Review in translational hematology

After chronic myelogenous leukemia: tyrosine kinase inhibitors in other hematologic malignancies

Martha Wadleigh, Daniel J. DeAngelo, James D. Griffin, and Richard M. Stone

Introduction

Tyrosine kinases phosphorylate proteins on tyrosine residues, producing a biologic signal that influences many aspects of cellular function including cell growth, proliferation, differentiation, and death. Constitutive or unregulated activity through mutation or overexpression of these enzymes is a common pathologic feature in many acute and chronic leukemias. Inhibition of tyrosine kinases represents a strategy to disrupt signaling pathways that promote neoplastic growth and survival in hematologic malignancies and likely in other neoplasias as well. This review focuses on tyrosine kinases that have been implicated in the pathogenesis of hematologic diseases other than chronic myelogenous leukemia and discusses the evidence for the use of small molecules to target these kinases. (Blood. 2005;105:22-30)

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Mutated tyrosine kinases in chronic myeloproliferative disorders

As noted, the successful targeting of BCR/ABL by imatinib mesylate has intensified the search for tyrosine kinases that play similar pivotal roles in the molecular pathogenesis of other malignancies. Chromosomal translocations that generate fusion genes involving tyrosine kinase receptors have been identified in a small fraction of myeloproliferative disorders (MPDs). These fusion genes represent ideal targets for tyrosine kinase inhibition because of their presumed central role in the neoplastic process of these disorders.

PDGFR-β

Platelet-derived growth factor receptor β (PDGFR-β) belongs to the type III receptor tyrosine kinase family characterized by a transmembrane domain, a juxtamembrane domain, a split kinase domain containing a kinase insert region and a C-terminal tail (Figure 1).49 Ligand binding to the receptor initiates the signaling cascade by inducing receptor dimerization, leading to activation of the kinase domain, and resulting in autophosphorylation. The autophosphorylated tyrosine residues then act as intracellular docking sites for second messengers involved in mitogenesis, cytoskeletal rearrangements, and chemotaxis.49,50

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PDGFR-β rearrangements are associated with chronic myelomonocytic leukemia (CMML), an MPD characterized by dysplastic monocytosis, bone marrow fibrosis, eosinophilia, and progression to AML. In 1994, Golub et al demonstrated that the chromosomal abnormality t(5;12)(q31-q33;p13) found in rare cases of CMML resulted in a fusion gene linking TEL (ETS variant of the kinase) and PDGFR-β, a transcription factor, with PDGFR-β activation is linked to the eosinophilia seen in these patients.5 Since the initial description, multiple PDGFR-β rearrangements have been identified, which characterize other cases of CMML (Table 2).6-12,51,53 These chromosomal rearrangements result in fusion proteins such that PDGFR-β activity is ligand independent and constitutively activated. The breakpoint of the PDGFR-β gene rearrangements is often the transmembrane and intracellular domains of the kinase, but the fusion gene partner replaces its extracellular ligand-binding domain and is likely to function to induce ligand-independent dimerization, and ultimately, constitutive activation of the kinase.54 The fusion protein ETV6-PDGFR-β alone causes hematopoietic cell lines to become growth factor independent55 and causes a CMML-like disorder in transgenic mice, implicating it in the molecular pathogenesis of the disorder.56,57

Imatinib mesylate inhibits PDGFR-β suggesting it is a rational therapy for patients with MPDs associated with activated PDGFR-β receptors. In preclinical models, imatinib mesylate inhibited cell lines expressing ETV6-PDGFRB58 as well as the RAB5-PDGFR-β fusion proteins.59 In mouse models of CMML transformed by ETV6-PDGFR-β, imatinib mesylate treatment resulted in statistically significant prolonged survival compared to controls.60 Given these results, imatinib mesylate was studied in patients with PDGFR-β translocations. In an updated report by Apperley et al, 9 patients with chromosomal translocations involving PDGFR-β (5q33) were treated with 200 to 800 mg imatinib mesylate daily.50,61 Five of the 9 patients had the ETV6-PDGFR fusion gene. All patients had leukocytosis and eosinophilia. At a median follow-up of 14 months, all patients responded rapidly. All but 1 patient had a complete cytogenetic response and 2 patients attained a complete molecular remission as defined by polymerase chain reaction (PCR) negativity for the ETV6-PDGFRB transcript.61 Similarly, a patient with CMML and the RAB5-PDGFR fusion gene also responded to imatinib mesylate treatment following relapse after stem cell transplantation.59 In contrast, imatinib mesylate has not been effective in patients with CMML without a PDGFR-β gene rearrangement.62

