Crenolanib Inhibits the Drug-Resistant PDGFRA D842V Mutation Associated with Imatinib-Resistant Gastrointestinal Stromal Tumors

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Abstract

**Purpose:** To determine the potential of crenolanib, a potent inhibitor of PDGFRA, to treat malignancies driven by mutant PDGFRA.

**Experimental Design:** The biochemical activity of crenolanib was compared with imatinib using a panel of PDGFRA-mutant kinases expressed in several different cell line models, including primary gastrointestinal stromal tumors (GIST) cells. The antiproliferative activity of crenolanib was also studied in several cell lines with PDGFRA-dependent growth.

**Results:** Crenolanib was significantly more potent than imatinib in inhibiting the kinase activity of imatinib-resistant PDGFRA kinases (D842I, D842V, D842Y, DI842-843IM, and deletion I843). For example, crenolanib was 135-fold more potent than imatinib against D842V in our isogenic model system, with an IC50 of approximately 10 nmol/L. The relative potency of crenolanib was further confirmed in BaF3 and primary GIST cells expressing PDGFRA D842V. In contrast, imatinib was at least 10-fold more potent than crenolanib in inhibiting the V561D mutation. For all other tested PDGFRA mutations, crenolanib and imatinib had comparable potency.

**Conclusions:** Crenolanib is a potent inhibitor of imatinib-resistant PDGFRA kinases associated with GIST, including the PDGFRA D842V mutation found in approximately 5% of GISTs. The spectrum of activity of crenolanib suggests that this drug is a type I inhibitor (inhibitor of activated conformation of kinase). Based in part on these results, a phase II clinical study of this agent to treat GIST with the PDGFRA D842V mutation has been initiated.

Introduction

Activating mutations of receptor tyrosine kinases KIT or PDGFRA are fundamental to the pathogenesis of most gastrointestinal stromal tumors (GIST). More than 80% of GISTs express mutated, constitutively active KIT receptor, another 5% to 10% express mutated PDGFRA, whereas 10% to 15% of cases have no mutations in either of these kinases (wild-type GIST, WT; refs. 1, 2).

The use of KIT/PDGFRa tyrosine kinase inhibitors (TKI) has transformed the treatment of localized and advanced GIST. Front-line treatment of metastatic GIST with imatinib produces high rates of clinical benefit (PR + SD) and progression-free survival in the range of 18 to 24 months. In addition, median overall survival of patients with metastatic GIST is now approximately 50 to 60 months (2–4). In the second-line setting, treatment with sunitinib increases progression-free and overall survival of patients with imatinib-resistant disease compared with placebo (5). Patients with primary KIT exon 11 mutations have the longest progression-free and overall survival during imatinib treatment (3, 6). Congruent with this observation, KIT exon 11–mutant kinases are potently inhibited by imatinib and sunitinib in vitro (7).

However, treatment of PDGFRA-mutant GIST with currently available PDGFRA TKIs (imatinib, sunitinib, and others) has yielded mixed results, with outcomes being closely correlated with the intrinsic sensitivity of different mutant PDGFRA oncoproteins to various inhibitors. In vitro, some PDGFRA-mutant kinases (e.g., V561D) are extremely sensitive to imatinib, and patients with these underlying imatinib-sensitive PDGFRA mutations seem to have similar clinical outcomes as patients with KIT exon 11 mutations (7–10).

In contrast, the most common PDGFRA mutation associated with GIST, D842V, is strongly resistant to inhibition...
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Translational Relevance

Approximately 5% to 10% of gastrointestinal stromal tumors (GIST) have pathogenic activating mutations of the PDGFRA receptor tyrosine kinase. The most common of these mutant kinases, PDGFRA D842V, is resistant to inhibition by imatinib or sunitinib, which are currently approved as first-line and second-line treatment of advanced GIST, respectively. In addition, there are other PDGFRA mutations that are also resistant to imatinib. Patients whose GIST have a PDGFR A D842V-mutant kinase have a markedly inferior progression-free and overall survival compared with patients with KIT-mutant GIST. In this study, we identified crenolanib as a potent inhibitor of PDGFRA D842V-mutant kinase with an IC₅₀ of approximately 10 nmol/L. Thus, crenolanib is 100- to 150-fold more potent than imatinib against PDGFRA D842V. In addition, crenolanib has similar potency against less common imatinib-resistant PDGFRA mutations. On the basis of these results, a phase II study of crenolanib for advanced PDGFRA D842V-mutant GIST has been initiated (NCT01243346).

