Prediction of Crizotinib-Midazolam Interaction Using the Simcyp Population-Based Simulator: Comparison of CYP3A Time-Dependent Inhibition between Human Liver Microsomes versus Hepatocytes

Jialin Mao,1 Theodore R. Johnson, Zhongzhou Shen, and Shinji Yamazaki
Pharmacokinetics, Dynamics, and Metabolism, Pfizer Worldwide Research and Development, San Diego, California

Received September 18, 2012; accepted November 5, 2012

ABSTRACT

Crizotinib (Xalkori) is an orally available potent inhibitor of multiple tyrosine kinases, including anaplastic lymphoma kinase and mesenchymal-epithelial transition factor. Objectives of the present study were as follows: 1) to characterize crizotinib time-dependent inhibition (TDI) potency for CYP3A in human liver microsomes (HLM) and cryopreserved human hepatocytes suspended in human plasma (HSP); 2) to characterize crizotinib enzyme induction potency on CYP3A4 in cryopreserved human hepatocytes; 3) to predict crizotinib steady-state plasma concentrations in patients (e.g., autoinhibition and autoinduction) using the mechanistic dynamic model, Simcyp population-based simulator; and 4) to predict a clinical crizotinib-midazolam interaction using the mechanistic dynamic model as well as the static mathematical model. Crizotinib inactivation constant (K_i) and maximum inactivation rate constant (k_{inac}) for TDI were estimated as, respectively, 0.37 μM and 6.9 h^{-1} in HLM and 0.89 μM and 0.78 h^{-1} in HSP. Thus, crizotinib inactivation efficiency (k_{inac}/K_i) was ∼20-fold lower in HSP relative to HLM. Crizotinib E_{max} and EC_{50} for CYP3A4 induction (measured as mRNA expression) were estimated as 6.4- to 29-fold and 0.47 to 3.1 μM, respectively. Based on these in vitro parameters, the predicted crizotinib steady-state area under plasma concentration-time curve (AUC) with HLM-TDI was 2.1-fold higher than the observed AUC, whereas that with HSP-TDI was consistent with the observed result (±1-fold). The increase in midazolam AUC with coadministration of crizotinib (21-fold) was significantly overpredicted using HLM-TDI, whereas the prediction using HSP-TDI (3.6-fold) was consistent with the observed result (3.7-fold). Collectively, the present study demonstrated the value of HSP to predict in vivo CYP3A-mediated drug-drug interaction.

Copyright © 2013 by The American Society for Pharmacology and Experimental Therapeutics
Chen et al., 2011; Kirby et al., 2011). Furthermore, it has been reported that cryopreserved human hepatocytes suspended in human plasma (HSP), compared with those in protein-free media, showed better DDI predictions for some of cytochrome P450 inhibitors (Mao et al., 2011, 2012).

Recently, there has been a growing interest in physiologically based pharmacokinetic (PBPK) models, in which each physiologic compartment (i.e., organ or tissue defined by a tissue volume) is connected with blood flow. The PBPK model provides disposition profiles to be predicted from physico-chemical properties of compounds (Jones et al., 2006; Lavé et al., 2007; Nestorov, 2007). This dynamic approach is increasingly being employed in drug discovery and development setting to predict pharmacokinetics (PK) and DDI potential in the clinic. Traditionally, DDI predictions have been performed with static mathematical models using various inhibitor concentrations such as hepatic inlet or outlet concentrations in total (protein bound plus unbound) or unbound form (Mayhew et al., 2000; Obach et al., 2007; Fahmi et al., 2009; Boulec and Barberan, 2011; Mao et al., 2012). In these reports, hepatic DDI magnitudes for RI were reasonably predicted using the projected unbound portal vein (or hepatic inlet) concentration ($C_{inlet,u}$), whereas the use of unbound systemic (or hepatic outlet) concentration ($C_{sys,u}$) yielded better prediction for TDI and EI compared with $C_{inlet,u}$. Based on advancements in the field, the Food and Drug Administration (FDA) has recently issued a revised draft DDI guidance for industry (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf), which emphasizes the use of an integrated approach such as static mathematical model or mechanistic dynamic model. The dynamic model used was a commercially available Simcyp population-based dynamic simulator (Jamei et al., 2009).

Materials and Methods

Materials. Crizotinib [(R)-3-[1-(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-ylamine; chemical purity >99%] was synthesized by Pfizer Worldwide Research and Development (San Diego, CA) (Cui et al., 2011). Pooled HLM from 50 individual donors (30 males and 20 females) were prepared and characterized at XenoTech LLC (Lenexa, KS). Cryopreserved HEP suspension (three males and two females) and InVitro GRO HT medium were obtained from Celsis In Vitro Technologies (Baltimore, MD). Human plasma (heparin as anticoagulant) was obtained from Lampire Biologic Laboratories (Pipersville, PA). HEP maintenance medium was obtained from Lonza (Walkersville, MD). Midazolam, 1’-hydroxymidazolam (1’-OH midazolam), and [13C6]1’-hydroxymidazolam (internal standard) were obtained from BD Gentest (Woburn, MA). Glucose-6-phosphate and glucose-6-phosphate dehydrogenase were from Sigma-Aldrich (St. Louis, MO). All other commercially available reagents and solvents were of either analytical or high performance liquid chromatography grade.

Crizotinib TDI Assay in HLM. A two-step incubation scheme was used to analyze the inhibition of midazolam hydroxylation by crizotinib in HLM. Crizotinib at final concentrations of 0.3–10 μM (0.1% of final DMSO concentration) was preincubated with HLM (0.5 mg/mL) and NADPH (1 mM) at 37°C for 0, 5, 10, and 20 minutes. The incubation mixture was diluted (1:10) with buffer containing NADPH (1 mM), and midazolam (10 μM) was added for an additional 3-minute incubation period to quantify 1’-hydroxymidazolam as the remaining CYP3A activity. The incubation was then terminated by the addition of 100 μL acetonitrile/methanol (3:1 v/v) containing 3% formic acid and the internal standard (0.1 μM). The incubations were performed in duplicate. Samples were centrifuged at 2000 g for 20 minutes, and an aliquot of the supernatant was analyzed by liquid-chromatography tandem mass spectrometry (LC-MS/MS).