**Table 1. Mutated tyrosine kinases in human malignancies**

<table>
<thead>
<tr>
<th>Tyrosine kinase</th>
<th>Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td>CML</td>
</tr>
<tr>
<td></td>
<td>ALL</td>
</tr>
<tr>
<td></td>
<td>AML</td>
</tr>
<tr>
<td>TEL-ABL</td>
<td>ALL</td>
</tr>
<tr>
<td></td>
<td>AML</td>
</tr>
<tr>
<td>PDGFR-β</td>
<td>CMML</td>
</tr>
<tr>
<td></td>
<td>AML</td>
</tr>
<tr>
<td>TEL-Jak2</td>
<td>ALL</td>
</tr>
<tr>
<td>ALK-1</td>
<td>Anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>RET</td>
<td>MEN2</td>
</tr>
<tr>
<td>EGFR</td>
<td>Papillary thyroid carcinoma</td>
</tr>
<tr>
<td></td>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td></td>
<td>Nonsmall cell lung cancer</td>
</tr>
<tr>
<td>TPR-TRK</td>
<td>Papillary thyroid cancer</td>
</tr>
<tr>
<td>TPR-MET</td>
<td>Gastric carcinoma</td>
</tr>
<tr>
<td>FLT3</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
</tr>
<tr>
<td></td>
<td>ALL</td>
</tr>
<tr>
<td>PDGFR-α</td>
<td>HES</td>
</tr>
<tr>
<td></td>
<td>GIST</td>
</tr>
<tr>
<td>c-KIT</td>
<td>AML</td>
</tr>
<tr>
<td></td>
<td>Mastocytosis/mast cell leukemia</td>
</tr>
<tr>
<td></td>
<td>GIST</td>
</tr>
<tr>
<td>c-met</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>c-fms</td>
<td>AML, MDS</td>
</tr>
<tr>
<td>FGFR</td>
<td>MPD/AML</td>
</tr>
</tbody>
</table>

MEN2 indicates multiple endocrine neoplasia 2 syndrome.

**Figure 1. Schematic depicting structure of receptor tyrosine kinase type III family.** Structure of receptor tyrosine kinase type III family, on the left, shows 5 immunoglobulin-like ligand-binding domains (JM indicates the juxtamembrane domain) and two split kinase domains. FLT3 receptor has mutations identified in the JM as well as in the second split kinase domain at codon 835. c-KIT has a mutation in the immunoglobulin extracellular domain at a highly conserved aspartate residue at codon 419 as well as in the split kinase domain at codon 816, which is frequently seen in mast cell leukemia or mastocytosis.

**Table 2. Fusion proteins and cytogenetic abnormalities involving PDGFR-β**

<table>
<thead>
<tr>
<th>Cytogenetic abnormality</th>
<th>Tyrosine kinase fusion protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(5;12)(q33;q13)</td>
<td>TEL-PDGFR-β</td>
</tr>
<tr>
<td>t(5;7)(q33:p11)</td>
<td>HIP1-PDGFR-β</td>
</tr>
<tr>
<td>t(5;10)(q33;p21)</td>
<td>H4-PDGFR-β</td>
</tr>
<tr>
<td>t(5;17)(q33;p13)</td>
<td>RAB5-PDGFR-β</td>
</tr>
<tr>
<td>t(5;14)(q33;p32)</td>
<td>CEV14-PDGFR-β</td>
</tr>
<tr>
<td>t(5;14)(q33;p24)</td>
<td>NIN-PDGFR-β</td>
</tr>
<tr>
<td>t(1;5)(q23; p33)</td>
<td>Myeloma-1-PDGFR-β</td>
</tr>
<tr>
<td>t(5;17)(q33; p11.2)</td>
<td>HCMOGT-PDGFR-β</td>
</tr>
</tbody>
</table>
This patient was later unresponsive to escalated doses of imatinib mesylate. These observations led Cools and colleagues to closely examine known imatinib mesylate targets for possible involvement in the pathogenesis of HES. One patient in this clinical series was noted to have a t(1;4) chromosomal translocation. This focused attention on the PDGFR-α locus, known to be located at chromosome 4q12. In an elegant example of molecular detective work, Cools et al found that the kinase domain of PDGFR-α was fused to a previously uncharacterized gene also found on chromosome 4, and subsequently named FIP1-like1 (FIP1L1). Further analysis of patient samples found that 9 of 16 patients contained the same novel fusion gene, 8 of whom were men. Interestingly, this fusion gene is not the result of a chromosomal translocation, but rather an interstitial deletion.

