Application of Physiologically Based Pharmacokinetic Modeling to the Understanding of Bosutinib Pharmacokinetics: Prediction of Drug–Drug and Drug–Disease Interactions

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ABSTRACT
Bosutinib is an orally available Src/Abl tyrosine kinase inhibitor indicated for the treatment of patients with Philadelphia chromosome–positive chronic myelogenous leukemia. Bosutinib is predominantly metabolized by CYP3A as the primary clearance mechanism. The main objectives of this study were to 1) develop physiologically based pharmacokinetic (PBPK) models of bosutinib; 2) verify and refine the PBPK models based on clinical study results of bosutinib single-dose–drug–drug interaction (DDI) with ketoconazole and rifampin, as well as single-dose–drug–disease interaction (DDZI) in patients with renal and hepatic impairment; 3) apply the PBPK models to predict DDI outcomes in patients with weak and moderate CYP3A inhibitors; and 4) apply the PBPK models to predict DDZI outcomes in renally and hepatically impaired patients after multiple-dose administration. Results showed that the PBPK models adequately predicted bosutinib oral exposures in patients after single- and multiple-dose administrations. The PBPK models also reasonably predicted changes in bosutinib exposures in the single-dose DDI and DDZI results, suggesting that the PBPK models were sufficiently developed and verified based on the currently available data. Finally, the PBPK models predicted 2- to 4-fold increases in bosutinib exposures by moderate CYP3A inhibitors, as well as comparable increases in bosutinib exposures in renally and hepatically impaired patients between single- and multiple-dose administrations. Given the challenges in conducting numerous DDI and DDZI studies of anticancer drugs in patients, we believe that the PBPK models verified in our study would be valuable to reasonably predict bosutinib exposures under various scenarios that have not been tested clinically.

Introduction
Understanding how certain extrinsic and intrinsic factors influence systemic exposures of new molecular entities (NMEs) in patients is crucial for drug development and regulatory decision making (Zhao et al., 2011; Huang and Rowland, 2012; Zhang et al., 2012; Chang et al., 2013). Changes in drug exposures by these factors can potentially lead to differences in therapeutic and/or adverse responses, such that it is critical to appropriately optimize dosing regimens of NMEs, particularly anticancer agents with narrow therapeutic windows. One important extrinsic factor is coadministered drugs, which may change NME exposures through inhibition or induction of drug-metabolizing enzymes and/or transporters [i.e., drug–drug interaction (DDI)]. The U.S. Food and Drug Administration (FDA), the European Medicines Agency, and the Japanese Pharmaceuticals and Medical Devices Agency issued individual DDI guidelines that underscore the predictive use of integrated mechanistic approaches such as a physiologically based pharmacokinetic (PBPK) model as a tool for quantitative DDI assessment (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf, and http://www.mhs.go.jp/mss/T140710-jimu.pdf, respectively). Since most drugs are mainly eliminated from the body through metabolism and excretion in the liver and kidneys, impaired organ function in the liver and kidneys [i.e., drug–disease interaction (DDZI)] is one important intrinsic factor that can modulate drug exposures. The FDA and the European Medicines Agency issued guidelines to investigate the pharmacokinetics of NMEs in renally and hepatically impaired patients (RIPs and HIPs, respectively), providing recommendations on study design and data analysis as well as impact on dosing and labeling (http://www.fda.gov/downloads/Drugs/guidancecomplianceregulatoryinformation/guidances/ucm072123.pdf, http://www.fda.gov/downloads/Drugs/Guidances/UCM204959.pdf, and http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003122.pdf, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500162133.pdf).

Bosutinib (Bosulif; Pfizer, New York, NY) is an orally available Src/Abl tyrosine kinase inhibitor and was recently approved globally for the treatment of adult patients with chronic, accelerated, or blast-phase disease interaction; FDA, U.S. Food and Drug Administration; HIP, hepatically impaired patient; HV, healthy volunteer; NME, new molecular entity; P450, cytochrome P450; PBPK, physiologically based pharmacokinetics; RIP, renally impaired patient; tmax, time to reach Cmax; USPI, U.S. prescribing information; Vss, steady-state volume of distribution.

ABBREVIATIONS: AUC, area under the plasma concentration-time curve; AUCR, area under the plasma concentration-time curve ratio; CL, plasma clearance; Clint, intrinsic clearance; CP, cancer patient; DDI, drug–drug interaction; DDZI, drug–disease interaction; FDA, U.S. Food and Drug Administration; HIP, hepatically impaired patient; HV, healthy volunteer; NME, new molecular entity; P450, cytochrome P450; PBPK, physiologically based pharmacokinetics; RIP, renally impaired patient; tmax, time to reach Cmax; USPI, U.S. prescribing information; Vss, steady-state volume of distribution.
Philadelphia chromosome–positive chronic myelogenous leukemia with resistance or intolerance to prior therapy (http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/203341s006lbl.pdf; Pfizer, 2016). Bosutinib is predominantly metabolized by CYP3A4 as the primary clearance mechanism in humans with minimal urinary excretion (<2% of the administered dose as the parent drug) (Syed et al., 2014; CDER, 2012). As one of the potential risk assessments for extrinsic factors, single-dose bosutinib DDI studies with a strong CYP3A inhibitor (ketocazole) and a strong CYP3A inducer (rifampin) were conducted in healthy volunteers (HVs) (Abbas et al., 2012a, 2015). In these studies, ketoconazole increased bosutinib’s maximum plasma concentration (Cmax) by approximately 3-fold and its area under the plasma concentration-time curve (AUC) by approximately 5-fold, whereas rifampin decreased Cmax and AUC by 86% and 94%, respectively. Accordingly, the U.S. prescribing information (USPI) advises to avoid concurrent use of bosutinib with strong or moderate CYP3A inhibitors and inducers (Pfizer, 2016). In addition, the FDA issued a postmarketing requirement to evaluate effects of moderate CYP3A4 inhibitors (e.g., erythromycin) on bosutinib exposures to recommend appropriate dosing regimens when bosutinib is used concomitantly with moderate CYP3A inhibitors (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf). To assess the potential risks of intrinsic factors, the effects of impaired organ function on single-dose bosutinib pharmacokinetics were investigated in RIPS and HIPS (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf). Increases in bosutinib exposures were approximately 1.5-fold in moderate and severe RIPS, whereas they were approximately 2-fold in HIPS with Child–Pugh scores A, B, and C (Pugh et al., 1973). Accordingly, the USPI recommends adjusting the dosage for RIPS and HIPS (Pfizer, 2016). However, changes in bosutinib exposures have not yet been evaluated in multiple-dose DDZI studies, since it is challenging to recruit patients in sufficient numbers for multiple-dose administration studies of anticancer drugs. There are also ethical concerns, with possible supratherapeutic exposures in such clinical studies.

