV</td>
<td>5</td>
<td>9.0 ± 0.8</td>
<td>13 ± 2</td>
<td>−</td>
<td>−</td>
<td>9.3 ± 0.8</td>
<td>17 ± 4</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td></td>
<td>25</td>
<td>−</td>
<td>−</td>
<td>0.62 ± 0.37</td>
<td>4.0 ± 2.0</td>
<td>12 ± 8</td>
<td>12 ± 3</td>
<td>65</td>
</tr>
<tr>
<td>PF02341066</td>
<td>Monkey</td>
<td>IV</td>
<td>5</td>
<td>34 ± 4</td>
<td>13 ± 1</td>
<td>−</td>
<td>−</td>
<td>2.5 ± 0.3</td>
<td>5.5 ± 0.2</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td></td>
<td>25</td>
<td>−</td>
<td>−</td>
<td>0.24 ± 0.11</td>
<td>6 ± 0</td>
<td>4.1 ± 1.7</td>
<td>12 ± 3</td>
<td>42</td>
</tr>
<tr>
<td>PF02341066</td>
<td>Human</td>
<td>PO</td>
<td>100</td>
<td>−</td>
<td>−</td>
<td>0.061 ± 0.034</td>
<td>2.5 ± 1.7</td>
<td>0.60 ± 0.22</td>
<td>10 ± 2</td>
<td>−</td>
</tr>
<tr>
<td>PF04217903</td>
<td>Rat</td>
<td>IV</td>
<td>2.5</td>
<td>8.2 ± 0.5</td>
<td>1.6 ± 0.1</td>
<td>−</td>
<td>−</td>
<td>5.1 ± 0.3</td>
<td>3.7 ± 0.0</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td></td>
<td>10</td>
<td>−</td>
<td>−</td>
<td>2.7 ± 0.2</td>
<td>0.8 ± 0.4</td>
<td>15 ± 2</td>
<td>3.6 ± 0.2</td>
<td>71</td>
</tr>
<tr>
<td>PF04217903</td>
<td>Dog</td>
<td>IV</td>
<td>2.5</td>
<td>12 ± 2</td>
<td>3.7 ± 0.7</td>
<td>−</td>
<td>−</td>
<td>3.4 ± 0.6</td>
<td>5.2 ± 0.3</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>PO</td>
<td></td>
<td>10</td>
<td>−</td>
<td>−</td>
<td>2.4 ± 0.8</td>
<td>2 ± 0</td>
<td>22 ± 1</td>
<td>5.1 ± 2.1</td>
<td>100</td>
</tr>
<tr>
<td>PF04217903</td>
<td>Human</td>
<td>PO</td>
<td>30</td>
<td>−</td>
<td>−</td>
<td>0.24 ± 0.14</td>
<td>1.2 ± 0.7</td>
<td>0.81 ± 0.20</td>
<td>6.6 ± 1.3</td>
<td>−</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. (n = 3 animals or 4 patients per group for PF02341066; n = 2 animals or 6 healthy volunteers per group for PF04217903). −: not applicable.
Table 2
In vitro binding data of PF02341066 and PF04217903 across species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>(f_{u,\text{plasma}})</th>
<th>(R_{BP})</th>
<th>(f_{u,\text{blood}})</th>
<th>(f_{u,\text{microsomes}})</th>
<th>(f_{u,\text{hepatocytes}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF02341066</td>
<td>Rat</td>
<td>0.057</td>
<td>0.880</td>
<td>0.065</td>
<td>0.116</td>
<td>0.144</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>0.043</td>
<td>0.850</td>
<td>0.051</td>
<td>0.091</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>Monkey</td>
<td>0.072</td>
<td>1.37</td>
<td>0.053</td>
<td>0.103</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>0.093</td>
<td>1.01</td>
<td>0.092</td>
<td>0.122</td>
<td>0.151</td>
</tr>
<tr>
<td>PF04217903</td>
<td>Rat</td>
<td>0.197</td>
<td>0.980</td>
<td>0.201</td>
<td>0.808</td>
<td>0.843</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>0.341</td>
<td>0.806</td>
<td>0.423</td>
<td>0.780</td>
<td>0.819</td>
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<tr>
<td></td>
<td>Human</td>
<td>0.164</td>
<td>0.897</td>
<td>0.183</td>
<td>0.792</td>
<td>0.830</td>
</tr>
</tbody>
</table>

Data represent the mean of triplicate determination. Unbound fraction in plasma \((f_{u,\text{plasma}})\), blood-to-plasma concentration ratio \((R_{BP})\) and microsomes \((f_{u,\text{microsomes}})\) were experimentally determined. Unbound fraction in whole blood \((f_{u,\text{blood}})\) and hepatocytes \((f_{u,\text{hepatocytes}})\) were calculated by the equations described in the section of Materials and Methods.
Table 3

