Resistance to c-KIT kinase inhibitors conferred by V654A mutation

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Abstract

Certain mutations within c-KIT cause constitutive activation of the receptor and have been associated with several human malignancies. These include gastrointestinal stromal tumors (GIST), mastocytosis, acute myelogenous leukemia, and germ cell tumors. The kinase inhibitor imatinib potently inhibits c-KIT and is approved for treatment of GIST. However, secondary point mutations can develop within the kinase domain to confer resistance to imatinib and cause drug-resistant relapse. A common mutation, which results in a V654A substitution, has been documented in imatinib-resistant GIST patients. We expressed c-KIT cDNA constructs encoding the V654A substitution and in combination with a typical activating exon 11 mutation characteristic of GIST, V560G, in factor-dependent FDC-P1 cells. The V564A substitution alone resulted in enhanced proliferation in c-KIT ligand (stem cell factor) but not factor independence. Cells expressing the double mutant were, like those expressing single V560G mutant c-KIT, factor independent. Analysis of cellular proliferation in the presence of imatinib showed that the V654A substitution alone conferred resistance. The difference in sensitivity was especially pronounced for cells expressing single mutant V560G c-KIT compared with double mutant V560G/ V654A c-KIT. The findings were supported by studies of c-KIT phosphorylation. Analysis of the crystal structure of imatinib in complex with the kinase domain of c-KIT predicts that the V654A substitution directly affects the binding of imatinib to the receptor. Alternative c-KIT inhibitors, nilotinib (AMN107) and PKC412, were also less active on V560G/V654A c-KIT than on the V560G single mutant; however, nilotinib, like imatinib, potently inhibited the V560G mutant. PKC412 strongly inhibited imatinib-resistant D816V c-KIT. [Mol Cancer Ther 2007;6(3):1159–66]

Introduction

c-KIT is classified as a type 3 receptor tyrosine kinase and is structurally similar to platelet-derived growth factor receptor, c-FMS, and FLT-3 (1). Distinguishing features of this family include an extracellular region composed of five immunoglobulin-like loops, which is followed by a single hydrophobic transmembrane domain that anchors the receptor to the plasma membrane. The intracellular region contains a negative-regulatory juxtamembrane domain (JMD) and a tyrosine kinase split by an interkinase region (2). The intracellular sequence into two regions, the ATP binding pocket and the phosphotransferase catalytic site. Binding of the cognate ligand, stem cell factor (SCF), induces receptor dimerization and autophosphorylation of specific tyrosine residues within the cytoplasmic domain. This creates docking sites for signal transduction molecules, which activate downstream pathways that regulate proliferation, survival, adhesion, migration, and differentiation (1). c-KIT is expressed in hematopoietic precursors, mast cells, germ cells, melanocytes, and interstitial cells of Cajal that reside within the gastrointestinal tract (2).

Specific mutations within the c-KIT gene cause constitutive phosphorylation and activation of the kinase domain resulting in uncontrolled cell proliferation. Such genetic abnormalities have been implicated in the pathogenesis of gastrointestinal stromal tumors (GIST), mastocytosis, as well as some cases of acute myelogenous leukemia and testicular seminoma (3). The first “gain-of-function” mutations in c-KIT were discovered in the human mast cell leukemia cell line HMC-1 (4). These cells contain a wild-type (WT) c-KIT allele and an allele that harbors two point mutations, which result in amino acid substitutions V560G and D816V in the intracellular JMD, and D816V within the activation loop of the kinase domain. Expression of the corresponding mouse c-KIT mutants, V559G and D814V, in mouse factor-dependent hematopoietic cell lines or normal bone marrow cells resulted in constitutive tyrosine phosphorylation, kinase activity, factor-independent growth, and tumorigenicity in mice (5–7). The D816V substitution has been identified in peripheral blood mononuclear cells of patients...
suffering from mastocytosis with an associated hematologic disorder (8). Corresponding mutations at codon 816 have also been detected in acute myelogenous leukemia (9, 10) and germ cell tumors (11). c-KIT mutations in acute myelogenous leukemia are largely confined to “core binding factor” leukemias that are characterized by t(8;21) or inv(16) (refs. 12, 13). Mutations have been shown to occur at multiple locations in the protein, with mutations at codon 816 accounting for about half (13, 14). Mutations within c-KIT are detected on ~80% of GIST specimens, which arise from transformation of the interstitial cells of Cajal. Most mutations are located within the intracellular JMD and include in-frame deletions and insertions of various sizes along with point mutations that result in amino acid substitutions (15). In addition, a small proportion of c-KIT mutations have also been detected within the extracellular JMD and kinase regions (16).

