The Structure of Dasatinib (BMS-354825) Bound to Activated ABL Kinase Domain Elucidates Its Inhibitory Activity against Imatinib-Resistant ABL Mutants

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Abstract

Chronic myeloid leukemia (CML) is caused by the constitutively activated tyrosine kinase breakpoint cluster (BCR)-ABL. Current frontline therapy for CML is imatinib, an inhibitor of BCR-ABL. Although imatinib has a high rate of clinical success in early phase CML, treatment resistance is problematic, particularly in later stages of the disease, and is frequently mediated by mutations in BCR-ABL. Dasatinib (BMS-354825) is a multitargeted tyrosine kinase inhibitor that targets oncogenic pathways and is a more potent inhibitor than imatinib against wild-type BCR-ABL. It has also shown preclinical activity against all but one of the imatinib-resistant BCR-ABL mutants tested to date. Analysis of the crystal structure of dasatinib-bound ABL kinase suggests that the increased binding affinity of dasatinib over imatinib is at least partially due to its ability to recognize multiple states of BCR-ABL. The structure also provides an explanation for the activity of dasatinib against imatinib-resistant ABL mutants. (Cancer Res 2006; 66(11): 5790-7)

Introduction

The Philadelphia chromosome is a result of translocation between human chromosomes 9 and 22 and fuses the c-ABL gene with the breakpoint cluster (BCR) gene (1). The resulting BCR-ABL fusion protein is a constitutively active, oncogenic tyrosine kinase that causes cell transformation and chronic myeloid leukemia (CML; ref. 2). BCR-ABL is also responsible for malignant transformation in 15% to 30% of patients with acute lymphoblastic leukemia (ALL; ref. 3). Imatinib (STI-571, Gleevec; Fig. 1B) has been established as frontline treatment for CML (4, 5). It is a drug that exerts its effect by binding to the ABL kinase domain and inhibiting the tyrosine kinase activity of BCR-ABL. This results in a normalization of peripheral WBC counts and a substantial reduction of the Philadelphia chromosome-positive clone of stem cells in bone marrow, effectively offering hematologic and cytogenetic responses in the clinic. Although response rates to imatinib treatment are high in early (chronic phase) CML, responses in more advanced CML and ALL are only short lived (6-8). Furthermore, acquired resistance to imatinib represents a growing problem at all stages of CML, most often due to point mutations in BCR-ABL. Other mechanisms of resistance, both BCR-ABL dependent and BCR-ABL independent, such as overexpression of other tyrosine kinases, may also occur (9, 10).

Restoration of BCR-ABL-mediated signaling in the leukemic cells of patients with acquired imatinib resistance is due to point mutations in the ABL kinase domain in 70% to 90% of patients with CML (11). The structure of ABL kinase with imatinib [Protein Data Bank (PDB) ID 1IEP; ref. 12] reveals that these mutations occur either at residues directly implicated in imatinib binding or, more commonly, at residues important for the ability of the kinase to adopt a specific inactive conformation favorable to imatinib (11, 13). Dasatinib [N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide; ref. 14; Fig. 1A] is a novel, orally available, small-molecule multitargeted kinase inhibitor that potently inhibits BCR-ABL and SRC family kinases and is 325-fold more potent than imatinib against cells expressing wild-type BCR-ABL (15).

Dasatinib also potently inhibits imatinib-resistant BCR-ABL mutants. In a previous study, dasatinib was tested against 15 clinically relevant imatinib-resistant BCR-ABL mutants using cell-based biochemical and biological assays; 14 of these mutants were effectively inhibited in the low nanomolar range (16). Importantly, all imatinib-resistant BCR-ABL mutations located in the phosphate-binding loop (P-loop) of ABL kinase, which have been associated with poor prognosis (17), were successfully targeted by dasatinib. Additionally, dasatinib blocked the kinase activity of imatinib-resistant BCR-ABL mutants where the mutations reside in the activation loop and other sites in the COOH-terminal lobe. The only imatinib-resistant BCR-ABL mutant that was resistant to dasatinib in this in vitro study was the T315I mutant. In addition to cellular assays, dasatinib has shown in vivo efficacy, with no apparent toxicity, when given orally to severe combined immunodeficient mice with established leukemias derived from i.v. injection of Ba/F3 cells expressing wild-type BCR-ABL or the most commonly encountered imatinib-resistant BCR-ABL mutants.

