Dasatinib (BMS-354825) Pharmacokinetics and Pharmacodynamic Biomarkers in Animal Models Predict Optimal Clinical Exposure


Abstract

Purpose: Chronic myeloid leukemia (CML) is caused by reciprocal translocation between chromosomes 9 and 22, forming BCR-ABL, a constitutively activated tyrosine kinase. Imatinib mesylate, a selective inhibitor of BCR-ABL, represents current frontline therapy for CML; however, emerging evidence suggests that drug resistance to imatinib may limit its long-term success. To improve treatment options, dasatinib (BMS-354825) was developed as a novel, oral, multi-targeted kinase inhibitor of BCR-ABL and SRC family kinases. To date, dasatinib has shown promising anti-leukemic activity in preclinical models of CML and in phase I/II clinical studies in patients with imatinib-resistant or imatinib-intolerant disease.

Experimental Design: The pharmacokinetic and pharmacodynamic biomarkers of dasatinib were investigated in K562 human CML xenografts grown s.c. in severe combined immunodeficient mice. Tumoral levels of phospho-BCR-ABL/phospho-CrkL were determined by Western blot.

Results: Following a single oral administration of dasatinib at a preclinical efficacious dose of 1.25 or 2.5 mg/kg, tumoral phospho-BCR-ABL/phospho-CrkL were maximally inhibited at ~3 hours and recovered to basal levels by 24 hours. The time course and extent of the inhibition correlated with the plasma levels of dasatinib in mice. Pharmacokinetic/biomarker modeling predicted that the plasma concentration of dasatinib required to inhibit 90% of phospho-BCR-ABL in vivo was 10.9 ng/mL in mice and 14.6 ng/mL in humans, which is within the range of concentrations achieved in CML patients who responded to dasatinib treatment in the clinic.

Conclusions: Phospho-BCR-ABL/phospho-CrkL are likely to be useful clinical biomarkers for the assessment of BCR-ABL kinase inhibition by dasatinib.

Dasatinib (BMS-354825) is a novel, oral, multi-targeted inhibitor of BCR-ABL and SRC family kinases rationally designed for the treatment of chronic myeloid leukemia (CML). CML is defined by the presence of BCR-ABL, a constitutively activated form of the ABL tyrosine kinase that has been linked to malignant transformation (1, 2). The BCR-ABL fusion gene typically arises from a t(9;22)(q34;q11) translocation in bone marrow progenitor cells that produces a shortened chromosome 22, known as the Philadelphia chromosome (3). Around 20% to 30% of adult acute lymphoblastic leukemia (ALL) cases are also associated with the Philadelphia chromosome (4).

Imatinib mesylate, an oral inhibitor of the BCR-ABL kinase, is the current frontline therapy for CML (5–7). Although impressive therapeutic responses have been achieved in treatment of CML in the chronic phase (6–12), response rates in patients with more advanced disease are lower, and these responses are generally transient (5–12). Moreover, emerging evidence indicates that a significant proportion of patients in all phases of disease fail to achieve an optimal response to imatinib due to innate or acquired resistance to imatinib. The underlying mechanisms of imatinib resistance include BCR-ABL gene mutations (the most common mechanism), overexpression of BCR-ABL, and activation of BCR-ABL–independent pathways, such as signaling through members of the SRC family kinases (5–7, 11–13).