Materials and Methods

DiscoveRx–KINOMEsCan: Kd ELECT

Crenolanib was profiled using the commercially available KINOMEsCan: Kd ELECT screening service (DiscoveRx–KINOMEsCan) as previously described (18, 19). KINOMEsCan: Kd ELECT is based on a competition-binding assay that quantitatively measures the ability of a compound to compete with an immobilized, active site-directed ligand. The assay was carried out by combining 3 components: DNA-tagged kinase, immobilized ligand, and a test compound. The ability of the test compound to compete with the immobilized ligand was measured via quantitative PCR of the DNA tag. Inhibitor binding constants (Kₐ values) were calculated from duplicate 11-point dose–response curves. Measurements were made under optimized conditions that generate true thermodynamic Kₐ values that facilitate direct comparison of inhibitor affinity across kinases.

Reagents and antibodies

Imatinib was obtained commercially from LC Laboratories and reconstituted in PBS to yield a 10 mmol/L stock. Crenolanib was obtained from AROG Pharmaceuticals, LLC and reconstituted in dimethyl sulfoxide (DMSO) to yield a 10 mmol/L stock. Working solutions of imatinib or crenolanib were diluted in growth media or PBS for use in experiments. For PDGFRA immunoprecipitation experiments, we used rabbit polyclonal anti-PDGFRA antibody (Santa Cruz Biotechnology, SC-20) and Protein A/G beads (Santa Cruz). For detection of phospho-PDGFRA and total PDGFRA, we used anti-phosphotyrosine monoclonal antibody (PY-20, 1:500; BD Transduction Labs) and anti-PDGFRA rabbit polyclonal antibody (Santa Cruz), respectively. KIT immunoprecipitation experiments were carried out as previously described (7). For detection of phospho- and total-KIT, we used rabbit polyclonal antibody to P-KIT Tyr 719 (Cell Signaling Technology; catalog no. 3391, 1:500) or total KIT (Santa Cruz; C-19, 1:500), respectively.

Cell lines

The BaF3 V561D and BaF3 D842V cell lines have been previously described (13). These cell lines were generated by transfection of mutant PDGFRA isoforms into BaF3 cells.
Clones were selected for G418 resistance and interleukin-3 independence. DNA from stable lines was checked to ensure that the expected mutant PDGFRα sequence was present, and expression was verified by immunoblotting for total and tyrosyl phosphorylated forms of PDGFRα. The GIST T1 cell line was a generous gift of Dr. Taguchi (Kochi University, Kochi, Japan). This cell line was established from an untreated GIST and expresses an exon 11 deletion constitutively active KIT kinase (20). The EOL-1 cell line was derived from a patient with chronic eosinophilic leukemia and expresses the constitutively activated FIP1L1-PDGFRα fusion kinase (DSMZ Tissue Bank; ref. 21). The H1703 non–small cell lung cancer (NSCLC) cell line was obtained from the American Tissue Type Collection. The H1703 cell line has been previously reported to have 24-fold amplification of the 4q12 region that contains the PDGFRα locus (22).

Biochemical assessment of PDGFRα/KIT kinase activity

Chinese hamster ovary (CHO) cells were transiently transfected with mutated KIT or PDGFRα cDNA constructs and treated with various concentrations of imatinib or crenolanib as previously described (7, 13). Experiments involving recombinant DNA were carried out using biosafety level 2 conditions in accordance with published guidelines. Protein lysates from cell lines were prepared and subjected to immunoprecipitation using anti-KIT or anti-PDGFRA antibodies followed by sequential immunoblotting for phospho-KIT and total KIT, or phosphotyrosine or total PDGFRA, respectively, as previously reported (7, 13). Densitometry was carried out to quantify drug effect using Photoshop 5.1 software, with the level of phospho-KIT or phospho-PDGFRA normalized to total protein. Densitometry and proliferation experimental results were analyzed using Calcsyn 2.1 software ( Biosoft) to mathematically determine the IC50 values. The Wilcoxon rank sum test was used to compare the IC50 values of imatinib and crenolanib for a given mutation.

