

KIT Mutations in Mastocytosis and Their Potential as Therapeutic Targets

Jason Gotlib, MD, MS

*Stanford Cancer Center, Stanford University School of Medicine,
875 Blake Wilbur Drive, Room 2327B, Stanford, CA 94305–5821, USA*

KIT tyrosine kinase

The proto-oncogene *KIT* on chromosome 4q12 encodes a 145-kd transmembrane glycoprotein, one member of the class of type III receptor tyrosine kinases (TKs), which also includes platelet-derived growth factor receptor (PDGFR)- α and - β , FLT3, vascular endothelial growth factor receptor 2 (VEGFR2), and the receptor for macrophage colony-stimulating factor (c-FMS) [1–3]. *KIT* and related family members are characterized by an extracellular ligand-binding domain composed of five immunoglobulin-like structures, a transmembrane domain, a juxtamembrane domain, two intracellular TK domains interrupted by a kinase insert, and a C-terminal domain [2,4].

KIT is expressed on mast cells and other hematopoietic lineages, on germ cells, and on the interstitial cells of Cajal, which serve as pacemaker cells in the gut [5–8]. In the normal state, binding of *KIT* ligand (steel factor, stem cell factor [SCF]) [9–11] results in receptor homodimerization, which, in turn, leads to autophosphorylation of tyrosine residues located in intracellular portions of the molecule. Initiation of receptor TK activity results in a cascade of signaling events via downstream effector molecules, including Src kinases, c-Jun N-terminal kinase (Jnk), mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription (STATs), and phosphatidylinositol 3 (PI3) kinase/AKT [12,13].

Kit mutations in human malignancies and systemic mastocytosis

Several human malignancies are associated with somatic activating mutations of *KIT*. Disease-related mutations tend to cluster within specific exons

E-mail address: jason.gotlib@stanford.edu

of the *KIT* gene. For example, genetic studies indicate that most gastrointestinal stromal tumors (GISTs) possess a *KIT* mutation in exon 9, 11, 13, or 17, with mutations with the juxtamembrane region of exon 11 being the most common [3,14,15]. In-frame deletion plus insertion mutations involving the highly conserved Asp419 codon in exon 8 within the extracellular domain of *KIT* are associated with core-binding factor acute myeloid leukemias (AMLs) t(8;21) (FAB AML-M2) and inv(16) (FAB AML-M4Eo) [16]. D816V and D816Y mutations within KIT exon 17 have also been found in M2 and M4Eo AMLs [17]. Codon 816 mutations have been reported in germ cell tumors of seminoma or dysgerminoma differentiation (D816H) and sinonasal natural killer/T-cell lymphoma (D816N) [3,18,19]. Most exon 17 *KIT* mutations in sinonasal natural killer/T-cell lymphoma are related to codon 825 Val→Ala (V825A) substitutions, however, or to mutations within exon 11 codons 559 and 561 [19].

The pathogenesis of systemic mastocytosis (SM) is associated with constitutive activation of KIT [20–23]. Albeit rare in cutaneous mastocytosis, in more than 80% of cases of SM, the deregulated TK activity of KIT is attributable to an aspartate-to-valine catalytic-type mutation within codon 816 (D816V) [6]. Additional *KIT* mutations, with an estimated frequency of less than 5%, have been reported in SM with or without associated hematologic clonal non-mast cell lineage disease, including D816Y, D816F, D816H, D820G, V560G, F522C, and V530I [24]. D816V and other *KIT* mutations are not considered to be the only transforming events in SM, because additional genetic “hits,” including non-KIT-dependent oncogenic events, likely contribute to the neoplastic proliferation of mast cells [24,25]. Fig. 1 is a schematic of the *KIT* gene and the relative location of mutations related to the pathogenesis of SM and other human malignancies.

Activating *KIT* mutations result in autophosphorylation of the receptor independent of ligand binding and stimulation of downstream signaling cascades (including those previously mentioned). Studies using PI3 kinase inhibitors and KIT mutants incapable of recruiting PI3 kinase indicate that PI3 kinase is critical to the transforming capacity of D816V KIT [26,27]. The oncogenic potential of D816V KIT is also mechanistically linked to aberrant degradation of SHP-1, a protein tyrosine phosphatase that normally acts as a negative modulator of KIT signaling [28].

Gain-of-function *KIT* mutations may be of the “regulatory” type, which regulate the kinase activity of KIT, or “catalytic” (alternatively referred to as “enzymatic pocket type”) mutations, which directly affect the sequence of the active enzyme site of KIT [29]. Regulatory-type mutations are commonly found in the juxtamembrane domain of receptor TKs. Disruptions of this domain by missense mutations, in-frame insertions, or in-frame deletions result in constitutive activation of the respective TK, because this region serves an autoinhibitory function [30]. In hypereosinophilic syndrome (HES), molecular breakpoints of the *FIP1L1-PDGFR*A rearrangement lie within exon 12 of *PDGFR*A, which encodes a portion of the juxtamembrane

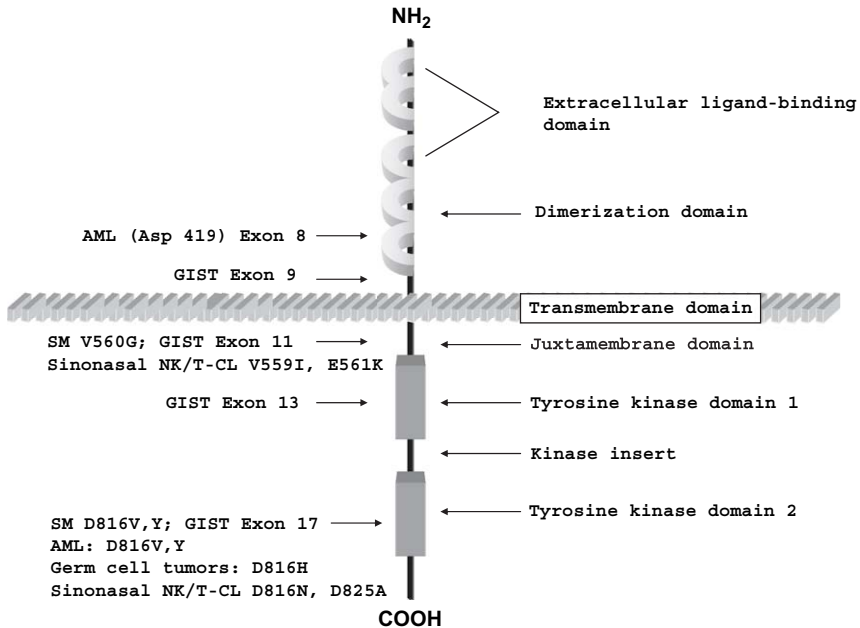


Fig. 1. KIT mutations in SM and other human malignancies. NK/T-CL, natural killer/T-cell lymphoma (*Adapted from* Heinrich M, Blanke CD, Druker BJ, et al. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002;20(6):1693; reprinted with permission from the American Society of Clinical Oncology).

domain [31]. Juxtamembrane internal tandem duplications in *FLT3* result in its constitutive activation in approximately 25% of cases of AML [32]. Mutation of the autoinhibitory juxtamembrane domain of *KIT* results in its constitutive activation in most cases of GISTs [14]. Akin to the golden principle in marketing, “location, location, location,” the site of these mutations has implications for targeted therapy with TK inhibitors. Preclinical and clinical data show that some agents have preferential activity against regulatory-type mutations (eg, imatinib), whereas others have equivalent or greater potency against catalytic-type mutations (eg, PKC412, dasatinib). Fig. 2 depicts the chemical structure of several TK inhibitors that exhibit activity against KIT and are further summarized in the following sections.

