TITLE: Prediction of Drug-Drug Interactions with Crizotinib as the CYP3A Substrate Using a Physiologically-Based Pharmacokinetic Model

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RUNNING TITLE: Prediction of Crizotinib Drug-Drug Interactions

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Number of Text Pages: 47
Number of Tables: 7
Number of Figures: 7
Number of References: 29
Number of Words in the Abstract: 250
Number of Words in the Introduction: 772
Number of Words in the Discussion: 1790
ABBREVIATIONS:

AUC, area under the plasma concentration-time curve; AUC\textsubscript{R}, fold-increase in AUC by an interacting drug; C\textsubscript{max}, maximum plasma concentration; C\textsubscript{maxR}, fold-increase in C\textsubscript{max} by an interacting drug; CL, plasma clearance; CL\textsubscript{int}, intrinsic clearance; DDI, drug-drug interaction; F\textsubscript{a}, fraction of dose absorbed from gastrointestinal tract; F\textsubscript{g}, fraction of dose that escapes intestinal first-pass metabolism; F\textsubscript{m}, fraction metabolized by an enzyme; f\textsubscript{u}, unbound fraction; K\textsubscript{i}, inhibition constant; K\textsubscript{i}, inactivation constant; k\textsubscript{inact}, maximum inactivation rate constant; NSI, nonstationary pharmacokinetic index; PBPK, physiologically-based pharmacokinetics; R\textsubscript{bp}, blood-to-plasma ratio; t\textsubscript{1/2,z}, apparent terminal half-life; t\textsubscript{max}, time to reach maximum plasma concentration; V\textsubscript{ss}, volume of distribution at steady-state
ABSTRACT

An orally available multiple tyrosine kinase inhibitor, crizotinib (Xalkori®), is a CYP3A substrate, moderate time-dependent inhibitor and weak inducer. The main objectives of the present study were to: 1) develop and refine a physiologically-based pharmacokinetic (PBPK) model of crizotinib based on clinical single- and multiple-dose results, 2) verify the crizotinib PBPK model based on crizotinib single-dose drug-drug interaction (DDI) results with multiple-dose coadministration of ketoconazole or rifampin, and 3) apply the crizotinib PBPK model to predict crizotinib multiple-dose DDI outcomes. We also focused on gaining insights into the underlying mechanisms mediating crizotinib DDIs using the dynamic PBPK model, Simcyp population-based simulator. First, PBPK-model-predicted crizotinib oral exposures adequately matched clinically observed results in the single- and multiple-dose studies. Second, the model-predicted crizotinib exposures sufficiently matched clinically observed results in the crizotinib single-dose DDI studies with ketoconazole or rifampin, resulting in the reasonably predicted fold-increases in crizotinib exposures. Finally, the predicted fold-increases in crizotinib exposures in the multiple-dose DDI studies were roughly comparable to those in the single-dose DDI studies, suggesting that the effects of crizotinib CYP3A time-dependent inhibition (net-inhibition) on the multiple-dose DDI outcomes would be negligible. Therefore, crizotinib dose-adjustment in the multiple-dose DDI studies could be possible based on the currently available single-dose results. Overall, we believe that the crizotinib PBPK model developed, refined and verified in the present study would be useful to adequately predict crizotinib oral exposures in other
clinical studies such as DDIs with weak/moderate CYP3A inhibitors/inducers and drug-
disease interactions in patients with hepatic or renal impairment.
INTRODUCTION

A clinically relevant drug-drug interaction (DDI) is generally considered a modification of pharmacological and/or toxicological responses of one substrate drug by another interacting drug. An evaluation of potential DDIs for new molecular entities (NMEs) are recognized as an important consideration in the drug discovery and development setting as well as the regulatory review process (Zhang et al., 2009; Rowland et al., 2011; Prueksaritanont et al., 2013). The US Food and Drug Administration (FDA) and the European Medicines Agency have recently issued DDI guidances (CDER, 2012; CHMP, 2012), which emphasize the use of an integrated mechanistic approach, such as a physiologically-based pharmacokinetic (PBPK) model, to quantitatively predict the magnitude of DDIs in the clinic. The dynamic modeling approach is increasingly being employed in all phases of drug discovery and development to evaluate potential DDI risks for NMEs (Boulenc and Barberan, 2011; Zhao et al., 2011; Huang and Rowland, 2012; Peters et al., 2012; Huang et al., 2013). Additionally, of keen interest by regulatory agencies is the use of mechanistic dynamic models to provide a deeper understanding of complex DDIs including simultaneous effects of two or more interacting drugs (e.g., inhibitors and inducers) on exposures of substrate drugs as well as drug-disease interactions in patients with hepatic or renal impairment (Zhao et al., 2011; Huang and Rowland, 2012; Huang et al., 2013; Varma et al., 2015).

Accordingly, the mechanistic dynamic modeling approach can provide an alternative to evaluate complex DDIs as FDA encourages study sponsors to use modeling and simulation to determine the best dosing strategy (Huang and Lesko, 2009; Rowland et al., 2011; Huang et al., 2013).
Crizotinib (PF02341066; Xalkori®) is an orally available small molecule inhibitor of multiple tyrosine kinases including anaplastic lymphoma kinase and mesenchymal-epithelial transition factor (CDER, 2011). Crizotinib has been reported as a CYP3A substrate, time-dependent inhibitor and inducer (Mao et al., 2013; Johnson et al., 2015). Accordingly, a clinical net-effect of crizotinib (as the interacting drug) on oral exposures (e.g., area under plasma concentration-time curve, AUC) of a CYP3A probe substrate, midazolam, was evaluated in cancer patients that received a single oral dose of midazolam (2 mg) before and after 28-day multiple oral administration of crizotinib 250 mg twice daily (Tan et al., 2010). The fold-increase in AUC ($AUC_R$) for midazolam was 3.7, suggesting crizotinib was a moderate CYP3A inhibitor. A reasonable prediction of midazolam $AUC_R$ with crizotinib has been reported using a PBPK modeling approach (Mao et al., 2013). In addition, crizotinib single-dose DDI studies (as the substrate drug) with a strong CYP3A inhibitor or inducer, i.e., ketoconazole (200 mg twice daily) or rifampin (600 mg once daily), were conducted in healthy volunteers (CDER, 2011; Xu et al., 2011a; Xu et al., 2011b). In these DDI studies, crizotinib $AUC_R$ was 3.2 with ketoconazole whereas that was 0.18 with rifampin. As mentioned above, crizotinib is mainly metabolized by CYP3A (as its own primary clearance), which is moderately inhibited and weakly induced by crizotinib itself, resulting in nonstationary pharmacokinetics during multiple-dose administration (CDER, 2011). Thus, a post-marketing requirement by FDA has been issued to conduct crizotinib “multiple-dose” DDI studies with strong CYP3A inhibitors and inducers to inform potential dose-adjustment (CDER, 2011). While this particular example may not be a rare complex DDI case, the number of factors to be considered to predict the outcome requires sophisticated
mechanistic models as similar to complex DDIs. Furthermore, it can be practically challenging to conduct multiple-dose DDI studies of anticancer drugs in a sufficient number of “patients” to be recruited and completed. There are also ethical concerns about the possibility of sub- and supra-therapeutic exposures of crizotinib in patients with concomitant multiple-dose administration of a strong CYP3A inhibitor or inducer.