Ex vivo assay using primary GIST cells

Surgical specimens of primary GIST from imatinib-naive patients were used for the primary cell cultures, as previously described (12). The DNA was isolated and PDGFRα D842V mutation was identified by direct sequencing, according to standard procedures. For Western immunoblotting, primary GIST cells obtained from collagenase-disaggregated tumor specimens were seeded in duplicate at 80% confluence in 25-mm diameter cell culture dishes (Corning Inc.) and grown in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS, 1.0 mmol/L nonessential amino acids, and 1.0 mmol/L sodium pyruvate for 24 hours at +37°C. Next, the cells were exposed to a crenolanib in different concentrations, or to vehicle alone (DMSO), and incubated for 2 hours at +37°C. After a wash in ice-cold PBS, cells were lysed and immunoblotted using anti-phospho-PDGFRα(Tyr754) and anti-PDGFRα antibodies.

Proliferation assays

Cells were added to 96-well plates at densities of 20,000 cells per well and incubated with imatinib or crenolanib for 72 hours before measuring cellular proliferation using a 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT)–based assay (Roche Molecular Biochemicals; ref. 13).

Results

DiscoveRx–KINOMEscan: Kd ELECT of crenolanib

Crenolanib was developed as an orally available inhibitor of PDGFR kinases with more than 100-fold selectivity versus a limited number of profiled kinases (VEGFR2, TIE-2, and FGFR-2). To better characterize the activity of crenolanib, we carried out competitive binding assays to quantify the affinity of crenolanib for a panel of recombinant kinases (DiscoveRx–KINOMEscan: Kd ELECT).

The results showed that crenolanib is a specific and potent inhibitor of class III receptor tyrosine kinases (RTK). The inhibitor-binding constant (Kd) of crenolanib for the wild-type receptors PDGFRα, PDGFRβ, and FLT3 was 3.2, 2.1, and 0.74 nmol/L, respectively (Table 1). In contrast to other known inhibitors of PDGFR (e.g., imatinib, sunitinib, and dasatinib), crenolanib has 25-fold more affinity for PDGFRα/B compared with KIT. Besides class III RTKs, crenolanib does not inhibit any other known tyrosine or serine/threonine kinases at clinically achievable concentrations. Notably, crenolanib was identified in this study as an extremely potent inhibitor of FLT3; the activity of crenolanib against FLT3 has not been previously reported.

<table>
<thead>
<tr>
<th>RTK</th>
<th>Crenolanib Kd (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS1R</td>
<td>30</td>
</tr>
<tr>
<td>FLT3</td>
<td>0.74</td>
</tr>
<tr>
<td>KIT</td>
<td>78</td>
</tr>
<tr>
<td>PDGFRα</td>
<td>2.1</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>3.2</td>
</tr>
</tbody>
</table>

NOTE: Crenolanib was profiled using the commercially available KINOMEscan: Kd ELECT screening service as previously described (18, 19). Kd measurements were based on a competition-binding assay that quantitatively measures the ability of a compound to compete with an immobilized, active-site directed ligand. Inhibitor binding constants (Kd values) were calculated from duplicate 11-point dose–response curves. Measurements were made under optimized conditions that generate true thermodynamic Kd values that facilitate direct comparison of inhibitor affinity across kinases.

Table 1. The inhibitor binding constant (Kd) of crenolanib for RTKs
In vitro measure of IC\textsubscript{50} activity of crenolanib or imatinib against specific mutants in an isogenic model system

The above results indicate that crenolanib is a fairly specific kinase inhibitor with only minimal predicted “off-target” effects. However, these binding assays are not always predictive of biochemical activity (23). Therefore, we sought to confirm these results in biochemical assays using full-length kinase. To determine the potential clinical efficacy of crenolanib in the treatment of GIST, we measured the activity of crenolanib against gain-of-function PDGFRA mutations associated with GIST. In particular, we analyzed the activity of crenolanib against imatinib-resistant PDGFRA oncoproteins.

Mutant isoforms corresponding to selected human PDGFRA mutations were created by site-specific mutagenesis and expressed in CHO cells by transient transfection as previously described (8, 13). All of the expressed mutants showed phosphorylation of tyrosine residues in the absence of PDGF-AA ligand, implying constitutive activation of their kinase domains. We directly compared the biochemical potency of imatinib and crenolanib to inhibit PDGFRA kinase domains. We directly compared the biochemical activity of crenolanib against specific mutants in an isogenic model system.