The phenylamino pyrimidine compound, imatinib, selectively regulates the activity of certain tyrosine kinases, in particular BCR-ABL, platelet-derived growth factor receptor, and c-KIT, through competitive inhibition at the ATP binding site. Although it binds with lower affinity, imatinib is also active against the colony-stimulating factor (CSF)-1 receptor, c-FMS, at therapeutically attainable concentrations (17). Imatinib interacts with the inactive conformation of the kinase domain and locks it into this position (18, 19). As a consequence, the transfer of phosphate groups to substrate molecules is prevented, and downstream signaling cascades normally generated by kinase activation are inhibited (20). Clinical trials for the treatment of chronic myelogenous leukemia and BCR-ABL—positive acute lymphoid leukemia showed superior efficacy and safety compared with traditional chemotherapeutic agents (21). In vitro studies showed that imatinib also inhibits the kinase activity of c-KIT (22). Promising results reported from phase I and II clinical trials led to the approval of imatinib for the treatment of malignant metastatic and/or unresectable GIST (23).

Despite the clinical success of imatinib in the treatment of GIST, numerous reports are confirming the emergence of resistance in secondary progressive tumors. This phenomenon of acquired resistance in GIST patients recapitulates treatment failure observed in advanced chronic myelogenous leukemia patients (24, 25) and is a major setback that must be addressed when developing targeted protein kinase inhibitors. As with BCR-ABL in chronic myelogenous leukemia, the tumors from the majority of these relapsing patients displayed new secondary point mutations in the exons of the c-KIT gene corresponding to the kinase domain (26–30). These missense mutations are believed to alter the structure of the kinase domain and consequently interfere with interactions between imatinib and the receptor (31). In particular, the occurrence of a missense mutation that results in the substitution of valine by alanine at codon 654 (V654A) has been documented in rapidly progressive imatinib-resistant samples from GIST patients in several studies (26–30, 32) and is the most common mutation associated with resistance (33).

We have examined the molecular mechanisms underlying the resistance to imatinib by expressing c-KIT containing the V564A substitution alone and in combination with a typical activating mutation found in GIST, V560G, in mouse factor-dependent FDC-P1 cells. Sensitivity of these mutant forms to imatinib and newly developed c-KIT kinase inhibitors nilotinib (AMN107) and PKC412 was examined in vitro and compared with the well-characterized imatinib-resistant D816V c-KIT mutant.

Materials and Methods

c-KIT Expression in FDC-P1 Cells

The nonadherent mouse factor-dependent early myeloid cell line, FDC-P1, was maintained in DMEM containing 10% FCS and 25 units/mL mouse granulocyte macrophage-CSF (GM-CSF). FDC-P1 lines expressing WT, V560G, and D816V mutant forms of human c-KIT have been described previously (34). To create the V654A mutation, QuikChange Site-Directed Mutagenesis (Stratagene, La Jolla, CA) was done on WT c-KIT in pBluescript. The oligonucleotide primers (Sigma Genosys, Castle Hill, New South Wales, Australia) were as follows: 5’-CTTGGTAATCACATGAA-TATGCGCAATCTACTGGAAGCC-3’ (forward) and 5’-GGCTCCAAGTATGTTGCAATATTGATGATTACCAAG-3’ (reverse). A 159-bp fragment spanning the V654A mutation was excised with PciI and ApalI and directionally cloned into the corresponding restriction sites in WT c-KIT in the defective retroviral expression vector pRUFneo. The V560G/V654A double mutant was generated by excising the PciI and ApalI fragment from pBluescript-V654A c-KIT and ligating it into the corresponding restriction sites of pRUFneo-V560G c-KIT.