Crizotinib TDI Assay in HSP. Crizotinib TDI potency was evaluated in HSP following a similar study design, as reported previously (Mao et al., 2011).

Briefly, the final crizotinib concentrations used in the incubation mixture were 0.13–100 μM (0.5% of final methanol concentration). Incubations were performed in duplicate. A total of 25 μL of stock cryopreserved HEP (prepared in human plasma as 2 × 10^6 cells/mL) was added to 50 μL of human plasma containing crizotinib and incubated for 0, 10, and 20 minutes (37°C, 5% CO2). HSP (three males and two females) at 3.5 × 10^6 cells/mL was added to the incubation mixture, followed by a 35-minute incubation period to quantify 1’-hydroxymidazolam as the remaining CYP3A activity. The final concentration of HEP was 0.5 × 10^6 cells/mL. Reactions were terminated by adding 200 μL of acetonitrile/methanol (3:1 v/v) containing the internal standard (0.15 μM). Samples were centrifuged at 2000 g for 20 minutes, an aliquot of the supernatant was analyzed by LC-MS/MS.

Crizotinib EI Assay in Cryopreserved HEP. Crizotinib CYP3A4 EI potency was evaluated in cryopreserved HEP from three donors (lot Hu4026, Hu8020, and HIE) using a procedure described previously (Fahmi et al., 2008). Briefly, cryopreserved HEP (3.5 × 10^6 viable cells per 0.5 mL of plating medium in each well of collagen I precoated 24-well plates) were incubated for 24 hours (37°C, 95% relative humidity, 5% CO2) before the addition of crizotinib (0.1% of final DMSO concentration). The cells were treated with crizotinib at final concentrations of 0.25–7 μM daily for 3 consecutive days. Quantification of CYP3A4 mRNA was performed using the TaqMan two-step reverse-transcription polymerase chain reaction method, and the relative quantity of the target CYP3A4 gene compared with the endogenous control was determined by the ΔΔCT method. An effect of crizotinib on cell viability was assessed using the WST-1 cell proliferation reagent.

LC-MS/MS Analysis for Midazolam Metabolite. Concentrations of the CYP3A-mediated metabolite of midazolam, 1’-hydroxymidazolam, in the in...
vitro TDI assays were determined by a LC-MS/MS method after the protein precipitation. The LC-MS/MS system consisted of Shimadzu LC-10ADvp pumps (Shimadzu, Columbia, MD), a CTC PAL autosampler (Leap Technologies, Carrboro, NC), and a Sciex API 4000 mass spectrometer (Sciex, Foster City, CA) equipped with a turbo ion spray source. Chromatographic separation was achieved by a reverse-phase column (Agilent Zorbax SB-Phenyl, 5 μm, 50 × 2.1-mm column; Agilent, Santa Clara, CA) with a mobile phase consisting of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B) at a flow rate of 0.8 ml/min. The injection volume was 5 μL. Analysts were eluted using a step gradient from 1 to 40% mobile phase B over 3 minutes, and then increased to 90% in the next 0.01 minutes. Mobile phase B was held at 90% from 3.01 to 3.50 minutes, and the column was re-equilibrated to 1% over another 0.5 minutes. The mass spectrometry was operated in the positive ionization mode using multiple reaction monitoring at specific parent ion-product ion transitions of m/z 342→203 for 1′-hydroxymidazolam and 345→171 for [13C3]1′-hydroxymidazolam. The dynamic range of the assay ranged from 7.5 to 2540 nM. The back-calculated calibration standard concentrations were within ±15% of their nominal concentrations with coefficients of variation of less than 15%. No significant carryover and matrix effects were observed in the study.

**Estimation of Crizotinib TDI Kinetic Parameters.** To determine TDI kinetic parameters (i.e., $K_I$ and $k_{inact}$) in the HLM assay, the apparent inactivation rate constant ($k_{obs}$) for each crizotinib concentration ([I]) was first estimated from the slope of initial linear decline of CYP3A remaining activity on a natural logarithmic scale over the preincubation time. Apparent $K_I$ and $k_{inact}$ values were then determined by solving the following nonlinear equation (Mayhew et al., 2000):

$$k_{obs} = k_{inact} \cdot [I] / (K_{I,app} + [I])$$ (1)

Subsequently, for prediction purpose, an estimate for $K_I$ was calculated from $K_{I,app}$ following the correction for in vitro nonspecific binding in microsomes (fu,mic), which was measured by the equilibrium dialysis method (Yamazaki et al., 2011).

In the HSP assay, the previously reported mathematical equations incorporating reversible (Eq. 2), irreversible (Eq. 3), or both reversible and irreversible inhibitions (Eq. 4) were employed to estimate the apparent reversible inhibition constant ($K_{I,app}$) and/or TDI parameters ($K_{I,app}$ and $k_{inact}$) by simultaneously fitting three IC50 curves obtained from different incubation times using a weighted nonlinear regression analysis (Mao et al., 2011):

$$V_f_I = \frac{[S] \cdot V_{max,1} - e^{-k_{obs} \cdot t}}{(K_{M,1} + [S]) \cdot V_C}$$ (2)

$$V_f_I = \frac{[S] \cdot V_{max,2} - e^{-k_{obs} \cdot t}}{(K_{M,2} + [S]) \cdot V_C}$$ (3)

$$V_f_I = \frac{[S] \cdot V_{max,3} - e^{-k_{obs} \cdot t}}{(K_{M,3} \cdot (1 + [I]) / K_{I,app} + [I]) \cdot V_C}$$ (4)

where $t$ is a total incubation time of a preincubation (0, 10, or 20 minutes) with an inhibitor, followed by a 35-minute incubation with midazolam. $V_f_I$ represents the 1′-OH midazolam formation rate at a given inhibitor concentration during total incubation time, $V_C$ represents the 1′-OH midazolam formation rate of vehicle control (no inhibitor) during total incubation time, $K_{M,1}$ is the Michaelis-Menten constant for 1′-OH midazolam formation in HSP (55 μM total corresponding to 2 μM free), $V_{max,1}$ represents maximum 1′-OH midazolam formation rate, [I] is the nominal inhibitor concentration, and [S] is the final incubation concentration of midazolam (30 μM).