Table 2. Biochemical IC\textsubscript{50} values for inhibition of kinase activity in cells expressing single mutation kinases

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Exon</th>
<th>Model</th>
<th>Imatinib</th>
<th>Crenolanib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIP1L1-PDGFR A</td>
<td>12</td>
<td>EOL-1</td>
<td>1 ± 0.2</td>
<td>21 ± 13</td>
<td>0.13</td>
</tr>
<tr>
<td>PDGFRA WT</td>
<td>None</td>
<td>CHO</td>
<td>9 ± 4</td>
<td>11 ± 10</td>
<td>0.62</td>
</tr>
<tr>
<td>PDGFRA V561D</td>
<td>12</td>
<td>CHO</td>
<td>32 ± 18</td>
<td>319 ± 75</td>
<td>0.006\textsuperscript{a}</td>
</tr>
<tr>
<td>PDGFRA N659K</td>
<td>14</td>
<td>CHO</td>
<td>56 ± 37</td>
<td>76 ± 67</td>
<td>0.77</td>
</tr>
<tr>
<td>PDGFRA RV560-561ERV</td>
<td>14</td>
<td>CHO</td>
<td>5 ± 3</td>
<td>44 ± 19</td>
<td>0.11</td>
</tr>
<tr>
<td>PDGFRA D842V</td>
<td>18</td>
<td>CHO</td>
<td>1,353 ± 311</td>
<td>9 ± 3</td>
<td>&lt;0.001\textsuperscript{a}</td>
</tr>
<tr>
<td>PDGFRA D842I</td>
<td>18</td>
<td>CHO</td>
<td>&gt;1,000</td>
<td>124 ± 36</td>
<td>0.02\textsuperscript{a}</td>
</tr>
<tr>
<td>PDGFRA D842Y</td>
<td>18</td>
<td>CHO</td>
<td>388 ± 137</td>
<td>88 ± 23</td>
<td>0.06</td>
</tr>
<tr>
<td>PDGFRA deletion DIMH842-845</td>
<td>18</td>
<td>CHO</td>
<td>21 ± 3</td>
<td>23 ± 9</td>
<td>0.75</td>
</tr>
<tr>
<td>PDGFRA D842-843I M</td>
<td>18</td>
<td>CHO</td>
<td>781 ± 342</td>
<td>10 ± 2</td>
<td>0.003\textsuperscript{a}</td>
</tr>
<tr>
<td>PDGFRA deletion I843</td>
<td>18</td>
<td>CHO</td>
<td>67 ± 43</td>
<td>197 ± 71</td>
<td>0.12</td>
</tr>
<tr>
<td>PDGFRA deletion HDSN8455-848P</td>
<td>18</td>
<td>CHO</td>
<td>266 ± 105</td>
<td>87 ± 52</td>
<td>0.30</td>
</tr>
<tr>
<td>PDGFRA D846Y</td>
<td>18</td>
<td>CHO</td>
<td>29 ± 13</td>
<td>18 ± 6</td>
<td>0.67</td>
</tr>
<tr>
<td>PDGFRA N848K</td>
<td>18</td>
<td>CHO</td>
<td>22 ± 7</td>
<td>39 ± 16</td>
<td>0.16</td>
</tr>
<tr>
<td>KIT exon 11 deletion</td>
<td>11</td>
<td>GIST-T1</td>
<td>8 ± 4</td>
<td>&gt;1,000</td>
<td>0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>KIT D816V</td>
<td>17</td>
<td>CHO</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>1.00</td>
</tr>
<tr>
<td>PDGFRA V561D</td>
<td>12</td>
<td>BaF3</td>
<td>13 ± 12</td>
<td>134 ± 71</td>
<td>0.03\textsuperscript{a}</td>
</tr>
<tr>
<td>PDGFRA D842V</td>
<td>18</td>
<td>BaF3</td>
<td>272 ± 163</td>
<td>2 ± 2</td>
<td>0.002\textsuperscript{a}</td>
</tr>
</tbody>
</table>

NOTE: The values for crenolanib and imatinib represent the biochemical IC\textsubscript{50} expressed in nmol/L units ± the SEM. Values represent the data from at least 3 replicate experiments per mutation.