Imatinib mesylate for systemic mastocytosis

Imatinib is a multi-TK inhibitor with activity against ABL, KIT, PDGFR α , and PDGFR β , and the product of the Abelson-related gene (*ARG*). Imatinib’s lack of specificity for a singular TK has translated into clinical benefit for several hematologic malignancies related to constitutive

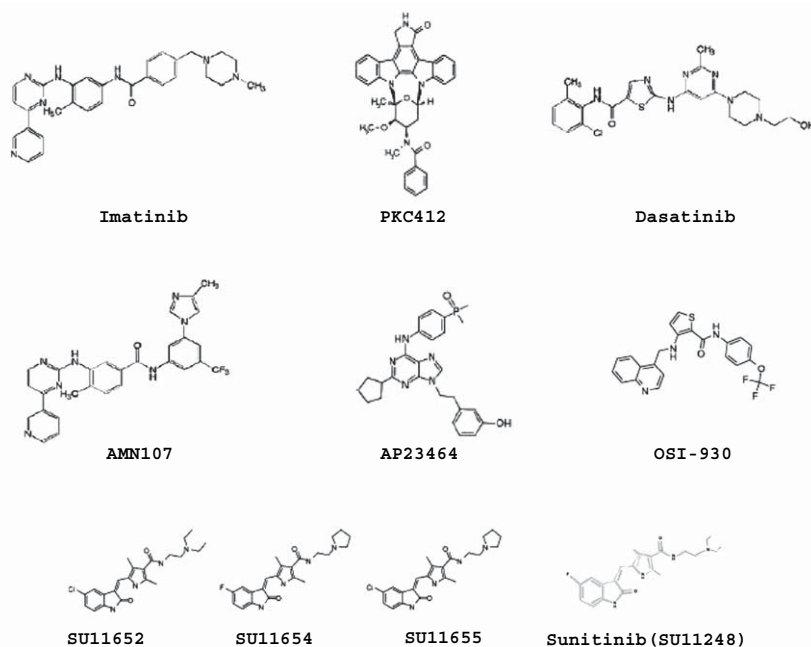


Fig. 2. Structure of KIT TK inhibitors under evaluation.

activation of the relevant TK: *BCR-ABL*-positive chronic myelogenous leukemia (CML) and acute lymphoblastic leukemia (ALL) [33,34], chronic myelomonocytic leukemia or myelodysplastic syndrome (MDS)/myeloproliferative disorder (MPD) with or without eosinophilia associated with rearrangements of *PDGFR* or *PDGFRB* [35], *FIP1L1-PDGFR*A-positive chronic eosinophilic leukemia (CEL) [31,36], dermatofibrosarcoma protuberans [37], and GISTs with activating mutations of *PDGFR*A or *KIT* [38,39].

In vitro studies

Although success with imatinib in *KIT*-mutated GISTs engendered optimism that imatinib would be effective against SM with the D816V *KIT* mutation, initial in vitro studies indicated otherwise [40–44]. When mature human mast cells derived from SCF-stimulated CD34-positive cord blood progenitors were treated with imatinib (1 μ mol), there was 70% inhibition of cell survival by 72 hours [41]. In contrast, imatinib had only a mild inhibitory effect on D816 *KIT*-positive bone marrow samples infiltrated with neoplastic mast cells. D816V *KIT*-bearing human and murine mast cell lines HMC-1, IC2KITV814, and P815 were resistant to the effects of imatinib, whereas FMA-3 cells, which carry a juxtamembrane *KIT* mutation, were inhibited by imatinib (0.1 μ mol) [41]. These findings of differential sensitivity

to imatinib have also been assessed using interleukin (IL)-3-dependent Ba/F3 cell lines transfected with various KIT mutants. KIT phosphorylation and cell growth was inhibited at 1 μmol in Ba/F3 cells transfected with wild-type KIT or a juxtamembrane KIT mutant (G559) [41]. No effect was seen in cells with D816V KIT (or the analogous murine mutation D814V KIT), even at concentrations up to 10 μmol .

Experiments using retrovirally transduced FDC-P1 factor-dependent murine early myeloid cells confirmed the sensitivity of wild-type and juxtamembrane V560G KIT transformants to imatinib and resistance to D816V KIT [42]. Western blot analysis of downstream signaling in these cells revealed that constitutive activation of ERK, AKT, and STAT3 by D816V KIT was not inhibited by imatinib at a dose of 5 μmol . SCF-induced phosphorylation of ERK 1/2, and AKT in wild-type KIT FDC cells as well as STAT3 in V560KIT FDC cells was inhibited by imatinib at a dose of 1 μmol [42].

These results parallel those by Akin and colleagues [43], who used human mast cell (HMC)-1 leukemia cell lines carrying the V560G *KIT* mutation alone (HMC-1⁵⁶⁰) or with D816V *KIT* as a second mutation (HMC-1^{560,816}). Imatinib (0.1–10 μmol) had no inhibitory effect on HMC-1^{560,816} cells but effectively suppressed the growth of HMC-1⁵⁶⁰ cells. These results were recapitulated in short-term cultures of mast cells isolated from bone marrow mononuclear cells from D816V *KIT*-positive patients with SM. Imatinib showed no preferential cytotoxicity against neoplastic mast cells in these individuals compared with effective reduction of mast cells from patients with wild-type *KIT* [43]. Mast cell numbers derived from patients with SM were similar in short-term cultures regardless of SCF supplementation, indicating a ligand-independent effect related to autonomous D816V KIT TK activation or autocrine or paracrine effects of SCF mediated by other mononuclear cells in culture [43].

Clinical studies

In vitro resistance of the D816V *KIT* mutation to imatinib has been borne out in case reports and small case series of patients with SM. In a Mayo Clinic study of 10 patients with SM, 5 had SM associated with eosinophilia; 2 (40%) of 5 were found to have the D816V *KIT* mutation and showed no response to imatinib at a dose of 400 mg/d, whereas 3 other patients experienced a complete remission [44]. The same investigators evaluated 14 patients with SM with associated eosinophilia. Three of these patients exhibited the D816V *KIT* mutation and were similarly refractory to imatinib [45]. In an Italian study of imatinib in 4 patients with SM, 3 showed no response and 1 had a transient response (<1 month), which was not further characterized [46]. The 1 patient without D816V *KIT* exhibited a decrease in bone marrow mast cell infiltration. In the M.D. Anderson case series of 18 patients with SM retrieved from a database spanning the years 1944 through 2002, 2 patients pretreated

with interferon- α experienced transient symptom improvement with imatinib dosed at a rate of 200 mg/d or twice daily [47]. A *KIT* mutation was not found in these patients. Imatinib resistance has also been reported in a patient with D816V *KIT*-positive aggressive SM [48]. Some benefit of imatinib in patients with SM has been described elsewhere, but characterization of the patients for *KIT* or other gene mutations was not cited; therefore, the mechanism of response in these individuals is not known [49].