Sequence analysis of the PDGFR-α kinase domain in the patient with clinical resistance to imatinib mesylate demonstrated that the fusion protein had acquired a substitution of isoleucine for tyrosine at codon 674 (T674I) in the ATP-binding region, an analogous position to the common T315I mutation found in BCR/ABL that confers clinical resistance to imatinib mesylate. This verifies that the fusion gene is the target for imatinib mesylate in HES. The inhibitory concentration of 50% (IC50) of imatinib mesylate in BA/F3 cell lines transformed with the novel fusion gene is 3.2 nM; thus FIP1L1-PDGFRα is more sensitive to imatinib mesylate than BCR/ABL, explaining why patients with HES responded to imatinib mesylate at doses of 100 mg/d, well below the established 400 mg/d used in patients with CML. Only 60% of the patients who responded to imatinib mesylate in this series had the novel fusion gene, suggesting that the remaining 40% of responders possibly contain other mutated tyrosine kinases yet to be identified.

An interesting molecular overlap has been identified between the rare disorders of HES and systemic mast cell disease (SMCD). The clinical observation that patients with HES who respond to imatinib mesylate tend to have elevated tryptase levels, combined with the evidence that 3 patients with SMCD and a peripheral myeloproliferative syndrome, a rare MPD with associated eosinophilia, had complete responses to imatinib mesylate, led investigators to search for evidence of the FIP1L1-PDGFRα fusion gene in these patients. Using fluorescent in situ hybridization (FISH), the novel fusion gene was found in all 3 responding patients. Thus, in this fraction of patients with SMCD, the therapeutic target of imatinib mesylate may not be related to c-KIT as previously thought but rather to FIP1L1-PDGFR-α.

### Other mutated kinases in chronic MPDs

Classic translocations resulting in novel fusion genes have been identified in several chronic MPDs and may be additional potential targets for small-molecule tyrosine kinase inhibitors. The 8p myeloproliferative syndrome, a rare MPD with associated eosinophilia that rapidly transforms into acute leukemia, has been linked to translocations involving chromosome 8. The classic translocation, t(8;13)(p11;q12), which was first described in this disorder, juxtaposes the fibroblast growth factor receptor (FGFR) to a zinc finger motif, generating a fusion protein with constitutive activity. In addition, other fusion proteins, such as TEL-ABL, or TEL-JAK2, or BCR-JAK2, are also potential targets for tyrosine kinase inhibition. In one brief report, a patient with an aggressive MPD characterized by a t(9;12) translocation (TEL-ABL) was treated with imatinib mesylate 600 mg daily and had a considerable, although transient, clinical response to this treatment. Therefore, patients with disorders characterized by these rare translocations may derive benefit from treatment with small-molecule inhibitors and this is an active area of investigation.

### Mutated tyrosine kinases in acute leukemias

#### FLT3

Recently, the Fms-like tyrosine kinase 3 (FLT3) receptor has been identified as a potential therapeutic target in acute myelogenous leukemia (AML). The FLT3 receptor also belongs to the type III class of receptor tyrosine kinases (Figure 1) and was cloned in 1991. It plays an important role in normal hematopoiesis as well as leukemogenesis. FLT3 is expressed on stem cell progenitors as well as in 70% to 100% of patients with AML, but it is uncommon in B-cell ALL, T-cell ALL, and CML. Its endogenous ligand is FLT3 ligand, a growth factor for immature myeloid cells and stem cells.

Mutations in FLT3 were first reported in 1996 when internal tandem duplications (ITDs), repeats of 5 to 40 or more amino acids in the juxtamembrane region, were discovered in patients with AML. Subsequent studies have demonstrated that these FLT3-ITD are found in approximately 25% of all cases of AML, 3% to 5% of myelodysplastic syndromes (MDSs), and infrequently in ALL. FLT3 mutations are more common in patients with AML with normal cytogenetics and t(15;17). The mutation is associated with increased peripheral blood leukocyte counts and a worse prognosis compared to patients without the mutation. In patients with t(15;17), FLT3 mutations are associated with a higher white blood cell count and an increased induction death rate and therefore a lower complete remission rate, but no increase in relapse rate. The loss of the wild-type FLT3 allele in conjunction with a FLT3-ITD has been shown to confer an even poorer prognosis.

