Dasatinib (BMS-354825), a Dual SRC/ABL Kinase Inhibitor, Inhibits the Kinase Activity of Wild-Type, Juxtamembrane, and Activation Loop Mutant KIT Isoforms Associated with Human Malignancies

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Abstract

Activating mutations of the activation loop of KIT are associated with certain human neoplasms, including the majority of patients with systemic mast cell disorders, as well as cases of seminoma, acute myelogenous leukemia (AML), and gastrointestinal stromal tumors (GISTs). The small-molecule tyrosine kinase inhibitor imatinib mesylate is a potent inhibitor of wild-type (WT) KIT and certain mutant KIT isoforms and has become the standard of care for treating patients with metastatic GIST. However, KIT activation loop mutations involving codon D816 that are typically found in AML, systemic mastocytosis, and seminoma are insensitive to imatinib mesylate (IC50 > 5-10 μmol/L), and acquired KIT activation loop mutations can be associated with imatinib mesylate resistance in GIST. Dasatinib (formerly BMS-354825) is a small-molecule, ATP-competitive inhibitor of SRC and ABL tyrosine kinases with potency in the low nanomolar range. Some small-molecule SRC/ABL inhibitors also have potency against WT KIT kinase. Therefore, we hypothesized that dasatinib might inhibit the kinase activity of both WT and mutant KIT isoforms. We report herein that dasatinib potently inhibits WT KIT and juxtamembrane domain mutant KIT autophosphorylation and KIT-dependent activation of downstream pathways important for cell viability and cell survival, such as Ras/mitogen-activated protein kinase, phosphoinositide 3-kinase/Akt, and Janus-activated kinase/signal transducers and activators of transcription. Furthermore, dasatinib is a potent inhibitor of imatinib-resistant KIT activation loop mutants and induces apoptosis in mast cell and leukemic cell lines expressing these mutations (potency against KIT D816Y > D816F > D816V). Our studies suggest that dasatinib may have clinical efficacy against human neoplasms that are associated with gain-of-function KIT mutations. (Cancer Res 2006; 66(1): 473-81)

Introduction

Gain-of-function mutations of the KIT receptor tyrosine kinase play an important role in oncogenesis of certain human malignancies, including the vast majority of gastrointestinal stromal tumors (GISTs; refs. 1, 2) and a subset of hematologic neoplasms (3–7) and germ cell tumors (8, 9). KIT is a class III receptor tyrosine kinase and is structurally characterized by an extracellular domain with five immunoglobulin-like repeats, a single transmembrane domain, a juxtamembrane domain, and a cytoplasmic tyrosine kinase domain. The kinase domain consists of the NH2-terminal (TK1) and COOH-terminal (TK2) lobes that are separated by a hydrophilic kinase insert. The TK2 domain contains the kinase activation loop, a critical hinged region of the kinase that must assume a particular conformation to allow full kinase activation (10).

Imatinib mesylate is a potent KIT tyrosine kinase inhibitor (11) and is now the standard frontline therapy for advanced GISTs (2, 12). Although imatinib is a potent inhibitor of the kinase activity of wild-type (WT) KIT and GIST-associated juxtamembrane domain mutant KIT isoforms, most KIT activation loop mutations are resistant to clinically achievable doses of imatinib (2, 11–16). Imatinib only binds to the inactive conformation of KIT; however, 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 (10, 17, 18). Activating KIT activation loop mutations are found in association with acute myelogenous leukemia (AML; ref. 5), mast cell disease (in particular systemic mastocytosis; refs. 4, 13), a subset of sinonasal natural killer/T-cell non-Hodgkin lymphoma (6, 7), seminoma/dysgerminoma (8, 9), and imatinib-resistant GIST (2, 12).

Dasatinib, formerly known as BMS-354825, is an ATP-competitive, dual SRC/ABL inhibitor (19). Notably, dasatinib can inhibit BCR-ABL activation loop mutations that are found in some chronic myelogenous leukemia (CML) patients with acquired clinical resistance to imatinib (20). Some small-molecule SRC/ABL inhibitors also have potency against KIT kinase (11, 14, 21, 22). Therefore, we hypothesized that dasatinib might inhibit the kinase activity of both WT and mutant KIT isoforms.