The available treatment options for patients with imatinib-failed disease are extremely limited; there is, therefore, an urgent need for improved treatment options for these patients. Dasatinib was designed to address the shortcomings of imatinib to provide an improved therapeutic option. Compared with imatinib, dasatinib has been shown to have 325-fold greater potency in cells transduced with wild-type BCR-ABL, which may be due in part to the ability of dasatinib binding both the active and inactive conformations of BCR-ABL (14). Furthermore, dasatinib has shown anti-leukemic activity in vitro and in vivo against preclinical models of human CML.
including those that are resistant to imatinib through a variety of mechanisms. When tested against patient-derived imatinib-resistant BCR-ABL mutation-positive cell lines, dasatinib showed activity against 18 of the 19 lines (13–17). Dasatinib’s increased potency has also been shown in a clinical setting in patients with imatinib-resistant or imatinib-intolerant disease. In phase I dose escalation studies, dasatinib induced complete and major cytogenetic responses in all phases of CML (chronic, accelerated, and blastic) and in Philadelphia chromosome–positive ALL (Ph⁺ ALL). Complete and partial hematologic responses and favorable tolerability were also reported (18). These promising results have been further confirmed in phase II clinical studies of dasatinib in patients with imatinib-resistant or imatinib-intolerant CML and Ph⁺ ALL (19–22). The results of phase II studies have indicated that dasatinib induces durable cytogenetic and hematologic responses in all phases of CML and Ph⁺ ALL and represents a well-tolerated therapeutic option post imatinib failure. Dasatinib has been proved by U.S. Food and Drug Administration for the treatment of CML in all phases and Ph⁺ALL in 2006.

To successfully develop molecularly targeted agents, such as dasatinib or imatinib, it is extremely valuable to establish pharmacodynamic biomarkers through preclinical studies that link key variables of drug activity from the molecular target to the clinical effects. In this respect, it is essential to understand the connections among (a) the expression or status of the molecular target and biological pathway; (b) achievement of the active plasma concentrations of drug; (c) the demonstration of drug activity against the intended molecular target (e.g., inhibition of an enzyme); (d) the modulation of the biochemical pathway in which the molecular target functions; (e) the induction of the downstream biological effects (e.g., anti-proliferation and/or survival benefit); and (f) the achievement of clinical responses (23, 24). Based on these considerations, the present study was designed to evaluate the following end points: (a) the relationship of pharmacokinetic with pharmacodynamic biomarkers at efficacious doses (i.e., pharmacokinetic versus in vivo modulation of tumoral phospho-BCR-ABL/phospho-CrkL) and (b) the potential of tumoral phospho-BCR-ABL/phospho-CrkL as biomarkers to assess target exposure (i.e., inhibition of BCR-ABL kinase).

### Materials and Methods

**Chemical reagents and antibodies.** Complete protease inhibitor tablets were from Roche Diagnostics (Indianapolis, IN). MicroBCA reagents were from Pierce (Rockford, IL). Rabbit polyclonal anti-phospho-ABL (pY412) antibody and anti-phospho-CrkL (pY207) antibody were purchased from Cell Signaling (Beverly, MA). Anti-rabbit-IgG antibody conjugated with horseradish peroxidase was purchased from BD Biosciences (Lexington, KY). Unless otherwise specified, all other chemicals and reagents were from Sigma (St. Louis, MO). Sterile buffers and solutions were obtained from Life Technologies (Carlsbad, CA). Sterile tissue cultures were obtained from Fisher Scientific Co. (Hanover Park, IL).

**Animals.** Female severe combined immunodeficient mice, 5 to 6 weeks of age, were obtained from Harlan Sprague-Dawley Co. (Indianapolis, IN) and maintained in an ammonia-free environment in a defined and pathogen-free colony. Animals were quarantined for −3 weeks before their use for tumor propagation and pharmacokinetic/pharmacodynamic study. They were given food and water ad libitum.

All studies were done in accordance with Bristol-Myers Squibb and the American Association for Accreditation of Laboratory Animal Care guidelines.

K562 human CML cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37°C/5% CO₂. Cells (2 × 10⁶) were s.c. implanted into the flanks of each severe combined immunodeficient mouse. Tumors were then allowed to grow to the predetermined size window (usually between 150-250 mg; tumors outside the range were excluded), and animals were evenly distributed to various treatment for pharmacokinetic/pharmacodynamic study.

**Drug formulation and administration.** For oral (p.o.) and i.v. administration, dasatinib was dissolved in a mixture of propylene glycol/water (50:50). The volume of administration was 0.01 mL/g for mice.