Mutations within the FLT3 activation loop of the kinase have also been identified. Approximately 7% of patients with AML have a substitution of aspartic acid at codon 835, most commonly for a tyrosine residue (D835Y), but other substitutions have been reported. In 2 patients, a 6–base pair (bp) insertion between codons 840 and 841 in the activation loop of FLT3 has been reported. Interestingly, although infants with mixed lineage leukemia (MLL) do not have FLT3-ITD mutations, a significant fraction has point mutations in the activation loop. These mutations may be at either codon 835 or 836. Infants with MLL gene translocation also tend to express wild-type FLT3 receptors at exceptionally high levels.

The result of either an FLT3-ITD mutation or an activating loop mutation is constitutive activation of the kinase. When wild-type and mutant FLT3 genes are transduced into interleukin 3 (IL-3)–dependent cell lines, including 32D and BA/F3 cells, the mutant FLT3-transfected cells (FLT3-ITD) become independent of growth factor. In contrast, the wild-type FLT3-transfected cells have minimal proliferation despite stimulation with FLT3 ligand. Furthermore, the cells expressing mutant FLT3-ITD demonstrate constitutive activation of signal transducer and activator of transcription 5 (STAT5) and mitogen-activated protein (MAP) kinases, the signaling pathways of FLT3. When FLT3-ITD mutants identical to those identified from primary human leukemia samples were transduced into primary mouse bone marrow cells using a retrovirus, an MPD but not overt leukemia developed. This suggests that FLT3-ITD is sufficient to induce a proliferative signal similar to that of BCR/ABL in chronic-phase CML but not sufficient to cause acute leukemia unless paired with other cellular events. The need
for a second cooperating mutation has been confirmed in a murine model using promyelocytic leukemia-retinoic acid receptor α (PML-RARA) transgenic mice transduced with an activated FLT3 allele.90 Introduction of the mutated FLT3 allele into the background of the PML-RARA mouse resulted in a more rapid transformation to leukemia, with a range of 62 to 299 days versus 8.5 months in mice without the mutated FLT3 allele.90

These preclinical studies demonstrated that the FLT3-ITD mutation is a potential therapeutic target in AML. The first studies to validate this concept used the compounds herbimycin a, AC1296, and AG1295.91-93 These compounds inhibited FLT3-ITD–transformed cells and prolonged the development of leukemia phenotypes in mice with FLT3-ITD–induced MPDs.

Newer FLT3 inhibitors have been developed that have shown promise in preclinical models and have moved to clinical trials in humans. At least 4 compounds are currently under development (Table 3). All have been found to be active in preclinical in vitro and animal models of FLT3-ITD disease.94,98,102,103 CEP-701 (Cephalon, West Chester, PA) is a novel indolocarbazole derivative that inhibits the autophosphorylation of wild-type and constitutively activated FLT3 in vitro with an IC50 of 2 to 3 nM.99 Results of the phase 1/2 trial using single-agent CEP-701 in patients with relapsed, refractory, or poor-risk AML and activating FLT3 mutations have recently been reported.99 The first 3 patients were treated with 40 mg by mouth twice a day, but ex vivo analysis showed incomplete inhibition of FLT3 autophosphorylation and no response was seen. The next 14 patients were treated with 60 mg twice a day, 3 of whom were escalated to 80 mg twice a day. Of these 14 patients, 4 had a decrease in peripheral blood leukemic blasts to less than 5%, with improvement in absolute neutrophil counts, and one patient had a decrease in bone marrow blasts to less than 5%. Grade 3 and 4 toxicities included febrile neutropenia, which occurred in 11 patients. More common less severe grade 1 and 2 side effects included nausea, emesis, and fatigue.