PBPK modeling is a powerful predictive approach to quantitatively extrapolate in vitro and in silico drug-dependent parameters to in vivo pharmacokinetics based on drug-independent physiologic parameters; thus, its application in drug development has increased in recent years, as illustrated by several excellent reviews (Lavé et al., 2007; Nestorov, 2007; Rowland et al., 2011; Jones and Rowland-Yeo, 2013; Jones et al., 2015). Consequently, growing emphasis is being placed on PBPK modeling to quantitatively predict the magnitude of in vivo DDIs and DDZIs of NMEs (Rowland et al., 2011; Huang and Rowland, 2012; Prueksaritanont et al., 2013; Wagner et al., 2015). Accordingly, it would be highly beneficial to develop a PBPK model of bosutinib to quantitatively predict effects of extrinsic and intrinsic factors on bosutinib exposures in patients. The main objectives of our study were to 1) develop PBPK models of bosutinib, 2) verify and refine the PBPK models based on currently available clinical results, 3) apply the PBPK models to predict DDI outcome in cancer patients (CPs) with weak and moderate CYP3A inhibitors, and 4) apply the PBPK models to predict DDZI outcomes in RIPS and HIPS after multiple-dose administration.

Materials and Methods

Clinical Pharmacokinetic Data

Detailed information about bosutinib clinical studies, such as single-dose pharmacokinetics in CPs, DDI studies with ketoconazole and rifampin in HVs, and DDZI studies with RIPS and HIPS, was previously reported (Cortes et al., 2011; Abbas et al., 2012b, 2013, 2015; Daud et al., 2012). Additional information about bosutinib pharmacokinetics is also available on the FDA website (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf). Briefly, bosutinib pharmacokinetics were determined in HVs (n = 12) and CPs (n = 3) after a single oral administration of a clinically recommended dose at 500 mg (Cortes et al., 2011; Hsyu et al., 2017). Bosutinib steady-state pharmacokinetics were determined in CPs (n = 3) after multiple-dose oral administration at 500 mg once daily (Cortes et al., 2011). A single-dose DDI study of bosutinib with ketoconazole in HVs (n = 24) was conducted in a two-way crossover design with a 14-day washout period (Abbas et al., 2012a). Each subject received a single oral dose of 500 mg bosutinib (day 2) either alone or with 5-day repeated oral doses of 400 mg ketoconazole once daily (days 1–5). A single-dose DDI study with rifampin was conducted in HVs (n = 24) (Abbas et al., 2015). Each subject received a single oral dose of 500 mg bosutinib (days 1 and 14) with 10-day repeated oral doses of 600 mg rifampin once daily (days 8–17). In a single-dose DDZI study with RIPS, 200 mg bosutinib was orally administered to moderate and severe RIPS (n = 8/group) defined as creatinine clearance of 30–50 and <30 ml/min, respectively, along with HVs (n = 8) as a control group (creatinine clearance >80 ml/min) (Abbas and Hsyu, 2016). In a single-dose DDZI study with HIPS, 200 mg bosutinib was orally administered to HIPS with Child–Pugh scores A, B, and C (n = 6 each), along with HVs (n = 9) as a control group (Abbas et al., 2013).