*In vivo blood clearance and in vitro hepatic clearance of PF02341066 and PF04217903 across species*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>CL_{blood} mL/min/kg</th>
<th>CL_{hep} mL/min/kg</th>
<th>ER</th>
<th>CL_{hep}' mL/min/kg</th>
<th>ER'</th>
<th>CL_{hep} mL/min/kg</th>
<th>ER</th>
<th>CL_{hep}' mL/min/kg</th>
<th>ER'</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF02341066</td>
<td>Rat</td>
<td>33</td>
<td>28</td>
<td>0.39</td>
<td>38</td>
<td>0.54</td>
<td>8.3</td>
<td>0.12</td>
<td>16</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>11</td>
<td>24</td>
<td>0.59</td>
<td>29</td>
<td>0.72</td>
<td>7.2</td>
<td>0.18</td>
<td>13</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Monkey</td>
<td>25</td>
<td>29</td>
<td>0.67</td>
<td>35</td>
<td>0.80</td>
<td>10</td>
<td>0.23</td>
<td>19</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>nd</td>
<td>7.9</td>
<td>0.40</td>
<td>9.3</td>
<td>0.46</td>
<td>3.0</td>
<td>0.15</td>
<td>4.5</td>
<td>0.22</td>
</tr>
<tr>
<td>PF04217903</td>
<td>Rat</td>
<td>8.3</td>
<td>7.3</td>
<td>0.10</td>
<td>22</td>
<td>0.32</td>
<td>5.2</td>
<td>0.07</td>
<td>18</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>15</td>
<td>8.6</td>
<td>0.22</td>
<td>13</td>
<td>0.34</td>
<td>9.9</td>
<td>0.25</td>
<td>16</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>nd</td>
<td>0.97</td>
<td>0.05</td>
<td>3.6</td>
<td>0.18</td>
<td>1.2</td>
<td>0.06</td>
<td>4.3</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data represent the mean of triplicate determination. In vivo blood clearance (CL_{blood}) was calculated from plasma clearance divided by blood-to-plasma concentration ratio. In vitro hepatic clearances were calculated by the well-stirred models (WS-I and WS-II) with and without all bindings (CL_{hep} and CL_{hep}', respectively). In vitro hepatic extraction ratios (ER and ER') were calculated by hepatic clearances (CL_{hep} and CL_{hep}', respectively) divided by hepatic blood flow.
Table 4
Predicted blood clearance and volume of distribution at steady-state of PF02341066 and PF04217903 in humans by different allometric scaling methods

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter</th>
<th>ALS-CL</th>
<th>ALS-MLP</th>
<th>ALS-BRW</th>
<th>ALS-CLint</th>
<th>ALS-Vss</th>
<th>ALS-Vss,u</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF02341066</td>
<td>CL-blood or Vss</td>
<td>8.0</td>
<td>3.9</td>
<td>2.6</td>
<td>14</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.729</td>
<td>1.12</td>
<td>1.69</td>
<td>0.813</td>
<td>0.991</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>0.961</td>
<td>0.933</td>
<td>0.951</td>
<td>0.713</td>
<td>1.00</td>
<td>0.987</td>
</tr>
<tr>
<td>PF04217903</td>
<td>CL-blood or Vss</td>
<td>22</td>
<td>7.8</td>
<td>4.3</td>
<td>7.6</td>
<td>6.3</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>1.17</td>
<td>1.52</td>
<td>2.07</td>
<td>1.06</td>
<td>1.25</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>r²</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

CL<sub>blood</sub>, blood clearance (mL/min/kg); V<sub>ss</sub>, volume of distribution at steady-state (L/kg); slope: allometric exponent; r², correlation coefficient; ALS-CL, simple allometry based on total blood clearance; ALS-MLP, allometry corrected with maximum life-span potential; ALS-BRW, allometry corrected with brain weights; ALS-CL<sub>int</sub>, simple allometry based on in vivo intrinsic clearance; slope: allometric exponent; r², correlation coefficient; ALS-V<sub>ss</sub>, simple allometry for V<sub>ss</sub>, ALS-V<sub>ss,u</sub>, simple allometry for V<sub>ss,u</sub>. 
Table 5