The ability of dasatinib to effectively inhibit the proliferation of cells expressing nearly all imatinib-resistant mutants tested to date suggests that this compound has significant therapeutic potential in the setting of imatinib-resistant CML. Indeed, a phase I dose escalating study has shown promising results in imatinib-resistant and imatinib-intolerant patients with all phases of CML and with Philadelphia chromosome-positive ALL (18). These phase I data are supported by preliminary results of four phase II trials in the
72 hours after infection by centrifugation and frozen at −70°C. (His)_{6}-ABL kinase protein expression was estimated to be 5 mg/L of culture.

**Purification of ABL kinase domain and formulation with imatinib and dasatinib.** The ABL kinase domain was purified and combined with inhibitors as reported previously (12) with minor modifications. Frozen cell paste from baculovirus-infected Sf9 cells was thawed and resuspended in 10 volumes of lysis buffer [50 mmol/L Tris-HCl (pH 8), 20 mmol/L NaCl, 10% (v/v) glycerol, 10 mmol/L DTT, Complete EDTA-free protease inhibitor cocktail (Roche, Indianapolis, IN)]. Cells were lysed by cavitation after nitrogen pressurization to 450 p.s.i. for 30 minutes at 4°C. The lysate was clarified by sedimentation at 100,000 × g_{max} for 40 minutes at 4°C. The supernatant was loaded onto a Q-Sepharose Fast Flow column equilibrated with 50 mmol/L Tris-HCl (pH 8), 20 mmol/L NaCl, 10% (v/v) glycerol, and 10 mmol/L DTT. The column was washed and eluted with a linear gradient of 20 to 500 mmol/L NaCl in 50 mmol/L Tris-HCl (pH 8), 10% (v/v) glycerol, and 15 mmol/L β-mercaptoethanol. (His)_{6}-ABL kinase was eluted in the gradient with 250 mmol/L NaCl and loaded onto a Ni-NTA Superflow (Qiagen, Valencia, CA) column equilibrated with 20 mmol/L imidazole. The column was eluted with a linear gradient of imidazole to 750 mmol/L. (His)_{6}-ABL kinase containing fractions were pooled and incubated at 50 μmol/L concentration at 4°C for 18 hours with 1 unit recombinant TEV (rTEV) protease (Invitrogen, Carlsbad, CA) per 3 μg protein to remove the (His)_{6} affinity tag. A 3× molar excess (150 μmol/L) of either imatinib or dasatinib was added from 15 mmol/L DMSO stocks to the completed rTEV digest. The ABL kinase inhibitor complex was purified and exchanged into the final buffer by chromatography on a Superdex 75 column in 20 mmol/L Tris-HCl (pH 8), 100 mmol/L NaCl, and 3 mmol/L DTT. The final yield of purified ABL kinase protein was ~2.7 mg/L of culture. Purified ABL kinase inhibitor complexes were concentrated to 5 or 10 mg/mL with Amicon-ultra 10,000 molecular weight cut-off centrifugal ultrafilters (Millipore, Billerica, MA) before crystallization.

**Table 1. Data collection and refinement statistics**

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*Data were collected from one cryomounted crystal. No visible decay of the diffraction pattern was observed over the course of the experiment.
Cocrystallization of ABL inhibitor complexes. All crystallization trials were by hanging-drop, vapor-diffusion method at 4°C. Equal volumes of protein inhibitor and reservoir solutions were mixed to form 2 μL drops. Cocrystals of ABL-dasatinib and ABL-imatinib could be grown spontaneously based on reported conditions (12), but the initial crystals of neither complex produced useful diffraction. Attempts to improve crystal quality by using crushed ABL-dasatinib crystals as microseeds were unsuccessful. In contrast, larger, separated, and more regularly shaped crystals of the ABL-imatinib complex were produced by streak microseeding (using a cat whisker) crushed ABL-imatinib crystals into fresh drops of 5 mg/mL ABL-imatinib mixed with reservoir solution from the initial hit. Seed-derived, ABL-imatinib crystals were crushed and used as microseeds to nucleate the growth of ABL-dasatinib crystals using the same procedure; diffraction quality crystals appeared in 3 days. The optimal reservoir solution was 22% (w/v) polyethylene glycol (PEG) 3350, 0.2 mol/L MgSO4, and 0.1 mol/L MES (pH 6.5). Crystals were transferred by cryoloop into the cryoprotectant [30% (w/v) PEG 3350, 0.2 mol/L MgSO4, and 0.1 mol/L MES (pH 6.5)] for 15 seconds and then relooped and flash cooled in liquid nitrogen.