MLN518 (CT53518) is a piperazinyl quinazoline. In a phase 1 trial, 40 patients with AML or myelodysplasia were treated with escalating doses of the compound.104 The dose-limiting toxicity of MLN518 was reversible, generalized weakness, which occurred in 3 of 9 patients treated at doses of 525 mg or more. This toxicity correlated with plasma concentrations more than 2000 nM, well above the level associated with inhibition of FLT3 autophosphorylation. Stabilization of peripheral blood counts, for longer than 5 months was seen in 2 patients with wild-type FLT3. In one patient with FLT3-ITD, the bone marrow blast count decreased from 80% to 15% with reductions of the peripheral blood blast count over the first 288 days of therapy. The phase 2 study of this drug will evaluate response at the maximum tolerated dose of 525 mg twice daily dose in patients with relapsed or refractory AML with confirmed FLT3-ITD. Of note, unlike CEP-701, MLN518 is not active against the D835Y mutation in the activation loop of FLT3.103

PKC412 (Novartis Pharmaceuticals, Basel, Switzerland), an N-benzoylstaurosporine, originally developed as a vascular endothelial growth factor receptor (VEGFR) and protein kinase C (PKC) inhibitor, is one of the more developed FLT3 inhibitors. A phase 1 trial of PKC412 in patients with advanced solid malignancies showed it to be a well-tolerated oral therapy. The most frequent treatment-related toxicities were nausea, vomiting, fatigue, and diarrrhea.95 A phase 2 trial of PKC412 at 75 mg by mouth 3 times a day was undertaken in patients with AML that expressed either a FLT3-ITD or an activation loop mutation.96,97 Patients had to have relapsed or refractory disease or not be candidates for cytotoxic chemotherapy. Of the first 14 patients treated, 12 had a more than 50% reduction in peripheral blasts compared to baseline including 2 patients who cleared blasts by day 29. Five patients had reduction in bone marrow blasts by more than 50%, of whom one had less than 5% blasts with normal peripheral counts on day 96 of treatment. Furthermore, a decrease in FLT3 autophosphorylation

### Table 3. FLT3 inhibitors in clinical development

<table>
<thead>
<tr>
<th>Tyrosine kinase inhibitor</th>
<th>Class</th>
<th>Receptor activity†</th>
<th>FLT3 IC50‡</th>
<th>Clinical trials</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC41294,95,97,113</td>
<td>PKC</td>
<td>KDR, PDGFR, KIT</td>
<td>528 nM</td>
<td>Phase 2: AML with/without FLT3-ITD</td>
<td>Nausea, emesis, fatigue</td>
</tr>
<tr>
<td>CEP-70198–102</td>
<td>Indolocarbazole</td>
<td>FLT3, TRKA, KDR, PDGFR</td>
<td>2–3 nM</td>
<td>Phase 2: AML with FLT3-ITD</td>
<td>Nausea, emesis, fatigue</td>
</tr>
<tr>
<td>CT5318103–105</td>
<td>Piperazinyl quinazoline</td>
<td>KIT, PDGFR, FLT3, FMS</td>
<td>170–220 nM</td>
<td>Phase 1: AML/MDS with/without FLT3-ITD</td>
<td>Generalized weakness, fatigue, nausea, vomiting</td>
</tr>
<tr>
<td>SU5416106–109</td>
<td>Indolinone</td>
<td>FLT3, KDR, PDGFR</td>
<td>250 nM</td>
<td>Phase 2: Refractory AML/MDS/MM</td>
<td>Fatigue, nausea, sepsis, bone pain</td>
</tr>
<tr>
<td>SU11248110,111</td>
<td>Indolinone</td>
<td>FLT3, KDR, PDGFR</td>
<td>10 nM</td>
<td>Phase 1: AML</td>
<td>Nausea, fatigue, cardiac dysfunction‡</td>
</tr>
</tbody>
</table>

MM indicates multiple myeloma.
†Receptor activity in descending order of potency.
‡Cardiac toxicity observed in AML patients with prior anthracycline use.
relative to total FLT3 protein occurred in 75% of patients’ blast
tissue, indicating the target was inhibited. A phase 2 trial was
also undertaken in patients with wild-type FLT3 and no significant
responses beyond transient hematologic improvement were seen.113
SU5416, SU6668, and SU11248 (Sugen, San Francisco, CA),
which are indolines, also have FLT3 inhibitor activity, whereas
SU6668 has no significant effect on FLT3 (IC50 > 50 μM).106,110 A
phase 1 study of SU11248 has been completed in patients with
AML.111 Five patients had FLT3 gene mutations (3 ITDs and 2
activating loop mutations). The investigators reported a decrease in
peripheral blast counts in some patients following a single dose of
SU11248; however, data on response rates are not yet available.

