Crenolanib (CP-668,596) Inhibits Phosphorylation of the Imatinib-Resistant D842V PDGFRADesignated Mutation Associated with Advanced GIST (Abstract 10012)

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Abstract (10012)

Background: 8-10% of gastrointestinal stromal tumors (GIST) have activating mutations of the platelet-derived growth factor receptor alpha (PDGFRα) gene. The most common PDGFRα mutation is the D842V mutation encoded by exon 18. This gain-of-function mutation results in constitutive tyrosine kinase activity. Currently approved tyrosine kinase inhibitors (TKIs), such as imatinib (IM) or sunitinib, have little to no in vitro activity against this mutation. Clinically, these drugs are ineffective for the treatment of patients whose GIST has a D842V mutation. In addition, the D842V mutation can develop as a secondary IM resistance mutation during treatment of patients with primary IM-sensitive GIST or hypereosinophilic syndrome. Crenolanib (formerly CP-688,596) is an orally bioavailable, highly potent and selective PDGFRα TKI. Phase I trials of crenolanib (CR) have shown a favorable toxicity profile, and achievable serum concentrations as high as 2,000 nM.

Methods: Mutant PDGFRα isoforms were expressed by transient transfection of Chinese hamster ovary cells. The transfected cells were treated with various concentrations of CR or IM. Protein lysates were immunoprecipitated with an anti-PDGFRα antibody, followed by sequential immunoblotting for activated and total PDGFRα. IC50 (50% inhibitory concentration) was measured densitometrically and compared with the PDGFRα expression bands and normalization using total PDGFRα expression.

Results: CR was effective in blocking the activity of single or compound PDGFRα D842V mutant kinases. In contrast, IM had no significant activity against these same mutant kinases.

Conclusions: GISTs with PDGFRα D842V mutations are clinically resistant to currently approved TKIs. CR blocks the kinase activity of PDGFRα D842V mutants (single and compound) at clinically achievable concentrations, providing a potential new therapeutic modality for GIST patients. Data from Phase I/II clinical trials of CR for treatment of GIST patients with primary or secondary PDGFRα D842V mutation is currently being initiated.

INTRODUCTION

• The vast majority of GISTs are caused by either KIT or PDGFRα gain-of-function mutations, with PDGFRα mutations being found in 5-8% of GISTs.

• The D842V mutation (encoded by exon 18), is found in up to two-thirds of GIST patients with primary PDGFRα mutations, but can also develop as a secondary drug resistance mutation.

• This gain-of-function mutation results in auto-phosphorylation and constitutive activation of PDGFRα kinase activity.

• Currently available TKIs like imatinib, sunitinib, sorafenib, and nilotinib have little to no clinically relevant in vitro activity against the D842V mutated PDGFRα (Figure 1).

• In a total of 33 documented cases of patients with the PDGFRα D842V mutation from international clinical trials, none responded to treatment with imatinib or sunitinib.

• The D842V mutation is thought to be a driver mutation, with KRAS and PDGFRα mutations being mutually exclusive.

• The D842V mutation is the most common activating mutation in GIST (8-10% of cases), with activating mutations in PDGFRα being found in 5-8% of GISTs.

• The D842V mutation is found in up to two-thirds of GIST patients with primary PDGFRα mutations, but can also develop as a secondary drug resistance mutation.

• This gain-of-function mutation results in auto-phosphorylation and constitutive activation of PDGFRα kinase activity.

• Crenolanib (FORMERLY CP-688,596)

• Crenolanib is an orally administered, benzimidazole compound that is highly selective and potent inhibitor of both PDGF receptors (PDGFRα and PDGFRβ).

• The chemical name of crenolanib besylate is 4-(1H-piperidinamine, 1H-[2H-[5H]-benzimidazole-1(3H)-yl]-6-quinolinyl)-monobenzenesulfonate, and its chemical abstracts service (CAS) registry number is 670220-03-6.

• Crenolanib has demonstrated activity in inhibiting the phosphorylation of PDGFRα in murine glial cells retrovirally mediated to overexpress PDGFRα.5

• Crenolanib has been evaluated in Phase I (single agent) and Phase Ib (in combination with axitinib and dacetaxel) trials.

• Crenolanib has shown a favorable toxicity profile, and achievable serum concentrations as high as 2,000 nM.6

METHODS

Recombinant kinase assays:

The activity of crenolanib against recombinant PDGFR D842V kinase was determined using a commercially available kinase screening service (Millipore IC50 profiler).

In vitro experiments

PDGFRα mutations were cloned by site-directed mutagenesis and all experiments were confirmed by bidirectional sequencing. CHO cells were transiently transfected with plasmids encoding cDNAs for wild-type or mutant proteins. Transfected cells were treated with either imatinib or crenolanib for 90 minutes in concentrations ranging from 0 to 1000 nM, in media containing 15% fetal bovine serum. The activation status (phosphorylation) of the PDGFRα protein was assayed by immunoprecipitation using an anti-PDGFRα antibody and sequential immunoblotting for phospho-PDGFRα (using anti-phosphotyrosine antibody) or total PDGFRα (anti-PDGFRα monoclonal antibody).

RESULTS

Figure 1. Inhibition of kinase activity (nM)

<table>
<thead>
<tr>
<th>Kinases</th>
<th>Crenolanib IC50</th>
<th>Imatinib IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFRα WT</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>PDGFRα D842V</td>
<td>7</td>
<td>716</td>
</tr>
<tr>
<td>PDGFRα V561D + D842V</td>
<td>18</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>PDGFRα T674I + D842V</td>
<td>24</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Figure 2. Inhibition of kinase activity (nM)

Figure 3. Western blot analyses of PDGFRα D842V transduced Ba/F3 cells after treatment with imatinib, nilotinib, sorafenib, and sunitinib for 90 min. None of the TKIs tested had inhibitory effect on the phosphorylation of the mutant PDGFRα.

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CONCLUSION/DISCUSSION

• Crenolanib is a unique TKI that blocks the kinase activity of PDGFRα D842V mutant at clinically achievable concentrations, even in the presence of the gatekeeper T674I mutation.

• Crenolanib may provide the first effective systemic therapy for GIST patients with primary or secondary PDGFRα D842V mutations as these activating mutations are clinically resistant to imatinib, sunitinib, and other commercially available tyrosine kinase inhibitors.

• A Phase II clinical study of crenolanib in patients with advanced gastrointestinal stromal tumors (GIST) with the D842V mutation in the PDGFRα gene has been initiated at Fox Chase Cancer Center and Oregon Health Sciences University (NCT01243346).

REFERENCES


5. AROG Pharmaceuticals, LLC. Crenolanib Investigator’s Brochure, 2011.