Given these challenges to conduct multiple-dose DDI studies, it would be highly beneficial to develop, refine and verify a PBPK model of crizotinib based on currently available data to quantitatively predict crizotinib exposures in multiple-dose DDI studies. The main objectives of the present study were: 1) to develop and refine crizotinib PBPK model based on the clinical single- and 28-day multiple-dose pharmacokinetic results, 2) to verify crizotinib PBPK model based on the results of crizotinib single-dose DDI studies with ketoconazole or rifampin, and 3) to apply the crizotinib PBPK model to predict crizotinib multiple-dose DDI outcomes. A commercially available Simcyp population-based dynamic simulator was used in the present study (Jamei et al., 2009). Furthermore, we investigated effects of crizotinib CYP3A net-inhibition on its oral exposures by comparing the prediction outcomes between the crizotinib PBPK models with and without the DDI parameters such as CYP3A time-dependent inhibition and induction. Consequently, we focused on gaining insights into the underlying mechanisms mediating crizotinib DDIs using the mechanistic dynamic modeling approach.
Materials and Methods

Clinical Pharmacokinetic Data

Detailed information about crizotinib clinical studies such as a single-dose oral bioavailability study, single-dose DDI studies with ketoconazole or rifampin and Phase I multiple-dose escalation studies were previously reported by Pfizer Oncology Business Unit (Pfizer Inc., San Diego, CA) (Tan et al., 2010; Xu et al., 2011b; Xu et al., 2011a; Xu et al., 2015). Additional information about crizotinib pharmacokinetics is also available in the FDA website (CDER, 2011). Briefly, in the single-dose oral bioavailability study, a single dose of crizotinib was administered to 14 healthy male adult volunteers either intravenously (50 mg for 2 hours) or orally (250 mg) in a two-way crossover design with a ≥14-day washout period. Plasma concentrations of crizotinib in subjects were determined up to 7 days postdose. The multiple-dose phase I study of crizotinib at the twice daily dose of 250 mg was conducted in 9 cancer patients. Plasma concentrations of crizotinib were determined on day -7 (single dose, n = 9), day 15 (n = 5) and day 28 (n = 5). The crizotinib single-dose DDI studies with ketoconazole or rifampin were conducted in 15 healthy male adult volunteers in a fasted state in a two-way crossover design with a ≥14-day washout period. In the DDI study with ketoconazole, a single oral dose of crizotinib (150 mg) was administered to healthy volunteers either as crizotinib alone or crizotinib on day 4 with coadministration of ketoconazole (200 mg twice daily with 12-h interval) from days 1 to 16. The lower dose of crizotinib (150 mg) relative to the recommended dose (250 mg) was selected in this study since an increase in crizotinib oral exposures was expected with coadministration of ketoconazole. In the DDI study with rifampin, a single oral dose of crizotinib (250 mg) was administered to healthy volunteers.
volunteers either as crizotinib alone or crizotinib on day 9 with coadministration of rifampin (600 mg once daily) from days 1 to 14. Plasma concentrations of crizotinib in all subjects were determined up to 14 days postdose in the DDI study with ketoconazole and up to 7 days postdose in the DDI study with rifampin.

**Crizotinib Input Parameters into Simcyp**

Physicochemical and pharmacokinetic parameters of crizotinib used for the present PBPK model are summarized in Table 1. Crizotinib hepatic microsomal intrinsic clearance ($CL_{\text{int,hep}}$) was back-calculated from the clinically observed plasma clearance ($CL$) using a retrograde model implemented in Simcyp version 13.1 (Jamei et al., 2009). Crizotinib intravenous and oral plasma CL estimates (geometric mean) were 46.8 and 108 L/h, respectively, in the single-dose oral bioavailability study (Xu et al., 2015). In the crizotinib single-dose DDI studies with ketoconazole and rifampin, crizotinib oral plasma CL estimates in the control groups (crizotinib alone) were 123 and 119 L/h, respectively (Xu et al., 2011a; Xu et al., 2011b). Crizotinib renal CL was estimated as 2.25 L/h based on urinary excretion (2.3% of the administered dose as parent drug) in a single oral-dose human mass-balance study with $[^{14}\text{C}]$crizotinib (Johnson et al., 2015). The fraction of dose absorbed from gastrointestinal tract ($F_a$) was estimated at approximately 0.5 since crizotinib excretion into feces was 53% of the administered oral dose (250 mg) in the human mass-balance study (Johnson et al., 2015). By taking into account renal CL, $F_a$ and $F_g$ (the fraction of dose that escapes intestinal first-pass metabolism), the back-calculated crizotinib $CL_{\text{int,hep}}$ values were 222 µL/min/mg microsomal protein from the intravenous CL (46.8 L/h) and 129 µL/min/mg microsomal protein from the oral CL (108 L/h) in the bioavailability study, and 146 µL/min/mg
microsomal protein from the mean oral CL estimate (121 L/h) in the control groups of the DDI studies. A fraction metabolized by CYP3A4 (\( f_{m,CYP3A4} \)) was estimated to be 0.8 based on the in vitro CYP phenotyping and the human mass-balance study (Johnson et al., 2011; Johnson et al., 2015); therefore, 80% of the back-calculated total CLint,hep was assigned to CYP3A4-mediated CLint while the remaining CLint,hep value was assigned to additional microsomal CLint,hep. Clinically observed crizotinib steady-state volume of distribution (\( V_{ss} \)) was 1772 L (= 25 L/kg) after a single intravenous 2-hour infusion; therefore, the predicted \( V_{ss} \) of 7.5 L/kg with the tissue-composition based model implemented in Simcyp (as the mathematical model 2) was adjusted to 25 L/kg using a Simcyp \( K_p \) scalar of 3.4 (Rodgers et al., 2005). Crizotinib time-dependent inactivation constant (\( K_i \)) and maximum inactivation rate constant (\( k_{inact} \)) for CYP3A4 were estimated as 0.89 µM and 0.78 h\(^{-1}\), respectively, in cryopreserved human hepatocytes suspended in human plasma (Mao et al., 2013). Crizotinib in vitro inhibitory effect on CYP3A as a reversible inhibitor was negligible (IC\(_{50}\) >30 µM) in human liver microsomes. Crizotinib CYP3A4 induction parameters, \( E_{max} \) and EC\(_{50}\), were estimated from three individual cryopreserved hepatocytes, and then normalized to 2.4 and 0.84 µM, respectively, with the positive control, rifampin (mean \( E_{max} \) of 90-fold and EC\(_{50}\) of 0.57 µM), by the induction calibrator of prediction tool box implemented in Simcyp.

**Simcyp Simulation**

Simcyp simulation of crizotinib plasma concentrations was performed by a full-PBPK model with a first-order absorption rate constant (\( k_a \)), that was set to predict clinically observed \( t_{max} \) (time to reach maximal plasma concentration, \( C_{max} \)). In the Simcyp \( Q_{gut} \) model (Yang et al., 2007), crizotinib blood flow term (\( Q_{gut} \)) was predicted to
be 4.0 L/h based on crizotinib physicochemical properties, and its unbound fraction in the
gut ($f_{u,gut}$) was assumed to be equal to an unbound fraction in blood ($f_{u,blood}$). From
Simcyp compound library of the interacting drugs, ketoconazole (sim-ketoconazole 200
mg bid), rifampin (sim-rifampicin), diltiazem (sim-diltiazem), erythromycin (sim-
erthyromycin), fluconazole (sv-fluconazole) and fluvoxamine (sv-fluvoxamine) were
used for the predictions of crizotinib DDI studies. Their Simcyp default DDI parameters
on CYP3A4 were as follows: ketoconazole competitive $K_i = 0.015$ µM ($f_{u,mic} = 0.97$);
 rifampin induction $E_{max} = 8$, $EC_{50} = 0.32$ µM and competitive $K_i = 10.5$ µM ($f_{u,mic} = 1$);
diltiazem competitive $K_i = 36.1$ µM, mechanism-based inhibition $k_{inact} = 0.702$ h$^{-1}$ and $K_i$
 $= 4.75$ µM ($f_{u,mic} = 1$); erythromycin competitive $K_i = 82$ µM ($f_{u,mic} = 0.909$), mechanism-
based inhibition $k_{inact} = 2.25$ h$^{-1}$ and $K_i = 23.2$ µM ($f_{u,mic} = 1$); fluconazole competitive $K_i$
 $= 10.7$ µM ($f_{u,mic} = 1$); fluvoxamine $K_i = 17.89$ µM ($f_{u,mic} = 0.441$). Rifampin $E_{max}$ was
set at 16 in the present study because it has been reported that a better correlation
between Simcyp-predicted and observed midazolam $AUC_R$ was observed among several
studies using rifampin $E_{max}$ of 16 than 8 (Almond et al., 2012).

All clinical trial simulations in Simcyp were performed with a virtual population
of healthy volunteers (fasted state) in 6 trials of 15 subjects (total 90 subjects), each aged
20 to 50 years with a female/male ratio of 0.5, whose CYP3A4 degradation rate constant
was 0.0193 h$^{-1}$ in the liver and 0.030 h$^{-1}$ in the intestine. The output sampling interval in
Simcyp simulation tool box was set to 0.2 hours in all simulations. Trial designs in the
clinical studies and the corresponding simulations were as follows:
Trial #1 (Single-dose intravenous pharmacokinetic study): A single intravenous dose of crizotinib (50 mg) was administered for 2 hours; plasma concentrations of crizotinib were simulated up to 7 days.

Trial #2 (Single-dose oral pharmacokinetic study): A single oral dose of crizotinib (250 mg) was administered; plasma concentrations of crizotinib were simulated up to 7 days.