*Simulated pharmacokinetic parameters of PF02341066 and PF04217903 in humans after oral administration of PF02341066 and PF04217903 at 100 and 30 mg, respectively*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Simulation Method</th>
<th>$C_{\text{max}}$</th>
<th>$t_{\text{max}}$</th>
<th>$AUC_{0-\infty}$</th>
<th>$t_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/mL</td>
<td>h</td>
<td>µg·h/mL</td>
<td>h</td>
</tr>
<tr>
<td>PF02341066</td>
<td>OBS</td>
<td>0.061</td>
<td>2.5</td>
<td>0.60</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>ALS–total</td>
<td>0.067 (1.1)</td>
<td>6.0 (2.4)</td>
<td>2.2 (3.7)</td>
<td>18 (1.8)</td>
</tr>
<tr>
<td></td>
<td>ALS–free</td>
<td>0.034 (1.8)</td>
<td>5.8 (2.3)</td>
<td>1.1 (3.7)</td>
<td>17 (1.6)</td>
</tr>
<tr>
<td></td>
<td>PBPK– IVIVE</td>
<td>0.16 (2.6)</td>
<td>1.7 (1.5)</td>
<td>1.1 (1.9)</td>
<td>12 (1.2)</td>
</tr>
<tr>
<td></td>
<td>PBPK– ALS</td>
<td>0.081 (1.3)</td>
<td>1.6 (1.6)</td>
<td>0.48 (1.2)</td>
<td>12 (1.1)</td>
</tr>
<tr>
<td>PF04217903</td>
<td>OBS</td>
<td>0.24</td>
<td>1.2</td>
<td>0.81</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>ALS–total</td>
<td>0.060 (4.0)</td>
<td>2.1 (1.8)</td>
<td>0.44 (1.8)</td>
<td>3.2 (2.0)</td>
</tr>
<tr>
<td></td>
<td>ALS–free</td>
<td>0.31 (1.3)</td>
<td>1.7 (1.5)</td>
<td>1.6 (1.9)</td>
<td>1.9 (3.4)</td>
</tr>
<tr>
<td></td>
<td>PBPK– IVIVE</td>
<td>0.17 (1.4)</td>
<td>0.9 (1.3)</td>
<td>1.8 (2.2)</td>
<td>11 (1.7)</td>
</tr>
<tr>
<td></td>
<td>PBPK– ALS</td>
<td>0.10 (2.3)</td>
<td>0.8 (1.5)</td>
<td>0.64 (1.3)</td>
<td>6.3 (1.0)</td>
</tr>
</tbody>
</table>

The accuracy of prediction is expressed as fold error (the ratio of predicted to observed or observed to predicted values) in parenthesis.

OBS, observed mean PK parameters; ALS–total, simulated PK parameters by allometric scaling for CL_{blood} and $V_{ss}$; ALS–free, simulated PK parameters by allometric scaling for CL_{int} and $V_{ss,u}$; PBPK– IVIVE, simulated PK parameters by PBPK modeling using hepatic CL predicted from IVIVE (WS-II); PBPK– ALS, simulated PK parameters by PBPK modeling using hepatic CL predicted from allometric scaling for CL_{int}.
Figure 1
Figure 2A

Plasma concentration (μg/mL)

Time after dosing (h)

0 6 12 18 24

10^{-3} 10^{-2} 10^{-1} 10^0

OBS

ALS-total

ALS-free

(A)
Figure 2B

Time after dosing (h)

Plasma concentration (μg/mL)

OBS
ALS- total
ALS- free

0 6 12 18 24

10^{-1}
10^{-2}
10^{-3}
10^{-4}

Figure 3A

(A) Rat IV

Plasma concentration
\((\mu g/mL)\)

Time after dosing (h)

OBS

PBPK
Figure 3B

Plasma concentration
(μg/mL)

Time after dosing (h)

10^{-3} 10^{-2} 10^{-1} 10^0 10^1

0 12 24 36 48

OBS PBPK

(B) Rat PO
Figure 3C

Plasma concentration ($\mu$g/mL) vs. Time after dosing (h)

- OBS
- PBPK

Logarithmic scale for plasma concentration.
Time after dosing (h) 0 1 2 2 4 3 6 4 8
Plasma concentration (μg/mL) 10^-3 10^-2 10^-1 10^0 10^1

(D) Dog PO

OBS

PBPK

Figure 3D
Figure 4A

Time after dosing (h) vs. Plasma concentration (μg/mL)

(A) Rat IV

- OBS
- PBPK
Figure 4B

(B) Rat PO

Plasma concentration
(μg/mL)

Time after dosing (h)

OBS

PBPK

10^1

10^0

10^{-1}

10^{-2}

10^{-3}

0 6 12 18 24
Figure 4C

Plasma concentration (μg/mL) vs. Time after dosing (h)

- OBS
- PBPK

(C) Dog IV

Log scale for concentration and linear scale for time.
Plasma concentration

(μg/mL)

Time after dosing (h)

0 6 12 18 24

(D) Dog PO

OBS

PBPK

Figure 4D
Figure 5A

Plasma concentration
(μg/mL)

Time after dosing (h)

0 6 12 18 24

10^{-3} 10^{-2} 10^{-1} 10^{0}

OBS PBPK-IVIVE PBPK-ALS

(A)
Supplemental Data

Article Title: Prediction of Oral Pharmacokinetics of cMet Kinase Inhibitors in Humans: Physiologically Based Pharmacokinetic Model versus Traditional One Compartment Model


Journal Title: Drug Metabolism and Disposition
Supplemental Data, Table 1.