Structural determination and refinement. Data collection was done at beamline X25 (National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY), with the wavelength tuned to 1.1 Å and equipped with a nitrogen cryostream set to 100 K and an ADSC Quantum 315 detector positioned at 240 mm (ADSC, Poway, CA). Diffraction images were processed with Denzo and Scalepack from the HKL suite (HKL, Charlottesville, VA; ref. 25). Intensities were converted to structure factor amplitudes and placed on an absolute scale with Truncate (26) from the CCP4 suite (27). Initial phases were calculated by molecular replacement by AMoRe (28), with the dimer from 1FPU used as the search model. A solution was not found when one monomer from 1FPU was used as the search model presumably because the noncrystallographic 2-fold rotation axis, which is nearly parallel with the X axis at approximately z = 3/8 and y = 1/2, is closely aligned with one of the crystallographic 2-fold screw axes. Instead, the dimer from 1FPU was used, although, in hindsight, the dimer from 1M52 would have been the better choice based on structural homology.

Coordinates. The refined coordinates and structure factors were deposited in the PDB (31) as ID 2GQG.

Results

Structure of ABL-dasatinib complex. We report the X-ray crystal structure of the human ABL kinase domain (residues 225-512) complexed with dasatinib in the PDB (31) as ID 2GQG.

![Figure 2. Structure of dasatinib complex. A, overview of the three-dimensional structure of ABL kinase with dasatinib. Stick, dasatinib; green, carbon atoms. Blue, NH2-terminal lobe of ABL kinase; orange, P-loop; magenta, helix α-C; red, hinge region; pink, COOH-terminal lobe; green, catalytic loop; yellow, activation loop. B, cut-away detailed view of dasatinib and nearby residues in the ATP-binding site. Green and blue, inhibitor molecules found in the two asymmetric units; gray, one protein structure with surface of residues displayed. Dashed lines, hydrogen bonds.](image-url)
helix called helix α-C. The COOH-terminal lobe is larger than the NH2-terminal lobe and is predominantly helical. The two lobes are connected through a single polypeptide strand (the linker/hinge region) that acts as a hinge about which the two domains can rotate with respect to one another on binding of ATP and/or substrate. The ATP-binding site is a deep cleft between the two lobes and sits beneath a highly flexible P-loop connecting strands β1 and β2. The P-loop contains a conserved glycine-rich sequence motif (GXXGxG), where x, usually tyrosine or phenylalanine, caps the site of phosphate transfer. In ABL kinase, this residue is Tyr253. The structure reveals that the P-loop is partially disordered, as indicated by high B-factors and broken electron density.

Dasatinib sits in the ATP site enveloped by the two lobes, with the aminothiazole moiety of the molecule occupying the site normally bound by the adenine group of ATP (Fig. 2B). The 2-chloro-6-methyl phenyl ring of dasatinib is orthogonal to the thiazole carbamido group and probes into a mostly hydrophobic pocket near Thr315 that is not occupied by ATP. The piperazine 2-chloro-6-methyl phenyl ring of dasatinib is orthogonal to the similar conformations to activated LCK (Fig. 3). Interestingly, despite the differences in phosphorylation state, the position and conformation of Tyr393 is similar between the two ABL molecules. The phosphorylated Tyr393 makes a hydrogen bond with the side chain of Arg386, whereas, in the unphosphorylated molecule, Tyr393 is not involved in hydrogen bonding. Instead, the unphosphorylated Tyr393 stacks with the hydrocarbon portion of the side chain of Arg386 which makes a hydrogen bond via its guanidinium group with the backbone carbonyl of Ser385.