Trial #3 (Single-dose DDI study with ketoconazole): A single oral dose of crizotinib (150 mg) was administered on day 4 with and without multiple oral coadministration of ketoconazole 200 mg twice daily for 16 days; plasma concentrations of crizotinib and ketoconazole were simulated during the drug treatment period.

Trial #4 (Single-dose DDI study with rifampin): A single oral dose of crizotinib (250 mg) was administered on day 9 with multiple oral coadministration of rifampin 600 mg once daily for 14 days; plasma concentrations of crizotinib and rifampin were simulated during the drug treatment period.

Trial #5 (Multiple-dose pharmacokinetic study): An oral dose of crizotinib (250 mg) was administered twice daily for 28 days; plasma concentrations of crizotinib were simulated during the drug treatment period.

Trial #6 (Multiple-dose DDI study with ketoconazole): An oral dose of crizotinib (150 mg) was administered twice daily for 28 days with and without multiple oral coadministration of ketoconazole 200 mg twice daily for 28 days; plasma concentrations of crizotinib and ketoconazole were simulated during the drug treatment period.
Trial #7 (Multiple-dose DDI study with rifampin): An oral dose of crizotinib (250 mg) was administered twice daily for 28 days with and without multiple oral coadministration of rifampin 600 mg once daily for 28 days; plasma concentrations of crizotinib and rifampin were simulated during the drug treatment period.

Trials #8 (Multiple-dose DDI study with a weak or moderate CYP3A inhibitor): An oral dose of crizotinib (150 mg) was administered twice daily for 28 days with and without multiple oral coadministration of diltiazem 120 mg twice a day, erythromycin 500 mg three times a day, fluconazole 200 mg once a day or fluvoxamine 50 mg once a day for 28 days; plasma concentrations of crizotinib, diltiazem, erythromycin and fluconazole were simulated during the drug treatment period.

These trial designs are also summarized in Table 2. Two Simcyp compound files of crizotinib with (on) and without (off) crizotinib DDI parameters such as CYP3A4 time-dependent inhibition (K_i and k_inact) and induction (E_max and EC_{50}) were used to investigate net-effects of crizotinib DDI potential on the simulation outcomes #3-7. That is, the on/off ratios in the simulation results suggest net-effects of crizotinib auto-inhibition/induction potential on crizotinib pharmacokinetics and DDI outcomes. In the single-dose trials #1 to 4, the area under the plasma concentration-time curve from time zero to infinity (AUC_{0-\infty}) was calculated from simulated plasma concentrations using the linear trapezoidal rule:

\[ AUC_{0-\infty} = \int_{0}^{\infty} + C_L/\lambda \]  \hspace{1cm} (1)
where $AUC_{0-L}$, $C_L$ and $\lambda$ represent the area under the plasma concentration-time curve from time zero to the last time point, 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.

Apparent terminal half-life ($t_{1/2,z}$) was calculated from $0.693/\lambda$ whereas $C_{\text{max}}$ and $t_{\text{max}}$ were obtained from Simcyp outputs. To understand crizotinib nonstationary pharmacokinetics, an index of nonstationary pharmacokinetics ($NSI$) was calculated from the area under the plasma concentration-time curve over dosing interval ($AUC_{0-\tau}$) at steady-state divided by $AUC_{0-\tau}$ in the corresponding single-dose studies. In the DDI studies, the fold-increases in $C_{\text{max}}$ ($C_{\text{maxR}}$) and $AUC_R$ (e.g., $AUC_{0-\tau}$ ratios in the single-dose studies and $AUC_{0-\tau}$ ratios in the multiple-dose studies) were calculated from the ratios of the simulated values in treatment groups relative to control groups. In addition, fractions metabolized/excreted ($f_m$) by each CL pathway at all simulation time points were individually calculated from simulated $CL_{\text{int}}$ value in each pathway divided by total $CL_{\text{int}}$ value in a Simcyp virtual population. Crizotinib $F_g$ values at all simulation time points were also individually calculated by the $Q_{\text{gut}}$ model using the simulated $Q_{\text{gut}}$, $f_{u,\text{gut}}$ and intestinal $CL_{\text{int}}$ in a Simcyp virtual population. Mean and median $f_m$ and $F_g$ values were then calculated along with percent coefficient of variation (CV%) and the 5th to 95th percentiles per each treatment day. All of these calculations on Simcyp output files were performed with Microsoft Excel 2007 (Microsoft, Redmond, WA). Geometric means of pharmacokinetic parameters in 6 simulation trials were compared to the clinically observed geometric mean. A goodness-of-fit between the model-predicted and observed plasma concentrations was assessed by a linear regression analysis ($r^2 > 0.9$) with
SigmaPlot 11 (Systat Software, Inc., San Jose, CA), together with visual inspection, while that on the pharmacokinetic parameters such as $C_{\text{max}}$ and AUC was evaluated with the predicted-to-observed ratio of $\leq \pm 50\%$. Plots of goodness-of-fit for the model-predicted and observed plasma concentrations were summarized in Supplemental Data, Figure S1.
RESULTS

Model Development and Verification

Prediction of Crizotinib Single-Dose Pharmacokinetics: Following a single intravenous 2-hour infusion of crizotinib 50 mg to healthy subjects, the observed crizotinib $C_{\text{max}}$, $AUC_{0-\infty}$ and $t_{1/2,z}$ (geometric mean) were 155 ng/mL, 1067 ng·h/mL and 39 hour, respectively (Table 3). As shown in Fig. 1A, crizotinib plasma concentrations were adequately predicted by the PBPK model ($r^2 = 0.983$). Consequently, the predicted crizotinib $C_{\text{max}}$ (167 ng/mL), $AUC_{0-\infty}$ (1258 ng·h/mL) and $t_{1/2,z}$ (45 hour) were within 20% of the observed results. Following a single oral administration of crizotinib 250 mg to healthy subjects, the observed crizotinib estimates for $C_{\text{max}}$, $AUC_{0-\infty}$ and $t_{1/2,z}$ were 100 ng/mL, 2321 ng·h/mL and 29 hour, respectively (Table 3). Crizotinib oral plasma concentrations were also reasonably predicted by the PBPK model ($r^2 = 0.977$) (Fig. 1B). The predicted crizotinib $C_{\text{max}}$ (103 ng/mL) and $AUC_{0-\infty}$ (2878 ng·h/mL) were within 20% of the observed results whereas $t_{1/2,z}$ (58 hours) was slightly over-predicted by 2-fold.

Prediction of Crizotinib Pharmacokinetics in Crizotinib Single-Dose DDI Study with Ketoconazole: In the crizotinib single-dose DDI study with ketoconazole, an oral dose of crizotinib 150 mg was administered to healthy volunteers either as crizotinib alone (control group) or crizotinib on day 4 with coadministration of ketoconazole 200 mg twice daily from days 1 to 16 (treatment group). Clinically observed crizotinib $C_{\text{max}}$ and $AUC_{0-\infty}$ (geometric mean) were, respectively, 66 ng/mL and 1260 ng·h/mL in the control group and 94 ng/mL and 3986 ng·h/mL in the treatment group, resulting in $C_{\text{maxR}}$ of 1.4 and $AUC_{R}$ of 3.2 (Table 4). PBPK model-predicted crizotinib plasma concentrations reasonably matched the observed results in both the control and treatment
groups ($r^2 = 0.946$ and 962, respectively) (Fig. 2A and 2B). The predicted crizotinib $C_{\text{max}}$ and $AUC_{0-\infty}$ in both the groups were within 20% of the observed results whereas the predicted $t_{1/2,z}$ values were roughly within 2-fold of the observed values in both the groups (Table 4). In addition, the simulation was performed without crizotinib DDI parameters (off) to investigate crizotinib CYP3A net-inhibition effects on the DDI outcomes. The predicted crizotinib $C_{\text{max}}$, $AUC_{0-\infty}$ and $t_{1/2}$ were comparable between the simulations on and off (Table 4). The predicted crizotinib $C_{\text{max}}R$ (1.6) and $AUC_R$ (3.6) in the simulation off were also comparable to those (1.6 and 3.4, respectively) in the simulation on. Time-courses of the predicted crizotinib hepatic and intestinal $CL_{\text{int}}$ in the simulation on are graphically presented in Fig. 3A and 3B, respectively. The hepatic and intestinal $CL_{\text{int}}$ values in the control group slightly decreased on day 4 when crizotinib was orally administered and thereafter returned to the baseline level by the end of study. In contrast, the hepatic and intestinal $CL_{\text{int}}$ values in the treatment group were significantly inhibited from the beginning of ketoconazole treatment, resulting in a little change in crizotinib $CL_{\text{int}}$ values on day 4. Based on the predicted time-courses of crizotinib median $f_{\text{m,CYP3A4}}$ and $F_g$ in a Simcyp virtual population of 90 subjects, ketoconazole-mediated $CL_{\text{int}}$ inhibition resulted in a decrease in $f_{\text{m,CYP3A4}}$ of 0.80 to 0.19 with an increase in $F_g$ of 0.94 to 1.0 on day 4 (data not shown). Overall, the Simcyp simulation reasonably predicted the effect of ketoconazole on the crizotinib exposures in the crizotinib single-dose DDI study.