Physiological parameters for the PBPK models in rats, dogs and humans

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Rat</th>
<th>Dog</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume (mL/kg)</td>
<td>Flow rate (mL/min/kg)</td>
<td>Volume (mL/kg)</td>
</tr>
<tr>
<td>Adipose</td>
<td>76</td>
<td>23</td>
<td>150</td>
</tr>
<tr>
<td>Brain</td>
<td>5.7</td>
<td>6.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Gut</td>
<td>27</td>
<td>52</td>
<td>37</td>
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<tr>
<td>Heart</td>
<td>3.3</td>
<td>16</td>
<td>7.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.3</td>
<td>47</td>
<td>5.5</td>
</tr>
<tr>
<td>Liver&lt;sup&gt;1&lt;/sup&gt;</td>
<td>40</td>
<td>70</td>
<td>32</td>
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<tr>
<td>Lung&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.0</td>
<td>332</td>
<td>8.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>404</td>
<td>92</td>
<td>457</td>
</tr>
<tr>
<td>Skeleton</td>
<td>41</td>
<td>41</td>
<td>81</td>
</tr>
<tr>
<td>Skin</td>
<td>190</td>
<td>19</td>
<td>91</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.0</td>
<td>8.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Testes</td>
<td>10</td>
<td>3.6</td>
<td>10</td>
</tr>
<tr>
<td>Rest of body</td>
<td>106</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>Arterial blood</td>
<td>27</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>Venous blood</td>
<td>54</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>-</td>
<td>9.6</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup> Sum of flow rates through the hepatic artery and portal vein; <sup>2</sup> Total blood flow rate; -: not applicable

Body weights are assumed to be 0.25, 12.5 and 70 kg for rats, dogs and humans, respectively.
Supplemental Data, Table 2.

Partition coefficients in tissues and resulting volume of distribution of PF02341066 and PF04217903 in rats, dogs and humans

<table>
<thead>
<tr>
<th>Tissues</th>
<th>PF02341066</th>
<th></th>
<th></th>
<th></th>
<th>PF04217903</th>
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<tbody>
<tr>
<td></td>
<td>Rat $K_p$</td>
<td>$V_d$</td>
<td>Dog $K_p$</td>
<td>$V_d$</td>
<td>Human $K_p$</td>
<td>$V_d$</td>
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<td>$V_d$</td>
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<td>–</td>
<td>0.045</td>
<td>–</td>
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<td>–</td>
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<tr>
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<td>–</td>
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<td>14</td>
<td>19</td>
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<tr>
<td>Observed $V_d$</td>
<td>13</td>
<td>13</td>
<td>-</td>
<td></td>
<td>1.6</td>
<td>3.7</td>
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</table>

$K_p$, estimated partition coefficients in tissues; $V_d$, predicted volume of distribution (L/kg); –, not applicable
**Supplemental Data, Table 3.**
*Simulated pharmacokinetic parameters of PF02341066 and PF04217903 in rats and dogs after intravenous or oral administration*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Dosing route</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µg·h/mL)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
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</thead>
<tbody>
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<td>PF02341066</td>
<td>Rat</td>
<td>IV</td>
<td>5</td>
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<td>–</td>
<td>3.5 (1.2)</td>
<td>7.3 (1.1)</td>
</tr>
<tr>
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<td>25</td>
<td>0.54 (1.0)</td>
<td>1.8 (2.6)</td>
<td>5.2 (1.1)</td>
<td>7.3 (1.0)</td>
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<tr>
<td></td>
<td>Dog</td>
<td>IV</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>8.0 (1.2)</td>
<td>14 (1.2)</td>
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<tr>
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<td>PO</td>
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<td>0.63 (1.0)</td>
<td>3.1 (1.3)</td>
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<td>Rat</td>
<td>IV</td>
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<td>–</td>
<td>–</td>
<td>5.7 (1.1)</td>
<td>1.7 (2.1)</td>
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<tr>
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<td></td>
<td>PO</td>
<td>10</td>
<td>2.3 (1.2)</td>
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<tr>
<td></td>
<td>Dog</td>
<td>IV</td>
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<td>–</td>
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<td>10 (2.2)</td>
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</table>

Pharmacokinetic parameters were obtained from simulated plasma concentration-time profiles by PBPK modeling. The accuracy of prediction is expressed as fold error (the ratio of predicted versus observed values) in parenthesis. –: not applicable