Efficacy of Crenolanib Against the PDGFRα Activating Mutation, D842V, Associated with Gastrointestinal Stromal Tumors

M. Heinrich1, D. Griffith1, A. Mckinley1, A. Presnell1, A. Ramachandran2, C. Muralidhara3, M. von Mehren3
1. Portland VA Medical Center and OHSU Knight Cancer Institute; 2. AROG Pharmaceuticals, LLC, Dallas, TX; 3. Fox Chase Cancer Center, Philadelphia, PA.

PDGFRα 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 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.
- 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 PDGFRα 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

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Ori</th>
<th>Patients who responded</th>
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<tbody>
<tr>
<td>Imatinib</td>
<td>B222 phase II</td>
<td>0/3</td>
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<tr>
<td>Imatinib</td>
<td>EORTC phase III</td>
<td>0/4</td>
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<tr>
<td>Imatinib</td>
<td>US phase III</td>
<td>0/4</td>
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<tr>
<td>Sunitinib</td>
<td>Phase I/II</td>
<td>0/4*</td>
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*3 patients with primary PDGFRα 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 mediatied to overexpress PDGFRα.
- Crenolanib has been evaluated in Phase I (single agent) and Phase Ib (in combination with axitinib and docecatin) 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).

Cellular Assay with Transiently Transfected CHO Cells

PDGFRα mutations were cloned by site-directed mutagenesis and all mutations were confirmed by bidirectional sequencing. CHO cells were transiently transfected with plasmids encoding CDNs 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).

Crenolanib Inhibits the Activity of PDGFRα D842V Mutation In:

Transiently Transfected CHO Cells

![Figure 2. Inhibition of autophosphorylation of D842V mutant PDGFRα transiently expressed in CHO cells by crenolanib or imatinib.](image)

Cellular assay with transiently transfected CHO cells

Crenolanib inhibits the activity of PDGFRα D842V mutation in:

Stably Transduced Ba/F3 cells

![Figure 3. Western blot expression of PDGFRα and p-PDGFRα of D842V-transfected Ba/F3 cells after treatment with crenolanib.](image)

Primary GIST Patient Cell Lines

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

Conclusions

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References

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