Prediction of Crizotinib Pharmacokinetics in Crizotinib Single-Dose DDI Study with Rifampin: In the crizotinib single-dose DDI study with rifampin, an oral dose of crizotinib 250 mg was administered to healthy volunteers either as crizotinib alone
(control group) or crizotinib on day 9 with coadministration of rifampin 600 mg once
daily from days 1 to 14 (treatment group). Clinically observed crizotinib $C_{\text{max}}$ and
$AUC_{0-\infty}$ (geometric mean) were, respectively, 102 ng/mL and 2192 ng·h/mL in the
control group and 32 ng/mL and 397 ng·h/mL in the treatment group, resulting in $C_{\text{max},R}$
of 0.31 and $AUC_{R}$ of 0.18 (Table 4). PBPK model-predicted crizotinib plasma
concentrations reasonably matched the observed results in both the control and treatment
groups ($r^2 = 0.988$ and 0.911, respectively) (Fig. 2C and 2D). The predicted crizotinib
$C_{\text{max}}$ and $AUC_{0-\infty}$ in both the groups were within 10% of the observed result whereas the
predicted $t_{1/2,z}$ values were within 2-fold of the observed values in the control and
treatment groups (Table 4). When the DDI simulation was performed without crizotinib
DDI parameters (off), the predicted crizotinib $C_{\text{max}}$, $AUC_{0-\infty}$ and $t_{1/2}$ were comparable to
the predicted values in the simulation on. The predicted crizotinib $C_{\text{max},R}$ (0.30) and
$AUC_{R}$ (0.15) in the simulation off were also comparable to those (0.31 and 0.15,
respectively) in the simulation on (Table 4). Time-courses of the predicted crizotinib
hepatic and intestinal $CL_{\text{int}}$ in the simulation on are presented in Fig. 4A and 4B,
respectively. The hepatic and intestinal $CL_{\text{int}}$ values in the treatment group significantly
increased from the beginning of rifampin treatment and then reached steady-state around
day 6. Thereafter, the $CL_{\text{int}}$ values slightly decreased on day 9 when crizotinib was orally
administered, and then returned to the steady-state level by the end of study. The
predicted time-courses of crizotinib median $f_{m,\text{CYP3A4}}$ and $F_g$ in a Simcyp virtual
population showed an increase in $f_{m,\text{CYP3A4}}$ of 0.80 to 0.96 with a decrease in $F_g$ of 0.94 to
0.73 on day 9 (data not shown). Overall, the Simcyp simulation sufficiently predicted the
effect of rifampin on the crizotinib exposures in the crizotinib single-dose DDI study.
Model Verification and Refinement

Prediction of Multiple-Dose Crizotinib Pharmacokinetics: Following 28-day multiple oral administration of crizotinib 250 mg twice daily to cancer patients, the observed crizotinib plasma concentration-time profiles were relatively flat during the dosing interval of 12 hours as presented in Fig. 5. Clinically observed crizotinib $C_{\text{max}}$ and $\text{AUC}_{0-\tau}$ (geometric mean) on day 28 were 328 ng/mL and 3054 ng·h/mL, respectively, with $\text{NSI}$ of 1.3 (Table 5). The PBPK-model over-predicted crizotinib plasma concentrations (Fig. 5A), resulting in predicted crizotinib $C_{\text{max}}$ (515 ng/mL) and $\text{AUC}_{0-\tau}$ (6165 ng·h/mL) that were, respectively, 1.6- and 2.0-fold higher than the observed results. The predicted $\text{NSI}$ (2.1) was also over-predicted by 1.6-fold. Thus, the predicted crizotinib oral exposures did not meet our criteria of goodness-of-model prediction ($\leq 50\%$), leading us to refine the model to improve the agreement between the observed versus predicted results. Based on a sensitivity analysis of crizotinib pharmacokinetic parameters, the PBPK model-predicted plasma concentrations were in good agreement with the observed results when $F_a$ was assumed to be 0.3 (Fig. 5B). The predicted $C_{\text{max}}$ (266 ng/mL) and $\text{AUC}_{0-\tau}$ (3182 ng·h/mL) were within 20% of the observed values (Table 5). The predicted $\text{NSI}$ (1.1) was also consistent with the observed result (1.3).

To further investigate crizotinib-mediated CYP3A net-inhibition potential during multiple-dose oral administration, steady-state crizotinib pharmacokinetic parameters were compared between the simulations on and off (Table 5). The predicted on/off ratios for crizotinib $\text{AUC}_{0-\tau}$ were 2.1 to 2.5, suggesting the crizotinib CYP3A net-inhibition effect on the accumulation of steady-state oral exposure was 2 to 3-fold (if $F_a$ was consistent during the multiple-dose administration). In addition, the time-courses of
crizotinib median $f_{m,CYP3A4}$ in the liver and $F_g$ in the intestine were predicted in the simulation on. Crizotinib $f_{m,CYP3A4}$ decreased from 0.80 on day 0 to 0.48 on day 28 during 28-day multiple-dose administration while $F_g$ increased from 0.94 on day 0 to 0.98 on day 28 (data not shown).

**Model Application**

**Prediction of Crizotinib Pharmacokinetics in Multiple-Dose DDI Study with Ketoconazole:** Following 28-day multiple oral administration of crizotinib 150 mg twice daily with and without coadministration of ketoconazole 200 mg twice daily, the predicted crizotinib $C_{max}$ and $AUC_{0-\tau}$ were, respectively, 114 ng/mL and 1359 ng·h/mL in the control group and 250 ng/mL and 2978 ng·h/mL in the treatment group, resulting in the predicted $C_{maxR}$ of 2.2 and $AUC_R$ of 2.2 (Table 6). Thus, the predicted $AUC_R$ in the multiple-dose simulation was 1.5-fold lower than that (3.4) in the single-dose simulation (Table 4). When crizotinib DDI parameters were not incorporated into the simulation (off), the predicted $C_{max}$ and $AUC_{0-\tau}$ in the treatment group (221 ng/mL and 2619 ng·h/mL, respectively) were 3.3-fold higher than those in the control group (66 ng/mL and 791 ng·h/mL, respectively). Thus, the predicted $AUC_R$ (3.3) in the simulation off was comparable to that (3.4) in the single-dose simulation. It is worth noting that the on/off ratio was diminished from 1.7 in the control group to 1.1 in the treatment group, leading to the lower $AUC_R$ of 2.2 in the simulation on.

Time-courses of the predicted crizotinib hepatic and intestinal $CL_{int}$ in the simulation on are graphically presented in Fig. 3C and 3D, respectively. The hepatic and intestinal $CL_{int}$ values in the control group gradually decreased and then reached steady-state around day 14 whereas those in the treatment group were significantly inhibited.
from the beginning of the treatment. At the end of treatment (i.e., day 28), the hepatic and intestinal $\text{CL}_{\text{int}}$ values were, respectively, 3- and 12-fold lower in the treatment group than in the control group. The hepatic $\text{CL}_{\text{int}}$ inhibition led to a decrease in crizotinib $f_{m,CYP3A4}$ of 0.80 (median) to 0.61 in the control group and to 0.07 in the treatment group on day 28 (Fig. 6A). In contrast, crizotinib $F_g$ increased from 0.94 (median) to 0.97 in the control group and to 1.0 in the treatment groups on day 28 (Fig 6B). The predicted crizotinib $f_{m,CYP3A4}$ and $F_g$ reached steady-state around day 14.