Because the conformations are similar between the two ABL molecules in the unit cell, the following discussions will refer only to the phosphorylated molecule. The kinase-conserved “DFG” motif, Asp363-Phe362-Gly363 (ABL numbering), which marks the NH2-terminal portion of the activation loop, is seen to adopt the same conformation as that found in the activated LCK structure. Optimal phosphate transfer requires the precise spatial arrangement of several catalytic residues that are conserved among all tyrosine kinases, including Asp363 and Asn368, which emanate from the catalytic loop. Asp363, the catalytic base, interacts with the

![Figure 3. Comparison of dasatinib complex with an activated kinase.](image-url)
attacking hydroxyl group of the substrate, whereas Asn\textsuperscript{368} engages in hydrogen bonding interactions that orient Asp\textsuperscript{363}. Asp\textsuperscript{361} of the DFG motif and Asn\textsuperscript{368} are also required for the binding of two divalent metal cations involved in coordination of the triphosphate group. All of these residues in the ABL-dasatinib structure superimpose well with the activated LCK structure. It is also observed that a conserved Lys–Glu (Lys\textsuperscript{271}–Glu\textsuperscript{286}) salt bridge, important for maintaining an active kinase conformation and orienting the lysine side chain for interaction with the ATP phosphates, is present and similarly arranged as in the LCK structure. Collectively, the above observations indicate that dasatinib can bind to the activated form of ABL kinase.

Structural comparison with other bound forms of ABL. To provide a structural explanation for the greater potency of dasatinib versus imatinib against BCR-ABL, structural comparisons were made between ABL-dasatinib and other bound forms of the enzyme. First, a structural comparison of the ABL-dasatinib complex was made with the imatinib-bound form of ABL (PDB ID 1IEP; ref. 12). Imatinib has been shown previously to bind to BCR-ABL only when the enzyme is in its inactive conformation (12, 23). The most striking difference between the two ABL structures is the conformation of the activation loop (Fig. 4). The activation loop in the imatinib-bound form folds back toward the ATP-binding site, forming interactions with both the P-loop and the inhibitor, thus resulting in an inhibitory kinase conformation. The differences in the activation loop conformation between the two forms of ABL begin at the DFG motif. Phe\textsuperscript{382} of the DFG motif in the imatinib-bound position points toward the ATP-binding site, whereas Tyr\textsuperscript{253} is not seen to interact with the activation loop conformation. The activation loop in the dasatinib-bound form is more extended, although the electron density was weak here. The Lys–Glu salt bridge is still present, and the Asp–Glu salt bridge is also maintained. The activation loop of the ABL-dasatinib structure is approximately 5 Å further out toward solvent-exposed protein compared with the imatinib-bound form. The orientation of the Tyr\textsuperscript{253} of the activation loop is also different, with the hydrophobic pocket occupied by Phe\textsuperscript{382}, as does the benzamidine group of dasatinib. The latter observation shows why the activation loop is able to exist in the active conformation in the presence of bound dasatinib but not in the presence of bound imatinib (Fig. 4). The P-loop bends toward imatinib to form close interactions with the kinase inhibitor, including an edge-to-face aromatic interaction between Tyr\textsuperscript{253} and the pyrimidine and pyridine rings. Compared with the imatinib-bound form, the P-loop in the dasatinib-bound structure is more extended, although the electron density was weak for this part of the protein. The hydroxyl moiety of Tyr\textsuperscript{253} seems to be important for maintaining an active kinase conformation and orienting the lysine side chain for interaction with the ATP phosphates, as is indicated by the electron density in this region.

Figure 4. A comparison of dasatinib complex with imatinib complex. A ribbon representation of the ABL-dasatinib complex (protein and dasatinib carbons, green) overlayed with the corresponding complex of ABL-imatinib (protein and imatinib carbons, purple). The activation loops are labeled. Note the diverging direction of the activation loop in the two structures. Phe\textsuperscript{382} of the DFG motif (activation loop) in a dot surface representation for each complex to show that dasatinib would not be able to bind to the active conformation of ABL because of a clash with Phe\textsuperscript{382} (as well as other activation loop residues) as found in the dasatinib-bound ABL conformation. Dasatinib, on the other hand, would be able to bind to the imatinib-bound activation loop conformation.

Based on modeling of dasatinib in the imatinib-bound and the PD173955-bound forms of ABL, there do not seem to be any major steric clashes that would preclude dasatinib from also binding to the inactive conformations found in these other ABL structures. However, a few unfavorable intermolecular contacts would exist with a small portion of the P-loop in each of the other ABL structures. This is most likely because the P-loop contours to allow Tyr\textsuperscript{253} to interact with imatinib and PD173955 (and also with Phe\textsuperscript{382} of the activation loop), whereas Tyr\textsuperscript{253} is not seen to interact with dasatinib in our structure. The P-loop is very flexible, and so with minor movement of the P-loop away from the PD173955-bound and imatinib-bound positions, it seems that dasatinib can also bind to these forms. Because the electron density of the P-loop of our structure is not very definitive, one would speculate that the P-loop is mobile even when dasatinib is bound, indicating that, for the most part, it is not forming a critical interaction. The lack of P-loop interactions by dasatinib may actually be advantageous because several imatinib-resistant ABL mutations appear in the P-loop. The above observations suggest that the increased wild-type ABL binding affinity of dasatinib over imatinib is at least partially due to the apparent ability of dasatinib to recognize multiple states of the enzyme.

