Population Pharmacokinetic and Pharmacodynamic Analysis of Bosutinib

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Summary: Bosutinib is an orally active, competitive inhibitor of Src/Abl tyrosine kinases. A population pharmacokinetic model was developed using data pooled from 3 studies of patients (n = 870) with solid tumors or Philadelphia chromosome–positive leukemia. Patients (aged 18–91 y, weighing 35–221 kg) who received bosutinib 50 to 600 mg orally with food each contributed 6–9 pharmacokinetic samples. The final pharmacokinetic model was a linear two-compartment model with first-order absorption, an absorption lag-time, and dose-dependent bioavailability. Oral absorption was relatively slow, with a half-time of 1.14 h and a lag-time of 0.87 h; time to peak concentration was 5–6 h. Apparent clearance was 120 L/h. The apparent volume of the peripheral compartment was large with a slow turnover; alpha and beta half-lives were 19 h and 290 days, respectively. All parameters were estimated with acceptable precision (standard error <30%). No tested covariate (protocol, baseline demographic/clinical characteristics, or laboratory results) explained the high inter-individual variability of bosutinib pharmacokinetics. Therefore, adjusting bosutinib dose for body size (weight, surface area) would not provide benefit over fixed dosing. Using this exposure model in pharmacodynamic assessment of one study, adverse event incidence was shown to be similar in overall and bosutinib-responsive populations.

Keywords: bosutinib; population pharmacokinetics; pharmacodynamics; pharmacokinetic model; study design

Introduction

Bosutinib (SKI-606) is an orally active, dual inhibitor of the Src and Abl tyrosine kinases, with minimal activity against platelet-derived growth factor receptor or c-KIT.1,2) In an initial phase I study in patients with solid tumors, bosutinib demonstrated promising tolerability and pharmacokinetics that confirmed a once-daily dosing regimen.3) A phase I/II study demonstrated high rates of complete hematologic and major cytogenetic response in patients with Philadelphia chromosome–positive (Ph+) leukemia in the second-line setting, following development of resistance/intolerance to imatinib,4) as well as in the third- and fourth-line settings following resistance/intolerance to imatinib and dasatinib and/or nilotinib.5) A phase III study of patients with newly diagnosed chronic phase (CP) chronic myeloid leukemia (CML) has demonstrated superior rates of major molecular response, similar rates of complete cytogenetic response, and fewer events of transformation to accelerated/blast phase compared with imatinib.6) These studies also demonstrated that bosutinib has an acceptable safety profile primarily characterized by transient and manageable gastrointestinal events.3,4) The pharmacokinetics of bosutinib are well characterized in healthy volunteers. Following administration of a single 500-mg dose of bosutinib with food, the median time-to-peak concentration (tmax) is 6 h. Bosutinib exhibits dose proportional increases in area under the concentration-time curve (AUC) and maximum observed concentration (Cmax), over the dose range of 200 to 800 mg. Bosutinib is highly bound to human plasma proteins in vitro (94%). Bosutinib has a mean apparent volume of distribution >5,000 L, suggesting extensive distribution to tissues. Bosutinib is

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primarily metabolized by CYP3A4 and <3% of the dose was excreted in the urine after a single oral dose.\(^7\)

To better understand the pharmacokinetics of bosutinib in patients, a population pharmacokinetic analysis was performed and a population model was developed using data pooled from three clinical studies in patients with solid tumors or CP CML patients who were either naive to treatment or were previously treated.\(^8\)

Additional objectives included identification of covariates that might account for the inter-individual variability in the pharmacokinetic parameters of bosutinib and evaluation of the performance of the developed pharmacokinetic model. The resulting pharmacokinetic model was utilized to examine the relationships between exposure and safety in the phase III study for all evaluable patients and for those patients who were classified as responders to bosutinib therapy. The resulting pharmacokinetic data also were used for a more extensive pharmacokinetic-pharmacodynamic analysis that was previously reported.\(^9\)

### Methods

**Study design:** The population pharmacokinetic model was developed using pooled data from three clinical studies of single-agent bosutinib, described below. All patients provided written informed consent prior to entry into their respective study. The study protocols were approved by institutional review boards at each site and conducted in accordance with the Declaration of Helsinki.

**Phase I study in solid tumors\(^3\)**

This study was a first-in-human, phase I trial in patients with advanced solid tumor malignancies, conducted in two parts. In Part 1 (dose escalation), oral doses of bosutinib 50 to 600 mg were administered once daily with food using a 3 + 3 design. In Part 2 (dose expansion), approximately 30 patients each with refractory colorectal, pancreas, or non–small cell lung cancer were treated with bosutinib 400 mg once daily with food, the recommended phase II dose. Blood samples were collected predose and at 1, 2, 3, 4, 6, 8, 24, and 48 h after a single dose on Day 1, and predose and at 1, 2, 3, 4, 6, 8, and 24 h postdose on Day 14 (on Days 14 and 15 after 14 days of continuous daily dosing).

