# Crenolanib (CP-868,596), a Highly Potent PDGFR Inhibitor, Inhibits Phosphorylation of the Imatinib-Resistant PDGFRα (D842V) Activating Mutation Associated with Advanced GIST

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## Abstract: 3586

Background: 8-10% of gastrointestinal stromal tumors (GIST) have activating mutations of the platelet-derived growth factor receptor alpha (PDGFRa) kinase. The most common PDGFRa mutation is the D842V mutation (encoded by exon 18). This gain-of-function mtuations results in auto-phosphorylation and constitutive activation of PDGFRa kinase activity. Type II receptor tyrosine kinase (RTK) inhibitors, such as imatinib and sunitinib, which only bind to the inactive conformation of the RTK, have little to no in vitro activity against this mutation. Clinically, these drugs are not effective for the treatment of GIST patients with D842V mutation. In addition, the D842V mutation can develop as a secondary imatinib resistance mutation during treatment of GISTs with primary imatinib-sensitive PDGFRa mutations (e.g. primary exon 12 mutations), or treatment of patients with hypereosinophillic syndrome with translocations involving PDGFRa.

Drug Background: Crenolanib is an orally bioavailable, highly potent and selective PDGFR TKI. Crenolanib is a benzimidazole compound that has $IC_{50}$s of 0.9 nM and 1.8 nM for PDGFRa and PDGFRb, respectively. Phase I trials of crenolanib have shown good oral bioavailability, a favorable toxicity profile, and achievable serum concentrations as high as 2000 nM.

Methods: Mutant PDGFRa isoforms were expressed by transient transfection of Chinese Hamster ovary cells. The transfected cells were treated with various concentrations of crenolanib before preparation of protein lystates. PDGFRa protein was assayed for activation status (phosphorylation) by immunoprecipitation using an anti-PDGFRa antibody, followed by sequential immunoblotting for phospho PDGFRa (using antiphosphotyrosine antibody) or total PDGFRa (anti-PDGFRa monoclonal antibody). $IC_{50}$ was measured by densitometry of the phospho PDGFRa bands and normalization using total PDGFRa expression (to correct for variations in loading of PDGFRa protein in the various lanes).

Results: Crenolanib inhibited the phosphorylation of wild type PDGFRa at an $IC_{50}$ of 10 nM and PDGFRa (D842V) with an $IC_{50}$ between 10 to 30 nM. Imatinib was ineffective in blocking PDGFRa (D842V) phosphorylation in these experiments ($IC_{50}$ > 1000 nM). Profiling of crenolanib against other GIST-relevant primary and secondary PDGFRa mutations is ongoing and will be reported.

Discussion: GIST due to D842V activating mutations of PDGFRA gene are clinically resistant to imatinib, sunitinib, and other commercially available tyrosine kinase inhibitors. Crenolanib blocks phosphorylation of D842V mutant of PDGFRa at clinically achievable concentrations, providing a potentially new therapeutic modality for GIST patients. A clinical trial in GIST patients with primary or secondary PDGFRa (D842V) mutation is being initiated.

## Introduction/Background

- Majority of GISTs are due to either KIT/PDGFRA 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 PDGFRA mutations developed either as a primary mutation or a secondary 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 in vitro activity against the D842V mutated PDGFRα RTK
- 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.

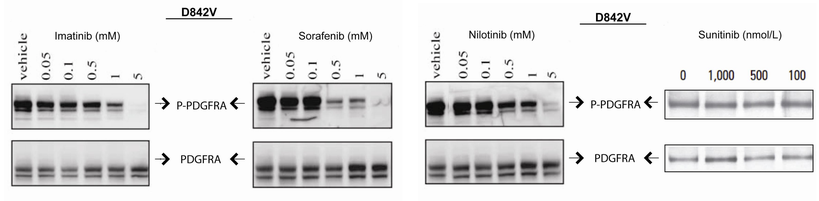


**Figure 1.** Western blot analyses of PDGFRA D842V-transduced Ba/F3 cells after treatment with imatinib, nilotinib, sorafenib, and sunitinib for 90 min. None of the TKIs tested had inhibitory effect on the phosphorylation of the mutant PDGFRα. [ adapted from Debiec-Rychter et. al., 2006, and Heinrich et. al., 2008]

## Clinical outcome of D842V-mutant GIST is poor with current therapies

- An international survey of GIST referral centers, documented that none of the nineteen assessable patients with the PDGFRA D842V mutation had an objective response to imatinib. 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.
- Similarly, other trials have also shown that patient with the D842V mutation do not respond to treatment with imatinib or sunitinib.
  - In the B222 phase II trial, none of the three patients with PDGFRA D842V mutation responded
  - In the EORTC phase III trial, 4 patients with known PDGFRA D842V mutation had no response
  - In the US phase III study, none 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 the primary PDGFRA D842V mutation, or in the one patient with a primary exon 12 mutation who had a secondary exon 18 D842V mutation

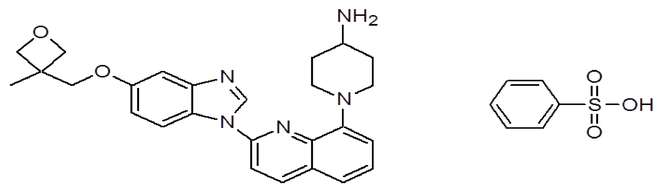


**Figure 2.** Chemical structure of crenolanib besylate, the benzenesulfonate salt of crenolanib that has been and will be used in clinical studies