Both constructs were verified by automated DNA sequencing and transfected into BOSC 23 packaging cells (35) using LipofectAMINE 2000 (Invitrogen, Mount Waverly, Victoria, Australia). Forty-eight hours posttransfection, the retroviral supernatants were harvested and filtered through a 0.45 μm filter. Retroviral infection was done by resuspending 10⁶ FDC-P1 cells in a suspension containing 3 mL DMEM/10% FCS and 1 mL retroviral supernatant with 8 μg/mL polybrene (Chemicon, Temecula, CA). After 48 h, the infectants were selected with 1 mg/mL G418 until uninfected control cells were dead. FDC-P1 (V654A KIT) were further selected by growth in 100 ng/mL recombinant human SCF (Peprotech, Rocky Hill, NJ). G418-resistant FDC-P1 (V560G/V654A KIT) cells were further selected for growth in the absence of GM-CSF. In both cases, the introduced c-KIT sequences were confirmed to be correct by isolation of genomic DNA from the cells following selection. PCR of the retroviral insert, and sequencing, FDC-P1 (WT KIT) and FDC-P1 (V654A KIT) were routinely maintained in mouse GM-CSF (as above). FDC-P1 (D816V KIT), FDC-P1 (V560G KIT), and FDC-P1 (V560G/V654A KIT) were maintained in the absence of factor. All cell populations were assessed for c-KIT expression by flow cytometry and Western blotting. All c-KIT constructs encode the more abundant GNNK isoform (34).
c-KIT Kinase Inhibitory Drugs
Imatinib was repurified from capsules obtained commercially (Novartis, Basel, Switzerland). Nilotinib (AMN107) and PKC412 were provided by Novartis.

Immunofluorescence Assay
Cell surface expression of c-KIT on FDC-P1 cells was examined by indirect immunofluorescence as described previously (36) and analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

Cell Proliferation Assay
To evaluate cell survival and growth, the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay was used. FDC-P1 cell lines were cultured in 96-well trays at a density of 2 × 10^4/mL in DMEM/10% FCS containing appropriate factors. In some experiments, imatinib, nilotinib, or PKC412 was added to cultures at the indicated concentrations. After 48 h, viable cells were measured using the CellTiter96 Aqueous Cell Proliferation Assay (Promega, Madison, WI).

Immunoprecipitation and Western Blot Analysis
FDC-P1 cells expressing WT or mutant forms of c-KIT grown in log phase were serum and, where appropriate, growth factor starved in DMEM supplemented with 0.1% bovine serum albumin at 2 × 10^5/mL for 2 h at 37°C. Cells were incubated in the presence or absence of various concentrations of imatinib, nilotinib, or PKC412 as indicated. Cells were resuspended to 2 × 10^5/mL in serum-free DMEM and, where indicated, stimulated with 100 ng/mL SCF at 37°C for 5 min following the addition of ice-cold PBS. Cells were pelleted at 220 × g and lysed in 1.2 mL of modified radioimmunoprecipitation assay buffer [1% NP40, 50 mmol/L Tris (pH 7.6), 150 mmol/L NaCl, 1 mmol/L EDTA, 0.25% sodium deoxycholate, 0.05% SDS] supplemented with 5 mmol/L sodium fluoride, 5 mmol/L tetrasodium pyrophosphate, 5 mmol/L sodium vanadate, 1 mg/mL leupeptin, 1 mg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride, and Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany) and incubated on ice for 30 min. Lysates were clarified by centrifugation at 15,000 × g for 15 min, and total protein content was determined by bicinchoninic acid analysis (Pierce Biotechnology, Rockford, IL). For immunoprecipitation, 1 mg total protein from each lysate, or 3 mg total protein for immunoprecipitation of D816V c-KIT, was incubated with 4 μg antihuman c-KIT monoclonal antibody KT4 and 25 μL of a 50% slurry of protein A-Sepharose (Amersham Biosciences, Castle Hill, New South Wales, Australia) at 4°C on a rotating platform for 2 h. Immunoprecipitates were washed four times in lysis buffer supplemented with 5 mmol/L sodium orthovanadate and 5 mmol/L sodium fluoride. After the final wash, the protein A-Sepharose pellet was resuspended in loading buffer containing 2-mercaptoethanol, boiled for 5 min, and subjected to SDS-PAGE on 8% gels. Gels were transferred onto Hybond enhanced chemiluminescence nitrocellulose membranes (Amersham Biosciences), blocked with 1% bovine serum albumin in TBS containing 0.1% Tween 20, and probed with anti-phosphotyrosine monoclonal antibodies 4G10 (Upstate Biotechnology, Lake Placid, NY) and PY20 (BD Biosciences) followed by horseradish peroxidase–labeled secondary antibody. Membranes were stripped in stripping buffer [62.5 mmol/L Tris (pH 6.5), 100 mmol/L 2-mercaptoethanol, 0.1% SDS] at 60°C for 30 min with agitation and reprobed for c-KIT using goat polyclonal anti–c-KIT antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoblots were visualized with enhanced chemiluminescence plus reagent on Typhoon Imaging System (Amersham Biosciences) and quantified with ImageQuant software.