The initial results of these early FLT3 inhibitor studies indicate
that these compounds have biologic activity against AML. How-
ever, few patients have achieved a complete or durable remission
with single-agent therapy. Diverse factors may contribute to the
rather modest activity of FLT3 inhibitors in AML. Incomplete
kinase inhibition has been seen in some cases. However, primary
resistance was also observed in the presence of complete kinase
inhibition, perhaps because FLT3 mutations are a late event in the
pathogenesis of AML, and thus may not be essential to leukemogen-
esis per se.99 This is in contrast to BCR-ABL, which is thought
to initiate CML. In addition, the patients studied were heavily
pretreated. In this setting, a low response rate is not unexpected, in
analogy to myeloid blast crisis of CML, where patients pretreated
for blastic transformation responded less well to imatinib mesylate
than patients without prior therapy.45 Nonetheless, similar to
imatinib mesylate, these agents are well tolerated with few side
effects and their toxicity profiles are well suited to combination
with cytotoxic chemotherapy. To have a significant clinical impact,
it is apparent that FLT3 inhibitors will need to be combined with
chemotherapy or even other targeted therapy, much in the way that
all-trans-retinoic acid (ATRA) is for acute PML.

c-KIT

c-KIT is another receptor tyrosine kinase in the type III subfamily
(Figure 1). It is expressed on hematopoietic progenitor cells, mast
cells, germ cells, and the pacemaker cells of the gut.114 c-KIT is
expressed in a variety of human malignancies and is mutated and
constitutively activated in gastrointestinal stromal tumor (GIST),
mastocytosis/mast cell leukemia, and AML. Activating
mutations can occur in many different exons of the c-KIT gene and
activation of signaling pathways leads to proliferation, differentia-
tion, migration, and survival of hematopoietic stem cells, mast
cells, melanocytes, and germ cells.28,115

Valine substituted for aspartic acid at codon 816 (D816V
mutation) in the activation loop of the kinase catalytic domain is
the most common activating mutation in c-KIT (Figure 1). It is
predominantly found in systemic mastocytosis or mast cell leuke-
emia but has also been detected in patients with MPDs and some
cases of AML.49,114 This mutation results in a 10-fold increase in
the specific activity and a 9-fold increase in ATP affinity.114
Although imatinib mesylate inhibits wild type c-KIT, the D816V
mutation is resistant to imatinib mesylate.116 This is likely related to
the increased affinity of the mutated c-KIT for ATP or to
conformational changes in the activation loop of the receptor that
inhibits binding of imatinib mesylate.

Recently, investigators demonstrated that PKC412 inhibits
BA/F3 cell lines stably transformed by the c-KIT mutations,
D816Y and D816V, with an IC50 of 20 and 30 nM, respectively.117
These results were extended clinically when a 48-year-old woman
with mast cell leukemia and a D816V mutation underwent
treatment with PKC412. Treatment with PKC412, 100 mg twice
daily, resulted in clinical improvement, with a reduction of mast
cells and a decrease in myeloblasts from 5% to 10% in the marrow
at diagnosis to less than 5% after 2 months of therapy. Further
follow-up and larger studies are warranted to determine the true
efficacy of PKC412 or other tyrosine kinase inhibitors in diseases
characterized by D816V c-KIT mutations.

Two isoforms of mutated c-KIT have been identified in AML,
both associated with the cytogenetic abnormalities of t(8;21) or
inv(16).28,29 One involves aspartate 816 previously mentioned; the
other involves a highly conserved aspartic acid residue at position
419, located in the extracellular domain (Figure 1).28 The D816
mutation is identical to that in mastocytosis and thus is not
amenable to targeted therapy with imatinib mesylate. It is unclear if
the other mutation is amenable to targeting with imatinib mesylate,
as it has not been reported to be an activating mutation.28 Targeting
of c-KIT mutations in AML is further inhibited by the fact that less
than 8% of AML patients have c-KIT mutations.28