In contrast to the experience with D816V *KIT*, imatinib has demonstrated efficacy in rare patients with SM with juxtamembrane *KIT* mutations. Akin and coworkers [50] described a patient with SM who responded to imatinib at a dose of 400 mg/d. After 3 months of therapy, bone marrow mast cell infiltration decreased from 50% to less than 10%, the serum tryptase level decreased from 173 to 20 ng/mL, and the patient experienced marked improvement of symptoms. Mutational analysis revealed a novel transmembrane *KIT* mutant Phe522Cys (F522C) [50]. Imatinib (1 μ mol) resulted in inhibition of autophosphorylation of Cos-7 cells transfected with F522C *KIT* as well as elimination of mast cells from the patient's marrow when placed in short-term 8-day culture. A novel K509I juxtamembrane *KIT* mutation was identified in a mother and daughter in a kindred of familial mastocytosis [51]. The daughter was treated with imatinib at a dose of 400 mg/d, resulting in improvement in liver function tests and serum tryptase levels, reduction in hepatosplenomegaly, and symptom improvement. Although these examples of mast cell disease with juxtamembrane *KIT* mutations, such as K509I and Phe522Cys, are rare, these cases highlight the importance of performing mutational analysis of the *KIT* gene to exploit the utility of imatinib whenever such circumstances are present.

***FIP1L1-PDGFR* α -positive systemic mastocytosis**

From the latter part of 2001 through 2003, several publications reported that empiric therapy of HES with imatinib at doses as low as 100 mg/d resulted in rapid and complete hematologic remission [36,52–56]. These results were reminiscent of the successful experience with imatinib in *BCR-ABL*-positive leukemias and implicated a dysregulated TK involving one of imatinib's known targets. A collaborative multicenter effort discovered that the therapeutic basis of response to imatinib was the drug's inhibition of a novel fusion TK, *FIP1L1-PDGFR* α [36]. The *FIP1L1-PDGFR* α genotype was subsequently found to cosegregate with a clinical phenotype consisting of myeloproliferative HES-MPD with tissue fibrosis, increased serum tryptase levels, and a historically poor prognosis (before the introduction of imatinib) [57].

The patients with HES-MPD in the latter study [57] fulfilled one minor diagnostic criterion for SM in that the bone marrow from most of these individuals exhibited greater than 25% atypical mast cells, although dense mast cell infiltrates (the major diagnostic criterion for SM) were generally not observed. Elevation of the serum tryptase was also noted in these

patients (a second minor criterion for a diagnosis of SM), although this finding is not specific to mast cell disease and may also be observed in other myeloid malignancies. Some debate exists as to whether *FIP1L1-PDGFR* is in fact related to the pathogenesis of eosinophilic subsets of SM or whether it relates more to a diagnosis of HES with associated morphologic finding of increased atypical mast cells within the bone marrow [58]. Using fluorescence in situ hybridization (FISH) for the *CHIC2* deletion as a surrogate for the *FIP1L1-PDGFR* gene fusion, clonality has been demonstrated in peripheral blood mononuclear cells, purified neutrophil and eosinophil fractions, a low proportion of B and T lymphocytes, and mast cell culture derived from bone marrow CD34 cells isolated from the marrow of one patient with HES-MPD [59,60]. These data are consistent with *FIP1L1-PDGFR*–mediated disease arising in a pluripotent hematopoietic progenitor. These clonality studies cannot clarify the semantic issue of whether these patients represent HES with increased bone marrow mast cells or SM with eosinophilia, however. This matter is best reserved for the consensus panels (eg, the World Health Organization) that classify these diseases.

With this background, the Mayo Clinic group detected deletion of the *CHIC2* locus in 3 imatinib-responsive patients in whom they diagnosed as having SM with eosinophilia. *FIP1L1-PDGFR* was not found in 2 other D816V *KIT*-positive patients with SM who failed to respond to imatinib [61]. A follow-up study evaluated the frequency of *FIP1L1-PDGFR* in a combined retrospective and prospective study of 89 patients with moderate to severe eosinophilia [62]. *FIP1L1-PDGFR* was detected in 11 patients: in 1 patient with an unclassified MPD, in 10 (56%) of 19 patients pathologically diagnosed as having SM with eosinophilia, but in none of the 57 patients classified as having HES. Seven of 11 patients found to be *FIP1L1-PDGFR*-positive were originally diagnosed as having HES based solely on clinical information but were reclassified as having SM with eosinophilia by pathologic review. In contrast to the dense clusters of mast cells typically observed in SM, the bone marrow of most of these *FIP1L1-PDGFR*-positive patients with SM with eosinophilia demonstrated loose aggregates of mast cells by tryptase immunostaining. The D816V *KIT* mutation was detected in 3 of 9 *FIP1L1-PDGFR*-negative patients with SM with eosinophilia but in none of the 9 patients who were positive for the fusion, indicating that the *KIT* mutation and *PDGFR* rearrangement are mutually exclusive oncogenic events in mast cell disease. Durable complete remissions were elicited in all 8 *FIP1L1-PDGFR*-positive patients with SM with eosinophilia treated with low-dose imatinib (100 mg/d) [62].

PKC412

PKC412 (N-benzoyl-staurosporine) is an alternative small molecule inhibitor of multiple type III receptor TKs, including FLT3, KIT, VEGFR2, PDGFR, and fibroblast growth factor receptor (FGFR) [63,64]. It is

currently under evaluation in phase II clinical trials, with or without cytotoxic chemotherapy, in patients with relapsed or newly diagnosed FLT3 mutated AML [65,66]. PKC412 also demonstrated activity in a patient with stem cell MPD with the constitutively activated *ZNF198-FGFR1* fusion TK [67].

In the HES study by Cools and colleagues [36], one patient with AML arising from CEL with *FIP1L1-PDGFR α* and a complex karyotype experienced a hematologic relapse after 5 months of imatinib treatment. Relapse was temporally related to the development of a new mutation in the ATP-binding region of PDGFR α , T674I. Sequence analysis revealed that this T674I mutation is in an analogous position to the T315I mutation that confers resistance to imatinib, dasatinib, and AMN107 in the context of CML. Cools and colleagues [36] subsequently demonstrated that as an alternative PDGFR α inhibitor with a chemical structure different from imatinib, PKC412 could effectively inhibit the FIP1L1-PDGFR α T674I mutant in vitro, and disease progression in an in vivo murine model of FIP1L1-PDGFR α T674I-induced myeloproliferation [68].