Bosutinib Input Parameters in PBPK Models

A commercially available dynamic PBPK model, Simcyp population-based simulator version 13.1 (Simcyp Limited, Sheffield, UK), was used for all simulations (Jamei et al., 2009). The physicochemical and pharmacokinetic parameters of bosutinib used for the PBPK models are summarized in Table 1. A fraction of the dose absorbed (Fa) at the 500-mg dose was estimated at approximately 0.7, since the recovery of bosutinib (as the parent drug) in feces was 30% of the administered oral dose in a single oral-dose human mass-balance study with [14C]bosutinib at 500 mg, and fecal recovery of bosutinib was unlikely confounded by biliary excretion of the unchanged drug and/or reversible metabolites based on the metabolic profiling results in the mass-balance study (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf). Accordingly, bosutinib Fa was set at 0.7 at the dose of 500 mg. In DDZI studies with RIPS and HIPS at the 200-mg dose, observed AUC estimates (993 and 714 ng·h/ml, respectively) in the control groups were 10%–40% lower than the dose-normalized AUC value (1104 ng·h/ml) calculated from the AUC estimate at 500 mg assuming a dose proportionality between 200 and 500 mg. Therefore, predicted Fa values in the DDZI studies with RIPS and HIPS were adjusted to 0.6 and 0.5, respectively, by the differences in dose-normalized AUC values. Bosutinib renal clearance was estimated to be 0.9 l/h based on urinary excretion (approximately 2% of the administered dose as the parent drug) in the human mass-balance study CDER, 2012. An input parameter of hepatic microsomal intrinsic clearance (CLint) in bosutinib PBPK models was back-calculated from the clinically observed oral plasma clearance (CL/Foral) using a retrograde model implemented in Simcyp. The back-calculated CLint values were 300 µl/min per mg microsomal proteins from the CL/Foral estimate of 200 l/h (189–207 l/h) at the oral doses of 500 mg in CPs (Cortes et al., 2011; Daud et al., 2012). The fraction of bosutinib metabolized by CYP3A4 (Fa,cYP3A4) was estimated as near unity based on the in vitro cytochrome P450 (P450) phenotyping and the human mass-balance study CDER, 2012. Therefore, the back-calculated CLint value was assigned to CYP3A4-mediated CLint in the PBPK models. Bosutinib steady-state volume of distribution (Vss) was predicted to be 15 l/kg, based on a single-species scaling with an exponent of unity from mice, rats, and dogs (12, 12–19, and 14 l/kg, respectively) after correction for species differences in unbound fractions in plasma (fu,plasm), which were 0.059, 0.061, and 0.041, respectively (Hosea et al., 2009; CDER, 2012). Therefore, the predicted Vss of 7.5 l/kg by the tissue composition–based model implemented in Simcyp (as the mathematical model 2) was adjusted to 15 l/kg using a Kr scalar of 2 (Rodgers et al., 2005).

To predict bosutinib plasma concentration-time profiles, the full-PBPK models implemented in Simcyp were used with the first-order absorption models (Simcyp, 2013). For DDZI prediction in HIPS, Foti (2014) previously reported that a full-PBPK model in Simcyp considerably overpredicted changes in drug oral exposures, which was likely due to physiologic parameters related to shunting of blood flow away from the liver. Therefore, we performed the bosutinib DDZI
prediction in HIPs by minimal-PBPK models with a single adjustment compartment (Simcyp, 2013). Bosutinib input parameters of a single adjustment compartment were as follows: $V_{ss}$ of 9.7 l/kg, with $k_{in}$ of 0.039 h$^{-1}$ and $k_{out}$ of 0.021 h$^{-1}$ based on pharmacokinetic analyses with two-compartment models.

**PBPK Modeling and Simulation**

Our modeling and simulation approaches were basically categorized into three main tiers: 1) model development, 2) model verification/refinement, and 3) model application. For model development, bosutinib PBPK models were developed based on in vitro and in vivo data, and bosutinib exposures were then predicted in HVs after a single oral administration. The predicted results were also compared between virtual populations of HVs and CPs because the single-dose DDI studies with ketoconazole and rifampin were conducted in HVs. For model verification and refinement, PBPK model-predicted bosutinib exposures were compared with clinically observed results in the single-dose DDI and DDZI studies to evaluate the predictive model performance. For model application, bosutinib single-dose DDI outcomes with weak and moderate CYP3A inhibitors and multiple-dose DDZI outcomes in RIPS and HIPs were predicted by the PBPK models. An outline of these simulation trials is summarized in Table 2. For bosutinib DDI prediction with weak and moderate CYP3A inhibitors, erythromycin (250 mg four times a day), fluconazole (200 mg once daily), fluvoxamine (50 mg once daily), and verapamil (120 mg three times a day) were used for PBPK modeling. The compound files of ketoconazole (sim-ketoconazole 400 mg daily), rifampin (sim-rifampicin), erythromycin (sim-erythromycin), fluconazole (sv-fluconazole), fluvoxamine (sv-fluvoxamine), and verapamil (sim-verapamil) from the Simcyp compound library were used for the DDI predictions. Default DDI parameters on CYP3A4 were as follows: ketoconazole [competitive $K_i = 0.015 \mu M (f_{umic} = 0.97)$], rifampin [induction $E_{max} = 16$, $EC_{50} = 0.32 \mu M$, and competitive $K_i = 10.5 \mu M (f_{umic} = 1)$], erthyromycin [competitive $K_i = 82 \mu M (f_{umic} = 0.909)$], mechanism-based inhibition $k_{inact} = 2.25 h^{-1}$, and $K_i = 23.2 \mu M (f_{umic} = 1)$], fluconazole [competitive $K_i = 10.7 \mu M (f_{umic} = 1)$], fluvoxamine [$K_i = 17.89 \mu M (f_{umic} = 0.441)$], and verapamil [mechanism-based inhibition $k_{inact} = 2.0 h^{-1}$ and $K_i = 2.21 \mu M (f_{umic} = 1)$].

Simulation of all clinical trials was performed in Simcyp with a virtual population in a fed state in 10 trials of 10 subjects (total of 100 subjects), as the USP1 recommends that bosutinib be taken with food. The output sampling interval in a Simcyp simulation tool box was set at 0.2 hours in all simulations. Virtual populations used from the Simcyp default population library were as follows: HVs; moderate and severe RIPS with glomerular filtration rates of 30–60 ml/min and <30 ml/min, respectively; and mild, moderate, and severe HIPs with Child–Pugh scores A, B, and C, respectively. Key features of the Simcyp virtual population of RIPS and HIPs are as follows (Johnson et al., 2010; Rowland Yeo et al., 2011; Simcyp, 2013): features for RIPS include 1) reduced kidney weight and blood flow, 2) reduced hepatic portal blood flow or portal hypertension with consequential blood shunting to bypass the liver with increased blood flow through the hepatic artery and mesentery.