We report herein that dasatinib potently inhibits WT KIT and juxtamembrane domain mutant KIT autophosphorylation and KIT-dependent activation of downstream pathways important for cell viability and cell survival, such as Ras/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/Akt, and Janus-activated kinase/signal transducers and activators of transcription (STAT). Furthermore, we show that dasatinib is a potent inhibitor of imatinib-resistant KIT activation loop mutants and induces apoptosis in mast cell and leukemic cell lines expressing these mutations.
Materials and Methods

Cell lines. The WT FLT3 Ba/F3 cell line, a murine interleukin 3 (IL-3)-dependent hematopoietic pro-B cell line, the Chinese hamster ovary cell line Chinese hamster ovary-K1, and the murine p815 mast cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The murine KIT D814Y mutant isoform expressed by the p815 cell line (23) is homologous to the human KIT D816Y mutation. The human hematopoietic growth factor–dependent M07e cell line was obtained from Dr. Hal Broxmeyer (Department of Microbiology and Immunology, Walthter Oncology Center, Indiana University School of Medicine, Indianapolis, IN). The human HMC-1.1 mast cell line expressing a KIT juxtamembrane domain mutant isoform (V560G) was kindly provided by Dr. Axel Muller (Division of Allergic Diseases, Department of Internal Medicine, Mayo Clinic, Rochester, MN). A spontaneously occurring subclone of the HMC-1.1 cell line, HMC-1.2 (24, 25), which has an additional mutation in the activation loop (D816V), was kindly provided by Dr. Akin (Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD). All cell lines were grown in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone, South Logan, UT), 1% penicillin G (10,000 units/mL), and streptomycin (10,000 μg/mL), 2 mmol/L L-glutamine (both Life Technologies-Invitrogen, Carlsbad, CA), 1% FCS containing supernatant (10%) from WEHI-3 cells (ATCC) was added to the growth medium for the parental Ba/F3 cell line. M07e cells were cultured using recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) as a growth supplement as previously described (11).

Site-directed mutagenesis and generation of a Ba/F3 cell line expressing mutant KIT. KIT cDNA was generously provided by Dr. Axel Muller (Department of Molecular Biology, Max Planck Institute for Biochemistry, Martinsried, Munich, Germany) and cloned into the pLXSN retroviral vector plasmid (BD Biosciences, San Diego, CA), the pCDNA3.1 vector plasmid, or the M5gNeo plasmid (BD, Heidelberg, Germany; ref. 11).

Antibodies and reagents. An anti-KIT rabbit polyclonal antibody, an anti-STAT3 mouse monoclonal antibody (both Santa Cruz Biotechnology, Santa Cruz, CA), an anti-AKT (polyclonal) rabbit antibody (Cell Signaling Technology, Beverly MA), and an anti-MAPK1/2 [extracellular signal-regulated kinase (ERK1/2)] rabbit monoclonal antibody (Upstate Biotechnology, Lake Placid, NY) were used at a 1:5,000 to 1:1,000 dilution.

Results

Dasatinib inhibits the kinase activity of WT and juxtamembrane domain mutant KIT isoforms. Dasatinib is a potential dual SRC/ABL kinase inhibitor that is currently in phase I/II clinical studies of CML and solid tumors. Based on structural homology considerations and prior descriptions of the activity of some SRC and/or ABL inhibitors against KIT (11, 14, 21, 22), we hypothesized that dasatinib might also inhibit KIT kinase activity. Indeed, dasatinib potently inhibited the ligand-dependent autophosphorylation of WT KIT kinase in the cytokine-dependent human myeloid leukemia cell line M07e with an IC50 of 1 to 10 nmol/L. Dasatinib also inhibited stem cell factor (SCF)–dependent proliferation of these cells with a similar IC50 (5-10 nmol/L). In comparison, the IC50 values for imatinib inhibition of autophosphorylation and proliferation were 50 to 100 nmol/L, respectively (Fig. 1A-B; ref. 31). Dasatinib had little effect on the GM-CSF-dependent proliferation of these cells (IC50 > 10,000 nmol/L), suggesting that the effect of dasatinib on SCF-dependent proliferation was due to its inhibition of KIT kinase rather than direct effects on downstream kinases (e.g., SRC family members) that might be common to both the KIT and GM-CSF receptors (Fig. 1B).