A corollary of the hypothesis that PKC412 would be effective for imatinib-resistant T674I-mediated hypereosinophilia was that it may also be useful for SM related to the imatinib-resistant D816V *KIT* mutation. This hypothesis was first corroborated by in vitro experiments. In contrast to imatinib, PKC412 effectively inhibited the growth of D816V *KIT*-transformed Ba/F3 cells with a cellular 50% inhibitory concentration (IC₅₀) of approximately 30 to 40 nmol [69,70]. Western blot analysis of lysates from PKC412-treated Ba/F3 cells demonstrated reduction of the phosphotyrosine content of KIT and the downstream effector STAT3 but not of STAT5 [69,70]. Previous reports showed that STAT3 is aberrantly phosphorylated by D816V KIT [71,72]. Although phosphorylation of STAT5 was not affected by PKC412, a recent study indicated that D816V KIT leads to persistent activation of STAT5 in neoplastic mast cells [73]. PKC412 could inhibit several KIT-transformed Ba/F3 cell lines, but the degree of inhibition was variable [69]. The IC₅₀s were in a lower range of 33 to 95 nmol for D816V and D816Y as well as for other mutations, including V560G, R634W, and N822K. The PKC412 IC₅₀s for other transforming *KIT* mutations were in a relatively higher range of 146 to 265 nmol [69].

The in vitro nanomolar inhibitory activity of PKC412 led Gotlib and colleagues [70] to treat a patient with mast cell leukemia (with an associated MDS-MPD) on a compassionate use basis. With PKC412 at a dose of 100 mg administered orally twice daily, the patient exhibited a major partial response, with significant resolution of liver function abnormalities, resolution of a portal vein thrombosis, and marked improvement in performance status. In addition, PKC412 treatment resulted in a significant decline in the percentage of peripheral blood mast cells and in the serum histamine level and was associated with a decrease in KIT phosphorylation and D816V *KIT* mutation frequency. The patient lost her response after 3 months of

therapy because of progression of her MDS-MPD to AML. Use of PKC412 in this case provided the first proof-of-principle in human beings that resistance of the D816V KIT TK to inhibition by imatinib could be circumvented with a structurally different small molecule inhibitor that targets the same protein [70].

The encouraging data from the single patient use of PKC412 in mast cell leukemia has led to a phase II trial of PKC412 in aggressive SM and mast cell leukemia. Based on the cooperative (additive) effects of PKC412 with other agents, including cladribine or AMN107 (see below) with HMC-1 cells that bear V560G and D816V *KIT* mutations [74], combinations of these drugs are likely to enter clinical trial testing in the near future.

Dasatinib

Dasatinib (BMS-354825) is an orally bioavailable dual SRC/ABL kinase inhibitor with greater than 300-fold potency against BCR-ABL compared with imatinib in preclinical studies [75]. Dasatinib is currently being evaluated in phase II trials of imatinib refractory and/or relapsed or intolerant *BCR-ABL*-positive CML and ALL. Two recent studies demonstrated that dasatinib also exerts potent activity against wild-type and juxtamembrane KIT mutants as well as against D816V KIT [76,77]. In an in vitro kinase assay, the IC_{50} s of dasatinib for wild-type and D816V KIT were 79 nmol and 37 nmol, respectively [75]. Growth inhibition assays using the HMC-1.1 cell line (V560G *KIT*-positive only) revealed that dasatinib and imatinib were similarly effective in blocking cellular proliferation (IC_{50} of 5–10 nmol), with the IC_{50} for induction of apoptosis being 14 nM for dasatinib and approximately 70 nM for imatinib [76]. In the two studies, the growth of the HMC-1.2 cell line (V560G and D816V *KIT*-positive) was inhibited by dasatinib in the high nanomolar range (eg, IC_{50} s of 490 nmol and 1200 nmol) but was resistant to imatinib [76,77]. Dasatinib has also been shown to be preferentially effective against enriched primary human neoplastic mast cells compared with other hematopoietic cells from patients with D816V *KIT*-positive SM, with increasing activity over the dose range of 0.1 to 1.0 μ mol; imatinib exhibited no growth-suppressive effects against neoplastic mast cells in this ex vivo culture system. Signaling assays have shown that induction of apoptosis and inhibition of cellular proliferation strongly correlate with blockade of KIT, MAP kinase, and AKT activation, and less so with inhibition of STAT3 phosphorylation [77].

D816V *KIT* or mutations in the kinase domain (KD) of BCR-ABL generate a kinase conformation that stabilizes the activation loop in the “open” or active conformation. Imatinib can only bind the inactive or “closed” form of KIT or BCR-ABL, explaining its relative lack of efficacy in D816V *KIT*-positive SM or CML with certain KD mutations [78–82]. In contrast, dasatinib can bind the active conformation of KIT and BCR-ABL and is not predicted to be affected by alterations in the activation

loop induced by mutations. These crystallographic and preclinical data provide a strong rationale for evaluating dasatinib in trials of mast cell disease.

AMN107, AP23464, and MLN518

AMN107 is a novel aminopyrimidine oral TK inhibitor that targets ABL, PDGFR, and KIT. AMN107 is active against many imatinib-resistant BCR-ABL mutants *in vitro* and *in vivo* and is approximately 25-fold more potent than imatinib against wild-type BCR-ABL [83]. In a study evaluating the *in vitro* profile of AMN107 against various BCR-ABL mutants and other constitutively activated kinases, the drug was found to inhibit KIT autophosphorylation and the proliferation of Ba/F3 cells transformed by the exon 11 juxtamembrane *KIT* deletion 560 to 561 with an IC_{50} of 20 to 30 nmol compared with 160 to 200 nmol for the exon 13 *KIT* mutation GIST882 [84]. D816V KIT was not screened in this analysis, however. Several preclinical lines of evidence indicate that AMN107 has only modest activity against D816V *KIT*. At a 1- μ mol concentration, imatinib and AMN107 strongly reduced KIT phosphorylation and induced apoptosis in the HMC-1.1 cell line, which bears only V560G *KIT*, but exhibited only mild effects on these end points with HMC-1.2 cells, which additionally carries D816V *KIT* [85]. In another study, AMN107 inhibited the proliferation of murine D814V and human D816V KIT-transformed Ba/F3 cells but only at relatively higher concentrations of 1 to 2 μ mol, with apoptosis of greater than 80% to 100% of cells observed in the 2- to 4- μ mol range [86]. Based on nanomolar activity against PDGFR α [84], it is predicted that AMN107 would exhibit activity in FIP1L1-PDGFR α -mediated SM and HES. A current phase II trial evaluating AMN107 in imatinib-refractory or -intolerant CML/ALL includes one arm that is enrolling SM patients.