For a virtual population of CPs, the demographic characteristics in a population file of HVs were changed based on those of CPs previously reported (e.g., age, body weights, plasma protein level, and hematocrit) (Cheeti et al., 2013).

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>531</td>
<td>Calculated</td>
</tr>
<tr>
<td>logP</td>
<td>3.1</td>
<td>Measured</td>
</tr>
<tr>
<td>$pK_a$ (monobase)</td>
<td>7.9</td>
<td>Measured</td>
</tr>
<tr>
<td>$k_{in,plasma}$</td>
<td>0.063</td>
<td>Measured</td>
</tr>
<tr>
<td>$B/P$</td>
<td>1.2</td>
<td>Measured</td>
</tr>
<tr>
<td>$F_s$</td>
<td>0.5–0.7</td>
<td>Estimated from the mass-balance study results</td>
</tr>
<tr>
<td>$k_{in}$ (h$^{-1}$)</td>
<td>0.13</td>
<td>Estimated from the clinical study results</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>1</td>
<td>Estimated from the clinical study results</td>
</tr>
<tr>
<td>$P_{eff}$ (10$^{-4}$ cm/s)</td>
<td>1.8</td>
<td>Calculated from physiochemical property</td>
</tr>
<tr>
<td>$Q_{sys}$ (l/h)</td>
<td>8.7</td>
<td>Calculated by Simcyp</td>
</tr>
<tr>
<td>$Q_{gut}$ (l/h)</td>
<td>1</td>
<td>Assumed (Simcyp default)</td>
</tr>
<tr>
<td>$V_{sys}$ (l/kg)</td>
<td>15</td>
<td>Predicted from nonclinical results</td>
</tr>
<tr>
<td>$Cl_{sys}$ (CYP3A4) (µl/min per mg protein)</td>
<td>300</td>
<td>Back-calculated from the observed $Cl/F_{oral}$</td>
</tr>
<tr>
<td>$Cl_{renal}$ (l/h)</td>
<td>0.9</td>
<td>Estimated from the two-compartment PK analysis</td>
</tr>
</tbody>
</table>

$^*$Distribution rate constants for a single adjustment compartment in minimal-PBPK models used for simulation in HIPs.

**TABLE 2**

Simulation outline of bosutinib DDI and DDZI studies for model verification and application

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Bosutinib Dose</th>
<th>Treatment Day</th>
<th>Precipitant</th>
<th>Dose</th>
<th>Treatment Day</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDI</td>
<td>HVs</td>
<td>500 mg</td>
<td>2</td>
<td>Ketoconazole</td>
<td>400 QD</td>
<td>1–5</td>
<td>Predicted versus observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td>Rifampin</td>
<td>600 QD</td>
<td>1–10</td>
<td>Predicted versus observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>Fluvoxamine</td>
<td>50 QD</td>
<td>1–8</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>Fluconazole</td>
<td>200 QD</td>
<td>1–8</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>Erythromycin</td>
<td>250 QID</td>
<td>1–8</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>Verapamil</td>
<td>120 TID</td>
<td>1–8</td>
<td>Predicted</td>
</tr>
<tr>
<td>DDZI</td>
<td>RIPS</td>
<td>200 mg</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Predicted versus observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–28</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Predicted versus observed</td>
</tr>
<tr>
<td>HIPS</td>
<td>200 mg</td>
<td>1</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Predicted</td>
</tr>
<tr>
<td></td>
<td>1–28</td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Predicted</td>
</tr>
</tbody>
</table>

Dashes indicate not applicable. QD, once daily; QID, four times a day; TID, three times a day.
Data are expressed as geometric means with percent coefficients of variation in parentheses (n = 3 in the observed results in CPs, n = 12 in the observed results in HVs, and n = 100 in the predicted results; 10 individuals × 10 groups).

<table>
<thead>
<tr>
<th>Population</th>
<th>Analysis</th>
<th>Single Dose</th>
<th>Multiple Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>AUC</td>
</tr>
<tr>
<td>CPs</td>
<td>Observed</td>
<td>97 (35)</td>
<td>2030 (23)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>74 (55)</td>
<td>1759 (58)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>HVs</td>
<td>Observed</td>
<td>95 (39)</td>
<td>2193 (38)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>80 (47)</td>
<td>2445 (65)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Dashes indicate not applicable.