**Pharmacokinetic analysis.** To characterize the pharmacokinetic variables of dasatinib, mice (n = 3 per time point) were bled by cardiac puncture, following a single i.v. administration of 5 mg/kg dasatinib at 0, 0.08, 0.25, 1, 3, 6, and 24 hours, or following a single p.o. administration at doses of 1.25, 2.5, and 5 mg/kg at 0, 0.5, 1, 3, 7, 17, and 24 hours. Plasma samples were collected and frozen at −80°C until analysis by high-performance liquid chromatography/mass spectrometry. In brief, plasma samples were deproteinized with acetonitrile; the supernatant was analyzed by high-performance liquid chromatography/mass spectrometry. The standard curve ranged from 1 to 5,000 ng/mL and was fitted with a quadratic regression weighed by reciprocal concentration (1/x). The limit of quantitation for the purposes of this assay was 1 ng/mL.

Pharmacokinetic data analysis was done by noncompartmental method using Kinetica (v4.0.2; Innaphase Corp., Philadelphia, PA). The maximum plasma concentration (C_max) and the time to reach C_max (T_max) were determined by visually inspecting the profiles of plasma drug levels versus time. The half-life of plasma drug elimination (1/β) was the ratio of 0.693 to the slope obtained by log-linear regression of the terminal phase of the drug plasma profile. The area under the plasma drug concentration curve (AUC) was estimated by the trapezoidal rule. Other pharmacokinetic variables, including the total body clearance (CL), steady-state volume of distribution (Vss), and oral bioavailability (F_p.o.) were calculated by equations as follows:

\[
CL = \frac{D_i}{AUC_i} \quad (A)
\]

\[
V_{ss} = \frac{D_i}{C_0} \quad (B)
\]

\[
F_{p.o} = \frac{AUC_{p.o}/D_{p.o}}{AUC_{i.v}/D_{i.v}} \quad (C)
\]

where D is the dose, and C_0 is the estimated initial plasma concentration by extrapolating drug concentration to zero after i.v. bolus administration.

**Western blot analysis of phospho-BCR-ABL and phospho-CrkL in tumors.** The pharmacodynamic studies were conducted in mice bearing K562 xenografts. Following a single p.o. administration of dasatinib at 1.25 and 2.5 mg/kg, tumors were surgically removed at

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**Optimizing Clinical Dosing Regimen with Biomarkers**

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specified time points of 0, 0.5, 1, 3, 7, 17, and 24 hours; immediately snap-frozen in liquid nitrogen; and stored at -80°C until analysis. Frozen tumor tissues were ground into powder under -80°C with a grinding device (WWR Scientific Products, South Plainfield, NJ) then lyzed in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitor cocktails. The total protein concentration of tumor lysate was determined using the MicroBCA method (Pierce). The denatured lysate was resolved on 12% SDS-PAGE gels and transferred to Immobilon-P polyvinyl membrane (Millipore Corp., Bedford, MA). To detect phospho-proteins, the membrane was incubated with rabbit polyclonal anti-phospho-BCR-ABL or anti-phospho-Crkl antibody (1:1,000 dilution) for 30 minutes at 37°C, followed by incubation with 0.1 μg/mL of anti-rabbit-IgG antibody conjugated with horseradish peroxidase. The phospho-proteins were visualized with Western blot chemiluminescence reagent Enhanced Chemiluminescence Plus (Amersham Pharmacia Biotech, Piscataway, NJ). The molecular sizes of BCR-ABL and Crkl were estimated by comparison with prestained protein precision markers (Bio-Rad Laboratories, Hercules, CA).

Pharmacokinetic and pharmacodynamic modeling. The pharmacokinetic and pharmacodynamic data for dasatinib generated in mice bearing K562 tumors were analyzed simultaneously using the SAAM II software (Seattle, WA). The pharmacokinetic data with i.v. administration were simultaneously fitted using a two-compartment model:

\[ V_c \frac{dC}{dt} = -k_{10} V_c C - k_{12} V_c C + k_{21} V_p C_p \]  

where \( k_{10} \) is the elimination rate constant; \( k_{12} \) and \( k_{21} \) are transfer rate constants between the central and peripheral compartments, respectively; \( V_c \) and \( V_p \) are the volumes of distribution of the central and peripheral compartments, respectively; and \( C \) and \( C_p \) are the drug concentrations of central and peripheral compartments, respectively.