Similar to PKC412 and dasatinib, the family of ATP-based inhibitors AP23464 and AP23848 shows nanomolar activity against KIT activation loop mutants, including D816V [87]. These effects were mediated by induction of G₀/G₁ cell cycle arrest and apoptosis. In addition, KIT signaling was attenuated by reductions of AKT and STAT3 phosphorylation [87]. Interestingly, wild-type and juxtamembrane *KIT* mutants were less sensitive to the effects of these inhibitors. As the authors of this study indicated, the TK profile of these ATP-based inhibitors is analogous to the relatively greater activity of gefitinib against epidermal growth factor receptor (EGFR) with p-loop and activating loop mutations versus the wild-type EGFR in the setting of non-small-cell lung cancer [87]. Lack of inhibition of hematopoietic colony formation (eg, burst forming unit-erythroid and colony forming unit-granulocyte macrophage) at concentrations that can inhibit D816V KIT [87] and the preferential sensitivity of activation loop mutants compared with wild-type KIT provide an exploitable therapeutic window for future clinical development of AP23464 and related compounds.

MLN518 is a quinazoline-based small molecule inhibitor of several type III receptor TKs, including wild-type KIT. MLN518 was also found to inhibit the proliferation of D816V KIT-transformed Ba/F3 cells with an IC₅₀ of 250 nmol and phosphorylation of D816V KIT with an IC₅₀ of 160 nmol [88]. Although these IC₅₀s are higher than those observed for PKC412 and dasatinib, with MLN518 dosed at 525 mg twice daily, the mean steady-state plasma trough level was reported to be in the range of 400 to 500 nmol, suggesting that clinical activity may be attainable in patients [88].

Other tyrosine kinase inhibitors of KIT

Additional TK inhibitors that have been evaluated against wild-type and mutant KIT include the indolinone compounds SU11652, SU11654, and SU11655 [89]. The related class of indolinone molecules (eg, SU4984, SU5416, SU5614, SU6577, SU6668) demonstrate activity against KIT in the range of .05 μmol to <5 μmol [3]. Sunitinib (SU11248), recently approved for imatinib-resistant GIST, also demonstrates activity against the juxtamembrane KIT mutant V559D (IC₅₀ of 70 nmol) and V559D/T670I (IC₅₀ of 100 nmol) [90]. The novel thiophene multikinase inhibitor OSI-930 shows nanomolar anti-KIT activity in preclinical studies using the HMC-1 cell line (V560G *KIT*) [91,92].

Table 1 summarizes the relative in vitro potencies of these TK inhibitors against various mutated KIT proteins and FIP1L1-PDGFRα based on in vitro IC₅₀ data (eg, kinase, cell proliferation, apoptosis assays) published to date. These in vitro data may not necessarily reflect the efficacy of these agents in human beings because of pharmacokinetic and pharmacodynamic issues related to these drugs.

Table 1
Estimated relative in vitro potencies (50% inhibitory concentration) of tyrosine kinase inhibitors against KIT and FIP1L1-PDGFRα

	KIT			FIP1L1-PDGFRα
	Wild type	V559D, V560G	D816V	
Imatinib	+/++	++/+++	—	++++
PKC412	+	++	+++	+
Dasatinib	++++	+++ /++++	++	N/A
AP23464	++	++	+++ /++++	N/A
AMN107	++	++ /+++	—	++
MLN518	+	+++	+	N/A
SU11652 and related indolinone compounds	++ /++++	++ /+++	+	N/A
OSI-930	++ /+++	++ /+++	N/A	N/A

50% inhibitory concentration potency key: (—): > 1 nmol; (—/+): 500 nmol to 1000 nmol; (+): 100 nmol to 500 nmol; (++) : 50 nmol to 100 nmol; (+++) : 10 nmol to 50 nmol; (++++): <10 nmol.
Abbreviation: N/A, not available.

Other candidate targets for molecular therapy against neoplastic mast cells

As cited in a previous review, “There is more to mast cell disease than codon 816 c-kit mutations” [25]. Other *KIT* mutations (described previously), the *FIP1L1-PDGfra* gene fusion, gene polymorphisms (eg, IL-4 receptor α chain) [93], and as yet undefined molecular lesions likely influence the phenotypic expression of mast cell disease.

Neoplastic mast cells exhibit aberrant expression of cell surface and intracellular signaling molecules, a potential plentiful source of targets for molecularly guided therapy. For example, CD25 is the IL-2 receptor α chain whose cell surface expression is strongly linked to mast cells bearing the D816V *KIT* mutation [94]. The role of CD25 in the pathogenesis of SM is not well defined; however, anti-CD25 therapy for SM is being investigated on a clinical trial basis. One example is denileukin diftitox (Ontak), a recombinant DNA-derived cytotoxic protein that includes sequences for IL-2 fused to diphtheria toxin fragments [95]. Denileukin diftitox is approved for use in cutaneous T-cell lymphoma.

Hsp 90 is a heat shock protein, which stabilizes many client proteins, including type III receptor TKs, such as FLT3 and KIT. 17-Allylamino-17-demethoxy-geldanamycin (17-AAG) is a derivative of geldanamycin that binds to hsp 90, resulting in decreased levels of mutated KIT and reduced phosphorylation of KIT, AKT, and STAT3 [96]. 17-AAG also demonstrated inhibition of patient-derived neoplastic mast cells. A phase II trial of 17-AAG is currently being led by the National Cancer Institute.

Nuclear factor (NF)- κ B is a dimeric transcription factor whose role as a mediator of KIT receptor signaling in neoplastic mast cell growth is now receiving more attention. The NF- κ B inhibitor IMD-0354 suppressed malignant mast cell proliferation, an effect associated with decreased expression of cyclin D3 and the retinoblastoma protein, resulting in cell cycle arrest and apoptosis [97]. Inhibition of the NF- κ B pathway may represent a novel therapeutic approach for mast cell disorders.

Table 2 lists additional molecules associated with neoplastic mast cells and the rationale for considering them as therapeutic targets in mastocytosis [26,27,98–102].

Summary

Deregulation of the KIT receptor TK by the prevalent activation loop mutation D816V has served as a focal point in therapeutic strategies aimed curbing neoplastic mast cell growth. Perhaps the most important development in this era of targeted therapy, and certainly relevant to KIT-driven diseases like mastocytosis, is the realization that small molecule inhibitors with varied chemical structure (eg, PKC412, dasatinib, AP23464) can circumvent the resistance of TKs to first-generation agents such as imatinib. Genuine opportunity now exists to effectively treat mastocytosis, and the

Table 2

Non-tyrosine kinase molecules associated with neoplastic mast cells: potential targets for molecular therapy