Results

Model Development

Prediction of Bosutinib Pharmacokinetics in CPs and HVs. After a single oral dose of 500 mg bosutinib, clinically observed bosutinib pharmacokinetics were comparable between CPs and HVs (Table 3). The PBPK model-predicted plasma concentration-time profiles of bosutinib adequately matched the observed results in both CPs and HVs after a single oral administration of 500 mg bosutinib (Fig. 1). Predicted C<sub>max</sub> and AUC values were comparable to the observed results, with P/O ratios of 0.8–1.1 (Table 3). In PBPK modeling, predicted hepatic and intestinal CL<sub>int</sub>, f<sub>u,plasma</sub> blood-to-plasma ratios (B/P), and unbound fractions in the blood (f<sub>u,blood</sub>) were also comparable between virtual populations of CPs and HVs (Supplemental Table 1). After multiple oral doses of 500 mg bosutinib once daily, the model-predicted plasma concentration-time profiles of bosutinib sufficiently matched the observed results, with a P/O ratio of 0.7 for C<sub>max</sub> and AUC (Table 3). Overall, these results suggested that bosutinib PBPK models were reasonably developed based on the in vitro and in vivo data.
Prediction of Bosutinib DDZI in RIPs. A single oral dose of 200 mg bosutinib was administered to moderate and severe RIPs, along with HVs as the control. The clinically observed \( C_{\text{max}} \) and AUC values were 33 ng/ml and 993 ng·h/ml in HVs, 42 ng/ml and 1404 ng·h/ml in moderate RIPs, and 44 ng/ml and 1575 ng·h/ml in severe RIPs, respectively. Thus, the \( C_{\text{max}} \) in HIPs, as the P/O ratios for \( C_{\text{max}} \) and the AUCR were 1.3 and 1.4 in moderate RIPs and 1.3 and 1.6 in severe RIPs, respectively (Table 5). The PBPK models reasonably predicted plasma concentrations of bosutinib in all of the groups (Fig. 3). The predicted \( C_{\text{max}} \) and AUC were comparable to the observed results, with P/O ratios of 0.9–1.1; as a result, the \( C_{\text{max}} \) and the AUC were adequately predicted, with P/O ratios of 1.1–1.2 (Table 5). The model-predicted hepatic CYP3A4 abundances in moderate and severe RIPs (3.6 and 3.0 \( \times 10^6 \) pmol/liver, respectively) were 50%–60% lower than those in HVs (7.1 \( \times 10^6 \) pmol/liver) (Supplemental Table 2). Consequently, the decreases in model-predicted individual CYP3A4 abundances in the liver were associated with the increases in model-predicted individual AUC values in the virtual populations (Supplemental Fig. 1). On the other hand, the predicted individual \( f_u,\text{blood} \) did not correlate well with the predicted individual AUC values. The difference in predicted \( f_u,\text{blood} \) values between HVs (0.053) and moderate to severe RIPs (0.059–0.069) was minimal (i.e., 1.1- to 1.3-fold) (Supplemental Table 2). Overall, the PBPK models sufficiently predicted the increases in bosutinib exposures in moderate and severe RIPs, which could be largely caused by the decrease in hepatic CYP3A4 abundances.

Prediction of Bosutinib DDZI in HIPs. A single oral dose of 200 mg bosutinib was administered to HIPs with Child–Pugh scores A, B, and C, along with HVs as the control. The clinically observed \( C_{\text{max}} \) and AUC values in HVs and HIPs with Child–Pugh A, B, and C were 32 ng/ml and 714 ng·h/ml, 78 ng/ml and 1720 ng·h/ml, 64 ng/ml and 1350 ng·h/ml, and 49 ng/ml and 1270 ng·h/ml, respectively. As a result, the observed AUCRs in HIPs with Child–Pugh A, B, and C were 2.4, 1.9, and 1.8, respectively, whereas the \( C_{\text{max}} \)Rs were 2.4, 2.0, and 1.5, respectively (Table 6). Thus, bosutinib exposures in HIPs were roughly 2-fold higher than those in HVs, with an apparent trend of slight decreases in the exposures with increasing severity of hepatic impairment. The PBPK models did not predict bosutinib exposures well in HIPs, as the P/O ratios for \( C_{\text{max}} \) and AUCR varied from 0.6 to 2.0 (Table 6).

To improve the predictive model performance, bosutinib \( f_u \) values in HIPs with Child–Pugh A, B, and C were back-calculated at 0.68, 0.35, and 0.23, respectively, based on the P/O ratios of AUC with an \( f_u \) of 0.5 in HVs. That is, the decrease in \( f_u \) in HIPs was assumed in PBPK modeling, as discussed later. Using the back-calculated \( f_u \) values, the PBPK models adequately predicted bosutinib exposures in HIPs with Child–Pugh A, B, and C (Fig. 4). The predicted AUC and AUCR were comparable to the observed results, with P/O ratios of 0.9–1.0, whereas the \( C_{\text{max}} \) and \( C_{\text{max}} \)Rs were slightly underpredicted, with P/O ratios of 0.5–0.8 (Table 6). In PBPK modeling, the predicted CYP3A4 abundances in virtual populations of HIPs with Child–Pugh A, B, and C decreased with increasing disease severity, with 4.0, 2.1, and 1.2 \( \times 10^6 \) pmol/liver, in the liver and 0.042, 0.032, and 0.022 \( \times 10^6 \) pmol/intestines in the intestines, respectively (Supplemental Table 2). These CYP3A4 abundances in the liver and intestines were 40%–80% and 20%–60% lower than those in HVs (7.1 \( \times 10^6 \) pmol/liver and 0.056 \( \times 10^6 \) pmol/intestines), respectively. The decreases in model-predicted individual CYP3A4 abundances in the virtual populations were associated with the increases in predicted individual AUC values (Supplemental Fig. 2). On the other hand, the predicted individual \( f_u,\text{blood} \) did not correlate well with the predicted AUC values. The difference in the predicted \( f_u,\text{blood} \) between HVs (0.053) and HIPs (0.062–0.10) was minimal (i.e., 1.2- to 1.9-fold) (Supplemental Table 2). Overall, the PBPK models sufficiently predicted the increases in bosutinib exposures in HIPs with Child–Pugh A, B, and C, assuming the decrease in \( f_u \).