## Crenolanib (CP-868,596)

- Crenolanib is an orally administered, benzimidazole compound that is a highly selective and potent inhibitor of both PDGF receptors (PDGFRα and PDGFRβ)
- Crenolanib has demonstrated activity in inhibiting the phosphorylation of PDGFRα in transient transfected CHO cells as well as in murine glial cells that are retrovirally mediated to overexpress PDGFRα
- Crenolanib has also been shown to inhibit PDGFRα in cell lines derived from patients with hypereosinophilic syndrome as well as in selected NSCLC cell lines
- Crenolanib besylate has been clinically evaluated in a dose-finding phase I study as a single agent at doses ranging from 60 mg BID to 340 mg QD. A phase Ib combination study with docetaxel with or without axitinib has also been completed
- Phase I (Lewis et. al., 2009) and Phase Ib (Michael et al., 2010) trials of crenolanib have shown a favorable toxicity profile, and achievable serum concentrations as high as 691ng/mL
- The chemical name of crenolanib besylate is 4-piperidinamine, 1-[2-[5-[(3-Methyl-3-oxetanyl) methoxy]-1H-benzimidazol-1-yl]-8-quinolinyl] – monobenzenesulfonate (fig. 2), and its chemical abstracts service (CAS) registry number is 670220-93-6

## Method

**Millipore $IC_{50}$ profiler Assay:**

Activated PDGFRα isoform (D842V) (h) was incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 250 μM GGMEDIYFEFMGGKKK, 10 mM MgAcetate and [γ-33P-ATP]. The reaction was initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 3% phosphoric acid solution. 10 μL of the reaction was then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

**In vitro experiments by Dr. Michael Heinrich at OHSU:**

PDGFRA mutations were cloned by site-directed mutagenesis of the respective wild-type cDNA. All mutations were confirmed by bidirectional sequencing. Chinese hamster ovary cells were transiently transfected with plasmids encoding cDNAs for wild-type or mutant proteins. Twenty-four hours after transfection, the cells were treated with either imatinib or crenolanib for 90 minutes in concentrations ranging from 0 to 1000 nM, in media containing 10% 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

Inhibition of phosphorylation of exon 18, PDGFRα mutation, D842V

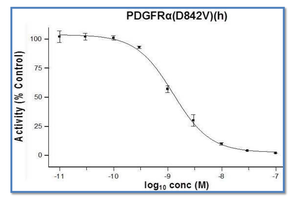


**Figure 3.** $IC_{50}$ Profiler results from Millipore demonstrate that crenolanib has an $IC_{50}$ of 1nM against the PDGFRα D842V kinase, determined as a percentage of the mean kinase activity in the positive control samples. Analysis is performed using XLFit version 5.1 (ID Business Solutions).

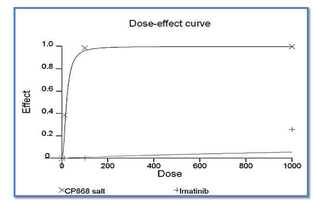


**Figure 4.** Experiments conducted by Dr. Michael Heinrich at OHSU demonstrate that crenolanib is highly potent inhibitor of phosphorylation of D842V mutant PDGFRα transiently expressed in CHO cells at an $IC_{50}$ of 6nM.

| | Millipore $IC_{50}$ Profiler Studies | In vitro studies by Michael Heinrich | |
|---|---|---|---|
| Kinases | Crenolanib $IC_{50}$ | Crenolanib $IC_{50}$ | Imatinib $IC_{50}$ |
| PDGFRα(D842V)(h) | 1 | 6 | >1000 |
| PDGFRα(V561D)(h) | 4 | 270 | 20 |
| PDGFRα(V561D+D842V)(h) | - | ongoing | >1000 |

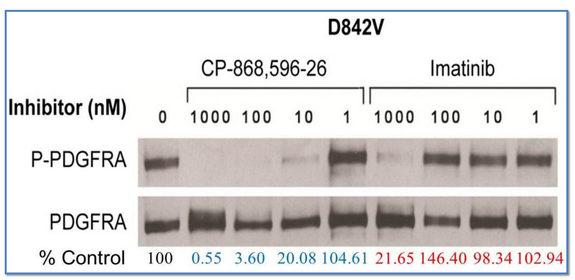


**Figure 5.** Inhibition of autophosphorylation of D842V mutant PDGFRα transiently expressed in CHO cells by crenolanib. Consistent with previous results, imatinib at doses of up to 1000 nM had little to no effect on the activity of PDGFRα D842V kinase, as assessed by measurement of PDGFRα autophosphorylation. In contrast, crenolanib was able to inhibit phosphorylation of PDGFRA D842V at an $IC_{50}$ of 6nm and an $IC_{90}$ of 25nm.

## Conclusion/Discussion:

- Crenolanib is a unique TKI that blocks phosphorylation of D842V mutant PDGFRα at clinically achievable concentrations
- Crenolanib may provide the first effective systemic therapy for GIST patients with the PDGFRA D842V mutation as this activating mutation is 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)

Knight Cancer Institute at Oregon Health & Science University

AROG PHARMACEUTICALS, LLC