The pharmacokinetic data with p.o. administration were simultaneously fitted using a two-compartment model with first-order absorption kinetics, which can be described by the following integrated equation:

\[ V_c \frac{dC}{dt} = k_o D_{p.o.} F_{p.o.} e^{-k_{stt}} - k_{10} V_c C - k_{12} V_c C + k_{21} V_p C_p \]  

where \( k_o \) is the absorption rate constant; \( D_{p.o.} \) is the oral dose; and \( F_{p.o.} \) is the oral bioavailability. The estimated pharmacokinetic variables were then used to link the pharmacokinetic model with the pharmacodynamic model to simulate the plasma concentration-time profiles.

The inhibition of tumoral phospho-BCR-ABL by dasatinib was used as the pharmacodynamic biomarker in pharmacodynamic modeling with an indirect sigmoid \( E_{\max } \) model. In the absence of drug, the rate of changes in the phospho-BCR-ABL level can be described by the following differential equation:

\[ \frac{dR_0}{dt} = k_{in} - k_{out} R_0 \]  

where \( R_0 \) is the baseline value of phospho-BCR-ABL; \( k_{in} \) is the zero-order rate constant for the formation of phospho-BCR-ABL; and \( k_{out} \) is the first-order rate constant of the dephosphorylation of phospho-BCR-ABL. In the absence of drug, the level of phospho-BCR-ABL was assumed to be at steady state, at which the rate of the dephosphorylation of BCR-ABL \((k_{out} R_0)\) is equal to the rate of the dephosphorylation of phospho-BCR-ABL \((k_{in} R_0)\):

\[ k_{in} = k_{out} R_0 \]  

In the presence of dasatinib, it was assumed that the reduction of phospho-BCR-ABL was primarily due to the inhibition by drug, instead of through the endogenous dephosphorylation process. The percentage of phospho-BCR-ABL relative to the baseline value \((R/R_0)\) in the presence of dasatinib was described by the following differential equation:

\[ \frac{d(R/R_0)}{dt} = \frac{k_{in}}{R_0} \left( 1 - \frac{E_{\max } C}{EC_{50} + C} \right) - k_{out} (R/R_0) \]  

where \( C \) is the plasma concentration of dasatinib after dosing; \( E_{\max } \) is the maximum inhibitory response and is assumed to be equal to 1 in the model; and \( EC_{50} \) is the drug concentration corresponding to 50% of the maximum inhibitory response.

Substituting Eq. 7 into Eq. 8 and assuming an \( E_{\max } \) of 1 yields the following:

\[ \frac{d(R/R_0)}{dt} = k_{out} \left( 1 - \frac{E_{\max } C}{EC_{50} + C} \right) - k_{out} (R/R_0) \]  

Eq. 1 was used to simultaneously fit the profiles of phospho-BCR-ABL versus time. Pharmacokinetic/pharmacodynamic model fitting was assessed by the minimization of the objective function, Akaike and Schwarz-Bayesian information criteria, visual inspection of the fitting, and residual plots.

Results

Pharmacokinetics of dasatinib in mice. The anti-leukemic efficacy of dasatinib has been extensively evaluated in severe combined immunodeficient mice bearing s.c. K562 xenografts at dose levels from 50 to 2.5 mg/kg/d (16). At the lowest dose tested (2.5 mg/kg/d), dasatinib substantially inhibited the growth of K562 tumors with both twice daily (BID) and once daily (QD) schedules. Therefore, 1.25 mg/kg/dose BID or 2.5 mg/kg/dose QD were considered to be the minimum efficacious doses.

To fully characterize the relationship between the pharmacokinetic and the pharmacodynamic biomarkers of dasatinib, a detailed pharmacokinetic study was conducted in mice given a single p.o. dose of 1.25, 2.5, or 5 mg/kg (Fig. 1A). The pharmacokinetic variables were derived and listed in Table 1. Following oral administration, dasatinib was rapidly absorbed with a T\(_{max}\) of 1 hour for all three oral doses, whereas the C\(_{max}\) and the AUC\(_{0-24\,\text{hours}}\) seemed dose dependent. The disposition of dasatinib was characterized in mice following a single i.v. administration at 5 mg/kg. Upon drug administration, the plasma level of dasatinib exhibited a biexponential decline, with a T\(_{1/2}\) of 0.79 hour (Fig. 1B). The CL was high, exceeding the hepatic blood flow. The V\(_{ss}\) was large, indicating a significant extravascular distribution for dasatinib in mice.