![Fig. 2. PBPK model-predicted and observed plasma concentrations of bosutinib in HVs after a single oral administration of 500 mg bosutinib with (red) and without (blue) coadministration of 400 mg ketoconazole once daily (A) and 600 mg rifampin once daily (B). The x-axis represents the time after dosing in hours, and the y-axis represents the predicted (lines) and observed (open circles) plasma concentrations in nanograms per milliliter on a logarithmic scale. The predicted and observed plasma concentrations are expressed as the mean (solid lines) with 5th and 95th percentiles (dashed lines) and the mean ± S.D., respectively.](https://example.com/fig2.png)
Model Application

Prediction of Bosutinib DDIs with Weak and Moderate CYP3A Inhibitors. Bosutinib DDIs with weak and moderate CYP3A inhibitors were predicted by the PBPK models. In these DDI predictions, a single oral dose of 500 mg bosutinib was administered to a virtual population of HVs on day 5 with and without 9-day repeated coadministration of fluvoxamine (50 mg once daily), erythromycin (250 mg four times a day), or verapamil (120 mg three times a day). The PBPK model-predicted plasma concentration-time profiles are graphically presented in Supplemental Fig. 3. The predicted bosutinib Cmax and AUC with CYP3A inhibitors were 84 ng/ml and 2603 ng·h/ml with fluvoxamine, 190 ng/ml and 8689 ng·h/ml with erythromycin, and 181 ng/ml and 7466 ng·h/ml with verapamil, respectively (Table 7). Correspondingly, the predicted CmaxR and AUCR by the moderate inhibitors were 2.3 and 3.4 with fluvoxamine, 2.5 and 4.0 with erythromycin, and 2.4 and 3.1 with verapamil, respectively, whereas those with the weak inhibitor, fluvoxamine, were 1.0. Thus, the increases in bosutinib exposures with moderate CYP3A inhibitors were predicted to be 2- to 4-fold. In addition, the model-predicted bosutinib fA,CYP3A decreased from 0.98 to 0.94–0.95 by the moderate CYP3A inhibitors, whereas the model-predicted Fh and Fp increased from 0.63 to 0.74–0.85 and 0.56 to 0.78–0.93, respectively.

Prediction of Multiple-Dose DDZIs in RIPS and HIPs. To obtain PBPK model-predicted bosutinib steady-state exposures in RIPS and HIPs, multiple oral doses of 200 mg bosutinib once daily were administered to virtual populations of moderate and severe RIPS, along with HVs (Supplemental Fig. 4), and to virtual populations of HIPs with Child–Pugh A, B, and C, along with HVs (Supplemental Fig. 5). In RIPS, the predicted CmaxR and AUCR on day 28 were 1.7 and 1.8 in moderate RIPS and 1.7 and 1.9 in severe RIPS, respectively (Table 8). Thus, the predicted increases in bosutinib steady-state exposures in moderate and severe RIPS were comparable to those after a single-dose administration. In HIPs, the predicted CmaxR and AUCR on day 28 were 2.3 and 2.5 for Child–Pugh A, 1.9 and 2.2 for Child–Pugh B, and 1.8 and 2.2 for Child–Pugh C, respectively (Table 8). Therefore, the predicted increases in bosutinib exposures in HIPs were also comparable between single- and multiple-dose administrations.

Discussion

We have developed and verified PBPK models to understand the effects of extrinsic and intrinsic factors on bosutinib pharmacokinetics. This practice has become common in drug development and regulatory decision making (Zhao et al., 2012; Huang et al., 2013; Sinha et al., 2014; Jones et al., 2015). Bosutinib PBPK models appear to be successful in providing predictive DDI and DDZI outcomes. However, some issues have been identified and warrant further discussion.

In the single-dose mass-balance study with HVs (500 mg), fecal recovery of bosutinib as the parent drug was approximately 30% of the administered dose CDER, 2012. Fecal recovery was considered to be a fraction of the unabsorbed dose (1 − Fh), because it was unlikely confounded by biliary excretion of the unchanged drug and/or reversible metabolites based on the metabolic profiling results (CDER, 2012). Thus, bosutinib Fh at the 500-mg dose was set at 0.7 in the PBPK models assuming that Fh was comparable between HVs and CPs. Baker et al. (2004) previously reported that CYP3A activity did not change with age, sex, and body size measurements in 134 CPs. Consistently, PBPK model-predicted exposures of the CYP3A probe substrate, midazolam, were comparable between HVs and CPs (Cheeti et al., 2013). In our study, model-predicted bosutinib exposures were also comparable between these populations (Table 3). In the DDZI studies (200 mg), bosutinib Fh was adjusted to 0.5–0.6 to sufficiently recover the observed exposures in the control groups. The Fh adjustment suggested that bosutinib Fh slightly decreased from the doses of 500 to 200 mg, although the differences might be within interindividual/study variability. Clinically observed increases in bosutinib exposures were supra-proportional at doses of 50–200 mg, whereas they were roughly dose proportional at the higher doses of 200–600 mg (Hsu et al., 2014; CDER, 2012). Since bosutinib is a substrate of P-glycoprotein, nonlinear-to-linear pharmacokinetics at the lower-to-higher doses would be considered mainly due to a saturation of intestinal P-glycoprotein–mediated efflux, resulting in increases in Fh at doses up to around 200 mg. Thus, the slight increase in
Fa from the doses of 200 to 500 mg could be consistent with the clinical findings.