Overexpression of tyrosine kinase as target
c-KIT

Although c-KIT is rarely mutated, it is expressed in approximately
60% to 80% of cases of AML and “overexpressed” in a fraction of
those cases.118-120 The addition of stem cell factor (SCF), the
endogenous ligand for c-KIT, results in the proliferation of KIT+
AML blast cell lines such as M07E.121 Because a significant
percentage of patients with AML overexpress wild-type c-KIT, and
imatinib mesylate has been shown to be selective inhibitor of
c-KIT, the use of imatinib mesylate in c-KIT+ AML has been
proposed. The rationale for treatment is based on the hypothesis
that high levels of receptor in vivo might confer a growth
advantage in the marrow because of the presence of abundant KIT
ligand present in stromal cells. In a phase 2 pilot study, 21 patients
with relapsed or refractory c-KIT+ AML were treated with
imatinib mesylate 600 mg/d.122 Five responses were seen; 2 patients had
a complete hematologic remission, 2 had partial responses (PRs),
and another patient had no evidence of leukemia. Western blotting
of primary blasts taken from patients confirmed that c-KIT was
activated, but mutational analysis did not identify any previously
identified c-KIT mutations. Furthermore, activity of imatinib
mesylate did not correlate with decreased c-KIT phosphorylation.
Therefore, it is difficult to determine the cause for the modest
activity of imatinib mesylate in this disease.

As noted, the small molecules SU5416 and SU6668 inhibit
c-KIT, as well as VEGFR-2, FGFR, FLT3, and PDGFR.107 In
preclinical models using M07E cells, a human myeloid leukemia
cell line, SU5416 and SU6668 inhibited tyrosine autophosphoryla-
tion of c-KIT in a dose-dependent manner. In addition, when used
on leukemic blasts from c-KIT+ patients, both compounds inhibited
SCF-induced phosphorylation of c-KIT and induced apopto-
sis.107 In Europe, SU5416 has been tested in patients with
refractory c-KIT+ AML.108,123 In this trial 43 patients with AML
whose leukemic blasts overexpressed c-KIT were treated with
twice-weekly infusions of 145 mg/m2 SU5416. Of 25 evaluable
patients, only one patient had a complete response, defined as less
than 5% blasts in the bone marrow, absence of circulating blasts in
the peripheral blood, but without normalization of blood counts,
and 7 patients had a PR (defined as reduction of blasts in blood or
bone marrow by at least 50%), which lasted 1 to 5 months. None of

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the patients who responded had FLT3-ITDs but the presence of FLT3 activating loop mutations was not evaluated and targeting of c-KIT was not demonstrated in ancillary studies.

**VEGF**

Similar to c-KIT in AML, the VEGFRs are tyrosine kinases that are overexpressed in hematologic malignancies. Their ligand, VEGF, is responsible for many endothelial cell functions, which are regulated through the receptors: VEGFR-1 (FLT1), VEGFR-2, and VEGFR-3 (FLT4).124 VEGF has been implicated in tumor angiogenesis and may play a role in the pathophysiology of hematologic malignancies by regulation of bone marrow angiogenesis through autocrine and paracrine mechanisms. Murine models using homoygous gene knockouts suggest differential roles for VEGFR-1 and VEGFR-2; VEGFR-2 expression is important in angiogenesis and vasculogenesis, whereas VEGFR-1 may be more important for endothelial organization and remodeling.125,126

Microvessel density is an indirect way of measuring angiogenesis and is elevated in the bone marrow of patients with MDS and AML and decreases following chemotherapy in responsive disease.127,128 Immunohistochemical analysis of bone marrow biopsies reveal that changes in microvessel density parallels changes in VEGF and VEGFR-2 expression in patients with AML implicating VEGF signaling in angiogenesis.129 Furthermore, cellular VEGF levels appear to correlate inversely to survival in patients with AML.130 As a result, a number of receptor tyrosine kinase inhibitors are being used in hematologic malignancies to block angiogenesis through VEGF inhibition. These include SU5416, SU11248, PKC412, PTK787, and others.131,132

In the large European study using SU5416 in patients with c-KIT+ AML, correlative studies were performed to assay VEGF inhibition.108 The investigators evaluated VEGF mRNA expression, measured by PCR and found that patients who responded to SU5416 expressed high levels of VEGF mRNA prior to treatment. In contrast, the nonresponders did not have high VEGF expression prior to treatment. Decreased microvessel density in the bone marrow of responders correlated with VEGF expression. Although these results are intriguing, it remains unclear whether the clinical activity seen with this compound is due to VEGF or c-KIT or FLT3 inhibition, or if these results are an epiphenomenon.