**Phase II study in Ph+ leukemia\(^4,5\)**

This was an open-label, two-part study to assess the safety and efficacy of bosutinib in leukemia. Enrolled patients had confirmed Ph+ leukemia and resistance or intolerance to prior imatinib (and possibly prior dasatinib and/or nilotinib). In Part 1 (dose escalation) of the study, continuous daily doses of bosutinib 400 to 600 mg with food were administered to determine the maximum tolerated dose; blood samples were collected on Day 1 predose and at 1, 2, 3, 4, 6, 8, 24, and 48 h after a single dose on Day 1; predose and at 6 h postdose on Day 8; and predose and at 1, 2, 3, 4, 6, 8, and 24 h postdose on Day 15. Part 2 evaluated the safety and efficacy of bosutinib 500 mg once daily with food (recommended phase II dose determined in Part 1 of the study). Blood samples in Part 2 were collected predose and at 2, 4, 6, and 24 h postdose on Day 1; and predose during Weeks 12, 24, and 36.

**Phase III study in CP CML\(^6\)**

This was a randomized, open-label, active-controlled (vs. imatinib) trial in patients with newly diagnosed (≤ 6 months) CP CML and no prior anti-leukemia treatment (except anagrelide or hydroxyurea). Bosutinib 500 mg was administered once daily with food, and blood samples were collected on Days 1 and 28 predose and at 3 and 6 h postdose, and predose during Weeks 8 and 12.

**Determination of bosutinib concentrations in pharmacokinetic samples:** For the phase I study in solid tumors and the phase I/II study in Ph+ leukemia, bosutinib concentrations in potassium (K3) EDTA plasma were measured using a liquid chromatography/tandem mass spectrometric (LC/MS/MS) assay (Wyeth Research, Collegeville, PA) over a linear range of 1.0 to 250 ng/mL (1/concentration\(^2\) weighting). Plasma samples (500 µL) were fortified with an internal standard (d\(_8\)-bosutinib, 50 µL, 100 ng/mL) followed by a saturated sodium borate solution (100 µL) and mixed. The samples were then extracted with 3 mL of ethyl acetate, shaken for 15 min on a horizontal shaker, and centrifuged at 3,000 rpm for 5 min at 4°C. After the aqueous layer was frozen using a dry ice/acetone bath, the organic solvent layer was removed and dried under a gentle nitrogen stream at 25°C in a TurboVap evaporator. Sample residues were reconstituted (150 µL) with a solution of mobile phase A:mobile phase B (80:20, where mobile phase A = acetonitrile:1% aqueous formic acid (10:90) and mobile phase B = 2% formic acid in acetonitrile), transferred to polypropylene tubes containing a 0.22 µM filter, and centrifuged at 10,000 rpm for 5 min at room temperature. Filtered extracts (10 µL) were injected onto an Aquasil C18 column (20 mm × 2.1 mm internal diameter, 3 µm; Thermo Scientific, Waltham, MA) heated to 40°C and eluted with a linear gradient (0%–98% B in 1.7 min followed by 98% B for 2.5 min; 0.2 mL/min flow rate) onto a Sciex API 3000 mass spectrometer (AB Sciex, Framingham, MA) equipped with a turbo ion spray interface. Detection was in positive ion mode using mass transitions m/z 530.3 > 141.2 amu for bosutinib and 538.5 > 149.3 amu for d\(_8\)-bosutinib, with a dwell time of 500 ms for both. Interassay precision (expressed as percent coefficient of variation [%CV]) was ≤ 10.6% and interassay accuracy (expressed as percent relative error) ranged from −9.0% to 3.6% across the quality controls that were assayed with the study samples.