Structural basis for activity against imatinib-resistant ABL mutations. The activity of dasatinib was previously assessed against 15 clinically relevant imatinib-resistant BCR-ABL mutants covering 12 different amino acid locations (M244V, G250E, Q252H/R, Y253F/H, E255K/V, T315I, F317L, M351T, E355G, F359V, H396R, and F486S; ref. 16). Several imatinib-resistant mutation sites are in
contact with imatinib, whereas others are speculated to be involved in stabilization of the specific inactive imatinib-bound conformation of ABL. Only the T315I mutant was clearly resistant to dasatinib, retaining kinase activity even in the presence of micromolar concentrations of the compound. The only other mutant showing any appreciable decrease in sensitivity was the F317L mutant, which required 3- to 5-fold higher concentrations of dasatinib to inhibit the growth of Ba/F3 cells. Interestingly, the Q252R mutant was consistently more sensitive to dasatinib compared with wild-type BCR-ABL.

The activity profile of dasatinib against the 15 imatinib-resistant BCR-ABL mutants is, for the most part, remarkably consistent with insight gained from an examination of the amino acid locations of the mutants in the crystal structure of ABL kinase with dasatinib (Fig. 5). Dasatinib is involved in a hydrogen bond with the side chain of T315, and the side chain methyl group of this amino acid residue is involved in van der Waals contact with the 2-chloro-6-methyl phenyl ring. As such, T315 is a critical contact residue for dasatinib. The resistance of the T315I mutant is most likely due to the loss of a phenyl ring. As such, T315 is a critical contact residue for dasatinib.

The difference in P-loop conformation between the dasatinib- and imatinib-bound forms of ABL can help explain the difference in activity between the two inhibitors against the M244V, G250E, Q252H/R, Y253F/H, and E255K/V mutants. A G250E mutation might be expected to reduce flexibility of the P-loop, thereby not allowing the P-loop to adopt the imatinib-induced conformation. The improved activity of dasatinib against the G250E mutant may result from the fact that P-loop interactions seem less important for dasatinib. Q252 points into solvent in the dasatinib-bound form of ABL, explaining why the Q252H/R mutants are not resistant to dasatinib, but it is unclear from the structure why the Q252R mutant would be more sensitive to dasatinib compared with wild-type BCR-ABL. In the imatinib-bound form, however, the side chain of Q252 contributes to the distorted P-loop conformation by packing up against Y253, which in turn interacts with imatinib and hydrogen bonds with N322. Y253 does not interact with dasatinib, but the hydroxyl of Y253 is seen to hydrogen bond with the backbone of R367, an interaction that must not be critical due to the maintenance of dasatinib activity against the Y253F mutant. E255 hydrogen bonds to its own backbone in the dasatinib-bound form, whereas, in the imatinib-bound form, it hydrogen bonds with the hydroxyl of Y257, stabilizing the imatinib-bound conformation of the P-loop. M244 is proximal to, and makes contacts with, the P-loop in both the dasatinib-bound and the imatinib-bound forms of ABL. Changes in the identity of this residue most likely result in changes in its interactions with the P-loop, potentially destabilizing the specific inactive ABL conformation that is a prerequisite for imatinib binding.

The conformation of H396, which resides in the activation loop, is slightly different between the two ABL molecules in the asymmetric unit cell of the dasatinib-bound form. In either molecule, there are no discernable interactions of this residue with protein or inhibitor, which may explain why the H396R mutation does not affect dasatinib activity. H396 in the imatinib-bound form, however, is involved in a hydrogen bond that stabilizes the inactive conformation of the activation loop. The position and conformation of the other COOH-terminal lobe mutation site residues, M351, E355, F359, and F486, are similar between the dasatinib- and imatinib-bound forms of ABL. However, it is postulated that these residues, which neighbor the activation loop, are critical for maintaining the particular inactive imatinib-bound conformation. F359 is also involved in van der Waals contacts with both imatinib and the activation loop in the imatinib-bound form. Dasatinib does not reside near this residue, explaining its potency against BCR-ABL with mutations at this site.