The ratio of $V_f_I$ and $V_C$, normalized the baseline for the enzyme activity at the designated incubation time. Model selection was based on a number of criteria such as Akaike information criterion, estimates for each parameters, and standard errors. Finally, an estimate for $K_{I,app}$ was converted to $K_I$ (as the input for Simcyp) following the correction for the unbound fraction in plasma (fu,plasma), which was measured by the equilibrium dialysis method (Yamazaki et al., 2011).

### TABLE 1

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>Parameters (units)</th>
<th>Parameters (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>450.34</td>
<td>CLint (μl/min/mg protein)</td>
<td>194</td>
</tr>
<tr>
<td>4.29</td>
<td>CLint,CYP3A8</td>
<td>54.8 &amp; 8.9</td>
</tr>
</tbody>
</table>
| 5.49             | CLint,others       | Time-dependent inhibition on CYP3A in HLM (log10)
| 0.093            | $K_I$ (μM)         | 0.37 |
| 1.1              | $k_{inact}$ (h⁻¹) | 0.69 |
| 0.085            | Time-dependent inhibition on CYP3A in HSP (log10) | $k_f$ (h⁻¹)
| 0.12             | $K_I$ (μM)         | 0.89 |
| 0.6              | $k_{inact}$ (h⁻¹) | 0.78 |
| 0.01–0.18        | CYP3A4 inhibition in HEPa (log10) | $k_e$ (h⁻¹)
| 4.0              | $E_{max}$ (fold)   | 2.4 |
| 0.085            | EC50 (μM)          | 0.84 |
| 25               | Reversible inhibition on CYP3A4 in HLM (μM) | $IC_{50}$ (μM) | >30

$^a$ The value of $p_{ka}$ was adjusted from 5.4 and 8.9 (diprotic base) to 7.6 (monprotic base) to predict clinically observed $V_{max}$ of 25 L/h.

$^b$ The value of $k_f$ was calculated by $f_{plasma}$ and $R_{app}$.
crizotinib $p_K_v$ values were adjusted from 5.4 and 8.9 (diprotic base) to 7.6 (monoprotic base) to predict the clinically observed $V_{ss}$ of 25 L/kg from the single intravenous infusion study (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf). Using the unadjusted $p_K_v$ values of 5.4 and 8.9, the predicted crizotinib $V_{ss}$ was 7.0 L/kg, which resulted in poor fits to the clinically observed single-dose intravenous infusion and oral plasma concentration profiles. Although a minimal PBPK model-implemented Simcyp reasonably predicted the areas under the plasma concentration-time curve (AUC), the maximum plasma concentration ($C_{max}$) was significantly underpredicted in both the single-dose intravenous infusion and oral plasma concentration profiles. Simulations for midazolam plasma concentration-time profiles were also performed by a full PBPK model using a modified Simcyp-default midazolam file with the adjusted $k_p$ of 2 h$^{-1}$ and $p_K_v$ values of 7.4 (ampholyte). Mathematical model 2 implemented in Simcyp was used to predict midazolam $V_{ss}$. These adjustments were required to simulate comparable midazolam plasma concentration-time profiles to the clinically observed results in patients ($n=14$) after a single oral administration of midazolam without coadministration of crizotinib (see above). In addition to these adjustments, the predicted liver to plasma partition coefficients ($k_p$) were set to unity from 22 and 1.3 for crizotinib and midazolam, respectively. These changes resulted in minimal effects on the predicted $V_{ss}$ values for both crizotinib (from 25.3 to 24.8 L/kg) and midazolam (from 1.0 to 1.1 L/kg).

Clinical trial simulation in Simcyp was performed with a virtual population of healthy volunteers in 8 trials of 10 subjects, each aged 18–65 years with a female/male ratio of 0.34, whose CYP3A4 degradation rate constant ($k_{deg}$) was 0.019 h$^{-1}$ in the liver and 0.030 h$^{-1}$ in the gastrointestinal (GI) tract. The output sampling interval in Simcyp simulation tool box was set to 0.2 h in all simulations. Trial designs used were as follows:

**Trial 1:** A single oral dose of 250 mg crizotinib was administered on day 1; crizotinib plasma concentration was simulated for 7 days.

**Trial 2:** Multiple doses of crizotinib, 250 mg twice daily with an interval of 12 hours, were orally administered for 28 days; crizotinib plasma concentration was simulated during crizotinib repeated administration.

**Trial 3:** A single oral dose of 2 mg midazolam was coadministered on day 28 with repeated oral doses of crizotinib (250 mg twice daily with an interval of 12 hours) for 28 days; plasma concentrations of midazolam and crizotinib were simulated during crizotinib repeated administration.

In these simulations, $C_{max}$, $t_{max}$ and AUC$_{0-\infty}$ were obtained from Simcyp output, whereas AUC$_{0-L}$ was calculated from simulated plasma concentrations using the linear trapezoidal rule:

$$AUC_{0-L} = AUC_{0-\infty} + C_L/\lambda_d$$

where AUC$_{0-L}$, $C_L$, and $\lambda_d$ represent the area under the plasma concentration-time curve from time zero to the last point in time, the plasma concentration at the last time point, and the elimination rate constant in the terminal phase of log plasma concentration-time curves determined by linear regression, respectively.