Structural Analysis
The X-ray crystallographic structures of c-KIT in the active and inactive conformations and complexed with imatinib (37, 38) were downloaded from the internet-based Research Collaboratory for Structural Bioinformatics Protein Data Bank4 (accession nos. 1T45, 1T46, and 1PKG). The structures were analyzed in DS Viewer Pro (Accelrys, Inc. San Diego).5

Results
Expression Levels of c-KIT in FDC-P1 Cells
FDC-P1 cells were infected with retrovirus encoding human V654A c-KIT or V654A/V560G c-KIT and then incubated in G418 to select for successfully transduced cells. V654A c-KIT–expressing cells were further selected for growth in human SCF in place of mouse GM-CSF. The V654A/V560G c-KIT–expressing cells were selected for factor-independent growth. The other cell lines were as described previously (34). The level of c-KIT on the surface of FDC-P1–derived cell lines was investigated using immunofluorescence and flow cytometric analysis (Fig. 1). The relative fluorescence of each cell line was compared with a negative antibody control. The results indicated that c-KIT was expressed at relatively high levels on FDC-P1 (WT KIT) and FDC-P1 (V560G KIT) cells. FDC-P1 (V654A KIT) and FDC-P1 (V560G/V654A KIT) cells displayed moderate levels, which contrasted with the relatively low surface expression of the D816V mutant c-KIT.

Preliminary Studies with V654A Mutant c-KIT
To confirm that the V654A substitution was capable of conferring imatinib resistance in this cell line model, FDC-P1 cells expressing WT or V654A c-KIT and maintained in SCF were tested for growth in the presence of increasing concentrations of imatinib by MTS assay, which quantitates the number of viable cells after a 48-h incubation period. The experiment also included imatinib-resistant FDC-P1 (D816V KIT) cells for comparison. The results, shown in Fig. 2A, confirmed that, under these conditions, the V654A mutation conferred a high degree of resistance to the drug. The proliferation of FDC-P1 (WT KIT) cells was inhibited by imatinib with a IC50 of 0.1 to 0.2 μmol/L as reported previously (34). In contrast, FDC-P1 (V654A KIT)

4 http://www.rcsb.org
5 http://www.accelrys.com
and FDC-P1 (D816V KIT) were resistant to inhibition by imatinib and in both cases displayed an IC50 of >5 μmol/L. This growth rate was significantly increased compared with FDC-P1 (WT KIT; P < 0.05, Student’s t test, three independent experiments). In addition, FDC-P1 (V654A KIT) cells were able to form colonies in the presence of 1 μmol/L imatinib in the semisolid medium methylcellulose, whereas cells expressing WT c-KIT did not (data not shown).

Certain oncogenic mutant forms of c-KIT, such as the D816V mutant, are intrinsically resistant to imatinib because they strongly favor the active conformation of the kinase domain, which cannot bind the drug (39). These activating mutations induce factor-independent growth of FDC-P1 cells. To assess this characteristic, FDC-P1–derived cell lines, as above, were cultured in increasing concentrations of SCF for 48 h and proliferation was evaluated by MTS assay. As expected, FDC-P1 (WT KIT) cells did not survive in the absence of factor, and their proliferation was dependent on increasing concentrations of SCF (Fig. 2B). In contrast, the proliferation of FDC-P1 (D816V KIT) cells remained constant, regardless of SCF concentration. Interestingly, the growth of FDC-P1 (V654A KIT) cells at low concentrations of SCF ranging from 1.5 to 12 ng/mL differed significantly from FDC-P1 cells expressing WT c-KIT (P < 0.05, Student’s t test, three independent experiments). However, the proliferation of these cells was still dependent on SCF. This indicated that the V654A mutation caused increased proliferation at low concentrations of SCF but did not confer constitutive activity.