Discussion

The results of this study provide the first view of the catalytic domain of wild-type ABL kinase in an active conformation, wherein the entire catalytic machinery is in the proper orientation and Tyr993 is phosphorylated. Our structure also provides insight into the molecular mechanism of inhibition of ABL kinase by dasatinib, which binds in the ATP-binding site and presumably displaces ATP. The observation that ABL can adopt the same active conformation independent of activation loop phosphorylation at Tyr993 may indicate that this conformation is inherently stable. The fact that the catalytic activity of the isolated ABL kinase domain, in the absence of regulatory domains, is not increased significantly by phosphorylation in the activation loop supports this notion (23).

Based on modeling of dasatinib in other conformations of ABL, we suggest that the greater ABL binding affinity of dasatinib over imatinib is at least partially due to the apparent ability of dasatinib to recognize multiple states of the enzyme.

Our X-ray analysis also sheds light on the favorable activity of dasatinib against clinically relevant imatinib-resistant BCR-ABL mutants (16). Several imatinib-resistant BCR-ABL mutants occur in the P-loop, which adopts a distorted conformation in the imatinib-bound form of ABL to make critical contacts with the inhibitor and...
the activation loop. Based on our structure, P-loop interactions do not seem to be as important for dasatinib binding to ABL. Several residues at the other mutation sites are speculated to be also involved in stabilization of the specific inactive imatinib-bound conformation of ABL. The observation that ABL conformational requirements are less stringent for dasatinib than for imatinib and that dasatinib is not involved in critical interactions with many of the mutated residues further explains the positive activity profile of dasatinib. The structure also reveals that a critical activation is made directly between dasatinib and Thr315 of the hinge region and explains the loss of activity against the T315I mutant.

This structure provides further experimental evidence that the activation loop of a kinase can, in general, adopt multiple conformations (35–37). Phosphorylation of the activation loop is believed to stabilize the extended or active conformation and/or destabilize the folded or inactive conformation(s), of which there could be many. If the etiology of many cancers involves the aberrant activation of kinases, then one may postulate that inhibiting the active kinase conformation may produce a more favorable outcome than inhibition that is dependent on an inactive kinase conformation. More evidence is needed to determine whether inhibiting multiple forms, only the activated form or only a particular inactive conformation of ABL, will result in superior inhibition, in vitro potency, and/or specificity. Some native kinases, such as KIT, exist in an autoinhibitory state due to the activation loop insertion into the kinase active site, thus disrupting formation of the activated structure (38). Interestingly, imatinib, a potent KIT inhibitor and now the standard frontline therapy for advanced gastrointestinal stromal tumors (39, 40) binds to an inactive conformation of KIT (38). However, most KIT activation loop mutations are resistant to clinically achievable doses of imatinib (41, 42), which can be explained by the findings that KIT activation loop mutations not only activate kinase activity but also stabilize the activation loop in a conformation that does not allow productive imatinib binding (38, 43). Dasatinib has recently been shown to be a potent inhibitor of wild-type KIT and imatinib-resistant KIT activation loop mutants (44), providing another example where an inhibitor that requires less stringent conformational requirements retains activity and where mutations that affect specific conformations may consequently alter the potency of inhibitors targeting these specific states. Dasatinib should contribute valuable information as it progresses through the clinic on the effectiveness of a drug not limited to the binding of one particular state.

Preclinical and clinical data obtained to date suggest that, for human leukemia that is driven by BCR-ABL, the apparent ability of dasatinib to bind to both the active and the inactive conformations of BCR-ABL affords this agent greater therapeutic potential compared with an agent, such as imatinib, which binds only to an inactive form of the enzyme. Phase I and II clinical experience has shown promising results for dasatinib in patients with imatinib-resistant and imatinib-intolerant disease (18–22). The lack of cross-resistance between dasatinib and imatinib, as evidenced by dasatinib retaining inhibitory activity against multiple imatinib-resistant forms of BCR-ABL in vitro, may result in dasatinib producing greater durability of response. Further follow-up of the phase II trials is awaited with interest.

In conclusion, the current study reveals that the potency and favorable profile of dasatinib against wild-type ABL and imatinib-resistant ABL mutants is at least partially due to its ability to recognize multiple states of BCR-ABL. Our findings highlight the importance of considering kinase enzyme conformation in the rational design of kinase inhibitors for cancer targets.

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