Molecule(s)	Basis for therapeutic consideration
PI3 kinase/AKT [26,27]	Critical to the transforming capacity of D816V KIT PI3 kinase/AKT inhibitors are in development
Bcl-2 [98]	Overexpressed in a case of mast cell leukemia Bcl-2 antisense compounds are in clinical trials for hematologic malignancies
CD52 [99]	Expressed on human bone marrow mast cells from SM patients Anti-CD52 monoclonal antibody Campath-1H is approved for the treatment of chronic lymphocytic leukemia
HO-1 [100]	Survival factor in human neoplastic mast cells expressing D816V <i>KIT</i>
CD44 [101]	Expressed on human neoplastic mast cells Anti-CD44 monoclonal antibody AD38 decreased proliferation of the human mast cell leukemia cell line HMC-1 through induction of apoptosis and cell cycle arrest
Mcl-1 [102]	Member of the Bcl-2 family of antiapoptotic proteins Mcl-1 antisense oligonucleotides (ASO) and mcl-1 siRNA reduce survival and increase apoptosis in HMC-1.2 cells carrying V560G and D816V <i>KIT</i> Cooperates with PKC412 and AMN107 in growth inhibition of HMC-1.2 cells

Abbreviations: siRNA, short interfering RNA; HO-1, heme-oxygenase-1.

arsenal consists of several orally bioavailable drugs with promising preclinical activity against D816V and other KIT mutants that promote mast cell growth. Because *KIT* mutations may not act as fully transforming oncogenic events in SM, it is prudent to evaluate combinations of TK inhibitors with drugs with activity in mast cell disease, such as cladribine, interferon- α , and corticosteroids. The identification of novel “drug-able” targets within mast cells should aid in the development of complementary therapies that promote enhanced cytotoxicity of mast cells through blockade of nonredundant signaling pathways. In addition, the generation of murine models that recapitulate human mastocytosis should accelerate preclinical testing of novel agents [103].

References

- [1] Yarden Y, Kuang W-J, Yang-Feng T, et al. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J* 1987;11(6):3341–51.
- [2] Reilly JT. Class III receptor tyrosine kinases: role in leukaemogenesis. *Br J Haematol* 2002;116(4):744–57.
- [3] Heinrich MC, Blanke CD, Druker BJ, et al. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J Clin Oncol* 2002;20(6):1692–703.
- [4] Rousset D, Agnes F, Lachaume P, et al. Molecular evolution of the genes encoding receptor tyrosine kinase with immunoglobulin-like domains. *J Mol Evol* 1995;41(4):421–9.

- [5] Ogawa M, Matsuzaki Y, Nishikawa S, et al. Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med* 1991;174(1):63–71.
- [6] Natali PG, Nicotra MR, Sures I, et al. Expression of c-kit receptor in normal and transformed human nonlymphoid tissues. *Cancer Res* 1992;52(22):6139–43.
- [7] Nocka K, Majumder S, Chabot B, et al. Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice—evidence for an impaired c-kit kinase in mutant mice. *Genes Dev* 1989;3(6):818–26.
- [8] Huizinga JD, Thuneberg L, Kluppel M, et al. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995;373(6512):347–9.
- [9] Williams DE, Eisenman J, Baird A, et al. Identification of a ligand for the c-kit proto-oncogene. *Cell* 1990;63(1):167–74.
- [10] Zsebo KM, Williams DA, Geissler EN, et al. Stem cell factor is encoded at the SI locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* 1990;63(1):213–24.
- [11] Huang E, Nocka K, Beier DR, et al. The hematopoietic growth factor KL is encoded by the SI locus and is the ligand of the c-kit receptor, the gene product of the W locus. *Cell* 1990;63(1):225–33.
- [12] Linnekin D. Early signaling pathways activated by c-Kit in hematopoietic cells. *Int J Biochem Cell Biol* 1999;31(10):1053–74.
- [13] Brizzi MF, Dentelli P, Rosso A, et al. STAT protein recruitment and activation in c-Kit deletion mutants. *J Biol Chem* 1999;274(24):16965–72.
- [14] Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279(5350):577–80.
- [15] Lux ML, Rubin BP, Biase TL, et al. KIT extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am J Pathol* 2000;156(3):791–5.
- [16] Gari M, Goodeve A, Wilson G, et al. c-Kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. *Br J Haematol* 1999;105(4):894–900.
- [17] Beghini A, Peterlongo P, Ripamonti CB, et al. C-Kit mutations in core binding factor leukemias. *Blood* 2000;95:726–7.
- [18] Tian Q, Frierson HFJ, Krystal GW, et al. Activating c-kit gene mutations in human germ cell tumors. *Am J Pathol* 1999;154(6):1643–7.
- [19] Hongyo T, Li T, Syaifudin M, et al. Specific c-kit mutations in sinonasal natural killer/T-cell lymphoma in China and Japan. *Cancer Res* 2000;60(9):2345–7.
- [20] Furitsu T, Tsujimura T, Tono T, et al. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. *J Clin Invest* 1993;92(4):1736–44.
- [21] Nagata H, Worobec AS, Oh CK, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci USA* 1995;92(23):10560–4.
- [22] Longley BJ, Tyrrell L, Lu SZ, et al. Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* 1996;12(3):312–4.
- [23] Longley BJ Jr, Metcalfe DD, Tharp M, et al. Activating and dominant inactivating c-KIT catalytic domain mutations in distinct clinical forms of human mastocytosis. *Proc Natl Acad Sci USA* 1999;96(4):1609–14.
- [24] O'Brien S, Tefferi A, Valent P. Chronic myelogenous leukemia and myeloproliferative disease. *Hematology (Am Soc Hematol Educ Program)* 2004;1:46–62.
- [25] Akin C. Clonality and molecular pathogenesis of mastocytosis. *Acta Haematol* 2005;114(1):61–9.
- [26] Chian R, Young S, Danilkovitch-Miagkova A, et al. Phosphatidylinositol 3 kinase contributes to the transformation of hematopoietic cells by the D816V c-KIT mutant. *Blood* 2001;98(5):1365–73.