For the phase III study in CP CML, bosutinib concentrations in potassium (K3) EDTA plasma were measured using an LC/MS/MS assay (PharmaNet USA, Princeton, NJ) over a linear range of 1.0 to 500 ng/mL (1/concentration\(^2\) weighting). Plasma samples (100 µL) were added to a 2.0 mL 96-well plate placed in an ice water bath and fortified with an internal standard (d\(_8\)-bosutinib, 50 µL, 50 ng/mL) followed by the addition of chilled (~4°C) carbonate buffer (pH 10, 100 µL) and mixed. The alkalized samples were extracted with 1 mL of methyl tert-butyl ether and mixed thoroughly; approximately 0.9 mL of the organic solvent layer was removed and dried under a nitrogen stream (40–60 psi) at 28°C with an EvapArray 96-well sample concentrator (Jones Chromatography, Lakewood, CO). Sample residues were reconstituted to 100 µL in water:methanol (50:50), vortexed, injected onto a YMC ODS-AQ column (50 × 2.0 mm internal diameter, 5 µm; Waters, Milford, MA) heated to 35°C, and eluted using a linear gradient (mobile phase A = 0.2% formic acid in water and mobile phase B = 0.2% formic acid in methanol; 15%–55% B in 1.5 min, followed by 55% B for 0.5 min; 0.4 mL/min flow rate) onto a Sciex API 4000 mass spectrometer (AB Sciex) equipped with a turbo ion spray interface. Detection was in positive ion mode using mass transitions m/z 530.3 > 141.2 amu for bosutinib and 538.2 > 149.3 amu for d\(_8\)-bosutinib, with a dwell time of 150 ms for both. Interassay precision (expressed as %CV) was ≤ 10.1% and interassay accuracy (expressed as percent relative error) ranged from −8.5% to 2.3% across the quality controls that were assayed with the study samples.

**Model development and selection:** Population modeling...
used NONMEM software, version 7 level 1.0 (Icon Development Solutions, Dublin, Ireland). To inform initial parameter estimates for the model, a noncompartmental analysis was conducted of the rich concentration-time data from the first dose of bosutinib in the first 20 patients of the phase I study in solid tumors.

Compartmental pharmacokinetic models were coded using the ADVAN2 and ADVAN4 subroutines of NONMEM, with estimation by the first-order conditional estimation (FOCE) method with eta-epsilon interaction.

The date and time of each dose were collected for each patient. Dose changes and dose interruptions were also captured and included in the model. Data with unconfirmed dosing information were excluded from the analysis.

Model selection

Models were selected on the basis of goodness-of-fit, as judged by several criteria. The likelihood ratio test was used to compare nested models with significance levels of \( p = 0.005 \) for structural models and forward addition of covariates, and \( p = 0.001 \) for backward deletion of covariates. The Akaike Information Criteria was used to compare for non-nested models and models with the same number of parameters, while various diagnostic plots were used to assess model performance. The condition number was used to assess co-linearity of a model and was calculated as the ratio of the largest to smallest value of the parameter correlation matrix. A condition number > 1,000 indicated model instability due to high co-linearity of the parameters.\(^{10}\) Minimization status was also considered, as well as reductions in inter-individual variability terms and parameter shrinkage. Conditional weighted residuals were used for model diagnostics.

All models were ranked by their NONMEM minimum objective function value, and their condition number, minimization status, and covariance step status were tabulated.

Step-wise model development

The model was developed and evaluated in multiple stages. Stage 1 of the model development systematically investigated the major structural components of the pharmacokinetic model. These included the use of untransformed or log-transformed data, one- and two-compartment models with first-order absorption, assessment of the benefit of an absorption lag (or not), identification of a residual error model (i.e., additive, proportional, or combined), and testing of different initial estimates of the absorption rate constant. For a 2-compartment model, the parameters were designated as apparent relative bioavailability (\( F \)), clearance (CL/F), central distribution volume (V2/F), inter-compartmental clearance (Q/F), peripheral distribution volume (V3/F), first-order absorption rate constant (KA), and absorption lag-time; a 1-compartment model did not have the parameters Q/F and V3/F. For these initial models, population inter-individual variability (exponential error model) was assigned for CL/F and V2/F only. Other models investigated at this stage included those with transit compartments representing the oral absorption process and a 3-compartment, first-order absorption model.

Stage 2 of the model development systematically examined the inter-individual variability error structure of the most appropriate structural model from Stage 1. Inter-individual variability was always assigned to CL/F and V2/F, but was added to other parameters singly and in combination. Each model was tried with and without covariance terms.

In Stage 3 of the model development, the diagnostic goodness-of-fit plots for the best model from Stage 2 were inspected for bias with respect to the influence of dose or study on residual distributions. Models were investigated where non-linearity was achieved by either CL/F or F changing with dose. A model with the F as a function of dose was adopted as follows:

\[
\text{LGT1} = \text{Effect of Dose} \times \log(\text{DOSE}/400) \\
F1 = \exp(\text{LGT1})/(1 + \exp(\text{LGT1})) \times 2
\]

The equations for the residual error models were:

If dose was \( \leq 100 \text{mg/day} \) : \( \epsilon_{ij} = \hat{C}_{ij} (1 + \varepsilon_{ij}) \)

If dose was \( > 100 \text{mg/day} \) : \( \epsilon_{ij} = \hat{C}_{ij} (1 + \varepsilon_{ij}) + \varepsilon_{ij} \)

The use of two separate residual variability terms was introduced early in the modeling process; at that time, the values were quite different. This difference diminished as the OMEGA matrix was fully described and the effect of dose on bioavailability was added. Separate residual error terms were kept because they had been incorporated throughout the model-building process; further, these terms were well estimated and did not over-parameterize the model.