In the DDI study with ketoconazole, the observed bosutinib AUCR was consistent with that expected from coadministration of a strong CYP3A4 inhibitor (i.e., 5-fold) (Zhao et al., 2009; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf). According to a postmarketing requirement, a clinical DDI study with a moderate CYP3A inhibitor, aprepitant (125 mg), was recently conducted in HVs (Hsyu et al., 2017). The observed bosutinib AUCR with aprepitant was approximately 2-fold, which appeared to be consistent with the PBPK model-predicted AUCR with the moderate CYP3A inhibitors in our study. In PBPK modeling, the predicted bosutinib $F_a$ and $F_g$ were approximately 0.6, which increased to near unity by coadministration of ketoconazole, suggesting that the increase in bosutinib exposures could be caused by CYP3A inhibition in both the liver and intestines. In the DDI prediction with moderate CYP3A inhibitors fluconazole, erythromycin, and verapamil, the predicted bosutinib $F_a$ and $F_g$ increased to 0.74–0.85 and 0.78–0.93, respectively. Thus, the moderate inhibitors could also inhibit CYP3A-mediated metabolism of bosutinib in both the liver and intestines. In the DDI study with rifampin, the predicted $F_a$ and $F_g$ decreased to 0.2, suggesting that the decrease in bosutinib exposures by rifampin could be

### TABLE 6
Clinically observed and PBPK model-predicted pharmacokinetic parameters of bosutinib in subjects with hepatic impairment after a single oral administration of 200 mg bosutinib

<table>
<thead>
<tr>
<th>Population</th>
<th>Analysis</th>
<th>PK Parameter</th>
<th>Ratio$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F_a$</td>
<td>$C_{max}$</td>
</tr>
<tr>
<td>HVs</td>
<td>Observed</td>
<td>32 (31)</td>
<td>4.0 (1.0–8.0)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>29 (43)</td>
<td>3.4 (2.6–4.7)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.9</td>
<td>–</td>
</tr>
<tr>
<td>HIPs</td>
<td>Observed</td>
<td>78 (52)</td>
<td>2.5 (0.5–4.0)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>39 (44)</td>
<td>2.0 (1.6–2.7)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Child–Pugh A</td>
<td>Observed</td>
<td>53 (42)</td>
<td>2.0 (1.6–2.7)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>36 (43)</td>
<td>2.0 (1.0–4.0)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Child–Pugh B</td>
<td>Observed</td>
<td>64 (35)</td>
<td>2.1 (1.6–2.9)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>38 (35)</td>
<td>2.1 (1.6–2.9)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>Child–Pugh C</td>
<td>Observed</td>
<td>49 (70)</td>
<td>1.5 (1.0–3.0)</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>34 (38)</td>
<td>2.0 (1.6–2.7)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Predicted</td>
<td>27 (34)</td>
<td>2.0 (1.6–2.7)</td>
</tr>
<tr>
<td></td>
<td>P/O</td>
<td>0.5</td>
<td>–</td>
</tr>
</tbody>
</table>

Dashes indicate not applicable.

$^a$Predicted $F_a$ was first fixed at 0.5 and then calculated from the simulation results with $F_a$ of 0.5.

$^b$Ratios of $C_{max}$ and AUC in HIPs to HVs.

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Fig. 4. PBPK model-predicted and observed plasma concentrations of bosutinib in HVs (A) and hepatically impaired patients with Child–Pugh scores A (B), B (C), and C (D) after a single oral administration of 200 mg bosutinib. The x-axis represents the time after dosing in hours, and the y-axis represents the predicted (lines) and observed (open circles) plasma concentrations in nanograms per milliliter on a logarithmic scale. The predicted and observed plasma concentrations are expressed as the mean (solid lines) with 5th and 95th percentiles (dashed lines) and the mean ± SD, respectively.
caused by CYP3A4 induction in both the liver and intestines. Thus, PBPK modeling could provide a quantitative framework with mechanistic insights to further understand in vivo DDIs.

The most obvious changes caused by chronic kidney disease are decreases in renal clearance. In addition, the disease is also associated with other changes such as reduced plasma protein binding and drug-metabolizing enzyme activity, particularly CYP3A (Sun et al., 2006; Dreischub and Lertora, 2008; Zhang et al., 2009). Thus, the pharmacokinetics of most drugs, including those that are primarily metabolized by CYP3A, should be evaluated in RIPs to provide appropriate dosing recommendation (http://www.fda.gov/downloads/Drugs/Guidances/UCM204959.pdf and http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500162133.pdf). The observed increases in bosutinib exposures in moderate and severe RIPs (i.e., approximately 1.5-fold) were comparable to those for the CYP3A probe substrate, midazolam (Vinik et al., 1983). Consistently, the observed increased blood flow through the hepatic artery and mesentery (Elbekai et al., 2004; Verbeeck, 2008). There appear to be considerable challenges to accurately incorporating these physiologic changes into PBPK models (particularly, portal-systemic blood shunting). As a result, PBPK models, specifically full-PBPK models in Simcyp, tend to overpredict drug exposures in HIPs (Foti, 2014).