Pharmacodynamics of dasatinib in mice bearing K562 human CML xenografts. The pharmacodynamic of dasatinib was evaluated in mice bearing K562 tumors following a single oral dose of 1.25 or 2.5 mg/kg. Western blot analysis showed that tumoral phospho-BCR-ABL was inhibited at both doses, but the extent and duration of the inhibition seemed different (Fig. 2A). The inhibition of phospho-BCR-ABL was further quantitated by optical densitometry (Fig. 3). At both 1.25 and 2.5 mg/kg, the maximum inhibition of phospho-BCR-ABL was achieved at ~3 hours after dose, and the inhibition was completely reversed by 24 hours. However, it seemed that phospho-BCR-ABL reached the basal level more quickly at 1.25 mg/kg than at 2.5 mg/kg. In general, the time course of tumoral phospho-BCR-ABL inhibition and recovery seemed
dose dependent and directly correlated with the plasma levels of dasatinib at 1.25 and 2.5 mg/kg (Fig. 3).

CrkL is one of the most well characterized downstream substrates of the BCR-ABL kinase; therefore, it may serve as an important pharmacodynamic biomarker of the active BCR-ABL kinase in CML, as exemplified in the clinical development of imatinib (6, 7, 25, 26). Hence, in addition to phospho-BCR-ABL, we also determined tumoral phospho-CrkL and observed that phospho-CrkL levels were inhibited at both 1.25 and 2.5 mg/kg. This paralleled the inhibition of phospho-BCR-ABL (Fig. 2B). As with phospho-BCR-ABL, the time course of phospho-CrkL inhibition and recovery was dose dependent and correlated with the plasma levels of dasatinib. Collectively, our data showed that dasatinib is able to modulate the activity of the BCR-ABL kinase in tumors, and that tumoral phospho-BCR-ABL/phospho-CrkL are mechanistically relevant biomarkers to support the clinical development of dasatinib for a therapeutic indication in CML patients.

Estimation of the efficacious plasma drug concentration by pharmacokinetic/biomarker modeling. Because dasatinib exhibited a biexponential decay in plasma levels following i.v. administration, a two-compartment model with first-order absorption kinetics was applied in pharmacokinetic modeling of oral dosing. The pharmacokinetic model was simultaneously fitted to the plasma data of dasatinib for 5 mg/kg i.v. and 1.25, 2.5, and 5 mg/kg p.o. (Fig. 4A and B). The $k_a$ (mean ± SD) and $k_{10}$ (mean ± SD) were estimated to be 0.3 ± 0.06 and

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<td>51</td>
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Table 1. Summary of pharmacokinetic variables of dasatinib in mice

Abbreviations: IV, i.v.; PO, oral.

Fig. 1. Plasma pharmacokinetics of dasatinib in mice following a single administration. Mice, upon drug administration, were bled by cardiac puncture at indicated time points. The blood was immediately centrifuged, and the plasma was collected and analyzed by liquid chromatography/mass spectrometry. Point, mean for three observations; bars, SD. A, p.o. administration: 5 mg/kg (●), 2.5 mg/kg (○), 1.25 mg/kg (▲). B, i.v. administration: 5 mg/kg (●).

Fig. 2. Western blot analysis of tumoral phospho-BCR-ABL (A) and phospho-CrkL (B) in mice bearing K562 human CML xenografts following a single p.o. administration of dasatinib at 2.5 mg/kg (a) and 1.25 mg/kg (b). Upon drug administration, tumors were surgically removed at the indicated time points, immediately snap-frozen in liquid nitrogen, and stored at −80°C until analysis. Tumor lysate was prepared in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitor cocktails and was further resolved on 12% SDS-PAGE gels. Phospho-BCL-ABL was detected with a rabbit polyclonal anti-phospho-BCR-ABL antibody (pY412; 1:1,000 dilution). Phospho-CrkL was detected with a rabbit polyclonal anti-phospho-CrkL antibody (pY207; 1:1,000 dilution). IP, immunoprecipitate; IB, immunoblot.