Gain-of-function mutations involving the KIT juxtamembrane domain occur in some cases of mast cell disease (24) and AML (5). In addition, KIT juxtamembrane domain mutations are found in approximately two thirds of GISTs, and this GIST subset has the best clinical response to imatinib (11, 12, 32). We tested the activity of dasatinib against KIT juxtamembrane domain mutations using the HMC-1.1 cell line, which is a spontaneously immortalized human mast cell leukemia cell line that expresses the KIT V560G mutant isoform (24). This particular mutation is one of the most common juxtamembrane domain point mutations found in GISTs (2). Dasatinib inhibited the kinase activity of KIT V560G in a dose-dependent manner with an IC50 of ~10 nmol/L, which is nearly identical to the previously reported results for imatinib (Fig. 2; refs. 11, 16). Imatinib potently inhibited cellular proliferation and induced apoptosis of this cell line in the low nanomolar range (Fig. 2; ref. 11). Therefore, we tested whether dasatinib had similar biological effects. Both dasatinib and imatinib inhibited cellular proliferation of HMC-1.1 cells with an IC50 of 5 to 10 nmol/L (Fig. 2B). As shown in Fig. 2C, dasatinib induced apoptosis of HMC-1.1 cells with an IC50 of 14 nmol/L, whereas the IC50 for imatinib was ~70 nmol/L. These data indicate that in HMC-1.1 cells, KIT kinase activation is required for cellular proliferation and/or ABL inhibitors against KIT (11, 14, 21, 22), we hypothesized that dasatinib might also inhibit KIT kinase activity. Indeed, dasatinib potently inhibited the ligand-dependent autophosphorylation of WT KIT kinase in the cytokine-dependent human myeloid leukemia cell line M07e with an IC50 of 1 to 10 nmol/L. Dasatinib also inhibited stem cell factor (SCF)–dependent proliferation of these cells with a similar IC50 (5-10 nmol/L). In comparison, the IC50 values for imatinib inhibition of autophosphorylation and proliferation were 50 to 100 nmol/L, respectively (Fig. 1A-B; ref. 31). Dasatinib had little effect on the GM-CSF-dependent proliferation of these cells (IC50 > 10,000 nmol/L), suggesting that the effect of dasatinib on SCF-dependent proliferation was due to its inhibition of KIT kinase rather than direct effects on downstream kinases (e.g., SRC family members) that might be common to both the KIT and GM-CSF receptors (Fig. 1B).

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and survival and dasatinib is at least as potent as imatinib for inhibiting the KIT V560G juxtamembrane domain mutation. Dasatinib inhibits the kinase activity of imatinib-resistant KIT activation loop mutations found in hematologic malignancies. Although imatinib potently inhibits the kinase activity of WT and juxtamembrane domain mutant KIT isoforms, it has minimal activity against KIT D816Y, D816F, or D816V mutant kinases (13). The inability of imatinib to inhibit these mutant isoforms is due to steric clash between imatinib and the “open” (or active) conformation of the KIT activation loop (19, 20). The predicted structural model of dasatinib binding to ABL suggests that changes in activation loop conformation might not significantly affect drug binding (19). We hypothesized that dasatinib would also be less sensitive to the KIT activation loop conformational changes than imatinib and, therefore, dasatinib would more potently inhibit KIT activation loop mutations involving codon 816. To test this hypothesis, we used a spontaneously generated subclone of the HMC-1.1 cell line HMC-1.2, which acquired the typical mastocytosis-associated D816V mutation on the same allele as the original V560G mutation (24, 25). KIT is constitutively autophosphorylated in this cell line but is resistant to treatment with clinically relevant doses of imatinib (13). Consistent with previous studies, the IC50 of imatinib for cellular proliferation of this cell line was >10,000 nmol/L (Fig. 3A-B; refs. 13, 24).

In contrast, dasatinib inhibited the kinase activity of KIT V560G/D816V in a dose-dependent manner with an IC50 of 5 to 10 nmol/L but dasatinib doses of 1,000 nmol/L had no significant effect on the GM-CSF-stimulated growth of these cells (IC50 > 10,000 nmol/L). The results from a single experiment are shown. A total of three experiments were done. Points, mean of three replicates; bars, SD. The dose-effect plots show the computed IC50 for the experimental results in (B, columns).