\textsuperscript{a}P < 0.05 by Wilcoxon rank sum test.
Figure 1. Sensitivity of single mutant PDGFRA isoforms to imatinib or crenolanib. CHO cells were transfected with PDGFRA constructs and 24 hours later were incubated for 90 minutes in the absence or presence of imatinib or crenolanib at the indicated concentrations. PDGFRA was immunoprecipitated from cell extracts and analyzed by immunoblotting using anti-PDGFRA (for total PDGFRA) and anti-phosphotyrosine (for phospho-PDGFRA) antibodies. Representative results from a minimum of 3 replicate experiments per mutant kinase are shown.
crenolanib nor imatinib had any significant biochemical activity against the D816V mutation, which is homologous to the PDGFRA D842V mutation associated with mastocytosis (IC50 > 1,000 nmol/L for both; ref. 15).

The molecular mechanisms underlying the secondary clinical progression of imatinib-sensitive PDGFRA-mutant GIST are poorly understood. However, one reported secondary mutation has been described—acquisition of a secondary PDGFRA D842V mutation (7). This same mutation has also been described as secondary drug resistance mutation in patients with hypereosinophilic syndrome who are treated with imatinib (24). In addition, the secondary gatekeeper mutation (PDGFRA T674I) has been reported in a patient with FIP1L1-PDGFRA–positive hematologic neoplasm treated with PDGFRA inhibitors (25).

We expressed the compound PDGFRA mutants V561D + T674I (exon 12 + gatekeeper), V561D + D842V (exon 12 + 18), and T674I + D842V (gatekeeper + exon 18) in CHO cells and compared the activity of imatinib or crenolanib against these compound-mutant kinases (Table 3, Fig. 2). Neither imatinib nor crenolanib had any activity against the V561D + T674I-mutant kinase (IC50 > 1,000 nmol/L for each). However, crenolanib has low nanomolar potency against the V561D + D842V-mutant kinase that is similar to its potency against the isolated D842V mutation. This was unexpected, as the isolated V561D mutation is relatively resistant to crenolanib. We also found that addition of the gatekeeper T674I mutation to the D842V mutation had no significant effect on the potency of crenolanib, indicating that crenolanib binding is not dependent upon the T674 residue.

Comparative activity of crenolanib and imatinib in cellular models

The above studies were conducted in a CHO transient transfection system that allows us to directly compare the activity of different TKIs on the same mutant kinase in an isogenic system. To extend these results, we also compared the potency of crenolanib and imatinib in a panel of PDGFRA-dependent cell lines. These cell lines allowed us to not only assess biochemical potency of the 2 inhibitors but also to measure the effect of PDGFRA kinase inhibition on cellular proliferation.

The EOL-1 cell line is derived from a patient with chronic eosinophilic leukemia and expresses the constitutively activated FIP1L1-PDGFRA fusion kinase (21). Both imatinib and crenolanib potently inhibit the kinase activity of the fusion oncogene with IC50 values of 1 and 21 nmol/L, respectively (P = 0.13; Fig. 3A). In addition, both compounds potently inhibit the proliferation of EOL-1 cells with a 50% growth inhibitory concentration of 0.2 pmol/L (Fig. 3B).

Table 3. Biochemical IC50 values for inhibition of PDGFRA kinase activity in CHO cells expressing compound-mutant kinases

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Exons</th>
<th>Imatinib</th>
<th>Crenolanib</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>V561D + T674I</td>
<td>12 + 14</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>0.99</td>
</tr>
<tr>
<td>V561D + D842V</td>
<td>12 + 18</td>
<td>&gt;1,000</td>
<td>26 ± 6</td>
<td>0.0002</td>
</tr>
<tr>
<td>T674I + D842V</td>
<td>14 + 18</td>
<td>&gt;1,000</td>
<td>24 ± 12</td>
<td>0.006</td>
</tr>
</tbody>
</table>

NOTE: The values for crenolanib and imatinib represent the biochemical IC50 expressed in nmol/L units ± the SEM. Values represent the data from at least 3 replicate experiments per mutation.
We also tested the comparative potency of imatinib and crenolanib to inhibit the activation of V561D- or D842V-mutant kinases expressed in BaF3 cells. Consistent with our results in the transient transfection model (CHO cells), imatinib is more potent than crenolanib in inhibiting the activity of V561D (IC$_{50}$ 11 vs. 85 nmol/L, $P = 0.05$). In addition, we confirmed that crenolanib is 100-fold more potent than imatinib for inhibiting activity of the D842V mutation (IC$_{50}$ 2 vs. 272 nmol/L, $P = 0.002$; Fig. 3A).