(continued...)
6.89 ± 1.3 h⁻¹, respectively, and the mean $V_c$ (mean ± SD) was 2.0 ± 0.4 L/kg. The intercompartment distribution constants $k_{12}$ (mean ± SD) and $k_{21}$ (mean ± SD) were 4.4 ± 0.8 and 2.7 ± 0.5 h⁻¹, respectively. The inhibition of tumoral phospho-BCR-ABL by dasatinib was used as the pharmacodynamic biomarker response in pharmacodynamic modeling. When the percentage of phospho-BCR-ABL inhibition was plotted versus the plasma concentrations of dasatinib at 1.25 and 2.5 mg/kg, hysteresis was observed, suggesting that there was a delay between the plasma concentration of drug and the inhibition of phospho-BCR-ABL (data not shown). Therefore, an indirect inhibitory $E_{\text{max}}$ model was applied in pharmacodynamic modeling of phospho-BCR-ABL inhibition. The pharmacodynamic model combined with the defined pharmacokinetic model was simultaneously fitted to all of the pharmacodynamic data, to estimate the active plasma concentration of dasatinib (Fig. 4C). The $E_{C50}$ (mean ± SD; the plasma concentration required to inhibit 50% of tumoral phospho-BCR-ABL) was 6.5 ± 1.9 ng/mL; $k_{\text{out}}$ (mean ± SD; the rate constant of the de-phosphorylation of phospho-BCR-ABL) was estimated as 2.52 ± 0.14 s⁻¹; $n$ (mean ± SD; the sigmoidicity factor) was estimated as 4.15 ± 0.21. The $E_{C90}$ was further derived to be ~10.9 ng/mL, at which 90% of inhibition of tumoral phospho-BCR-ABL was assumed. The human $E_{C90}$ was estimated to be 14.6 ng/mL after correcting for differences in plasma protein binding between mice and humans.

**Discussion**

Recent progress in understanding of the molecular basis of cancer has redefined the landscape for cancer drug discovery, enabling the development of molecular targeted therapies. To maximize the potential for rational development of these...
therapies, decision-making should be closely coupled to the identification, validation, and implementation of mechanism-based biomarkers (24). The use of biomarkers to facilitate the development of novel anticancer agents now has clear precedents, as exemplified with the development of trastuzumab (Herceptin), gefitinib (Iressa), and imatinib (Gleevec; refs. 6, 27–31).

In phase I/II trials of novel molecularly targeted agents, it is essential to establish the active drug concentration in plasma required to effectively inhibit the therapeutic target. To facilitate the clinical development of dasatinib, we estimated the active drug concentration using the pharmacokinetic/pharmacodynamic modeling approach. This approach was based on our previous studies on cetuximab (Erbitux), in which the predicted active drug plasma concentrations from the preclinical data correlated well with the concentrations achieved in cancer patients who responded to cetuximab treatment (32). In the present study, we estimated an EC$_{90}$ (the concentration at which 90% of tumoral phospho-BCR-ABL is expected to be inhibited) of 10.9 and 14.6 ng/mL for mouse and human, respectively. The expectation is that if dasatinib achieved plasma levels $\geq$14.6 ng/mL in CML patients, the BCR-ABL kinase should be almost completely inhibited, and antileukemic activity should be expected.

Previous studies have shown that, in order for molecularly targeted agents to produce antitumor effects, it is essential to maintain the plasma drug concentration above a critical threshold required to effectively inhibit the target (32–34). To this end, we simulated the plasma profiles for both 1.25 mg/kg/dose BID and 2.5 mg/kg/dose QD and further compared the time plasma levels were above the EC$_{90}$ for mice (i.e., 10.9 ng/mL) for both regimens. The time above the EC$_{90}$ was $\approx$ 8 hours for 1.25 mg/kg/dose BID and 6 hours for the 2.5 mg/kg/dose QD regimen (Fig. 5A). For the 1.25 mg/kg/dose QD regimen, the time above the EC$_{90}$ was $\approx$ 3 hours. Because dasatinib did not meet antitumor activity criteria at 1.25 mg/kg/dose QD in the preclinical efficacy study (data not shown), the plasma level above the EC$_{90}$ probably needs to be maintained for $>3$ hours to induce significant inhibition of tumor growth, and the minimum time above the EC$_{90}$ should be 6 to 8 hours to achieve the clinical efficacy. Optimal clinical doses and schedules should be further justified in combination with clinical response, safety information, and pharmacokinetic/biomarker evaluation in the clinical setting.