Figure 1. Dasatinib potently inhibits kinase activity of the WT KIT cell line M-07e. A, M-07e cells were treated with varying concentrations of dasatinib or imatinib for 90 minutes before preparation of cellular lysates. Immunoblotting for phosphorylated (PY20 antibody) and total forms of KIT were done to evaluate the inhibitory effect of dasatinib or imatinib on KIT activation (autophosphorylation). KIT autophosphorylation in these cells is SCF dependent and both dasatinib or imatinib inhibit phosphorylation of KIT with IC50 values of 1 to 10 nmol/L (dasatinib) and 100 to 1,000 nmol/L (imatinib), respectively. B, M-07e cells were treated with dasatinib or SCF (100 ng/mL) or GM-CSF (100 ng/mL) for 72 hours and cellular proliferation was measured using an XTT-based assay. Dasatinib inhibited the proliferation of SCF-stimulated M-07e cells with an IC50 of 5 to 10 nmol/L but dasatinib doses of 1,000 nmol/L had no significant effect on the GM-CSF-stimulated growth of these cells (IC50 > 10,000 nmol/L). The results from a single experiment are shown. A total of three experiments were done. Points, mean of three replicates; bars, SD. The dose-effect plots show the computed IC50 for the experimental results in (B, columns).
cells was strongly correlated with inhibition of cellular proliferation and induction of apoptosis. Notably, high-dose imatinib (>1,000 nmol/L) inhibited KIT autophosphorylation. A dose of 1,200 nmol/L imatinib inhibited the proliferation of p815 cells by 30% but did not significantly induce programmed cell death.

Effects of different amino acid substitutions of KIT aspartic acid 816 (D816) on sensitivity to dasatinib. Our results with the HMC-1.2 and p815 cell lines suggested that dasatinib might have different potency against D816Y than against D816V mutations. Alternatively, these results could reflect differences in activity of dasatinib against human or murine KIT and/or differences in drug uptake by the different cell lines. To address this issue, we generated isogenic factor–independent Ba/F3 cell lines expressing systemic mastocytosis–associated codon 816 mutations with an interchange of aspartic acid to valine (D816V), tyrosine (D816Y), or phenylalanine (D816F). Dasatinib inhibited the autophosphorylation of human KIT D816V and D816F with an IC₅₀ of ~100 nmol/L. However, the IC₅₀ for inhibition of autophosphorylation of the KIT D816Y mutation was significantly lower (IC₅₀ 1-10 nmol/L; Fig. 4A).

In comparison, imatinib in doses of up to 10,000 nmol/L did not significantly inhibit KIT autophosphorylation in D816V and D816F cells. However, D816Y cells were moderately sensitive to imatinib therapy with doses of >1,000 nmol/L but <10,000 nmol/L completely inhibit KIT autophosphorylation (Fig. 4B). These findings are in agreement with our results for the murine p815 (D814Y) cell line shown in Fig. 3D-F.

Dasatinib is 1 log more potent against WT or juxtamembrane domain mutant KIT isoforms than activation loop mutant KIT isoforms. Our results suggested that dasatinib was less active against KIT activation loop mutant isoforms than against WT or juxtamembrane domain mutant KIT. However, we were unable to directly compare this in our isogenic Ba/F3 system, as we could not establish an IL-3-independent cell line expressing WT or juxtamembrane domain mutant KIT. Thus, we could not directly compare WT KIT to activation loop mutant KIT in the
same cellular context. Therefore, we did additional experiments in which we transiently transfected CHO-K1 cells with expression vectors encoding WT or mutant KIT isoforms. Transfected cells were treated with dasatinib and biochemically analyzed as described above. Consistent with our previous results, dasatinib inhibited the autophosphorylation of SCF-stimulated WT KIT (analogous to M-07eE) or juxtamembrane domain mutant KIT (analogous to the mutation in HMC-1.1) with an IC_{50} of 1 to 10 nmol/L, whereas the IC_{50} for inhibition of autophosphorylation of the KIT D816V and D816H mutations [reported in <5% of systemic mastocytosis (33) and 7% of seminoma cases (8)] was ~100 to 500 nmol/L (Fig. 4C). Some lane-to-lane variation in total KIT expression is apparent in Fig. 4; such differences are partially attributable to variations in efficiency of immunoprecipitation between experimental conditions and also to the previously described inverse association between kinase activation and protein degradation (i.e., KIT turnover is increased when the kinase is activated; ref. 34).