Reduced covariance models were also tried as indicated by the precision of the covariance terms (imprecise estimates were removed) and by the observed relationship between the random error terms for the parameters.

Additionally, the most appropriate model from Stage 3 was analyzed for the effect of each covariate (i.e., baseline characteristics and laboratory results) on the model, using power and linear models for continuous covariates and binary relationship for categorical covariates.

Example of a power model:

\[
\text{CL}_{ij} = \text{CL}_{pop} \left( \frac{\text{Covariate}_i}{\text{Mean Value}} \right)^\theta
\]

Example of a linear model:

\[
\text{CL}_{ij} = \text{CL}_{pop} + \theta \left( \frac{\text{Covariate}_i}{\text{Mean Value}} \right)
\]

Example of a binary model:

\[
\text{CL}_{ij} = \text{CL}_{pop} (1 + \theta \text{Covariate}_i)
\]

In all cases, CL\(_{ij}\) represents the individual clearance, CL\(_{pop}\) is the population mean clearance and \( \theta \) is the covariate effect. For the binary model, the covariate value is 0 for the reference (ex: male) and 1 for non-reference (ex: female) value.

Covariates evaluated included age, weight, body surface area, body mass index, sex, race, protocol, Eastern Cooperative Oncology Group (ECOG) performance status score, creatinine clearance, total bilirubin, alanine aminotransferase, aspartate aminotransferase, dose, and albumin. The covariate analysis proceeded by separately examining the influence of each covariate alone on the variability terms associated with CL/F, V2/F, V3/F, or KA. The resulting covariate models (if nested) were ranked by the \( p \) value–based comparison of the likelihood ratio test statistic with the base model; those with a \( p \) value \( < 0.005 \) were further assessed. A covariate relationship was not included in the model if the covariate parameter could not be estimated with a precision of <30% or if the covariate did not explain \( \geq 5\% \) of the associated inter-individual variability for the parameter; those covariates that changed the associated parameter by \( \geq 20\% \) were considered clinically important. The best covariate relationship was incorporated in the model and the influence of the remaining covariates were re-
examined in a forward inclusion step-wise manner. This process continued until no more suitable covariate relationships could be found.

In a separate analysis, the covariates for body size (weight and body surface area) were examined specifically with respect to the benefit of adjusting kinetics (and hence dose) for body size, using a power model with a coefficient of 1 on the parameters CL/F, V2/F, Q/F, and V3/F simultaneously. An allometric model for body weight was also examined, where the coefficient was fixed to 0.75 for CL/F and Q/F and to 1 for V2/F and V3/F. When applied separately to each parameter, weight and body surface area were not significant covariates. Adjusting all parameters for a standard allometric model did not improve the objective function.

Model evaluation and analysis: The predictive performance of the final model was evaluated using a visual predictive check. The final population model was used to simulate 200 replicates of the original observed dataset based on the final parameter values. Three types of visual predictive check plots were constructed:

1. The time-course of bosutinib concentrations for the observed data, the median, and 90% confidence intervals of the simulated concentrations were plotted on study and dose. Separate plots were constructed for the loading (Day ≤ 3) and maintenance (Day > 3) phases. The predictive performance of the model was considered acceptable if the majority of original data points were contained inside the predicted confidence intervals, with no significant systematic deviation between simulated and observed data.

2. Because the current recommended bosutinib dose is 500 mg/day, particular attention was given to the performance of the model for this dose regimen. The observed and simulated bosutinib concentrations at 2, 6, and 24 h after a dose in the maintenance phase were compared using box plots and summary statistics.

3. Three key covariates (i.e., body size, renal function, and liver function) were examined for their influence on the steady-state bosutinib concentration at 6 h after a 500 mg dose using box plots and summary statistics.

To determine the inter-individual variability in bosutinib exposure expected for a fixed bosutinib dose in a population of patients, the final model was used to simulate the range of concentrations expected at steady-state for a dose of 500 mg/day. A total of 1,000 patients were simulated using a database with sample times from 0 to 24 h at 0.5-h intervals. In addition, the distribution of the AUC expected for bosutinib doses of 200, 300, 400, and 500 mg/day was determined by simulation.