Geometric means of PK parameters in eight simulation trials were compared with the clinically observed geometric mean to assess Simcyp prediction accuracy. In addition to the Simcyp outputs of 5th and 95th percentiles of all 80 subjects, the geometric mean values of 5th and 95th percentiles were separately performed in the prediction of crizotinib-midazolam interaction with Simcyp using CRZ-HSP. Clinical trial simulation in Simcyp was the same as trial 3, as described above, and the PK parameters (e.g., $C_{max}$, $t_{max}$, AUC$_{0-L}$, and AUC$_{0-\infty}$) and geometric mean values of 5th and 95th percentiles among eight simulation trials were either obtained or calculated from Simcyp outputs, as described above.

**Prediction of Crizotinib-Midazolam Interaction with a Static Mathematical Model.** A static mathematical model (Fahmi et al., 2008) was used to predict the fold increase in midazolam AUC$_{0-L}$ (AUC$_{0-L}$) following coadministration with crizotinib:

$$AUC_R = \left( \frac{1}{A_0 \times B_0 \times C_0 \times f_{a,u} \times (1 - f_{a,u})} \right) \times \left( \frac{1}{A_2 \times B_2 \times C_2 \times (1 - F_2) + F_2} \right)$$

$$A = \frac{k_{deg} \times I}{k_{deg} + \frac{I}{[I] + K_I}}$$

$$B = 1 + \frac{d \times E_{max} \times [I]}{[I] + EC_{50}}$$

$$C = \frac{1}{I + \frac{[I]}{K_I}}$$

where A, B, and C represent TDI, EI, and RI, respectively, and the subscripts $h$ and $g$ denote liver and GI tract, respectively.

Crizotinib input parameters and CYP3A4 $k_{deg}$ values in the liver and GI tract used for the static model were exactly the same as Simcyp input parameters to compare in vivo DDI predictions between the dynamic and static models. Therefore, the proposed empirical scaling factor for induction (i.e., $d$) in the static model was set to unity since the EI parameters were already normalized with rifampin data for the dynamic model.

The availability in the GI tract ($F_g$) and $f_{a,GPIA4}$ for midazolam were set as 0.63 and 0.93, respectively (Ernest et al., 2005; Obach et al., 2006). Crizotinib steady-state unbound average plasma concentrations ($C_{a,\infty}$), which were calculated from AUC$_{0-\infty}$ divided by the dosing interval of 12 hours, followed by the correction for $f_{a,GPIA4}$ were used as the hepatic inhibitor concentrations ($[I]_M$) in the static model (as consistent with a full PBPK model with hepatic $k_p$ of unity). The intestinal inhibitor concentrations ($[I]_I$) were calculated from crizotinib parameters summarized in Table 1 using the following equation (Rostami-Hodjegan and Tucker, 2004):

$$[I]_I = D \cdot k_a \cdot F_a \cdot f_{a,GPIA4} \cdot Q_{out}$$

where $D$, $k_a$, $F_a$, $f_{a,GPIA4}$ and $Q_{out}$ represent the dose amount per dose (μmol), the first-order absorption rate constant (h$^{-1}$), the fraction of dose absorbed, the unbound fraction in the GI tract, and the enterocyte blood flow (L/h), respectively.

**Sensitivity Analysis for Crizotinib Hepatic $k_p$ and CYP3A4 $k_{deg}$ with Simcyp.** The sensitivity analyses for crizotinib hepatic $k_p$ value ranging from 1 to 24 and hepatic CYP3A4 $k_{deg}$ from 0.063 to 0.0063 h$^{-1}$ (i.e., $1/f_{1/2}$ of 1–100 h) were separately performed in the prediction of crizotinib-midazolam interaction with Simcyp using CRZ-HSP. Clinical trial simulation in Simcyp was the same as trial 3, as described above, and the PK parameters (e.g., $C_{max}$, $t_{max}$, AUC$_{0-L}$, and AUC$_{0-\infty}$) and geometric mean values of 5th and 95th percentiles among eight simulation trials were either obtained or calculated from Simcyp outputs, as described above.
In Vitro Effects of Crizotinib on CYP3A. Crizotinib TDI kinetic parameters for CYP3A4 were determined in HLM and HSP using midazolam as a CYP3A probe substrate. Estimated \( K_{\text{d,app}} \) and \( k_{\text{inact}} \) in HLM were 3.0 \( \mu M \) and 6.9 h\(^{-1} \), respectively (Fig. 2). Crizotinib IC\(_{50}\) values in HSP were adequately described by the irreversible model (Eq. 3) with \( K_{\text{d,app}} \) of 9.6 \( \mu M \) and \( k_{\text{inact}} \) of 0.78 h\(^{-1} \) (Fig. 3). The estimates for \( K_{\text{d}} \) in HLM and HSP following the correction for in vitro nonspecific binding (e.g., \( f_{\text{brain}} \) and \( f_{\text{plasma}} \), respectively) were 0.37 and 0.89 \( \mu M \), respectively. Thus, the \( K_{\text{d}} \) values in HSP was approximately 3-fold higher than that in HLM, whereas the \( k_{\text{inact}} \) from HSP was ~9-fold lower than that from HLM, resulting in the 20-fold difference in the inactivation efficiency (\( k_{\text{inact}}/K_{\text{d}} \)) between HLM and HSP (19 and 0.87 h\(^{-1} \times \mu M^{-1} \), respectively). These \( K_{\text{d}} \) and \( k_{\text{inact}} \) values were used for Simcyp simulation (Table 1).

In cryopreserved HEP from three donors, crizotinib concentration-dependently induced CYP3A4 mRNA expression with estimated \( E_{\text{max}} \) of 6.4- to 29-fold and \( E_{\text{50}} \) of 0.47–3.1 \( \mu M \). It should be noted that relatively low cell viability (<0.7 ratio to vehicle control sample) was observed at higher crizotinib concentrations (≥5 \( \mu M \)); thus, the accuracy of EI parameter estimation might have been impacted. Following the normalization with rifampin data (positive control), mean crizotinib \( E_{\text{max}} \) of 2.4-fold and \( E_{\text{50}} \) of 0.84 \( \mu M \) were used for Simcyp simulation (Table 1).