**Effect of the V654A Substitution in Combination with V560G, an Activating Mutation Characteristic of GIST, on Drug Sensitivity**

Clinically, the V654A substitution occurs in the context of GIST with primary activating mutations in exon 9 or exon 11, resulting in relapse of the disease in patients treated with imatinib. Exon 11 encodes the negative-regulatory intracellular JMD of c-KIT, and such mutations greatly increase sensitivity to imatinib due to increased access to its binding site (34, 38, 40, 41). In comparison with the V560G single mutant, FDC-P1 cells expressing the V560G/V645A mutant were much more resistant (approximate imatinib IC50 of 150 nmol/L compared with 15 nmol/L) and more resistant than the cells expressing WT c-KIT (Fig. 3B). The
greater sensitivity of cells expressing the V654A mutant alone in these experiments compared with those shown in Fig. 2 was found to be due to taking cells directly from maintenance in GM-CSF (i.e., without preconditioning in SCF) into the inhibitor assays. This has no effect on the results obtained with cells expressing the constitutively active mutants, which were grown in the absence of added growth factors throughout. In all cases, cells cultured in GM-CSF, and consequently independent of c-KIT signaling, were minimally affected by imatinib (Fig. 3A).

The inhibitory effects of alternative inhibitors of c-KIT, nilotinib and PKC412, were tested to determine whether the V654A mutation also caused resistance to these drugs. As shown in Fig. 3D, V560G mutant c-KIT was more sensitive to nilotinib than was WT c-KIT, a result similar to that with imatinib. Nilotinib sensitivity of cells expressing the V560G/V654A mutant was reduced compared with those harboring the V560G mutant, although they remained more sensitive than cells expressing WT c-KIT. The effect of the V654A mutation alone was less pronounced than for imatinib. As with imatinib, the D816V mutant kinase domain mutation conferred a high level of resistance to nilotinib, although slight inhibition was seen at the highest level tested (500 nmol/L). Nilotinib did not affect the growth of cells in GM-CSF except for cells expressing the double mutant where inhibition was reproducibly seen at the highest level of drug (Fig. 3C). In contrast, the staurosporine derivative PKC412 was much less specific, displaying considerable inhibition of cell survival and/or proliferation of all cell lines irrespective of whether growth was dependent on GM-CSF or c-KIT signaling (Fig. 3E). For c-KIT–dependent growth, in contrast to imatinib and nilotinib, the activating V560G mutation had a minimal effect on sensitivity. Similarly, the V654A mutation did not appreciably affect sensitivity to PKC412. The notable feature of the PKC412 data was the sensitivity of the kinase domain mutant, D816V, to this drug (Fig. 3F).

**Effect of Kinase Inhibitors on c-KIT Phosphorylation**

c-KIT–expressing FDC-P1 cells were incubated in the presence of various concentrations of imatinib, nilotinib, or PKC412 for 2 h under serum-free conditions and then lysed and immunoprecipitated with c-KIT antibody. FDC-P1 (WT KIT) and FDC-P1 (V654A KIT) cells were pulsed with...
SCF for 5 min before lysis. Immunoprecipitates were electrophoresed, then Western blotted, and analyzed for tyrosine-phosphorylated and total c-KIT (Fig. 4A). Data were quantitated and phosphorylated receptor levels were adjusted for total c-KIT and expressed as a percentage of control phosphorylation in the absence of inhibitor (Fig. 4B).

The results generally supported the cell proliferation data with the V654A substitution decreasing imatinib sensitivity in the context of both WT and V560G mutant c-KIT. Like imatinib, nilotinib (but not PKC412) more potently inhibited V560G c-KIT than WT c-KIT phosphorylation. However, inhibition by nilotinib was less influenced by the V654A substitution, especially in the context of WT c-KIT. PKC412 inhibition was not influenced by the V654A change alone. In contrast to the proliferation results, phosphorylation of V560G c-KIT was less inhibited by PKC412 than WT c-KIT, particularly in the double mutant. As reported previously, phosphorylation of D816V mutant c-KIT was not influenced by imatinib up to 1 μmol/L. It was weakly inhibited by nilotinib and potently inhibited by PKC412.

Discussion

It has become apparent that point mutations in the kinase domain are a major mechanism of drug-resistant relapse in cancer patients treated with small-molecule protein kinase inhibitors (31, 42). For inhibitors, such as imatinib that target the inactive conformation of the kinase (19, 38), two general mechanisms can lead to resistance (43). First,
mutations that strongly favor the active conformation of the kinase domain can preclude drug binding by an indirect (conformational) mechanism. An example of this is the D816V substitution in the activation loop of the kinase domain of c-KIT (39). Such mutant receptors confer constitutive kinase activity and the ability to grow in the absence of added growth factors on normally factor-dependent cells (34, 43). Second, kinase domain mutations that directly affect drug binding can confer resistance. Examples of this are the T315I substitution in ABL and the homologous T670I substitution in c-KIT (24, 29). Such mutations do not necessarily result in constitutive kinase activity.