Similar to the biochemical data, crenolanib is significantly more potent than imatinib for inhibiting proliferation of the BaF3 D842V cell line, with IC$_{50}$ values of 22 and 1,510 nmol/L, respectively (Fig. 3B).

PDGFRA genomic amplification leading to constitutive PDGFRA activation has been reported in a number of malignancies including NSCLC and gliomas (22, 26–28). We tested the activity of imatinib and crenolanib against the H1703 NSCLC cell line that has been previously reported to have 24-fold amplification of the 4q12 region that contains the PDGFRA locus. This cell line is dependent upon PDGFRA kinase activity for proliferation, as evidenced by inhibition of growth by short hairpin RNA against PDGFRA or PDGFRA TKIs (imatinib, sunitinib; ref. 22). Both imatinib and crenolanib inhibited PDGFRA activation in this cell line with IC$_{50}$ values of 93 and 26 nmol/L, respectively (Fig. 3B, $P = 0.4$). Both drugs also inhibited the proliferation of these cells in a similar dose range (data not shown).

Finally, we tested the biochemical activity of crenolanib in 2 primary GIST cells with PDGFRA D842V mutation by ex vivo assay. Crenolanib, at a concentration of 10 nmol/L, completely inhibits PDGFRA D842V activation, as assessed by autophosphorylation (Fig. 4). Due to the limited number of primary cells available, we did not carry out comparative studies against imatinib nor conducted experiments to assess the effect of crenolanib on cellular proliferation. Notably, in previously published studies using these cells, concentrations of imatinib up to 5,000 nmol/L had only a minimal effect on PDGFRA D842V autophosphorylation (29).
Figure 4. Crenolanib inhibits PDGFRα D842V in primary GIST cells by ex vivo assay. Two independently established primary GIST cell lines were exposed to a crenolanib in different concentrations, or to vehicle alone (DMSO), and incubated for 2 hours at 37°C. Cell extracts were analyzed by Western blotting using anti-PDGFRα (for total PDGFRα) and anti-phospho-PDGFRα (Tyr754) antibodies.

Discussion

TKI inhibitor therapy of GIST has revolutionized the treatment of advanced metastatic or unresectable GIST. Before the introduction of TKI therapy, there was no effective medical therapy for GIST as these tumors are uniformly resistant to chemotherapy. However, the benefits of TKI therapy strongly correlate with the underlying biology of the tumor. Notably, the best results are seen for treatment of KIT exon 11–mutant GISTs that express mutant kinases that are extremely sensitive to in vitro inhibition by imatinib or sunitinib (2).

Although KIT is the most commonly mutated kinase in GIST, approximately 5% to 10% of GISTS express exclusively a mutated PDGFRα kinase (2). The response of patients with PDGFRα-mutant GIST to TKIs correlates with intrinsic sensitivity of the mutant kinase expressed by tumor cells. Patients whose GIST have an imatinib-sensitive PDGFRα mutation (e.g., PDGFRα exon 12 V561D mutation) seem to have similar clinical outcomes as patients whose tumor has a KIT exon 11 mutation. In contrast, the most common PDGFRα mutation associated with GIST (PDGFRα exon 18 D842V) has been shown to be resistant to imatinib and sunitinib in vitro. Consequently, patients with PDGFRα D842V–mutant GIST treated with conventional GIST TKIs have a very low rate of clinical benefit, and the median overall survival for these patients is approximately 1 year, compared with the median of 4 to 5 years seen for patients with KIT exon 11–mutant GIST. In addition to the PDGFRα D842V mutation, there are also a number of less common PDGFRα mutations that also are resistant to imatinib in vitro (6, 8–10, 13, 14).