- [27] Shivakrupa R, Bernstein A, Watring N, et al. Phosphatidylinositol 3'-kinase is required for growth of mast cells expressing Kit catalytic domain mutant. *Cancer Res* 2003;63(15):4412–9.
- [28] Piao X, Paulson R, Van Der Geer P, et al. Oncogenic mutation in the KIT receptor tyrosine kinase alters substrate specificity and induces degradation of the protein tyrosine phosphatase SHP-1. *Proc Natl Acad Sci USA* 1996;93(25):14665–9.
- [29] Longley BJ, Reguera MJ, Ma Y. Classes of c-KIT activating mutations: proposed mechanisms of action and implications for disease classification and therapy. *Leuk Res* 2001;25(7):571–6.
- [30] Irusta PM, Luo Y, Bakht O, et al. Definition of an inhibitory juxtamembrane WW-like domain in the platelet-derived growth factor beta receptor. *J Biol Chem* 2002;277(41):38627–34.
- [31] Gotlib J, Cools J, Malone JM, et al. The FIP1L1-PDGFR α fusion tyrosine kinase in hyper-eosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. *Blood* 2004;103(8):2879–91.
- [32] Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood* 2002;100(5):1532–42.
- [33] Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001;344(14):1031–7.
- [34] Druker BJ, Sawyers CL, Kantarjian H, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001;344(14):1038–42.
- [35] Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. *N Engl J Med* 2002;347(7):481–7.
- [36] Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* 2003;348(13):1201–14.
- [37] Rubin BP, Schuetze SM, Eary JF, et al. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J Clin Oncol* 2002;20(17):3586–91.
- [38] Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002;347(7):472–80.
- [39] Heinrich MC, Corless CL, Duensing A, et al. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003;299(5607):708–10.
- [40] Ma Y, Zeng S, Metcalfe DD, et al. The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. *Blood* 2002;99(5):1741–4.
- [41] Zermati Y, De Sepulveda P, Féger F, et al. Effect of the tyrosine kinase inhibitor STI571 on the kinase activity of wild-type and various mutated c-kit receptors found in mast cell neoplasms. *Oncogene* 2003;22(5):660–4.
- [42] Frost MJ, Ferrao PT, Hughes TP, et al. Juxtamembrane mutant V560GKit is more sensitive to imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol Cancer Ther* 2002;1(12):1115–24.
- [43] Akin C, Brockow K, D'Ambrosio C, et al. Effects of tyrosine kinase inhibitor STI571 on human mast cells bearing wild-type or mutated c-kit. *Exp Hematol* 2003;31(8):686–92.
- [44] Pardanani A, Elliott M, Reeder T, et al. Imatinib for systemic mast-cell disease. *Lancet* 2003;362(9383):535–7.
- [45] Pardanani A, Brockman S, Reeder T, et al. Treatment-relevant molecular classification of systemic mast cell disease associated with eosinophilia. *Blood* 2003;102(11):918a.

- [46] Rondoni M, Soverini S, Malagola M, et al. Imatinib seems to be ineffective in patients with systemic mastocytosis carrying the Asp816Val c-kit mutation. *Blood* 2003;102(11):335b–6b.
- [47] Hennessy B, Giles F, Cortes J, et al. Management of patients with systemic mastocytosis: review of the M.D. Anderson Cancer Center Experience. *Am J Hematol* 2004;77(3):209–14.
- [48] Musto P, Falcone A, Sanpaolo G, et al. Inefficacy of imatinib-mesylate in sporadic, aggressive systemic mastocytosis. *Leuk Res* 2004;28(4):421–2.
- [49] Jabbour E, Kantarjian H, Verstovsek S, et al. Imatinib mesylate for patients (pts) with hypereosinophilic syndrome (HES) and systemic mastocytosis (SM): a phase II trial. *Blood* 2004;104(11):271b.
- [50] Akin C, Funo C, Yavuz AS, et al. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 2004;103(8):3222–5.
- [51] Zhang LY, Smith ML, Schultheis B, et al. A novel K509I mutation of KIT identified in familial mastocytosis—in vitro and in vivo responsiveness to imatinib therapy. *Leuk Res* 2006;30(4):373–8.
- [52] Schaller JL, Burklund GA. Case report: rapid and complete control of idiopathic hypereosinophilia with imatinib mesylate. *MedGenMed* 2001;3(5):9.
- [53] Gleich GJ, Leiferman KM, Pardanani A, et al. Treatment of hypereosinophilic syndrome with imatinib mesilate. *Lancet* 2002;359(9317):1577–8.
- [54] Ault P, Cortes J, Koller C, et al. Response of idiopathic hypereosinophilic syndrome to treatment with imatinib mesylate. *Leuk Res* 2002;26(9):881–4.
- [55] Pardanani A, Reeder T, Porrata LF, et al. Imatinib therapy for hypereosinophilic syndrome and other eosinophilic disorders. *Blood* 2003;101(9):3391–7.
- [56] Cortes J, Ault P, Koller C, et al. Efficacy of imatinib mesylate in the treatment of idiopathic hypereosinophilic syndrome. *Blood* 2003;101(12):4714–6.
- [57] Klion AD, Noel P, Akin C, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. *Blood* 2003;101(12):4660–6.
- [58] Tefferi A, Pardanani A, Li CY. Hypereosinophilic syndrome with elevated serum tryptase versus systemic mast cell disease associated with eosinophilia: 2 distinct entities? *Blood* 2003;102(8):3073–4.
- [59] Tefferi A, Lasho TL, Brockman SR, et al. FIP1L1-PDGFRa and c-kit D816V mutation-based clonality studies in systemic mast cell disease associated with eosinophilia. *Haematologica* 2004;89(7):871–3.
- [60] Lemery SJ, Robyn JA, McCoy P, et al. Lineage analysis of the FIP1L1-PDGFRa fusion gene in myeloproliferative hypereosinophilic syndrome. *Blood* 2004;104(11):670a–1a.
- [61] Pardanani A, Ketterling RP, Brockman SR, et al. CHIC2 deletion, a surrogate for *FIP1L1-PDGFRa* fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib therapy. *Blood* 2003;102(9):3093–6.
- [62] Pardanani A, Brockman SR, Patemoster SF, et al. FIP1L1-PDGFRa fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. *Blood* 2004;104(10):3038–45.
- [63] Fabbro D, Ruetz S, Bodis S, et al. PKC412—a protein kinase inhibitor with a broad therapeutic potential. *Anticancer Drug Res* 2000;15(1):17–28.
- [64] Weisberg E, Boulton C, Kelly LM, et al. Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412. *Cancer Cell* 2002;1(5):433–43.
- [65] Stone RM, DeAngelo DJ, Klimek V, et al. Patients with acute myeloid leukemia and an activating mutation in FLT3 respond to a small molecule FLT3 tyrosine kinase inhibitor, PKC412. *Blood* 2005;105(1):54–60.
- [66] Stone RM, Fischer T, Paquette R, et al. Phase IB study of PKC412, an oral FLT3 kinase inhibitor, in sequential and simultaneous combinations with daunorubicin and cytarabine (DA) induction and high-dose cytarabine consolidation in newly diagnosed patients with AML. *Blood* 2005;106(11):121a–2a.