Prediction of Crizotinib Pharmacokinetics in Crizotinib Multiple-dose DDI Study with Rifampin: Following 28-day multiple oral administration of crizotinib 250 mg twice daily with and without coadministration of rifampin 600 mg once daily, the predicted crizotinib $C_{\text{max}}$ and $AUC_{0-\tau}$ were, respectively, 225 ng/mL and 2694 ng·h/mL in the control group and 24 ng/mL and 275 ng·h/mL in the treatment group with the large inter-subject variability of ~120% (Table 6). The predicted $C_{\text{max}}R$ and $AUC_R$ were 0.11 and 0.10, respectively. Thus, the predicted $AUC_R$ was roughly comparable between the single- and multiple-dose simulations. When crizotinib DDI parameters were not incorporated into the simulation (off), the predicted $C_{\text{max}}$ and $AUC_{0-\tau}$ were, respectively, 110 ng/mL and 1319 ng·h/mL in the control group and 15 ng/mL and 176 ng·h/mL in the treatment group. Thus, the predicted $AUC_R$ was comparable between the simulations on and off (0.10 and 0.13, respectively).

Time-courses of the predicted crizotinib hepatic and intestinal $\text{CL}_{\text{int}}$ in the simulation on are graphically presented in Fig. 4C and 4D, respectively. The hepatic and intestinal $\text{CL}_{\text{int}}$ values in the control group gradually decreased whereas those in the treatment group significantly increased from the beginning of the treatment to around
day 6 and then slightly decreased during the rest of the treatment period. The hepatic and intestinal CL_int values were 4 to 6-fold higher in the treatment group than in the control group on day 28. The rifampin-mediated CYP3A induction led to an increase in crizotinib f_m,CYP3A4 of 0.80 (median) to 0.96 on day 28 (Fig. 6C) while crizotinib F_g slightly decreased from 0.94 to 0.80 on day 28 (Fig. 6D). The predicted crizotinib f_m,CYP3A4 and F_g reached steady-state around day 14.

Prediction of Crizotinib Pharmacokinetics in Multiple-Dose DDI Study with a Weak or Moderate CYP3A Inhibitor: Following 28-day multiple oral administration of crizotinib 150 mg twice daily with and without coadministration of diltiazem (120 mg twice a day), erythromycin (500 mg three times a day), fluconazole (200 mg once a day) or fluvoxamine (50 mg once a day), the predicted crizotinib C_max and AUC_0-τ were, respectively, 123 ng/mL and 1476 ng·h/mL with diltiazem, 179 ng/mL and 2141 ng·h/mL with erythromycin, 199 ng/mL and 2378 ng·h/mL with fluconazole, and 114 ng/mL and 1368 ng·h/mL with fluvoxamine (Table 7). Correspondingly, the predicted AUC_R in the treatment groups with diltiazem, erythromycin and fluconazole were 1.1, 1.6, 1.8 and 1.0, respectively.
DISCUSSION

It has become common to utilize PBPK modeling to contribute to our understanding of DDI mechanisms. We have sought to develop and verify a PBPK model that could achieve this goal for crizotinib. The present study clearly illustrates the challenges associated with in developing such a model for a substrate drug that has time-dependent inhibition and induction properties when administered in combination with an interacting drug that is a strong inhibitor or inducer. Despite the complexity of these DDI mechanisms, the crizotinib PBPK model described appears successful in providing plausible predictions of multiple-dose DDI outcomes based on available single-dose DDI data along with single- and multiple-dose pharmacokinetic results. However, some issues identified in the present study remain and warrant further discussion.

In the single-dose oral bioavailability study, crizotinib oral bioavailability was estimated to be 0.43 with an $F_a \times F_g$ value of 0.96 in healthy subjects at an oral dose of 250 mg relative to an intravenous dose of 50 mg assuming linear pharmacokinetics (Xu et al., 2015). Using the model-predicted $F_g$ value of 0.94, $F_a$ was calculated to be ~0.9 in this study. However, the recovery of crizotinib (as parent drug) in feces of healthy subjects in the single-dose mass-balance study (250 mg) was 53% of the administered dose, and it was unlikely that this result was confounded by biliary excretion of the parent drug and/or reversible metabolites (Johnson et al., 2015). Therefore, the fecal recovery of crizotinib in the mass-balance study was considered as the fraction of dose unabsorbed (1-$F_a$). The discrepancy between $F_a$ estimates from these studies has been considered to be attributed to nonlinear pharmacokinetics between the oral dose of 250 mg versus the intravenous dose of 50 mg. Based on these findings, crizotinib $F_a$ was set to 0.5 in the
single-dose simulations, resulting in model-predicted crizotinib plasma concentrations that reasonably matched the observed results in the single-dose studies including the DDI studies. In contrast, crizotinib steady-state plasma concentrations in cancer patients were over-predicted by approximately 2-fold (Table 5), which led us to refine the model for the multiple-dose pharmacokinetic simulation. Following a sensitivity analysis of crizotinib pharmacokinetic parameters, the model-predicted results were in good agreement with the observed results when $F_a$ was assumed to be 0.3, suggesting that crizotinib $F_a$ might decrease from 0.5 to 0.3 during multiple-dose administration. Given the relatively large dose amount (250 mg), a twice-daily dosing regimen and different plasma concentration-time profiles at steady-state (i.e., relatively flat profiles) compared to single-dose administration, it could be possible that the absorption process became saturated following multiple-dose administration. Alternatively, crizotinib $F_a$ may differ between healthy subjects (single-dose) and cancer patients (multiple-dose), due, e.g., to its pH-dependent solubility and/or relatively low permeability with an efflux potential, i.e., a substrate of P-glycoprotein (CDER, 2011). Furthermore, crizotinib has been suggested as a moderate CYP3A inhibitor given the clinically observed DDI result with midazolam (i.e., $AUC_R$ of 3.7) following multiple-dose administration of crizotinib 250 mg twice daily (Tan et al., 2010). Based on the comparison of crizotinib steady-state simulations between on and off (without an interacting drug), crizotinib CYP3A net-inhibition potential (i.e., on/off ratio) was predicted as 2 to 3-fold (Table 5). This moderate on/off ratio appeared consistent with the midazolam $AUC_R$ when taking into account the difference in $f_m,CYP3A4$ and $F_g$ between crizotinib (0.8 and 0.9, respectively) and midazolam (0.9 and 0.6, respectively) (Wang, 2010). Conversely, the observed
crizotinib NSI of 1.3 suggested that crizotinib CYP3A net-inhibition potential was negligible to weak (Table 5). This discrepancy could also be explained by a possible decrease in $F_a$ following multiple-dose administration. That is, the lower than anticipated NSI may be a result of the net-effect of the opposing mechanisms of crizotinib-mediated CYP3A net-inhibition and a decrease in $F_a$. Additionally, when crizotinib steady-state plasma concentrations were simulated with only crizotinib CYP3A induction parameters ($E_{\text{max}}$ and $EC_{50}$) without its inhibition parameters ($K_i$ and $k_{\text{inact}}$), the simulated plasma concentrations were within 10% of the result of simulation off (data not shown). Thus, it is unlikely that crizotinib-mediated CYP3A induction can offset its CYP3A inhibition following multiple-dose administration.

In the single-dose DDI prediction with ketoconazole, the predicted crizotinib $C_{\text{max}}$ and AUC$_{0-\infty}$ in both the control and treatment groups were comparable between the simulations on and off (Table 4), suggesting a negligible effect of crizotinib CYP3A net-inhibition on the single-dose DDI results. These findings were expected based on the overall crizotinib net-inhibition mechanism (i.e., moderate time-dependent inhibition with weak induction). In the multiple-dose DDI prediction with ketoconazole, crizotinib CYP3A net-inhibition could theoretically be considered as additive to ketoconazole-mediated inhibition. The predicted $C_{\text{max}}$ and AUC$_{0-\tau}$ in the control group were 1.7-fold higher in the simulation on than off due to the effect of crizotinib-mediated CYP3A net-inhibition (Table 6). In contrast, the predicted crizotinib $C_{\text{max}}$ and AUC$_{0-\tau}$ in the treatment groups were comparable between the simulations on and off, which was most likely due to strong (or near-complete) CYP3A inhibition by ketoconazole.

Correspondingly, the diminished on/off ratios from the control group (1.7) to the
treatment group (1.1) resulted in the 1.5-fold lower predicted $C_{maxR}$ and $AUC_{R}$ in the simulation on (2.2) than off (3.3). As shown in Fig. 3, the predicted crizotinib hepatic and intestinal $CL_{int}$ values were significantly inhibited by ketoconazole (treatment group) relative to crizotinib (control group). As a result, the predicted crizotinib $f_{m,CYP3A4}$ in the treatment group decreased from 0.80 to 0.07 while the predicted $F_g$ increased from 0.94 to 1.0, suggesting a near-maximal CYP3A inhibition following multiple-dose administration of ketoconazole 200 mg twice daily, which appears consistent with a previous report (Zhao et al., 2009). To confirm this hypothesis, crizotinib DDI prediction was performed with a higher ketoconazole dose of “400 mg twice daily” (Supplemental Data, Table S1). As expected, the predicted crizotinib $C_{max}$ and $AUC_{0-\tau}$ in the treatment group were roughly comparable to those with ketoconazole at the dose of 200 mg twice daily. Thus, the inhibition of CYP3A-mediated crizotinib clearance following multiple-dose administration of ketoconazole 200 mg twice daily appears to be nearly complete.