**Dasatinib inhibits KIT-dependent activation of downstream signaling pathways.** We studied the effects of inhibition of KIT kinase by dasatinib on the activation status of KIT-dependent downstream signaling pathways, including MAPK, AKT, and STAT3. Figure 5 shows representative Western blots for factor-independent Ba/F3 cells expressing human KIT D816V, D816F, or D816Y mutations. For comparison, we also analyzed the effects of dasatinib of activation of MAPK1/2, AKT, and STAT3 in HMC-1.1, HMC-1.2, and p815 cells. MAPK1/2 (ERK1/2), STAT3, and AKT are constitutively activated in these cells. The phosphorylation of STAT3 and MAPK1 was potently and completely inhibited in dasatinib-treated Ba/F3 D816V/Y/F cells with IC_{50} values that were similar to those required for inhibition of KIT autophosphorylation. STAT3 activation was potently but incompletely inhibited in dasatinib-treated p815 and HMC-1.2 cells. In contrast to the other cell lines, STAT3 activation was only minimally inhibited in dasatinib-treated HMC-1.1 cells. AKT activation was potently but incompletely inhibited in all tested cell lines using dasatinib doses of 10 to 1,000 nmol/L. Similarly, MAPK2 activation was also less potently inhibited than MAPK1 (Fig. 5). Notably, dasatinib-induced inhibition of proliferation and induction of apoptosis are strongly correlated with inhibiting the activation of KIT, MAPK (MAPK1 > MAPK2), and AKT. In contrast,

![Figure 3](https://www.aacrjournals.org/doi/10.1158/0008-5472.CAN-05-2897)

Figure 3. Dasatinib potently inhibits kinase activity of human KIT V560G/D816V and murine KIT D814Y isoforms. HMC 1.2 (human KIT V560G/D816V; A-C) or p815 (murine KIT D814Y; D-F) cells were treated with varying concentrations of dasatinib or imatinib as described above. The potency of the reagents was evaluated by sequentially immunoblotting for phosphorylated and total forms of KIT (A and D), an XTT-based assay to assess inhibition of cellular proliferation (B and E), and a flow cytometry-based assay of apoptosis induction (C and F). Representative experimental results from a total of three. Columns (B and E), mean of three replicates; bars, SD. The dose-effect plots (C and F) indicate the computed IC_{50} for the experimental results shown immediately to the left of the plot. A to C, HMC1.2: Dasatinib potently inhibits autophosphorylation of V560G/D816V KIT, whereas imatinib was inactive in the same dose range. However, HMC-1.2 cells need an ~1 log higher concentration of dasatinib to inhibit proliferation and induce apoptosis compared with the concentration required to inhibit KIT autophosphorylation. D to F, p815: Dasatinib potently inhibited autophosphorylation of KIT and consequently lead to inhibition of cellular proliferation and induction of apoptosis. In contrast, high-dose imatinib (1,000 nmol/L) partially inhibited the autophosphorylation of D814Y but had only a moderate inhibitory effect on cellular proliferation and did not induce apoptosis in the tested dose range.
STAT3 activation does not correlate with cellular proliferation and/or avoidance of apoptosis.

Effects of dasatinib on cellular proliferation and survival of isogenic cells expressing KIT D816 F/V/Y. Consistent with the results of our biochemical studies, dasatinib inhibited the proliferation of Ba/F3 KIT D816V and D816F cells with an IC₅₀ of 100 to 150 nmol/L, and Ba/F3 KIT D816Y cells with an IC₅₀ of 5 nmol/L. In contrast, imatinib had no significant inhibitory effect on the growth of these three cell lines (IC₅₀ > 10,000 nmol/L; Fig. 6A). Dasatinib also potently induced apoptosis of the Ba/F3 KIT D816V and D816F cell lines with calculated IC₅₀ values of 220 and 120 nmol/L, respectively. The IC₅₀ for induction of apoptosis of the Ba/F3 D816Y cell line was 20 nmol/L (Fig. 6B). Therefore, dasatinib was at least 1 log more potent against KIT D816Y than against KIT D816V/F. Addition of murine IL-3 (5 ng/mL) to Ba/F3 D816V cells prevented dasatinib-induced apoptosis [77% viable cells when cultured with dasatinib 1000 nmol/L + IL-3 versus 0.1% viable cells when cultured in 1,000 nmol/L. dasatinib and no IL-3 (data not shown)].