In the clinical DDZI study with HIPs, the observed apparent, slight but consistent, decrease in bosutinib exposures with increasing severity of hepatic impairment was not expected from the results of other CYP3A substrates (Verbeeck, 2008; Johnson et al., 2010). Therefore, the PBPK models did not predict bosutinib exposures well in HIPs (when \( F_a \) was fixed at 0.5) (Table 6). In PBPK modeling, the predicted CYP3A4 abundances in the liver and intestines of virtual populations decreased with increasing disease severity by 1.8- to 6.2-fold and 1.3- to 2.5-fold, respectively, relative to HVs (Supplemental Table 2). The model-predicted individual CYP3A4 abundances in RIPs were inversely associated with bosutinib exposures (Supplemental Fig. 2). These findings suggested that some other factors in HIPs could (in part) offset the increases in bosutinib exposures caused by the decrease in CYP3A-mediated clearance. For plasma protein binding, the clinically observed bosutinib ex vivo \( f_{u,\text{plasma}} \) values were comparable between HVs and HIPs (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203341Orig1s000ClinPharmR.pdf). The PBPK model-predicted individual \( f_{u,\text{blood}} \) did not correlate well with the predicted AUC values, although the model-predicted \( f_{u,\text{blood}} \) values in HIPs were 1.2- to 1.9-fold higher than those in HVs (Supplemental Fig. 2; Supplemental Table 2). Thus, the changes in \( f_{u,\text{blood}} \) did not appear to offset the increase in bosutinib exposures due to the decrease in CYP3A abundances.

In addition to reducing drug-metabolizing enzymes and plasma proteins, cirrhosis also increases gastrointestinal absorption due to congestion and decreased blood flow in the intestinal mucosa (Elbekai et al., 2004; Verbeeck, 2008). Consistently, the observed bosutinib \( t_{\text{max}} \) decreased with the increasing disease severity from 4.0 in HVs to 2.5, 2.0, and 1.5 hours in HIPs with Child–Pugh A, B, and C, respectively, in parallel with the decrease in observed \( C_{\text{max}} \)Rs of 2.4, 2.0, and 1.5, respectively (CDER, 2012). Therefore, the absorption of bosutinib could possibly be altered in patients with varying degrees of hepatic impairment. In contrast, the observed apparent terminal half-life was approximately 2-fold longer in HIPs (86–113 hours) than HVs.

### Table 7

<table>
<thead>
<tr>
<th>Precipitant</th>
<th>PK Parameter</th>
<th>Ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_{\text{max}}$</td>
<td>AUC $</td>
<td>C_{\text{max}R}$</td>
</tr>
<tr>
<td>ng/ml</td>
<td>ng h/ml</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>84 (42)</td>
<td>2603 (51)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>190 (34)</td>
<td>8689 (40)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>210 (36)</td>
<td>10,197 (50)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>181 (41)</td>
<td>7466 (63)</td>
</tr>
</tbody>
</table>

$^a$Ratios of \( C_{\text{max}} \) and AUC in the treatment group (bosutinib with CYP3A inhibitor) to the control group (bosutinib alone).

It is well known that cirrhosis not only reduces the expression of drug-metabolizing enzymes and transporters but also changes hepatic architecture, leading to the development of blood shunting to bypass the liver with increased blood flow through the hepatic artery and mesentery (Elbekai et al., 2004; Verbeeck, 2008). There appear to be considerable challenges to accurately incorporating these physiologic changes into PBPK models (particularly, portal-systemic blood shunting). As a result, PBPK models, specifically full-PBPK models in Simcyp, tend to overpredict drug exposures in HIPs (Foti, 2014). Accordingly, we performed the DDZI prediction in HIPs with minimal-PBPK models, which had fewer physiometric parameters, especially those related to blood shunting (Simcyp, 2013; Foti, 2014).

### Table 8

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>PK Parameter</th>
<th>Ratio$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F_a$</td>
<td>$C_{\text{max}}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ng/ml</td>
<td>h</td>
</tr>
<tr>
<td>RIs</td>
<td>HVs</td>
<td>0.60</td>
<td>57 (51)</td>
</tr>
<tr>
<td></td>
<td>Moderate RIs</td>
<td>0.60</td>
<td>95 (57)</td>
</tr>
<tr>
<td></td>
<td>Severe RIs</td>
<td>0.60</td>
<td>98 (57)</td>
</tr>
<tr>
<td>HIPs</td>
<td>HVs</td>
<td>0.50</td>
<td>52 (49)</td>
</tr>
<tr>
<td></td>
<td>Child–Pugh A</td>
<td>0.68</td>
<td>120 (60)</td>
</tr>
<tr>
<td></td>
<td>Child–Pugh B</td>
<td>0.35</td>
<td>97 (57)</td>
</tr>
<tr>
<td></td>
<td>Child–Pugh C</td>
<td>0.23</td>
<td>94 (51)</td>
</tr>
</tbody>
</table>

Dashes indicate not applicable.

$^b$Predicted \( F_a \) was fixed at the values used for the single-dose simulation.

$^b$Ratios of \( C_{\text{max}} \) and AUC in RIPs or HIPs to HVs.
In conclusion, our study demonstrates that bosutinib PBPK models have adequately been developed, verified, and refined based on currently available data such as single-dose DDI and DDZI studies; therefore, the models can be applied to predict bosutinib exposures in single-dose DDI studies with other P450 inhibitors and multiple-dose DDZI studies. The DDI prediction suggested 2- to 4-fold increases in bosutinib exposures by moderate CYP3A inhibitors. The DDZI prediction suggested that the fold increases in bosutinib exposures in RIPS and HIPS would be comparable to single- and multiple-dose administrations. Given the challenges in conducting numerous DDI and DDZI studies of anticancer drugs in CPs, it would be highly beneficial to develop PBPK models to quantitatively predict their exposures under various scenarios that have not yet been tested clinically. We believe that bosutinib dose adjustments in CPs could be reasonably recommended by the PBPK models developed and verified in this study.

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Authorship Contributions

Participated in research design: Ono, Yamazaki. Performed data analysis: Ono, Yamazaki. Wrote or contributed to the writing of the manuscript: Ono, Hsyu, Abbas, Loi, Yamazaki.

References


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