Taken together, these findings suggest that, when co-administered with ketoconazole, the effect of crizotinib CYP3A net-inhibition on its oral exposures would be negligible following either single- or multiple-dose administration of crizotinib.

In the single-dose DDI prediction with rifampin, the predicted crizotinib $C_{max}$ and $AUC_{0-\infty}$ in both the control and treatment groups were comparable between the simulations on and off (Table 4), suggesting a negligible effect of crizotinib CYP3A net-inhibition on the single-dose DDI outcome, as anticipated from on crizotinib net-inhibition mechanism. Unexpectedly, the observed apparent $t_{1/2,z}$ in the treatment group (48 hour) was slightly longer than that in the control group (33 hour). In contrast, the predicted $t_{1/2,z}$ (33 hour) in the treatment group was shorter than that (55 hour) in the
control group as would be expected from rifampin-mediated CYP3A induction (Table 4). These findings might suggest that the interaction of crizotinib with rifampin could result from not only CYP3A induction but also some other mechanism(s) such as transporter-mediated distribution and/or excretion. Crizotinib has not been identified as a substrate of any uptake transporters; however crizotinib is a substrate of P-glycoprotein (CDER, 2011), which is also induced by rifampin (Paine et al., 2002; Kim et al., 2008). Therefore, P-glycoprotein may be, in part, play a role in the crizotinib-rifampin interaction (e.g., increase in entero-hepatic circulation). In the multiple-dose DDI predictions with rifampin, crizotinib-mediated CYP3A net-inhibition could theoretically diminish rifampin-mediated CYP3A induction to some extent. However, the predicted $C_{\text{max}}$ and AUC$_{0-\tau}$ values in both the control and treatment groups were approximately 2-fold higher in the simulation on than off, resulting in the comparable predicted $C_{\text{max}R}$ and $AUC_R$ between the simulations on and off (Table 6). Consistently, there appeared negligible to minimal effects of crizotinib on the predicted hepatic and intestinal CL$_{\text{int}}$ when the CL$_{\text{int}}$ was markedly induced following multiple-dose coadministration of rifampin (Fig. 4). Additionally, the multiple-dose DDI simulation was performed with a lower rifampin E$_{\text{max}}$ of 8. As expected, the difference in the predicted $C_{\text{max}R}$ and $AUC_R$ between the E$_{\text{max}}$ of 8 (0.26 to 0.28) and 16 (0.10 to 0.13) was 2 to 3-fold due to the difference in the E$_{\text{max}}$ values (Supplemental Data, Table S1). However, the predicted $C_{\text{max}R}$ and $AUC_R$ were still comparable between the simulation on and off with E$_{\text{max}}$ of 8, suggesting that the effects of crizotinib on the multiple-dose DDI outcomes could be negligible even though rifampin E$_{\text{max}}$ was decreased to its half. Collectively, these simulations suggest that the effect of crizotinib-mediated CYP3A net-inhibition on its
oral exposure would be negligible on the multiple-dose DDI outcomes when coadministered with rifampin.

In the DDI prediction for substrate drugs, hepatic $f_{m,CYP3A}$ and intestinal $F_g$ are considered as the most important parameters (Fahmi et al., 2008). The present simulation results suggested that 1) crizotinib hepatic $f_{m,CYP3A}$ decreased from 0.80 (median) to 0.48 following multiple-dose administration of crizotinib 250 mg twice daily, 2) coadministration of crizotinib (150 mg twice daily) with ketoconazole (200 mg twice daily) resulted in the decrease in the $f_{m,CYP3A}$ to 0.07, and 3) coadministration of crizotinib (250 mg twice daily) with rifampin (600 mg once daily) resulted in the increase in the $f_{m,CYP3A}$ to 0.96 (Fig. 7). The changes in hepatic $f_{m,CYP3A}$ would lead to the significant $f_m$ changes in other pathways such as non-CYP3A metabolic clearance and renal excretion. In contrast, the gut contribution to crizotinib systemic DDIs was considered minimal because of the predicted high $F_g$ (0.94). The predicted crizotinib $F_g$ increased to 0.98 following multiple-dose administration of crizotinib alone and to 1.0 by coadministration with ketoconazole, while it decreased to 0.80 by coadministration with rifampin. The mechanistic dynamic modeling approach affords an opportunity to gain such an insight into the underlying mechanisms mediating DDIs as a function of time.

Overall, the present study demonstrated that the crizotinib PBPK model reasonably predicted plasma concentrations of crizotinib in humans after a single intravenous infusion, single- and 28-day multiple oral administration. Furthermore, the PBPK model adequately predicted the fold-increases in crizotinib oral exposures in humans after a single oral administration of crizotinib with multiple-dose coadministration of ketoconazole or rifampin. These results suggest that the crizotinib
PBPK model described has been sufficiently developed, refined and verified based on the clinically observed results, and can be applied to predict crizotinib multiple-dose DDI outcomes. The multiple-dose DDI predictions suggest that crizotinib net-inhibition activity on CYP3A may have a negligible contribution to DDI outcomes when crizotinib is coadministered with strong CYP3A inhibitors or inducers such as ketoconazole and rifampin. Therefore, recommendations for crizotinib dose-adjustment in multiple-dose DDI scenarios could be possible based on the currently available single-dose DDI results. Overall, we believe that the present crizotinib PBPK model can be useful to predict crizotinib exposures in other clinical studies such as DDIs with weak/moderate CYP3A inhibitors/inducers and drug-disease interactions in patients with hepatic or renal impairment.
Acknowledgment

We acknowledge Akintunde Bello, Weiwei Tan and Huiping Xu, (Clinical Pharmacology, Pfizer, San Diego, CA) for valuable discussion about clinical pharmacokinetics of crizotinib and Bhasker Shetty and Paolo Vicini (Pharmacokinetics, Dynamics and Metabolism, Pfizer, San Diego, CA) for excellent inputs for the draft manuscript.
Authorship Contribution

Participated in research design: Yamazaki

Conduct experiments: na

Performed data analysis: Yamazaki

Wrote or contribute to the writing of the manuscript: Johnson, Smith and Yamazaki
References


CDER (2011) Application Number: 202570Orig1s000, Clinical Pharmacology and Biopharmaceutics Review(s).


Footnotes

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Legends for Figures

Fig. 1. Observed and PBPK model-predicted plasma concentrations of crizotinib in healthy subjects after a single intravenous (A) or oral administration (B). Crizotinib was administered intravenously (50 mg for 2-h infusion) or orally (250 mg) to 14 healthy subjects in the single-dose oral bioavailability study. Crizotinib plasma concentrations were predicted in a virtual population of healthy subjects (n =15 per group × 6 groups, total 90 subjects) with a full-PBPK model implemented in the Simcyp simulator. The x-axis represents the time after dosing in hours and the y-axis represents the observed (○) and PBPK model-predicted (—) plasma concentrations in nanograms per milliliter on a logarithmic scale. The observed and predicted plasma concentrations are expressed as mean ± SD and mean (solid line) with 5th and 95th percentiles (dashed line), respectively.

Fig. 2. Observed and PBPK model-predicted plasma concentrations of crizotinib in healthy subjects after a single oral administration of crizotinib with and without coadministration of ketoconazole (A and B) or rifampin (C and D). A single oral dose of crizotinib was administered to 15 healthy subjects either as crizotinib 150 mg alone (A) or crizotinib 150 mg on day 4 (B) of 16-day multiple coadministration of ketoconazole 200 mg twice daily or either as crizotinib 250 mg alone (C) or crizotinib 250 mg on day 9 (D) of 14-day multiple coadministration of rifampin 600 mg once daily. Crizotinib plasma concentrations were predicted in a virtual population of healthy subjects (n =15 per group × 6 groups, total 90 subjects) with a full-PBPK model implemented in the Simcyp simulator. The x-axis represents the time after a single oral administration of crizotinib in hours and the y-axis represents the observed (○) and PBPK model-predicted (—) plasma concentrations of crizotinib in nanograms per milliliter.
milliliter on a logarithmic scale. The observed and predicted plasma concentrations are expressed as mean ± SD and mean (solid line) with 5th and 95th percentiles (dashed line), respectively.