Discussion

Crystal structures of imatinib bound to the kinase domains of ABL or KIT indicate that imatinib binds to the ATP-binding site of these kinases only when the activation loop of the kinase is in the inactive or "closed" conformation (17, 18, 35–37). One mechanism of acquired resistance to imatinib in CML is the development of mutations of the BCR-ABL activation loop that stabilize the kinase in the active conformation, thus preventing imatinib binding (e.g., V379I, L387M, and H396R; ref. 38). Structural studies of the pyrido[2,3-d]pyrimidine class of dual SRC-ABL inhibitors show that these compounds also bind to the ATP-binding site in ABL but without regard for the position of the activation loop, which can be in either the active or inactive conformation (19, 21, 22, 35). Notably, these compounds can inhibit the kinase activity of certain imatinib-resistant BCR-ABL activation loop mutant isoforms. Based on these observations, Shah et al. (20) profiled the activity of dasatinib against a panel of cell lines expressing WT or imatinib-resistant BCR-ABL. Dasatinib inhibited the kinase activity of 14 of 15 imatinib-resistant BCR-ABL mutants, including all tested activation loop mutants. Therefore, dasatinib is predicted to bind to the ATP-binding site of BCR-ABL irrespective of the conformation of the activation loop (20).

Gain-of-function point mutations of the KIT activation loop are associated with certain human neoplasms, including systemic mast cell disorders (13, 33), AML (5), seminoma/dysgerminoma (8, 39), and GIST (both primary and imatinib-resistant GIST; refs. 12, 40). In the case of mast cell disorders, seminoma, and AML, the most frequent KIT mutation is the replacement of the normal aspartic acid residue at codon 816 of the activation loop with a valine residue (D816V). The D816V mutation results in constitutive activation of KIT kinase activity and is predicted to help stabilize the activation loop in the active conformation. In addition to D816V, other mutations involving codon 816 have been reported in systemic mast cell disorders (D816Y and D816F; refs. 13, 33).
AML (D816Y; refs. 5, 41), and/or seminomas (D816Y and D816H; refs. 8, 9, 42). Consistent with the structural model of imatinib binding to KIT, the kinase activity of all of these mutants is resistant to imatinib (2, 8, 13).

Based on previous reports of the activity of some small-molecule compounds against KIT activation loop mutations (21, 43) and specifically, of some SRC and/or ABL inhibitors against KIT (19), we hypothesized that dasatinib might also inhibit the kinase activity of KIT. In our studies, we found dasatinib to be a potent inhibitor of WT KIT with an IC₅₀ for inhibition of autophosphorylation and cellular proliferation of 5 to 10 nmol/L. In comparison, the IC₅₀ for inhibition of autophosphorylation and proliferation in these same cells by imatinib was 10- to 20-fold higher (~100 nmol/L; ref. 11).

The IC₅₀ for dasatinib inhibition of KIT autophosphorylation that we obtained using cell-based assays of full-length KIT is very similar to that reported by Lombardo et al. (19) using cell-free assays of kinase domain–only recombinant KIT enzyme.

Juxtamembrane domain mutations of KIT are commonly associated with human GISTs (summarized in ref. 12) and a minority of cases of systemic mastocytosis (33) and AML (5, 44). Dasatinib also potently inhibits KIT juxtamembrane domain mutations with an IC₅₀ of 1 to 10 nmol/L. Notably, dasatinib had similar potency to imatinib for inhibition of KIT autophosphorylation and cellular proliferation in a mast cell line expressing juxtamembrane domain mutant KIT (HMC-1.1) and was even more potent than imatinib for inducing apoptosis of this cell line.

Dasatinib is a much more potent inhibitor of KIT activation loop mutants than imatinib, with IC₅₀ values for inhibition of autophosphorylation of KIT D816 mutants in the range of 10 to 100 nmol/L. Interestingly, the potency of the dasatinib against KIT kinase is differentially influenced by various activation loop mutations. Notably, KIT D816Y is 10-fold more sensitive to dasatinib than KIT D816V/F. In addition, KIT D816F is 2-fold more sensitive to dasatinib compared with KIT D816V.

Our results suggest that the conformation of the KIT activation loop does influence dasatinib potency, perhaps due to secondary changes in the ATP-binding pocket that influence drug binding. Alternatively, the different activation loop mutations might have

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Table: Dasatinib-mediated inhibition of activation loop mutant KIT kinase activity blocks the activation of major downstream pathways.

