Crenolanib (CP-868,596) Inhibits Phosphorylation of the Imatinib-Resistant D842V PDGFRA Activating 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 (PDGFRA) gene. The most common PDGFRA 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 hypereosinophillic syndrome. Crenolanib (formerly CP-868,596) is an orally bioavailable, highly potent and selective PDGFR 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-PDGFRA antibody, followed by sequential immunoblotting for activated- and total-PDGFRA. IC_{50} (50% inhibitory concentration) was measured by densitometry of the phosphoPDGFRA bands and normalization using total PDGFRA expression.

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

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

INTRODUCTION

- The vast majority of GISTs are caused by either KIT or PDGFRA gain-of-function mutations, with PDGFRA 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 PDGFRA mutations, but can also develop as a secondary drug resistance mutation.
- This gain-of-function mutation results in auto-phosphorylation and constitutive activation of PDGFRA kinase activity.
- Currently available TKIs like imatinib, sunitinib, sorafenib, and nilotinib have little to no clinically relevant in vitro activity against the D842V mutated PDGFRA (Figure 1).
- In a total of 33 documented cases of patients with the PDGFRA D842V mutation in international clinical trials, none responded to treatment with imatinib or sunitinib.

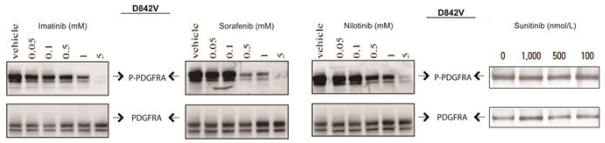


Figure 1. Western blot analyses of PDGFRA D842V-transduced Ba/F3 cells after treatment with imatinib, nilotinib, sorafenib, and sunitinib for 90 min. None of the TKIs tested had inhibitory effect on the phosphorylation of the mutant PDGFRα.[1,2]

CLINICAL OUTCOME OF D842V-MUTANT GIST IS POOR WITH CURRENT THERAPIES

- An international survey of GIST referral centers for patients with the PDGFRA D842V mutation, documented that none of the nineteen assessable patients had an objective response to imatinib.[3] The median progression-free survival was only 2.8 months, and the median survival was only 12.7 months. The survival of these patients is much shorter than the median survival of imatinib-sensitive GIST patients which is greater than 3 years.[4]
- Similarly, other trials have also shown that patients with the D842V mutations do not respond to treatment with imatinib or sunitinib.
  - In the B222 phase II trial, 0 of 3 patients with PDGFRA D842V mutations responded to imatinib.
  - In the EORTC phase III trial, 0 of 4 patients with known PDGFRA D842V mutations responded to imatinib.
  - In the US phase III study, 0 of the 4 patients with PDGFRA D842V mutation responded to imatinib treatment.
  - In the phase I/II trials of sunitinib, there were no responses in the 3 patients with primary PDGFRA D842V mutations, or in the one patient with a primary exon 12 mutation who had a secondary exon 18 D842V mutation.

CRENOLANIB (FORMERLY CP-868,596)

- Crenolanib is an orally administered, benzimidazole compound that is highly selective and potent inhibitor of both PDGF receptors (PDGFRA and PDGFRB).
- The chemical name of crenolanib besylate is 4-piperidinamine, 1-[2-[5-[(3-Methyl-3-oxetanyl) methoxy]-1H-benzimidazol-1-yl]-8-quinolinyl] - monobenzenesulfonate, and its chemical abstracts service (CAS) registry number is 670220-93-6.[5]
- 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[6] (single agent) and Phase Ib[7] (in combination with axitinib and docetaxel) 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

PDGFRA mutations were cloned by site-directed mutagenesis and all mutations 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, followed by sequential immunoblotting for phospho-PDGFRα (using anti-phosphotyrosine antibody) or total PDGFRα (anti-PDGFRα monoclonal antibody).

RESULTS

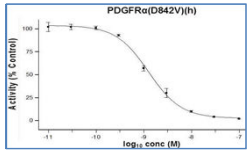


Figure 2. IC_{50} Profiler results from Millipore demonstrate that crenolanib has an IC_{50} of 1nM against recombinant human PDGFRA D842V kinase. Data are expressed s a percentage of the residual kinase activity compared with mock treated kinase.

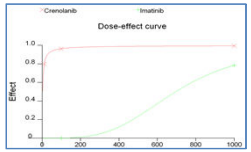
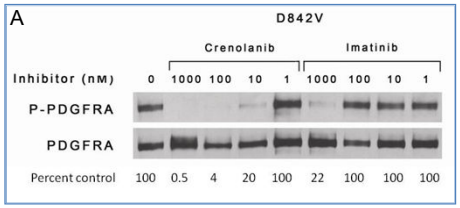


Figure 3. Representative data demonstrating that crenolanib is highly potent inhibitor of phosphorylation of D842V mutant PDGFRA in cells. The biochemical IC_{50} for inhibition of PDGFRA D842V transiently expressed in CHO cells was 7 nM.

	Inhibition of kinase activity (nM)	
Kinases	**Crenolanib IC_{50}**	**Imatinib IC_{50}**
PDGFRA WT	**11**	**9**
PDGFRA D842V	**7**	**716**
PDGFRA V561D + D842V	**18**	**>1000**
PDGFRA T674I + D842V	**24**	**>1000**



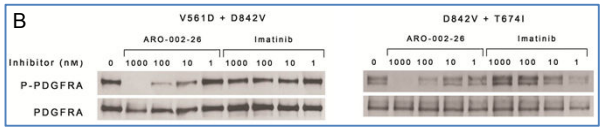


Figure 4. Inhibition of autophosphorylation of D842V mutant PDGFRA transiently expressed in CHO cells by crenolanib or imatinib. (A) Consistent with previous results, imatinib at doses of up to 1000 nM had only a moderate effect on the activity of PDGFRA D842V kinase, as assessed by measurement of PDGFRA autophosphorylation. In contrast, crenolanib was able to inhibit phosphorylation of PDGFRA D842V at an IC_{50} of 7nm and an IC_{50} of 25nm. (B) Crenolanib was also effective against compound PDGFRA D842V mutant kinases.

CONCLUSION/DISCUSSION

- Crenolanib is a unique TKI that blocks the kinase activity of PDGFRA 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 PDGFRA 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 PDGFRA gene has been initiated at Fox Chase Cancer Center and Oregon Health Sciences University (NCT01243346).

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