**Predicted Crizotinib Plasma Concentrations with Simcyp.**

Clinically observed crizotinib \( C_{\text{max}} \), \( t_{\text{max}} \), and AUC\(_{0\rightarrow r}\) were 87 ng/mL, 4 hours, and 1817 ng·h/mL, respectively, in cancer patients (\( n = 8 \)) after a single oral administration of 250 mg crizotinib (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf). By the Simcyp simulation with CRZ-HLM, the predicted \( C_{\text{max}} \) (89 ng/mL) was within 10% of the observed value, whereas the AUC\(_{0\rightarrow r}\) (3901 ng·h/mL) was overpredicted by 1.7-fold (Table 2). As shown in Fig. 4A, the predicted crizotinib plasma concentrations were slightly, but consistently higher than the observed concentrations after \( t_{\text{max}} \). In contrast, the predicted AUC\(_{0\rightarrow r}\) (1745 ng·h/mL) by Simcyp with CRZ-HSP was consistent with the observed value (<10%), whereas the predicted \( C_{\text{max}} \) (42 ng/mL) was underpredicted by ~2-fold (Table 2). It remained unclear why the \( C_{\text{max}} \) was underpredicted. One of the possible reasons could be the effect of multidrug-resistance transporter protein, P-glycoprotein, on the absorption and export of crizotinib since crizotinib was reported to be a substrate of P-glycoprotein (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202570Orig1s000ClinPharmR.pdf). The predicted crizotinib plasma concentrations with CRZ-HLP were in good agreement with the observed profiles after \( t_{\text{max}} \) (Fig. 4B).

Clinically observed crizotinib steady-state \( C_{\text{max}} \), \( t_{\text{max}} \), and AUC\(_{0\rightarrow r}\) were 328 ng/mL, 4 hours, and 3054 ng·h/mL, respectively, in cancer patients (\( n = 5 \)) after 28-day repeated oral administration of 250 mg crizotinib twice daily (see above). The predicted \( C_{\text{max}} \) (543 ng/mL) and AUC\(_{0\rightarrow r}\) (6494 ng·h/mL) by Simcyp with CRZ-HLM were overpredicted by 1.7- and 2.1-fold, respectively (Table 2; Fig. 4C). In contrast, the Simcyp-predicted crizotinib steady-state plasma concentrations with CRZ-HSP were in good agreement with the observed concentrations (Fig. 4D). The predicted \( C_{\text{max}} \) (273 ng/mL) and AUC\(_{0\rightarrow r}\) (3258 ng·h/mL) were within 20% of the observed values (Table 2). Additionally, the effect of crizotinib TDI or EI on crizotinib steady-state plasma concentrations was examined in a virtual population of healthy volunteers by Simcyp using CRZ-HSP with/without TDI and/or EI parameters. The predicted \( C_{\text{max}} \) (288 ng/mL) and AUC\(_{0\rightarrow r}\) (3432 ng·h/mL) with only TDI parameters were comparable to the predicted values (273 ng/mL and 3258 ng·h/mL) with both TDI and EI parameters (within 5%), whereas those with only EI parameters (128 ng/mL and 1519 ng·h/mL) were comparable to the predicted values (133 ng/mL and 1587 ng·h/mL) without TDI and EI parameters (within 5%). The predicted \( C_{\text{max}} \) and AUC\(_{0\rightarrow r}\) with only TDI parameters were ~2-fold higher than the predicted values without TDI and EI parameters. Based on these comparisons, crizotinib steady-state plasma concentrations appeared to be accumulated by 2-fold due to TDI with a minimal EI effect.

**Predicted Crizotinib-Midazolam Interaction with Simcyp.**

Clinically observed and Simcyp-predicted pharmacokinetic parameter estimates of crizotinib in humans after a single or 28-day repeated oral administration of crizotinib (Fig. 4B).

<table>
<thead>
<tr>
<th></th>
<th>Single Dose (250 mg)</th>
<th>Repeated Doses (250 mg bid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C_{\text{max}} ) ng/mL</td>
<td>AUC(_{0\rightarrow r}) ng·h/mL</td>
</tr>
<tr>
<td>Observed</td>
<td>87 (34)</td>
<td>1817 (33)</td>
</tr>
<tr>
<td>Predicted HLM(^a)</td>
<td>89 (43)</td>
<td>3901 (57)</td>
</tr>
<tr>
<td>P/O ratio(^a)</td>
<td>1.0</td>
<td>2.1</td>
</tr>
<tr>
<td>HSP(^a)</td>
<td>42 (52)</td>
<td>1745 (72)</td>
</tr>
<tr>
<td>P/O ratio(^a)</td>
<td>0.48</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean (% coefficient of variation) for single dose (\( n = 8 \)) and 28-day repeated doses (\( n = 5 \)).

\(^a\) Simcyp simulation was performed using TDI parameters from either HLM or HSP.

\(^b\) Calculated mean ratio of predicted to observed pharmacokinetic parameters.
plasma concentration-time profiles were much higher than the observed profiles (Fig. 5A). The midazolam $C_{\text{max,R}}$ and $AUC_{R}$ were 3.0 and 21, respectively (Table 3; Fig. 6A), resulting in the 1.5- and 5.6-fold overpredictions of $C_{\text{max,R}}$ and $AUC_{R}$, respectively. In contrast, the predicted midazolam plasma concentration-time profiles with CRZ-HSP were relatively consistent with the observed profiles (Fig. 5B). The midazolam $C_{\text{max,R}}$ and $AUC_{R}$ (1.9 and 3.6, respectively) were in good agreement with the clinically observed results (Table 3; Fig. 6B). The 90% CI for predicted $C_{\text{max}}$ (1.4–2.9) was relatively consistent with the observed CI, whereas that for predicted $AUC_{0-\infty}$ (1.6–8.8) was slightly larger than the observed result.