When predicting clinically efficacious doses based on preclinical data, traditional allometric dose scaling may be problematic due to confounding interspecies factors as shown in our previous study of cetuximab (32). In contrast, the efficacious drug concentration of cetuximab predicted using biomarkers was within the range of concentrations achieved in cancer patients who achieved clinical responses (32). In the present study, the preclinical efficacious dose of 2.5 mg/kg/d, if simply scaled up based on the formula recommended by Freireich et al. (35) with an assumption of an average body weight of 70 kg, would be equivalent to a clinical dose of 25 mg/day. In phase I trials of dasatinib in CML patients few patients responded at lower doses; however, 70 mg BID or 140 mg QD seemed to be the optimal dose for most patients (36, 37). The dose of 25 mg/day is obviously well below the optimal dose of dasatinib observed in the clinic. In contrast, the estimated human EC$_{90}$ of 14.6 ng/mL is more predictive of the clinical efficacious regimen. Plasma concentrations of dasatinib in CML patients receiving the 70 mg BID or the 140 mg QD regimens are above the EC$_{90}$ of 14.6 ng/mL for $\approx$ 8 to 10 hours. This is slightly longer than the 6 to 8 hours minimum time requirement predicted from the preclinical model (Fig. 5B). These data suggest that optimal target inhibition is achieved on either a 140 mg QD or 70 mg BID regimen (36, 37).

Dasatinib overcomes multiple mechanisms of imatinib resistance, including BCR-ABL kinase overexpression, point...
mutations, and activation of SRC family kinases (16). Furthermore, dasatinib has comparable in vitro efficacy against imatinib-sensitive wild-type K562 and imatinib-resistant K562-R tumors (16). These data suggest that the active plasma concentration and the optimal time above the active plasma concentration may be similar for both imatinib-sensitive and imatinib-resistant CML tumors. This is very much relevant to the clinical development of this agent because the anti-leukemic activity of dasatinib is being explored in patients with imatinib-resistant or imatinib-intolerant disease. It is also important to note that the K562 cell line used to calculate the efficacious concentration of dasatinib in this study represents a blast crisis model of CML and has previously been shown to have a much higher dasatinib IC_{50} (0.5–1.0 nmol/L) compared with some patient-derived CML cell lines (IC_{50} <0.05 nmol/L; ref. 16). Therefore, the estimated efficacious concentration presented here may be greater than that required by some patients with CML, particularly in the first-line setting. However, because clinical experience with dasatinib to date has been in the second-line setting of pretreated patients with all phases of (mainly resistant) disease, the use of this in vitro model seems appropriate to guide the clinical study to achieve maximum responses of dasatinib in those patient populations.

In conclusion, the findings from the current study support a total daily dasatinib dose of 140 mg/d, given by either QD or BID dosing. Based on efficacy, biomarker response, and toxicity in phase I studies, the BID regimen was chosen to further evaluate the therapeutic potential of dasatinib in the phase II “START” (SRC/ABL Tyrosine Kinase Inhibition Activity: Research Trials of dasatinib) program. Results from this program have shown that this dosing regimen is indeed highly effective in overcoming resistance or intolerance to imatinib, inducing substantial cytogenetic and hematologic responses in all phases of CML and Ph+ ALL (19–22, 36, 37).

**References**

34. Cancer Therapy: Preclinical

**Cancer Therapy: Preclinical**

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Dasatinib (BMS-354825) Pharmacokinetics and Pharmacodynamic Biomarkers in Animal Models Predict Optimal Clinical Exposure

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