Furthermore, large studies have not been able to demonstrate a clear relationship between changes in microvessel density and clinical response133 and the best assay for measuring changes in angiogenesis remains unclear. Recently, circulating endothelial cells (CECs) have been shown to be a promising, noninvasive surrogate marker for angiogenesis in preclinical studies using murine models of cancer, as well as in several clinical studies of angiogenesis inhibitors.134-136

**Problems in the development of tyrosine kinase inhibitors as therapeutic agents**

The most important issue pertaining to tyrosine kinase inhibition is whether a putative target is relevant in the pathogenesis of the disease in question. Mutation-independent overexpression, as seen with VEGF and c-KIT, may well represent an epiphenomenon and may not be important in the neoplastic pathophysiology. As a result, inhibition of the overexpressed target might have little clinical benefit. Furthermore, a mutation may be a later mutational event in the pathophysiology of the disease and inhibition of this “secondary” target may only inhibit the proliferative thrust, but not delete the leukemic clone. Ancillary studies from the clinical trial of CEP-701 demonstrated that despite more than 85% FLT3 inhibition in 2 patients there was no clinical impact on disease.99 Other studies suggest that FLT3 mutational status changes from diagnosis to relapse and different FLT3 mutation clones may be dominant from one stage of disease to another.137,138 If an FLT3-ITD is a later event, then there will be FLT3+, or wild-type leukemic clones, and thus FLT3 inhibition may preferentially select for these clones causing resistance. Key to further development of these inhibitors will be the identification and verification of their therapeutic targets given their broad specificity.

Resistance remains one of the greatest challenges facing the development of tyrosine kinase inhibitors. Imatinib mesylate resistance in patients with CML serves as a cautionary note for other drugs under development. The mechanism of action of these small-molecule inhibitors is thought to be similar; that is, blocking of ATP binding to the kinase domain.139,140 Thus, mechanisms of imatinib mesylate resistance in CML may be analogous to resistance to other small-molecule inhibitors. In patients with CML, point mutations in BCR/ABL have been identified at the time of resistance that are not always detectable in samples taken prior to the initiation of therapy.141,142,143 These point mutations predominantly occur in the kinase domain, and more than a dozen different mutations have been reported.141-143,145 This same mechanism of resistance has been demonstrated in HES as well as with FLT3 inhibitor resistance, in vitro.146 Data are emerging from the FLT3 inhibitor clinical trials, which will help to determine clearly the mechanisms of resistance to these small molecules.

Strategies to overcome resistance may include the combination of small molecules with chemotheraphy or even other signal transduction inhibitors. For example, the development of FLT3 inhibitors in AML will probably require a combination of small molecules with chemotherapy for full activity. Preclinical studies demonstrate that the schedule of administration and drug combinations are important factors for efficacy.147 Giving a tyrosine kinase inhibitor prior to chemotherapy could prevent DNA synthesis, thereby making the leukemic blasts relatively resistant to cell cycle–active chemotherapy agents. In addition, inhibitors with different binding specificities could be combined. In vitro studies have demonstrated that different FLT3 mutations have varying sensitivities to the different FLT3 inhibitors.147 This suggests that molecular analysis of relapse samples may be useful in determining which inhibitor to use or how best to combine inhibitors.

**Conclusion**

Tyrosine kinase inhibition as a therapeutic strategy has been proven successful by the treatment of chronic phase CML with imatinib mesylate. This rationale has been translated successfully to other chronic hematologic malignancies such as CMML and HES that may arise from a mutation of a single tyrosine kinase. This strategy is more complicated when extended to advanced diseases such as AML. In some cases, tyrosine kinases are not mutated but overexpressed resulting in increased autocrine signaling through these receptors. Targeting this mechanism of signal transduction activation has not resulted in overwhelming benefit or response, as exemplified by c-KIT, wild-type FLT3, and VEGF inhibition. Furthermore, if an FLT3 inhibitor works it will be important to determine if efficacy is only due to inhibition of activating FLT3 mutations or due to inhibition of other targets. Ancillary studies
may help to determine which targets are inhibited, but there is no substitute for clinical trials in this regard. Furthermore, given the low frequency of complete remissions with single-agent therapy, FLT-3 inhibitors will need to be combined with cytoreductive chemotherapy. As the use of tyrosine kinases in chronic and acute leukemias has expanded, new targets such as the FIP1L1-PDGFR-α rearrangement in HES and SMCD have been discovered. The search is on to identify other novel targets. Moleculally targeted therapy remains a promising area of development that will continue to expand, as we understand more about the molecular pathogenesis of hematologic malignancies.

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