To estimate the time to steady-state for bosutinib, the final pharmacokinetic model was coded in the Berkeley Madonna program (Robert Macey and George Oster, University of California at Berkeley, CA), an efficient differential equation-solving program that allowed the effect of different bosutinib dose regimens to be examined expeditiously. The Madonna model was used to simulate the bosutinib concentrations expected during and after dosing for a dose regimen of 500 mg/day for 120 days, with the time to reach steady-state inferred graphically from the resulting plot.

Model utilization for pharmacodynamic analysis: For the phase III study in Ph+ CP CML,60 relationships between exposure and safety were analyzed for all evaluable patients (n = 245), and for patients who had complete cytogenetic response (CCyR), major molecular response (MMR), and complete hematologic response (CHR) at 1 year. Adverse events (AEs) included in the analysis were diarrhea, thrombocytopenia, rash, alanine aminotransferase (ALT) increase, aspartate aminotransferase (AST) increase, nausea, vomiting and neutropenia. The frequency of the incidence of an AE (grade ≥ 1) was plotted against binned AUC values derived from the final population pharmacokinetic model (minimum, 20 patients per bin) to yield probability curves for each AE.

Results

Patient characteristics: A total of 903 patients were included in the pooled data set, with 870 patients contributing to the pharmacokinetic analyses. Key patient characteristics are summarized in Table 1. Each patient contributed an average of 6 to 9 pharmacokinetic samples. Across the 3 clinical studies, enrolled patients were 53% male, aged 18 to 91 years old, and had a baseline weight range of 35 to 221 kg. Most leukemia patients (97% for the phase I/II study in imatinib-resistant/intolerant CP CML and 100% for the phase III study in newly diagnosed CP CML) had an ECOG performance status score of 0 or 1, indicating that they were asymptomatic or symptomatic but completely ambulatory, respectively. The ages and male:female ratios of patients enrolled in the phase III study reporting CCyR, MMR, and CHR were similar among cohorts and similar to those of the total phase III population.

Final population pharmacokinetics model: The final model of bosutinib pharmacokinetics after oral administration was a linear two-compartment model with first-order absorption and an absorption lag-time of 0.9 h and a dose-dependent bioavailability. The oral absorption of bosutinib was relatively slow, with an absorption half-time of 1.14 h. Terms describing between-patient variability were included on CL/F, V2/F, V3/F and KA, with a term describing the correlation between CL/F and V2/F. The proportional residual unexplained variability was 30% at doses ≤ 100 mg/day and 32% at doses > 100 mg/day.

Parameters of the population pharmacokinetics model were estimated with acceptable precision (percent standard error < 30%;

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Phase I in solid tumors (n = 145)</th>
<th>Phase I/II in imatinib-resistant/ intolerant CP CML (n = 513)</th>
<th>Phase III in newly diagnosed CP CML (n = 245)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic samples, n</td>
<td>1,362</td>
<td>4,317</td>
<td>1,558</td>
</tr>
<tr>
<td>Mean samples per patient, n</td>
<td>9.39</td>
<td>8.42</td>
<td>6.36</td>
</tr>
<tr>
<td>Mean age (range), y</td>
<td>58.9 (19–83)</td>
<td>51.7 (18–86)</td>
<td>47.1 (19–91)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>67 (46)</td>
<td>266 (52)</td>
<td>145 (59)</td>
</tr>
<tr>
<td>Race, n (%)&lt;</td>
<td>White</td>
<td>123 (85)</td>
<td>337 (66)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (1)</td>
<td>86 (17)</td>
<td>64 (26)</td>
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<tr>
<td>Other</td>
<td>7 (5)</td>
<td>59 (12)</td>
<td>24 (10)</td>
</tr>
<tr>
<td>ECOG performance status, n (%)&lt;</td>
<td>0</td>
<td>344 (67)</td>
<td>182 (74)</td>
</tr>
<tr>
<td>1</td>
<td>150 (29)</td>
<td>63 (26)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17 (3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Median weight (SD), kg</td>
<td>77.7 (22.5)</td>
<td>77.2 (20.9)</td>
<td>70.7 (16.4)</td>
</tr>
</tbody>
</table>

CP, chronic phase; CML, chronic myeloid leukemia; ECOG, Eastern Cooperative Oncology Group.
<Percentages may not total 100% because of rounding.
The apparent volume of the peripheral compartment was large with a slow turnover. Time-to-peak concentration was 5 to 6 h, while the estimated alpha and beta half-lives were 19 h and 290 days, respectively, with 68% and 32% of AUC attributable to the alpha and beta half-lives, respectively.