<table>
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Figure 5. Dasatinib-mediated inhibition of activation loop mutant KIT kinase activity blocks the activation of major downstream pathways. Cell lines expressing KIT activation loop mutations were treated with varying concentrations of dasatinib for 90 minutes before isolation of cellular protein lysates. Representative experimental results from a total of three. Two hundred micrograms of protein lysate from each cell line were immunoblotted for phosphorylated (p-STAT3, p-AKT, and p-MAPK1/2) and total forms of STAT3, AKT, and MAPK1/2. Downstream pathways affecting phosphorylation of AKT, STAT3, and MAPKs were activated in all untreated cell lines. Treatment with dasatinib lead to a marked decrease in the concentration of activated forms of STAT3, AKT, and MAPK1/2.
In our studies, inhibition of KIT kinase in HMC-1.1 (human mastocytosis) and p815 (murine mastocytosis) resulted in inhibition of cellular proliferation and induction of apoptosis. This suggests that therapeutic inhibition of KIT kinase would be effective for human mastocytosis that is associated with KIT D816 mutations. There are other lines of evidence to support this hypothesis: (a) KIT kinase inhibition is developmentally required for mast cell formation (1, 3, 4); (b) imatinib-induced inhibition of an alternative oncogenic kinase (FIP1L1-PDGFRA) results in marked clinical responses in variant systemic mastocytosis associated with this genomic alteration (45); (c) inhibition of KIT D816V by the kinase inhibitor PKC412 resulted in hematologic and clinical improvement in a patient with mast cell leukemia (46).

It should be noted that the HMC-1.2 cell line (human mastocytosis with KIT V560G/D816V) was less sensitive to the antiproliferative effects of dasatinib. Dasatinib potently inhibited KIT in these cells but this seemed to be insufficient to inhibit cellular proliferation or induce apoptosis. We speculate that other secondary oncogenic events, which developed during the prolonged cell passaging that gave rise to this cell line, are responsible for the apparent “disconnect” between inhibiting KIT kinase and effects on cellular proliferation and survival.

Dasatinib is currently in phase I/II trials for CML. Based on the preliminary reports of these studies, it seems that this drug is safe, well tolerated, and efficacious in the setting of imatinib-resistant CML (47). The pharmacokinetic data from these trials indicate that drug levels required to inhibit KIT activation loop mutations shown in the present studies can be safely achieved in the systemic circulation of patients. Based on our studies, we predict that dasatinib would have biological and clinical activity against human diseases associated with KIT activation loop mutations, including systemic mastocytosis (33), AML (5, 44), CDDP-resistant/refractory (39), seminoma/dysgerminoma (8, 9), and imatinib-resistant GIST (12).

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Figure 6. Dasatinib inhibits cellular proliferation and induces apoptosis of Ba/F3 KIT D816V/F/Y cells in a dose-dependent manner. Ba/F3 KIT D816V/F/Y cells were treated with dasatinib, imatinib, or vehicle only for 48 to 72 hours before measuring cellular proliferation using an XTT-based assay (A), or apoptosis using an Annexin V-propidium iodide flow cytometry–based assay (B). Representative experiment results from a total of three. Columns (A), average of three replicates; bars, SD. The dose-effect plots indicate the computed IC_{50} for the experiments shown immediately to the left of the plot. A, dasatinib inhibited the proliferation of Ba/F3 KIT D816V/F/Y cells with IC_{50} values of 150 nmol/L (D816V), 100 nmol/L (D816F), and 5 nmol/L (D816Y), respectively. In contrast, imatinib had little or no antiproliferative effects using doses of up to 10,000 nmol/L. B, dasatinib potently induced apoptosis in Ba/F3 KIT D816V/F/Y cells with IC_{50} values of 220 nmol/L (D816V), 120 nmol/L (D816F), and 15 nmol/L (D816Y), respectively. In contrast, 1,000 nmol/L imatinib did not induce apoptosis of any of these cell lines.
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Dasatinib (BMS-354825), a Dual SRC/ABL Kinase Inhibitor, Inhibits the Kinase Activity of Wild-Type, Juxtamembrane, and Activation Loop Mutant KIT Isoforms Associated with Human Malignancies

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