We tested the activity of crenolanib, a potent PDGFRα/B TKI to inhibit a panel of PDGFRα-mutant kinases. Crenolanib was significantly more potent than imatinib in inhibiting the kinase activity of imatinib-resistant PDGFRα kinases (D842L, D842V, D842Y, D842-843IM, and deletion 1843). For example, crenolanib was 135-fold more potent than imatinib at D842L kinase activity in our isogenic model system. The relative potency of crenolanib was further confirmed in BaF3 and primary GIST cells expressing PDGFRα D842V. In contrast, imatinib was 6- to 10-fold more potent than crenolanib in inhibiting the V561D mutation. For all other tested PDGFRα mutations, crenolanib and imatinib had comparable kinase activity.

The D842V mutation is the most common primary PDGFRα mutation found in GIST but can also develop as a secondary resistance mutation following imatinib treatment of PDGFRα-mutant tumors (7). Crenolanib potently inhibited the activity of the compound V561D + D842V-mutant kinase and retained activity when the gatekeeper T674I mutation was added to the D842V mutation.

Zhang and colleagues have classified TKIs into type I and type II inhibitors. Type I inhibitors can only bind to the active kinase conformation (30). In contrast, type II inhibitors, such as imatinib and sunitinib, can only bind to the inactive conformation of the kinase and function by preventing the enzyme from assuming the active conformation (30, 31). Of note, the D842V mutation is felt to stabilize the PDGFRα activation loop in the active conformation; this conformation cannot bind imatinib or sunitinib (15, 32). However, in this study, we found that this mutation is very potently inhibited by crenolanib.

The spectrum of activity of crenolanib suggests that it is a type I inhibitor. The ability of both imatinib and crenolanib to inhibit certain mutations with equivalent potency (e.g., PDGFRα D842V-845D), suggests that these particular mutant kinases are in an equilibrium with roughly equal populations of inactive and active kinase isoforms, thus allowing either type I and II inhibitors to bind and thereby inhibit kinase activity. On the basis of this model, the relative potency of imatinib and crenolanib against PDGFRα-mutant isoforms may provide insight into how different mutations affect the distribution of protein isoforms between the inactive and active state. In addition, further structure-based studies of crenolanib and related compounds may aid in the development of even more potent type I PDGFRα kinase inhibitors.

We hypothesize that crenolanib may be useful for the treatment of GIST with an associated PDGFRα mutation. In phase I–II studies, orally administered crenolanib was well tolerated and exhibited favorable pharmacokinetic properties. The dose-limiting toxicities included hematuria, increased liver function tests, and nausea/vomiting (16, 17). Based in part on these results and the prior clinical experience with crenolanib, a multicenter phase II study of crenolanib for treatment of PDGFRα D842V–mutant GIST has been initiated (NCT01243346).

In addition to a subset of GIST, genomic alterations resulting in PDGFRα or PDGFRβ activation have been reported in other human malignancies, including myeloproliferative disorders (e.g., FIP1L1-PDGFRα, BCR-PDGFRα, and ET16-PDGFRβ), gliomas (amplification of PDGFRα), dermatofibrosarcoma protuberans (activation of PDGFRβ due to paracrine secretion of COL1A1-PDGFB fusion protein), and NSCLC (genomic amplification of PDGFRα). Also, PDGFRβ activation may contribute to tumor angiogenesis either directly (endothelial cells) or indirectly (support of pericytes). Therefore, crenolanib may be useful in the treatment of cancers that have abnormal activation of PDGFRα or PDGFRβ. On the basis of prior studies of the role of PDGFRα in the pathogenesis/biology of gliomas, several phase II studies of crenolanib for
treatment of pediatric or adult glioma have been initiated (NCT01229644, NCT01393912; refs. 22, 26–28, 33, 34).

Disclosure of Potential Conflicts of Interest
M.C. Heinrich has received commercial research grants from AROG, Novartis, Imclone, and Ariad, and honoraria from speakers bureau of Novartis. He also has ownership interest (including patents) from MolecularMD and is a consultant and an advisory board member of Novartis, Pfizer, and MolecularMD. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
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References
33. Appelmann I, Liersch R, Kessler T, Mesters RM, Berdel WE. Angiogenesis inhibition in cancer therapy: platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) and their receptors: biological functions and role in malignancy. Recent Results Cancer Res 2010;180:51–81.
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