- [67] Chen J, DeAngelo DJ, Kutok JL, et al. PKC412 inhibits the zinc finger 198-fibroblast growth factor receptor 1 fusion tyrosine kinase and is active in the treatment of stem cell myeloproliferative disorder. *Proc Natl Acad Sci USA* 2004;101(40):14479–84.
- [68] Cools J, Stover EH, Boulton CL, et al. PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFR α -induced myeloproliferative disease. *Cancer Cell* 2003;3(5):459–69.
- [69] Gowney JD, Clark JJ, Adelsperger J, et al. Activation mutations of human c-Kit resistant to imatinib are sensitive to the tyrosine kinase inhibitor PKC412. *Blood* 2005;106(2):721–4.
- [70] Gotlib J, Berubé C, Gowney JD, et al. Activity of the tyrosine kinase inhibitor PKC412 in a patient with mast cell leukemia with the D816V KIT mutation. *Blood* 2005;106(8):2865–70.
- [71] Casteran N, De Sepulveda P, Beslu N, et al. Signal transduction by several KIT juxtamembrane domain mutations. *Oncogene* 2003;22(30):4710–22.
- [72] Ning ZQ, Li J, Arceci RJ. Signal transducer and activator of transcription 3 activation is required for Asp(816) mutant c-Kit mediated cytokine-independent survival and proliferation in human leukemia cells. *Blood* 2001;97(11):3559–67.
- [73] Gleixner KV, Mayerhofer M, Aichberger KJ, et al. PKC412 inhibits in vitro growth of neoplastic human mast cells expressing the D816V-mutated variant of KIT: comparison of AMN107, imatinib, and cladribine (2CdA) and evaluation of cooperative drug effects. *Blood* 2006;107(2):752–9.
- [74] Sonneck K, Mayerhofer M, Gleixner KV, et al. Detection of activated STAT5 in neoplastic mast cells in patients with systemic mastocytosis: role of c-kit D816V. *Blood* 2005;106(11):981a.
- [75] Shah NP, Tran C, Lee FY, et al. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 2004;305(5682):399–401.
- [76] Shah NP, Lee FY, Luo R, et al. Dasatinib (BMS-354825) inhibits KIT^{D816V}, an imatinib-resistant activating mutation that triggers neoplastic growth in the majority of patients with systemic mastocytosis. *Blood* 2006;108(1):286–91.
- [77] Schittenhelm MM, Shiraga S, Schroeder A, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. *Cancer Res* 2006;66(1):473–81.
- [78] Schindler T, Bornmann W, Pellicana P, et al. Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. *Science* 2000;289(5486):1938–42.
- [79] Nagar B, Bornmann WG, Pellicana P, et al. Crystal structures of the kinase domain of c-Abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 2002;62(15):4236–43.
- [80] Mol CD, Lim KB, Sridhar V, et al. Structure of a c-kit product complex reveals the basis for kinase transactivation. *J Biol Chem* 2003;278(34):31461–4.
- [81] Mol CD, Fabbro D, Hosfield DJ. Structural insights into the conformational selectivity of STI-571 and related kinase inhibitors. *Curr Opin Drug Discov Dev* 2004;7(5):639–48.
- [82] Mol CD, Dougan DR, Schneider TR, et al. Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. *J Biol Chem* 2004;279(30):31655–63.
- [83] Shah NP. Loss of response to imatinib: mechanisms and management. *Hematology (Am Soc Hematol Educ Program)* 2005;183–7.
- [84] Weisberg E, Manley PW, Breitenstein W, et al. Characterization of AMN107, a selective inhibitor of native and mutant bcr-abl. *Cancer Cell* 2005;7(2):129–41.
- [85] Verstovsek S, Akin C, Giles FJ, et al. Effects of AMN107, a novel aminopyrimidine tyrosine kinase inhibitor, on human mast cells bearing wild-type or mutated codon 816 c-kit. *Blood* 2005;106(11):985a.
- [86] Von Bubnoff N, Gorantla SHP, Kancha RK, et al. The systemic mastocytosis-specific activating cKit mutation D816V can be inhibited by the tyrosine kinase inhibitor AMN107. *Leukemia* 2005;19(9):1670–1.

- [87] Corbin AS, Demehri S, Griswold IJ, et al. In vitro and in vivo activity of ATP-based kinase inhibitors AP23464 and AP23848 against activation-loop mutants of Kit. *Blood* 2005; 106(1):227–34.
- [88] Corbin AS, Griswold IJ, La Rosée P, et al. Sensitivity of oncogenic KIT mutants to the kinase inhibitors MLN518 and PD180970. *Blood* 2004;104(12):3754–7.
- [89] Liao AT, Chien BB, Shenoy N, et al. Inhibition of constitutively active forms of mutant kit by multitargeted indolinone tyrosine kinase inhibitors. *Blood* 2002;100(2):585–93.
- [90] Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc Natl Acad Sci USA* 2005;102(31):11011–6.
- [91] Petti F, Thelemann A, Kahler J, et al. Temporal quantitation of mutant Kit tyrosine kinase signaling attenuated by a novel thiophene kinase inhibitor OSI-930. *Mol Cancer Ther* 2005; 4(8):1186–97.
- [92] Garton AJ, Crew APA, Franklin M, et al. OSI-930: a novel selective inhibitor of kit and kinase insert domain receptor tyrosine kinases with antitumor activity in mouse xenograft models. *Cancer Res* 2006;66(2):1015–24.
- [93] Daley T, Metcalfe DD, Akin C. Association of the Q576R polymorphism in the interleukin-4 receptor alpha chain with indolent mastocytosis limited to the skin. *Blood* 2001;98(3): 880–2.
- [94] Sotlar K, Horny H-P, Simonitsch I, et al. CD25 indicates the neoplastic phenotype of mast cells—a novel immunohistochemical marker for the diagnosis of systemic mastocytosis (SM) in routinely processed bone marrow biopsy specimens. *Am J Surg Pathol* 2004; 28(10):1319–25.
- [95] Eklund JW, Kuzel TM. Denileukin diftitox: a concise clinical review. *Expert Rev Anticancer Ther* 2005;5(1):33–8.
- [96] Fumo G, Akin C, Metcalfe DD, et al. 17-Allyamino-17-demethoxygeldanamycin (17-AAG) is effective in down-regulating mutated, constitutively activated KIT protein in human mast cells. *Blood* 2004;103(3):1078–84.
- [97] Tanaka A, Konno M, Muto S, et al. A novel NF- κ B inhibitor, IMD-0354, suppresses neoplastic proliferation of human mast cells with constitutively activated c-kit receptors. *Blood* 2005;105(6):2324–31.
- [98] Cerveró C, Escribano L, San Miguel JF, et al. Expression of bcl-2 by human bone marrow mast cells and its overexpression in mast cell leukemia. *Am J Hematol* 1999;60(3):191–5.
- [99] Santos DD, Hatjiharissi E, Tournilhac O, et al. CD52 is expressed on human mast cells and is a therapeutic target for the anti-CD52 monoclonal antibody Campath-1H in Waldenstrom's macroglobulinemia and mast cell disorders. *Blood* 2004;104(11):304b.
- [100] Kondo R, Mayerhofer M, Gleixner K, et al. Heme-oxygenase-1 (HO-1): a novel kit D816V-dependent target in neoplastic human mast cells (HMC-1). *Blood* 2005;106(11):983a.
- [101] Boehm A, Florian S, Gleixner K, et al. Inhibition of growth of neoplastic mast cells by CD44 mAb A3D8 is associated with G1 cell cycle arrest and apoptosis. *Blood* 2005; 106(11):982a.
- [102] Aichberger KJ, Mayerhofer M, Gleixner KV, et al. Identification of Mcl-1 as a novel target in neoplastic mast cells and demonstration of cooperative growth-inhibitory effects of mcl-1 antisense oligonucleotides, PKC412, and AMN107. *Blood* 2005;106(11):982a.
- [103] Demehri S, Corbin AS, Loriaux M, et al. Murine model of aggressive systemic mastocytosis: a disease caused by oncogenic kit. *Blood* 2004;104(11):666a–7a.