Sensitivty Analysis for Crizotinib Hepatic $k_{p}$ and CYP3A4 $k_{\text{deg}}$ with Simcyp. The sensitivity analysis for crizotinib hepatic $k_{p}$ values of 1–24 revealed that the midazolam $AUC_{R}$ with coadministration of crizotinib varied markedly from 3.6 to 13, while the predicted crizotinib $AUC_{0-\infty}$ values were within 2-fold (3258–5434 ng/h/ml) (Supplemental Fig. 1). Therefore, DDI prediction with a full PBPK model in Simcyp was highly sensitive to the hepatic $k_{\text{deg}}$ values relative to $C_{\text{max,R}}$. The $t_{1/2}$ of 30–40 hours yielded the best prediction of the midazolam $AUC_{R}$. Therefore, the hepatic $k_{\text{deg}}$ value of 0.019 h$^{-1}$ ($t_{1/2}$ of 36 h) used in this study was within a range of reasonable predictions on both the crizotinib steady-state plasma concentrations and crizotinib-midazolam interaction.

Predicted Crizotinib-Midazolam Interaction by Static Models. The predicted midazolam $AUC_{R}$ values with the static mathematical model using either clinically observed or Simcyp-predicted crizotinib $C_{\text{ave,u}}$ are summarized in Table 4. Using the clinically observed crizotinib $C_{\text{ave,u}}$, the predicted midazolam $AUC_{R}$ values with HLM-TDI and HSP-TDI were 17 and 3.2, respectively. Thus, the midazolam $AUC_{R}$ with HLM-TDI was overpredicted by ~5-fold, whereas the prediction with HSP-TDI was consistent with the observed $AUC_{R}$. In the static model, the midazolam $AUC_{R}$ in the liver (11–12) with HLM-TDI was much higher than that with HSP-TDI (2.5–2.6), whereas the $AUC_{R}$ in the intestines was relatively comparable between HLM-TDI and HSP-TDI (1.6 and 1.3, respectively). This comparable intestinal
AUCR between HLM-TDI and HSP-TDI appeared to be largely due to midazolam $F_{e}$ of 0.63, which resulted in the maximal effect of 1.6-fold increase. Using the Simcyp-predicted crizotinib $C_{ave,u}$ by HLM-TDI and HSP-TDI, the predicted midazolam AUCR values were 19 and 3.3, respectively. Thus, the prediction results of crizotinib-midazolam interaction with the static model using the Simcyp-predicted crizotinib $C_{ave,u}$ were relatively consistent with those with the Simcyp full PBPK models with $k_{inact}$ of unity.

In addition, the predicted midazolam AUCR values with Simcyp static model ($R_{ss}$) were 13 and 2.3 with HLM-TDI and HSP-TDI, respectively. These AUCR values were ~1.5-fold lower than the predicted values with the Simcyp full PBPK models (i.e., 21 and 3.6, respectively). The difference in the predicted ratios between the $R_{ss}$ and full PBPK predictions appeared to be largely due to the crizotinib TDI effects on the accumulation of crizotinib steady-state plasma concentrations, which was not taken into account for the Simcyp-$R_{ss}$ prediction, in which the inhibitor concentration was calculated from the input PK parameters such as $D$ and $CL$.

**Discussion**

In the present study, the estimated in vitro inactivation efficiency ($k_{inact}/K_I$) for crizotinib TDI potency was ~20-fold lower in HSP relative to HLM. Consistent with the present results, the known CYP3A inhibitors (e.g., diltiazem, erythromycin, and verapamil) were also reported to be less potent in HEP compared with HLM, and the difference in TDI potency has yielded an impact on in vivo DDI prediction (Xu et al., 2009; Chen et al., 2011). The advantages in utilization of HEP over HLM are considered to be that HEP are intact cellular systems containing not only a full complement of phase I/II-metabolizing enzymes, but also functional cell membranes. The estimated $k_{inact}$ in HEP is generally considered an apparent hybrid kinetic parameter consisting of the following: 1) the rate of diffusion through the cell membrane; 2) the total metabolic consumption rate (including sequential metabolism); and 3) the intrinsic enzyme inactivation rate. Therefore, these factors could be important for some drugs showing substantially different intra- versus extracellular concentrations and/or multiple metabolic pathways, particularly those associated with non-CYPs (Zhao, 2008).

Crizotinib was primarily metabolized by CYP3A (Johnson et al., 2011a), and its passive permeability was relatively low (~1 x 10^{-6} cm/s) in the recently reported permeability assay system using low-efflux Madin-Darby canine kidney cells (Di et al., 2011). Crizotinib was also reported to be a substrate of P-glycoprotein, as mentioned above. Thus, these factors could potentially lead to differences in intra- versus extracellular concentrations, and, correspondingly, to differences in the estimated TDI potency between HLM versus HSP.

**TABLE 3**

Clinically observed and Simcyp-predicted pharmacokinetic parameter estimates of midazolam in humans before and after 28-day repeated oral administration of crizotinib (twice daily doses of 250 mg)

<table>
<thead>
<tr>
<th>Crizotinib Administration</th>
<th>$C_{max}$ ng/mL</th>
<th>AUC$_{0-\infty}$ ng$h$/mL</th>
<th>Fold Increase in Midazolam Oral Exposure$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>13 (39)</td>
<td>32 (41)</td>
<td>2.0 1.4–2.9 3.7 2.6–5.1</td>
</tr>
<tr>
<td>Post</td>
<td>26 (44)</td>
<td>117 (61)</td>
<td></td>
</tr>
<tr>
<td>Predicted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>10 (38)</td>
<td>27 (90)</td>
<td>1.5 1.2–1.8 2.1 1.4–2.9</td>
</tr>
<tr>
<td>Post</td>
<td>30 (39)</td>
<td>547 (70)</td>
<td></td>
</tr>
<tr>
<td>HLM$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.77</td>
<td>3.83</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>1.2</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>P/O ratio$^b$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.2</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>1.9</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>HSP$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.72</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>0.94</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean (% coefficient of variation) for pre- and post-doses of crizotinib ($n = 14$ and 8 patients, respectively).

