EFFICACY OF CRENOLANIB AGAINST THE PDGFRA ACTIVATING MUTATION, D842V, ASSOCIATED WITH GASTROINTESTINAL STROMAL TUMORS

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PDGFRA MUTATIONS ACCOUNT FOR 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.
- Current drug therapies for GIST such as imatinib, sunitinib, sorafenib and nilotinib have no effect on GIST with the D842V mutation at clinically achievable concentrations.
- 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. The median progression-free survival was only 2.8 months. The median survival was only 12.7 months, which is much shorter than the median survival of imatinib-sensitive GIST patients which is greater than 3 years.

PATIENTS WITH D842V MUTATIONS IN GIST DO NOT RESPOND TO IMATINIB OR SUNITINIB

Therapy	Trial	Patients who responded
Imatinib	B222 phase II	0/3
Imatinib	EORTC phase III	0/4
Imatinib	US phase III	0/4
Sunitinib	Phase I/II	0/4*

*3 patients with primary PDGFRA D842V mutations, 1 patient with a primary exon 12 mutation and a secondary exon 18 D842V mutation

Table 1. Clinical responses to imatinib or sunitinib in patients with D842V mutation

CRENOLANIB BESYLATE (CP-868,596-26)

- Oral, mutant specific inhibitor of PDGFRα
- 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.

RECOMBINANT PDGFRα ASSAY

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

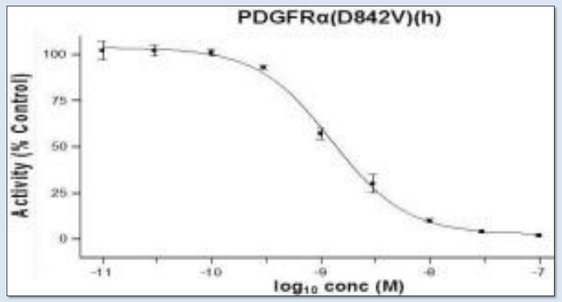

Figure 1. IC_{50} Profiler results from Millipore demonstrate that crenolanib has an IC_{50} of 1nM against recombinant human PDGFRA D842V kinase, Data are expressed s a percentage of the residual kinase activity compared with mock treated kinase.

CELLULAR ASSAY WITH TRANSIENTLY TRANSFECTED CHO CELLS

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).

PDGFRα Mutation	Crenolanib (nM)		Imatinib (nM)	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}
D842V	9	44	>1000	>1000
V561D+D842V	40	100	>1000	>1000
T674I+D842V	70	205	>1000	>1000
V561D+T674I	>1000	>1000	>1000	>1000

Table 2. IC_{50} and IC_{90} values of crenolanib and imatinib in transient transfected CHO cells with various PDGFRα mutations.

CRENOLANIB INHIBITS THE ACTIVITY OF PDGFRA D842V MUTATION IN:

Transiently Transfected CHO cells

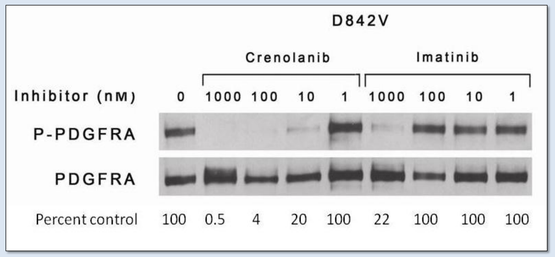

Figure 2. Inhibition of autophosphorylation of D842V mutant PDGFRA transiently expressed in CHO cells by crenolanib or imatinib. The biochemical IC_{50} for inhibition of PDGFRA D842V transiently expressed in CHO cells by crenolanib was 10 nM.

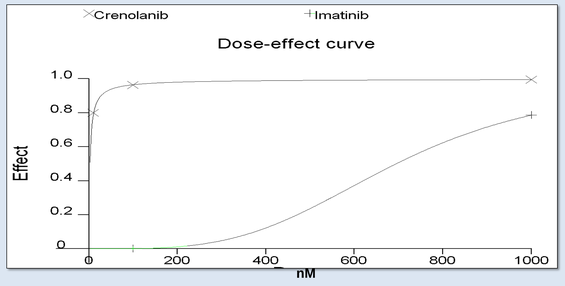

Figure 5. Representative data demonstrating that crenolanib is highly potent inhibitor of phosphorylation of D842V mutant PDGFRA in cells. The biochemical IC_{50} for inhibition of PDGFRA D842V transiently expressed in CHO cells was 7 nM.

Stably Transduced Ba/F3 cells

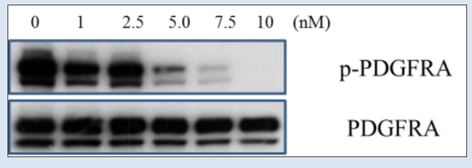

Figure 3. Western blot expression of PDGFR and p-PDGFR of PDGFRA D842V-transduced Ba/F3 cells after treatment with crenolanib. The IC_{50} of crenolanib in PDGFRA D842V stably transduced Ba/F3 cells was 10 nM.

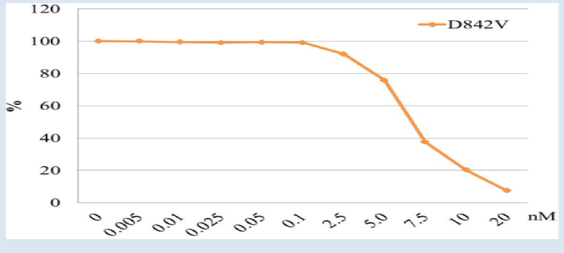

Figure 6. Proliferation assay of PDGFRA D842V-transduced Ba/F3 cells after treatment with crenolanib. Crenolanib has an IC_{50} of 7.5 nM in these cells.

PRIMARY GIST PATIENT CELL LINES

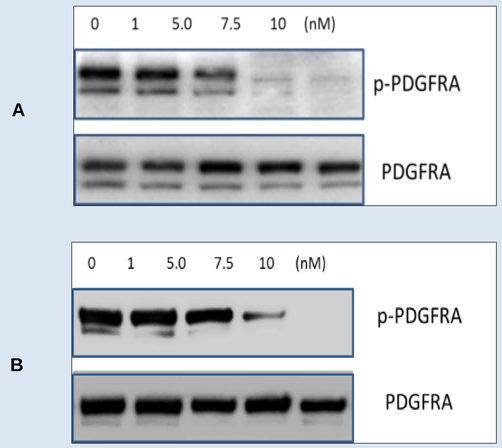

Figure 4. Western blot expression of PDGFR and p-PDGFR of two Imatinib-resistant primary GIST cell lines (A and B) after treatment with crenolanib. Inhibition of auto-phosphorylation is seen at 7.5-10 nM.

CONCLUSIONS

- Crenolanib inhibits PDGFRA phosphorylation at nanomolar concentrations in transiently transfected CHO cells, stably transduced Ba/F3 cells and primary GIST patient cell lines.
- Crenolanib is a unique TKI that blocks the kinase activity of PDGFRA D842V mutant at clinically achievable concentrations.
- 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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