Dose-dependency in F was observed, which was most noticeable at lower doses. The relative F for doses >300 mg/day was within 11% of the reference value (F = 1 for the 400 mg/day dose). The model appropriately predicted individual bosutinib concentrations for most patients, suggested by the close and even distribution of points to the line of identity in a plot of observed versus individual-predicted concentrations (Fig. 1A). However, there are a noticeable number of observations with low observed concentrations but high predicted concentrations; these may reflect patients with unexpectedly low concentrations due to missed doses. The points were also evenly distributed around the line of identity (F = 1) for the all-evaluable-patient population (Fig. 1B).

Inter-individual pharmacokinetic variability: The final population pharmacokinetics model showed high inter-individual variability, consistent with the high variability observed in bosutinib absorption. None of the covariates in the database (baseline characteristics [i.e., age, weight, body surface area, body mass index, sex, race, ECOG Performance Status score, protocol] and laboratory results [i.e., creatinine clearance, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and albumin]) improved the performance of the pharmacokinetic model. Therefore, multivariate covariate models were not considered, and backward deletion of covariates was not necessary (Fig. 2). In addition, adjusting the bosutinib dose for covariates representing body size (i.e., weight and body surface area) was not found to be necessary because body size was not found to be predictive of bosutinib exposure.

Model simulation: A visual predictive check of the time-course of bosutinib concentrations during the maintenance phase showed acceptable agreement between observed and simulated concentrations (Fig. 3), although model simulations also showed that a dose of bosutinib 500 mg/day could produce highly variable individual concentrations. The coefficient of variation of the predicted bosutinib concentrations at 2, 6, and 24 h after administration of bosutinib 500 mg/day (maintenance phase) were 65%, 63%, and 69%, respectively, and were in agreement with the observed values. However, within individuals, differences between peak and trough bosutinib concentrations were relatively small, with a population fluctuation ratio (Cmax/Cmin at steady-state) of 152%.

Bosutinib approached steady-state after 4 days, but concentrations were predicted to rise slowly during the maintenance phase due to the large apparent peripheral distribution volume. This behavior is characteristic of a drug that has a large peripheral distribution volume with a slow turnover. A summary of the predicted mean and %CV of AUC, Cmax, and Cmin for a range of doses is provided in Table 3. Median Cmax at steady-state was 215 ng/mL (405 nM) for bosutinib 500 mg/day, which was in excess of previously reported half-maximal inhibitory concentration (IC50) values (i.e., 0.53–10.6 ng/mL or 1–20 nM). The model-based accumulation index (Cmax [steady-state]/Cmax [first dose]) was 2.45, which is in agreement with the 2- to 3-fold increase inferred from the noncompartmental analysis. Median AUC at steady state was 4,322 ng·h/mL for bosutinib 500 mg/day.

Model utilization for pharmacodynamic analysis: For model utilization, all evaluable patients (n = 245) and patients who had treatment responses at 1 year of CCyR (n = 171), MMR (n = 98) and CHR (n = 175) were included in this analysis. Bosutinib exposure was similar among the different response populations (Table 4). The incidence of diarrhea was higher for the all-evaluable-patient population compared with patient subgroups who responded to bosutinib when the AUC for bosutinib was relatively low (Fig. 4A). Compared with patients who responded to bosutinib, the incidence of nausea (Fig. 4B) and neutropenia (Fig. 4C) in the all-evaluable-patient population was generally higher, independent of AUC. In general, the incidence of AEs including ALT increase (Fig. 4D), thrombocytopenia, rash, AST increase, and vomiting (data not shown), as a function of AUC, were similar for the all-evaluable-patient population versus the patient subgroups who responded to bosutinib.

Discussion

A population pharmacokinetic model of bosutinib was successfully developed from pharmacokinetic data collected in three clinical studies of bosutinib monotherapy. The final population pharmacokinetic model was a linear two-compartment model with first-order absorption and an absorption lag time and a dose-dependent bioavailability.