$^a$ Fold increase in $C_{max}$ ($C_{max,t}$) and AUC$_{0-\infty}$ ($AUC_{t}$) with coadministration of crizotinib with 90% CI.

$^b$ Calculated predicted to observed pharmacokinetic parameter ratio.

$^c$ Simcyp simulation was performed using TDI parameters from either HLM or HSP.
our findings, other investigators (Obach et al., 2007; Fahmi et al., 2009) also described in Materials and Methods. Consistent with the projected/calculated Csys,u value, we intended to use crizotinib kp concentration for DDI prediction by adjusting hepatic cell metabolism (i.e., unity), as described in Materials and Methods. Accordingly, we observed the clinically observed crizotinib-midazolam interaction was more significant on midazolam AUCR with coadministration of crizotinib. The observed and predicted AUCR values in each trial are expressed as geometric mean with 90% CI. The solid and dashed lines represent geometric mean and 90% CI, respectively, of all trials.

Mathematical models used in the Simcyp-PBPK model are the well-stirred and Qgut models in the liver and GI tract, respectively (Yang et al., 2007; Jamei et al., 2009). The well-stirred model assumes that drug distribution into liver is perfusion limited without any diffusion delay; therefore, unbound drug concentration within liver is in equilibrium with unbound drug concentration in liver outlet (i.e., Csys,u). Accordingly, we intended to use crizotinib Csys,u as the inhibitor concentration for DDI prediction by adjusting hepatic kp value (i.e., unity), as described in Materials and Methods. Consistent with our findings, other investigators (Obach et al., 2007; Fahmi et al., 2009; Boulenc and Barberan, 2011) reported that the use of Csys,u (e.g., Cmax0 or Csys,u) as the inhibitor concentration yielded the most accurate DDI predictions for TDI and EI by the static models. When the projected/calculated Cinlet,u was used, the DDI predictions for TDI and EI were generally overpredicted, while those for RI were more accurately predicted. These findings may suggest that RI mainly occurs during the first-pass metabolism process, whereas TDI and EI continue to take place beyond the first-pass metabolism. Consistently, the clinically observed crizotinib-midazolam interaction was more significant on midazolam AUCR than Cmax,kR, and the DDI prediction using crizotinib Csys,u yielded a reasonable prediction for both midazolam AUCR and Cmax,R. Thus, crizotinib Csys,u appears to be a more appropriate inhibitor concentration than Cinlet,u for the prediction of crizotinib-midazolam interaction.

The Qgut model in the GI tract is basically constructed by the well-stirred model, in which the blood flow term (i.e., Qgut) is a hybrid parameter consisting of a compound-dependent permeability through enterocyte membrane and a physiologic villous blood flow. Crizotinib Qgut value was estimated to be 4 L/h based on a calculated projected human jejunum permeability (Peff,man) value of 0.58 × 10−4 cm/s derived from its physicochemical properties. This Peff,man value was within a moderate range among 17 compounds reported (Yang et al., 2007). Since crizotinib Fg was estimated to be ~0.9 by the Qgut model, its moderate Peff,man did not appear to be a limiting factor for the first-pass metabolism in the GI tract. In contrast, the experimentally measured crizotinib in vitro permeability (1 × 10−6 cm/s) seemed to be lower than the value expected from its physicochemical properties, suggesting that the calculated Peff,man value might be overpredicted. However, crizotinib permeability can be taken into account to estimate TDI kinetic parameters in HSP because this system contains intact cell membranes. We therefore believe that a perfusion-limited model is appropriate for PK and/or DDI prediction when the kinetic parameters were determined in an in vivo system containing cell membranes. On the other hand, a diffusion rate through the cell membrane would likely be an important factor for PBPK model of low permeable compounds when their kinetic parameters were determined in assay systems without functional cell membranes (e.g., HLM). In such a case, a diffusion-limited model would be likely required for their PK and/or DDI prediction. Moreover, when compounds are metabolized by multiple enzymes, such as microsomal and cytosolic enzymes, HEP would be more appropriate to determine TDI kinetic parameters by taking into account an overall in vivo metabolic pathway. It has been reported that multiple enzymes (e.g., CYP3A and aldehyde oxidase) were involved in the crizotinib metabolism (Johnson et al., 2011a); therefore, this might be another factor that contributed to the difference in the TDI kinetics between HLM and HSP. These considerations are graphically summarized in Fig. 7. For PBPK model-based DDI prediction, the selection of either a perfusion- or diffusion-limited model should be carefully considered based upon each inhibitor’s property and in vitro assay system used.

![Fig. 6. Clinically observed and Simcyp-predicted fold increase in midazolam AUCR with coadministration of crizotinib. Midazolam (2 mg) was orally administered to cancer patients before (n = 14) and after (n = 8) twice daily doses of 250 mg crizotinib for 28 days. The x-axis represents the observed (Obs) and predicted 8 clinical trials (1–8) of 10 individuals by Simcyp with TDI parameters from HLM (A) and HSP (B), and the y-axis represents the fold increase in midazolam AUCR (AUCR) with coadministration of crizotinib. The observed and predicted AUCR values in each trial are expressed as geometric mean with 90% CI. The solid and dashed lines represent geometric mean and 90% CI, respectively, of all trials.](image-url)

### TABLE 4

<table>
<thead>
<tr>
<th>TDI</th>
<th>Crizotinib Cmax,unl</th>
<th>Predicted Fold Increase in Midazolam AUCR</th>
<th>Static Mathematical Model</th>
<th>Simcyp Rg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/mL Total</td>
<td>nM Free</td>
<td>Liver</td>
<td>Gut</td>
</tr>
<tr>
<td>HLM</td>
<td>255</td>
<td>53</td>
<td>11</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>541</td>
<td>112</td>
<td>12</td>
<td>1.6</td>
</tr>
<tr>
<td>HSP</td>
<td>255</td>
<td>53</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>56</td>
<td>2.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