The V654A c-KIT kinase domain mutation has been associated with imatinib-resistant GIST relapse in several studies (26–30, 33). We have examined this substitution in the context of WT c-KIT to better characterize its specific functional effects. We confirmed V654A c-KIT resistance to imatinib using cell proliferation and c-KIT autophosphorylation assays. The V654A mutation did not lead to constitutive kinase activity in either the receptor phosphorylation assay (data not shown) or the cell proliferation assay, although it did enhance the response to low levels of SCF (Fig. 2B). This suggests that the V654A substitution did not markedly favor the active conformation of the kinase domain but rather conferred resistance primarily by directly impeding imatinib binding. The V654A side chain contacts imatinib directly in the crystal structure (38), and the loss of this hydrophobic interaction in the V654A mutant is predicted to decrease imatinib binding affinity. As published recently by others (32, 44), our modeling confirmed this prediction (data not shown). In addition, the imatinib resistance of V654A c-KIT may be due in part to an enhanced ability to adapt the active conformation. This is manifested in the increased sensitivity of the mutant to low concentrations of SCF and can also be rationalized by analysis of the c-KIT crystal structures (37, 38). Amino acid 654 is located on a loop directly before the αC helix. This helix must move considerably to attain its position in the active conformation. We suggest that the less bulky alanine side chain allows greater freedom of movement in this part of the protein, thus facilitating conformational change.

The majority of GIST have mutations in exon 11 affecting the negative-regulatory intracellular JMD of c-KIT, whereas smaller proportions have mutations in exon 9 encoding the extracellular juxtamembrane region or in platelet-derived growth factor receptor α (16). Exon 11 mutations confer enhanced sensitivity to imatinib (34, 41), and patients with these mutations have the best response to imatinib therapy (45). Because the exon 13 V654A mutation seems to confer imatinib resistance in GIST, we have also examined this substitution in combination with the JMD V560G (exon 11) mutation. The double mutant displayed reduced imatinib sensitivity compared with WT c-KIT and, especially, V560G c-KIT in both cell proliferation and autophosphorylation assays.

To evaluate potential second-line kinase inhibitors, we tested the effects of nilotinib and PKC412 on the panel of c-KIT–expressing cell lines. Nilotinib is a phenylamino-pyrimidine compound related to imatinib. It has shown efficacy in imatinib-resistant chronic myelogenous leukemia associated with several kinase domain mutants of BCR-ABL (46). Like imatinib, nilotinib potently inhibited JMD mutant (V560G) c-KIT in both autophosphorylation and cell proliferation assays. In contrast to imatinib, it inhibited V560G/V564A mutant c-KIT to a similar extent to WT c-KIT in both assays. These two features suggest that nilotinib would be highly suitable for treatment of GIST with exon 11 mutant c-KIT and may be less susceptible to resistance arising from the V654A mutation. Other studies have reported varying results on the efficacy of nilotinib in inhibiting activation loop mutant D816V c-KIT (47, 48).

In our hands, this mutant displayed increased sensitivity to nilotinib compared with imatinib, but this was insufficient to be of likely therapeutic value. The indoline inhibitor sunitinib (SU11248) was also shown to inhibit c-KIT with exon 11 + V654A mutations in GIST cells treated ex vivo and in Ba/F3 cells (49). In the GIST specimen, the inhibition was less than observed in another specimen with a distinct exon 11 mutation alone, suggesting that the activity of this drug may also be reduced by the secondary V654A mutation. However, direct comparisons with c-KIT having the same primary mutation and also with WT c-KIT in the same cellular background are required.

The staurosporine derivative, PKC412, displayed more cytotoxicity unrelated to c-KIT inhibition in the cell proliferation assay and was a weaker inhibitor of V560G and V560G/V564A c-KIT than the other two compounds in both assays. In contrast, others found that the drug did inhibit phosphorylation of c-KIT Y703 in GIST cells harboring the V654A mutation together with an exon 9 mutation (27). The differences may be due to the different conformational effects of the exon 9 and exon 11 mutations on PKC412 binding. Notably, PKC412 was an effective inhibitor of D816V mutant c-KIT. Inhibition of D816V mutant c-KIT by PKC412 has also been reported by others (40, 48, 50), and the drug holds promise for the treatment of cases of mastocytosis and acute myelogenous leukemia harboring this mutation.

Overall, the data further show the need for careful mapping of mutations in target kinases for selection of the most appropriate drugs for individual patients.

References
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