The model was characterized by high inter-individual variability in kinetics. Multiple factors, including inconsistent compliance in dosing, pH-dependent solubility, saturable oral absorption, and metabolism via CYP3A4 likely contributed to the high inter-individual variability. The possibility of inconsistent dosing compliance is supported by the database and the discrepancy in observed versus individual predicted concentrations. There was a number of observations where the observed concentration is noticeably less than the model-predicted concentration, while the opposite was not observed (Fig. 1); this may be reflective of an unrecorded break in dosing prior to these pharmacokinetic samples or taking bosutinib without food, as a previous study indicated that food intake increases bosutinib exposure by approximately 1.5- to 2-fold. The observed inter-individual variability may also be due

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Table 2. Population pharmacokinetic model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>%SE</th>
<th>%</th>
<th>%SE</th>
<th>%</th>
<th>%SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F, L/h</td>
<td>120</td>
<td>5.33</td>
<td>59.9</td>
<td>11.6</td>
<td>12.4</td>
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<tr>
<td>V2/F, L</td>
<td>4,917</td>
<td>2.48</td>
<td>47.7</td>
<td>10.2</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>Q/F, L/h</td>
<td>56.4</td>
<td>10.6</td>
<td>51.4</td>
<td>15.5</td>
<td>54.0</td>
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<tr>
<td>V3/F, L</td>
<td>388,300</td>
<td>28.0</td>
<td>211.4</td>
<td>15.5</td>
<td>154.0</td>
<td></td>
</tr>
<tr>
<td>KA, L/h</td>
<td>0.610</td>
<td>5.35</td>
<td>104.4</td>
<td>9.54</td>
<td>24.7</td>
<td></td>
</tr>
<tr>
<td>t1/2, h</td>
<td>0.871</td>
<td>1.82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect of dose on F</td>
<td>1.25</td>
<td>17.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

%SE, percent standard error; ETA, empirical Bayes prediction of the inter-individual random effect; CL, clearance; F, apparent bioavailability; V2, central distribution volume; Q, inter-compartmental clearance; V3, peripheral distribution volume; KA, first-order absorption rate constant; t1/2, absorption lag-time.

Value of parameter for the reference dose of bosutinib 400 mg/day.
Fig. 1. Observed versus individual-predicted (A) and population-predicted (B) concentrations
The diagonal black line has a slope of 1; the red line is a Loess-smoothed line for the data; the horizontal black line is the lower limit of quantitation.

Fig. 2. Covariate effects on the empirical Bayes estimates of the inter-individual random effect on the final model
ETA1, empirical Bayes prediction of inter-individual random effect for clearance; BMI, body mass index.

Fig. 3. Visual predictive checks of observed versus simulated bosutinib concentrations during the maintenance phase across studies and bosutinib doses
Observed data were compared with 200 datasets of simulated data using the final pharmacokinetic model parameters. Plots are subset by study and bosutinib dose. Blue dots represent observed data; the solid black line is median of the observed data; the dashed black lines are the 5th and 95th percentiles of the observed data; the red line is the median of the simulated data; the grey shaded area represents the 5th to 95th percentiles of the simulated data. The model shows good predictive performance, as the majority of observed data fall within the 90% confidence intervals of the simulated data.
Table 3. Summary of model-generated bosutinib pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Median (range)</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, ng/mL-h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>1,645</td>
<td>1,376 (124–9,213)</td>
<td>1,080</td>
<td>66</td>
</tr>
<tr>
<td>300</td>
<td>2,721</td>
<td>2,294 (281–13,369)</td>
<td>1,707</td>
<td>63</td>
</tr>
<tr>
<td>400</td>
<td>4,087</td>
<td>3,374 (560–24,637)</td>
<td>2,683</td>
<td>66</td>
</tr>
<tr>
<td>500</td>
<td>5,216</td>
<td>4,322 (735–22,326)</td>
<td>3,316</td>
<td>64</td>
</tr>
<tr>
<td>Cmax, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>80.6</td>
<td>69.3 (6.8–406)</td>
<td>49.3</td>
<td>61</td>
</tr>
<tr>
<td>300</td>
<td>134</td>
<td>116 (12.9–579)</td>
<td>77.7</td>
<td>58</td>
</tr>
<tr>
<td>400</td>
<td>201</td>
<td>170 (41.7–1,151)</td>
<td>124</td>
<td>62</td>
</tr>
<tr>
<td>500</td>
<td>255</td>
<td>215 (43.9–1,019)</td>
<td>152</td>
<td>59</td>
</tr>
<tr>
<td>Cmin, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>56.6</td>
<td>46.0 (3.0–363)</td>
<td>41.3</td>
<td>73</td>
</tr>
<tr>
<td>300</td>
<td>93.2</td>
<td>76.7 (7.6–539)</td>
<td>65.4</td>
<td>70</td>
</tr>
<tr>
<td>400</td>
<td>140</td>
<td>112 (8.6–926)</td>
<td>102</td>
<td>73</td>
</tr>
<tr>
<td>500</td>
<td>180</td>
<td>147 (16.2–841)</td>
<td>126</td>
<td>70</td>
</tr>
</tbody>
</table>

%CV, percent coefficient of variance; AUC, area under the concentration-time curve; Cmax, maximum observed concentration; Cmin, minimum observed concentration.