- **-** not applicable.
- Static mathematical models used TDI parameters from either HLM or HSP.
- Average steady-state plasma concentrations (Cavunl) in total (ng/mL) and unbound (nM) forms were calculated from either the observed or Simcyp-predicted AUCavunl (listed in Table 2) divided by the dosing interval of 12 h.
- Clinically observed crizotinib Cinlet,u was used for the prediction of crizotinib-midazolam interaction with the static model.
- Simcyp-predicted crizotinib Cmax,unl was used for the prediction of crizotinib-midazolam interaction with the static model.
should also be important to select/simulate appropriate surrogate concentrations of both object and precipitant drugs as the unbound concentrations at target sites. In vivo DDI predictions are expected to be more accurate based on plasma concentration profiles predicted by the PBPK model compared with a fixed inhibitor concentration (e.g., \( C_{max,u} \) and \( C_{ave,u} \)) used for the static model. In the prediction of crizotinib-midazolam interaction, it would be worth noting that the Simcyp minimal and full PBPK models (with hepatic \( k_p \) of unity) yielded comparable DDI predictions when the predicted crizotinib concentrations in plasma were comparable between these models (Supplemental Table 1). Moreover, these DDI predictions were roughly comparable to the predictions from the static models such as a Simcyp-static model (i.e., \( R_u \)) and a static mathematical model (Supplemental Table 1). Therefore, there might not be significant differences in DDI prediction between dynamic and static models if the factors related to DDI were appropriately incorporated into these models. However, these comparable predictions for crizotinib-midazolam interaction could be largely due to the relatively flat crizotinib plasma concentration-time profiles (i.e., \( C_{max} \approx C_{ave} \)) during dosing interval, as shown in Fig. 4.

Several other factors related to TDI mechanism should also be considered for the in vivo DDI prediction. Quantitative measurement of in vivo CYP3A4 \( k_{deg} \) in humans remains a challenge as \( k_{deg} \) is often associated with an uncertainty in the DDI prediction (Obach et al., 2007; Rowland Yeo et al., 2011). Not surprisingly, a wide range of hepatic \( k_{deg} \) values has been reported for CYP3A4 as turnover \( t_{1/2} \) of 10–140 h (Yang et al., 2008; Grimm et al., 2009; Xu et al., 2009; Chen et al., 2011). We used the Simcyp default \( k_{deg} \) values of 0.019 h\(^{-1}\) (\( t_{1/2} = 36 \) h) in the liver and 0.030 h\(^{-1}\) (\( t_{1/2} = 23 \) h) in the intestines. Based on the sensitivity analysis for hepatic CYP3A4 \( k_{deg} \) values ranging from 0.693 to 0.00693 h\(^{-1}\), that is, \( t_{1/2} \) of 1–100 h, the hepatic \( k_{deg} \) value of 0.019 h\(^{-1}\) (\( t_{1/2} = 36 \) h) used in this study was within a range of reasonable predictions on both the crizotinib steady-state plasma concentrations and crizotinib-midazolam interaction (Supplemental Fig. 2). Another important parameter is the fraction metabolized by a drug-metabolizing enzyme, i.e., \( f_{mo,CYP3A4} \). The estimated crizotinib \( f_{mo,CYP3A4} \) was ~0.8 based on the in vitro CYP phenotyping study and the human mass-balance study with \(^{14}\)C]crizotinib (Johnson et al., 2011a, 2011b); the \( f_{mo,CYP3A4} \) of 0.8 used in the present study yielded reasonable PK/DDI predictions.

Despite these reasonable DDI predictions, the method used in the present study included potential limitations that should be addressed. First, neither in vitro nonspecific binding nor any decrease in crizotinib concentration during incubation was considered for the correction of in vitro crizotinib EI parameters (e.g., \( E_{max} \) and \( EC_{50} \)), as previously reported (Fahmi et al., 2008). In contrast, the obtained TDI parameters in HLM and HSP were corrected for the in vitro nonspecific binding. Secondly, and possibly more importantly, the physiologic CYP3A4 kinetic (or systems) parameters (i.e., \( k_p \) and \( k_{deg} \)) for the TDI prediction were assumed to remain constant in the presence of inhibitor, and an additional enzyme degradation pathway was introduced to reach new steady-state concentration as \( k_{obs} \) (Eq. 1). This assumption may not be valid when EI occurs in parallel to TDI. In fact, there appears to be limited understanding of underlying simultaneous in vitro to in vivo extrapolation for TDI and EI (and its possible interplay). Moreover, although TDI and EI of CYP3A are known to occur in both liver and GI tract, their magnitudes may be different in these organs. Despite these limitations, a PBPK modeling approach for DDI prediction affords an opportunity to gain greater insight into the underlying mechanisms mediating these complex interactions as a function of time with the effects of intrinsic factors such as organ dysfunction, age, and genetics.

Acknowledgments

The Authors greatly acknowledge Deepak Dalvie, Sascha Freiwald, Lance Goulet, Caroline A. Lee, Danielle Smith and Evan B. Smith [Pharmacokinetics, Dynamics, and Metabolism (PDM), Pfizer, San Diego, CA] for the bioanalytical and biotransformation assays, and Sherri Boldt, Odette A. Fahmi and Gregory S. Walker (PDM, Pfizer, Groton, CT) for the bioanalytical and induction assays. The Authors also thank Weiwei Tan and Keith D. Wilher [Clinical Pharmacology, Pfizer, San Diego, CA] for valuable discussion about clinical pharmacokinetic data, and Odette A. Fahmi [PDM, Pfizer, Groton, CT], R. Scott Obach [PDM, Pfizer, Groton, CT], Bill J. Smith [PDM, Pfizer, San Diego, CA], and Bhasker Shetty [PDM, Pfizer, San Diego, CA] for excellent inputs for the draft manuscript.

References


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