**Fig. 3. PBPK model-predicted crizotinib intrinsic clearance in liver (A and C) and intestine (B and D) of healthy subjects following a single oral administration of crizotinib with and without 16-day multiple oral administration of ketoconazole (A and B) or following 28-day multiple oral administration of crizotinib with and without ketoconazole (C and D).** A full-PBPK model simulation was performed in a Simcyp virtual population of healthy subjects (n = 15 per group × 6 groups, total 90 subjects) following a single oral administration of crizotinib (150 mg) alone or crizotinib (150 mg) on day 4 of 16-day multiple oral coadministration of ketoconazole (200 mg twice daily) and following 28-day multiple oral administration of crizotinib (150 mg twice daily) with and without coadministration of ketoconazole (200 mg twice daily). The x-axis represents the treatment period in days and the y-axis represents PBPK model-predicted crizotinib mean intrinsic clearance in liver (A and C) and intestines (C and D) in litter per hour.

**Fig. 4. PBPK model-predicted crizotinib intrinsic clearance in liver (A and C) and intestine (B and D) of healthy subjects following a single oral administration of crizotinib with and without 14-day multiple oral administration of rifampin (A and B) or following 28-day multiple oral administration of crizotinib with and without rifampin (C and D).** A full-PBPK model simulation was performed in a Simcyp virtual population of healthy subjects (n = 15 per group × 6 groups, total 90 subjects) following a
single oral administration of crizotinib (250 mg) alone or crizotinib (250 mg) on day 9 of
14-day multiple oral coadministration of rifampin (600 mg once daily) and following 28-
day multiple oral administration of crizotinib (250 mg twice daily) with and without
coadministration of rifampin (600 mg once daily). The x-axis represents the treatment
period in days and the y-axis represents PBPK model-predicted crizotinib mean intrinsic
clearance in liver and intestines in litter per hour.

**Fig. 5. Observed and PBPK model-predicted plasma concentrations of crizotinib in
cancer patients following 28-day multiple oral administration.** Crizotinib (250 mg
twice daily dose) was orally administered to 5 cancer patients for 28 days. Crizotinib
plasma concentrations were predicted in a virtual population of healthy subjects (n =15
per group × 6 groups, total 90 subjects) with a full-PBPK model implemented in the
Simcyp simulator assuming crizotinib F_{a} of 0.5 (A) or 0.3 (B). The x-axis represents the
time after dosing in hours and the y-axis represents the observed (○) and PBPK model-
predicted (—) plasma concentrations in nanograms per milliliter on a logarithmic scale.
The observed and predicted plasma concentrations are expressed as mean ± SD and mean
(solid line) with 5^{th} and 95^{th} percentiles (dashed line), respectively.

**Fig. 6. PBPK model-predicted crizotinib f_{m,CYP3A4} (A and C) and F_{g} (B and D) in a
Simcyp virtual population of healthy subjects following 28-day multiple oral
administration of crizotinib with and without ketoconazole (A and B) or rifampin (C
and D).** A full-PBPK model simulation was performed in a Simcyp virtual population of
healthy subjects (n =15 per group × 6 groups, total 90 subjects) following 28-day
multiple oral administration of crizotinib (150 mg twice daily) with and without
coadministration of ketoconazole (200 mg twice daily) or following 28-day multiple oral administration of crizotinib (250 mg twice daily) with and without coadministration of rifampin (600 mg once daily). The x-axis represents the treatment period in days and the y-axis represents PBPK model-predicted median \( f_{m,CYP3A4} \) or \( F_g \) with 10\(^{th}\), 25\(^{th}\), 75\(^{th}\) and 90\(^{th}\) percentiles in fraction.

**Fig. 7. Summary of PBPK model-predicted crizotinib \( f_m \) changes in a Simcyp virtual population of healthy subjects following 28-day multiple oral administration of crizotinib with and without ketoconazole or rifampin.** Crizotinib fractions \( f_m \) metabolized/eliminated by each pathway were predicted in a Simcyp virtual population of healthy subjects (\( n = 15 \) per group \( \times 6 \) groups, total 90 subjects) following 28-day multiple oral administration of crizotinib 250 mg twice daily (crizotinib), 28-day multiple coadministration of crizotinib 150 mg twice daily with ketoconazole 200 mg twice daily (crizotinib with ketoconazole) and 28-day multiple coadministration of crizotinib 250 mg twice daily with rifampin 600 mg once daily (crizotinib with rifampin). Data are expressed as median \( (n = 90) \) in each clearance pathway.
**TABLE 1**

Physicochemical and pharmacokinetic parameters of crizotinib used for PBPK model

<table>
<thead>
<tr>
<th>Parameters (units)</th>
<th>Crizotinib</th>
<th>Parameters (units)</th>
<th>Crizotinib</th>
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<tbody>
<tr>
<td>Molecular weight</td>
<td>450</td>
<td>CL(_{\text{int}}) (μL/min/mg protein)(^c)</td>
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<td>logP</td>
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<td>pK(_a)</td>
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<td>CL(_{\text{int,others}})</td>
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<td>f(_u,\text{plasma})</td>
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<td>R(_{\text{bp}})</td>
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<td>Time-dependent inhibition on CYP3A</td>
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<td>F(_a)</td>
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<td>k(_a) (h(^{-1}))(^a)</td>
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<td>k(_{\text{inact}}) (h(^{-1}))</td>
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<td>Q(_{\text{gut}}) (L/h)</td>
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<td>E(_{\text{max}}) (fold)</td>
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<td>V(_{\text{ss}}) (L/kg)</td>
<td>25</td>
<td>EC(_{50}) (μM)</td>
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\(^a\) The value of k\(_a\) was adjusted to simulate the clinically observed t\(_{\text{max}}\) of approximately 4 hours.  
\(^b\) The value of f\(_u,\text{gut}\) was assumed as f\(_u,\text{blood}\) calculated by f\(_u,\text{plasma}\) and R\(_{\text{bp}}\).  
\(^c\) The values of CL\(_{\text{int}}\) in human liver microsomes were back-calculated from the clinically observed CL estimates using a retrograde model implemented in Simcyp.
## TABLE 2
Simulation outline of crizotinib single- and multiple-dose pharmacokinetic and drug-drug interaction studies

<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>Dose (mg)</th>
<th>Treatment day</th>
<th>Coadministration</th>
<th>Dose (mg)</th>
<th>Treatment day</th>
<th>Analysis</th>
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<td><strong>Single-dose oral bioavailability study</strong></td>
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</tr>
<tr>
<td>IV</td>
<td>50</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Pred vs Obs</td>
</tr>
<tr>
<td>PO</td>
<td>250</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Pred vs Obs</td>
</tr>
<tr>
<td><strong>Single-dose drug-drug interaction studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>150</td>
<td>4</td>
<td>Ketoconazole</td>
<td>200 bid</td>
<td>1 - 16</td>
<td>Pred vs Obs</td>
</tr>
<tr>
<td>PO</td>
<td>250</td>
<td>9</td>
<td>Rifampin</td>
<td>600 qd</td>
<td>1 - 14</td>
<td>Pred vs Obs</td>
</tr>
<tr>
<td><strong>Multiple-dose pharmacokinetic and drug-drug interaction studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO</td>
<td>250 bid</td>
<td>1 - 28</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Pred vs Obs</td>
</tr>
<tr>
<td>PO</td>
<td>150 bid</td>
<td>1 - 28</td>
<td>Ketoconazole</td>
<td>200 bid</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
<tr>
<td>PO</td>
<td>250 bid</td>
<td>1 - 28</td>
<td>Rifampin</td>
<td>600 qd</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
<tr>
<td>PO</td>
<td>150 bid</td>
<td>1 - 28</td>
<td>Diltiazem</td>
<td>120 bid</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
<tr>
<td>PO</td>
<td>150 bid</td>
<td>1 - 28</td>
<td>Erythromycin</td>
<td>500 tid</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
<tr>
<td>PO</td>
<td>150 bid</td>
<td>1 - 28</td>
<td>Fluconazole</td>
<td>200 qd</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
<tr>
<td>PO</td>
<td>150 bid</td>
<td>1 - 28</td>
<td>Fluvoxamine</td>
<td>50 qd</td>
<td>1 - 28</td>
<td>Pred</td>
</tr>
</tbody>
</table>

IV, intravenous administration; PO, oral administration; qd, once a day; bid, twice a day; tid, three times a day; Pred, predicted; Obs, observed.
TABLE 3
Clinically observed and PBPK model-predicted pharmacokinetic parameters of crizotinib in healthy subjects following a single intravenous or oral administration of crizotinib

<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>Single Intravenous 2-hour Infusion (50 mg)</th>
<th>Single Oral Administration (250 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; ng/mL</td>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; ng·h/mL</td>
</tr>
<tr>
<td>Observed</td>
<td>155 (19)</td>
<td>1067 (18)</td>
</tr>
<tr>
<td>Predicted</td>
<td>167 (11)</td>
<td>1258 (23)</td>
</tr>
<tr>
<td>P/O&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (n = 14 for the observed; n = 15 per group × 6 groups for the predicted).