Fig. 4. Relationships between bosutinib exposure and specified adverse events (grade ≥1) of diarrhea (A), nausea (B), neutropenia (C), and increased ALT (D) in patients overall (red) and in those who responded to bosutinib therapy with CCyR (blue), MMR (green), or CHR (purple).

ALT, alanine aminotransferase; CCyR, complete cytogenetic response; CHR, complete hematologic response; MMR, major molecular response.

Table 4. Bosutinib exposure in phase III study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC, ng/mL-h</th>
<th>Cmax, ng/mL</th>
<th>Cmin, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCyR (n = 171)</td>
<td>4,530 ± 2,350</td>
<td>232 ± 129</td>
<td>148 ± 80.9</td>
</tr>
<tr>
<td>MMR (n = 98)</td>
<td>4,750 ± 2,210</td>
<td>242 ± 109</td>
<td>156 ± 79.0</td>
</tr>
<tr>
<td>CHR (n = 175)</td>
<td>4,540 ± 2,340</td>
<td>232 ± 128</td>
<td>156 ± 80.4</td>
</tr>
</tbody>
</table>

%CV, percent coefficient of variance; AUC, area under the concentration-time curve; Cmax, maximum observed concentration; Cmin, minimum observed concentration; CCyR, complete cytogenetic response; CHR, complete hematologic response; CP, chronic phase; CML, chronic myeloid leukemia; MMR, major molecular response.

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to high variability in the oral absorption of bosutinib as a low solubility drug. Because the solubility of bosutinib is pH-dependent,\(^1\) with bosutinib becoming more soluble at lower pH, varying gastric pH levels in individual patients may contribute to variable dissolution rates, and subsequently variable absorption. In addition, *in vitro* data suggest that bosutinib is a substrate of P-glycoprotein,\(^7\) which moves bosutinib back into the gut lumen. The saturable process shows more variability than absorption by passive diffusion. Further, bosutinib is predominantly metabolized by the CYP3A4 hepatic enzyme,\(^12,13\) which is well known for its variable expression and activity,\(^14\) possibly contributing to variability in absorption through first pass metabolism and elimination.

In practice, highly variable drug exposure may not necessarily be associated with highly variable drug effects if the majority of the concentrations achieved are near the maximum drug effect (*i.e.*, well above the IC\(_{50}\)). This appears to be the case for bosutinib, since previously reported IC\(_{50}\) values for bosutinib of 1 to 20 nmol/mL for inhibition and 28 to 150 nmol/mL for apoptosis in imatinib-sensitive cell lines\(^3\) are much lower than the estimated average steady-state concentration of 347 nmol/mL (184.1 ng/mL) for bosutinib 500 mg/day. Furthermore, despite variable exposures, bosutinib demonstrated acceptable safety and tolerability across clinical studies.\(^3–6\)

Despite the observed high inter-individual variability, none of the evaluated covariates met the criteria for inclusion in the model. There was also minimal pharmacokinetic benefit of adjusting the bosutinib dose for baseline demographic and clinical characteristics and laboratory results, because of both the lack of influence observed in the covariate analyses for these parameters and the general high inter-individual variability. However, relative bioavailability was influenced by dose (lower bioavailability at lower doses), and this was incorporated into the final structural population pharmacokinetic model. The lower F inferred for low doses could be accounted for by saturable active transport of bosutinib into the gut lumen, which shows more variability than absorption by passive diffusion.

The model was used to relate steady-state bosutinib exposure to relevant clinical endpoints. The results of the present pharmacodynamic analyses show that the incidence of AEs among variously defined treatment responders was equal to, or no worse than, the incidence among the all-evaluable-patient population. In a separate analysis using this same model in a pooled population of bosutinib-treated patients with CP CML that included both newly diagnosed and previously treated patients (but not patients with solid tumors, as in the current study),\(^7\) exposure-response relationships were noted for diarrhea and, more weakly, for rash incidence (but not severity); no relationship was found for nausea, vomiting, neutropenia, thrombocytopenia, or elevated ALT or AST. In terms of efficacy, exposure-response relationships were noted for patients with newly diagnosed CP CML for CCyR, MMR, and CHR at year 1, though similar relationships with efficacy outcomes were not observed in patients who had previously received tyrosine kinase inhibitor therapy.

Overall, the population pharmacokinetic model of bosutinib showed acceptable descriptive and predictive performance, and was suitable for deriving individual exposure metrics. This model was useful in pharmacodynamic analyses for investigating relationships between bosutinib exposure at steady-state with safety and efficacy observed in clinical trials.\(^8\)

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**References**