<sup>a</sup>Ratio of the predicted to observed results.
### TABLE 4
Clinically observed and PBPK model-predicted pharmacokinetic parameters of crizotinib in healthy subjects following a single oral administration of crizotinib with and without multiple coadministration of ketoconazole 200 mg twice daily or rifampin 600 mg once daily

<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>Control Group</th>
<th>Treatment Group</th>
<th><strong>Fold-Increase</strong>&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td>t&lt;sub&gt;1/2,z&lt;/sub&gt;</td>
</tr>
<tr>
<td>Crizotinib with ketoconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>66 (35)</td>
<td>1260 (25)</td>
<td>37 (12)</td>
</tr>
<tr>
<td>Predicted (on)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57 (35)</td>
<td>1422 (47)</td>
<td>51 (22)</td>
</tr>
<tr>
<td>P/O&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.87</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Predicted (off)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56 (35)</td>
<td>1335 (45)</td>
<td>50 (21)</td>
</tr>
<tr>
<td>on/off&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Crizotinib with rifampin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>102 (33)</td>
<td>2192 (27)</td>
<td>33 (21)</td>
</tr>
<tr>
<td>Predicted (on)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95 (35)</td>
<td>2499 (48)</td>
<td>55 (23)</td>
</tr>
<tr>
<td>P/O&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.93</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Predicted (off)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94 (35)</td>
<td>2256 (45)</td>
<td>53 (22)</td>
</tr>
<tr>
<td>on/off&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (n = 15 for the observed; n = 15 per group × 6 groups for the predicted).

<sup>a</sup> Fold-increase in C<sub>max</sub> and AUC<sub>0-∞</sub> of the treatment group (crizotinib with ketoconazole or rifampin) relative to control group (crizotinib alone); <sup>b</sup>The simulation results with (on) and without (off) crizotinib DDI parameters; <sup>c</sup> Ratio of the predicted to observed results; <sup>d</sup> Ratio of the predicted results on over off.
<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>$F_a^a$</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$\text{AUC}_{0-\tau}$ (ng·h/mL)</th>
<th>$\text{NSI}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>–</td>
<td>328 (25)</td>
<td>3054 (32)</td>
<td>1.3</td>
</tr>
<tr>
<td>Predicted (on)</td>
<td>0.5</td>
<td>515 (49)</td>
<td>6165 (49)</td>
<td>2.1</td>
</tr>
<tr>
<td>$P/O^d$</td>
<td>1.6</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted (off)</td>
<td>0.5</td>
<td>209 (44)</td>
<td>2500 (44)</td>
<td>–</td>
</tr>
<tr>
<td>$on/off^e$</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted (on)</td>
<td>0.3</td>
<td>266 (52)</td>
<td>3182 (52)</td>
<td>1.1</td>
</tr>
<tr>
<td>$P/O^d$</td>
<td>0.81</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted (off)</td>
<td>0.3</td>
<td>126 (44)</td>
<td>1500 (44)</td>
<td>–</td>
</tr>
<tr>
<td>$on/off^e$</td>
<td>2.1</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (n = 5 for the observed; n = 15 per group × 6 groups for the predicted).

- $F_a^a$: Crizotinib fraction absorbed used as the simulation input parameter;
- $\text{NSI}^b$: An index of nonstationary pharmacokinetics calculated by $\text{AUC}_{0-\tau}$ at steady-state divided by $\text{AUC}_{0-\infty}$ for the single-dose results (i.e., 2321 and 2878 ng·h/mL for the observed and predicted results, respectively, in Table 3);
- $P/O^d$: The simulation results with (on) and without (off) crizotinib DDI parameters;
- $on/off^e$: Ratio of the predicted to observed results;
- $on/off^e$: Ratio of the predicted results on over off; –: not applicable.
TABLE 6
PBPK model-predicted pharmacokinetic parameters of crizotinib in healthy subjects following 28-day multiple oral administration of crizotinib with and without coadministration of ketoconazole or rifampin

<table>
<thead>
<tr>
<th>Crizotinib</th>
<th>Control Group</th>
<th>Treatment Group</th>
<th>Fold-Increase&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crizotinib with ketoconazole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted (&lt;i&gt;on&lt;/i&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114 (57)</td>
<td>250 (38)</td>
<td>2.2 (47)</td>
</tr>
<tr>
<td>Predicted (&lt;i&gt;off&lt;/i&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66 (45)</td>
<td>221 (38)</td>
<td>3.3 (33)</td>
</tr>
<tr>
<td>&lt;i&gt;on/off&lt;/i&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7</td>
<td>1.1</td>
<td>0.66</td>
</tr>
<tr>
<td>Crizotinib with rifampin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted (&lt;i&gt;on&lt;/i&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225 (54)</td>
<td>24 (118)</td>
<td>0.11 (68)</td>
</tr>
<tr>
<td>Predicted (&lt;i&gt;off&lt;/i&gt;)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110 (45)</td>
<td>15 (65)</td>
<td>0.14 (48)</td>
</tr>
<tr>
<td>&lt;i&gt;on/off&lt;/i&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0</td>
<td>1.6</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (<i>n = 15 per group × 6 groups</i>). Dose levels were crizotinib 150 mg twice daily and ketoconazole 200 mg twice daily in the crizotinib-ketoconazole interaction and crizotinib 250 mg twice daily and rifampin 600 mg once daily in the crizotinib-rifampin interaction.

<sup>a</sup>Fold-increase in <i>C<sub>max</sub></i> and <i>AUC<sub>0-τ</sub></i> of the treatment group (crizotinib with ketoconazole or rifampin) relative to control group (crizotinib alone);<sup>b</sup>The simulation results with (<i>on</i>) and without (<i>off</i>) crizotinib DDI parameters; <sup>c</sup>Ratio of the predicted results <i>on</i> over <i>off</i>.
### TABLE 7
PBPK model-predicted pharmacokinetic parameters of crizotinib in healthy subjects following 28-day multiple oral administration of crizotinib with and without coadministration of less potent CYP3A inhibitors

<table>
<thead>
<tr>
<th>Crizotinib with a less potent CYP3A inhibitor</th>
<th>Control Group&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Treatment Group</th>
<th>Fold-Increase&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC&lt;sub&gt;0-\infty&lt;/sub&gt;</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>ng/mL</td>
<td>ng·h/mL</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>123 (56)</td>
<td>1476 (57)</td>
<td>1.1 (6.2)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>179 (44)</td>
<td>2141 (44)</td>
<td>1.6 (26)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>199 (48)</td>
<td>2378 (48)</td>
<td>1.8 (26)</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>114 (56)</td>
<td>1368 (57)</td>
<td>1.0 (0.6)</td>
</tr>
</tbody>
</table>

Data are expressed as geometric mean with percent coefficient of variation (CV%) in parentheses (n = 15 per group \( \times \) 6 groups). Dose levels were crizotinib 150 mg twice daily with diltiazem 120 mg twice a day, erythromycin 500 mg three times a day, fluconazole 200 mg once a day or fluvoxamine 50 mg once a day.

<sup>a</sup> Mean values in the control groups of these simulation results.

<sup>b</sup> Fold-increase in C<sub>max</sub> and AUC<sub>0-\infty</sub> of the treatment group (crizotinib with CYP3A inhibitor) relative to control group (crizotinib alone).
Figure 3
Figure 4
Figure 7: Pie charts showing the distribution of renal, CYP3A, and other factors before and after treatment with Crizotinib.

- **Baseline**:
  - Renal: 0.06
  - CYP3A: 0.80
  - Others: 0.14

- **Crizotinib**:
  - Renal: 0.15
  - CYP3A: 0.48
  - Others: 0.37

- **Crizotinib with Rifampin**:
  - Renal: 0.03
  - CYP3A: 0.96
  - Others: 0.02

- **Crizotinib with Ketoconazole**:
  - Renal: 0.21
  - CYP3A: 0.07
  - Others: 0.72