Crenolanib is a unique benzimidazole tyrosine kinase inhibitor (TKI) known to preferentially bind to class III receptor tyrosine kinases (RTKs) at nanomolar concentrations (Table 1). It has been evaluated in phase I and phase II trials in solid tumors and is currently being evaluated in a phase II trial in adult glioma, a phase II trial in gastrointestinal stromal tumors and a phase I trial in pediatric glioma.

### Binding Constants

<table>
<thead>
<tr>
<th>RTK</th>
<th>Kd (nM)</th>
</tr>
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<tbody>
<tr>
<td>FLT3</td>
<td>0.74</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>2.1</td>
</tr>
<tr>
<td>PDGFR</td>
<td>3.2</td>
</tr>
<tr>
<td>CFS1R</td>
<td>30</td>
</tr>
<tr>
<td>KIT</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 1. Binding constants of crenolanib for the class III RTKs PDGFR, FLT3, KIT and CFS1R. Crenolanib has nanomolar potency for these wild type kinases.

### Competition Binding Assay

The effect of ABL1 A-loop phosphorylation on crenolanib affinity was investigated in a competition binding assay. The relative affinities of TKIs for phosphorylated and non-phosphorylated ABL1 have been previously used to accurately distinguish type I from type II inhibitors. The KINOMEScan assay combined a DNA-tagged kinase and an immobilized ligand with crenolanib. Crenolanib’s ability to compete with the immobilized ligand was measured via quantitative PCR of the DNA tag (see Figure 1).5

### TKIs Can Be Classified as Type I or Type II

Depending on the mechanism of binding to the kinase, tyrosine kinase inhibitors can be classified as type I or type II inhibitors. The characteristics of each type of inhibitor are listed in Table 2, below. A schematic of the kinase inhibitor binding modes is given in Figure 2, below. Resistant mutations to imatinib, sunitinib, quizartinib, and sorafenib are mutations that render the kinase domain of the RTK constitutively phosphorylated. Inhibitors that target the phosphorylated kinase thus may have potential use in treating diseases that harbor these mutations.

### Phosphorylation

<table>
<thead>
<tr>
<th>TKIs</th>
<th>Crenolanib, PIK-39</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crenolanib, PIK-39</td>
</tr>
</tbody>
</table>

Table 2. Type I and type II TKIs differ in the conformation of the kinase they prefer to bind. Type I TKIs preferentially bind to phosphorylated “active” kinase while type II TKIs preferentially bind to non-phosphorylated “inactive” kinase.5,4

### Affinity of Crenolanib for FLT3

Crenolanib’s difference in binding affinities for the non-autoinhibited and autoinhibited states of FLT3 also indicate that the molecule functions as a type I inhibitor. As is shown in Figure 2, crenolanib has a Kd of 0.63mM for non-autoinhibited FLT3 and a Kd of 6.7nM for autoinhibited FLT3. Crenolanib thus has an approximately 10-fold affinity shift between the non-autoinhibited and autoinhibited states of FLT3. This value is within the range of affinity shifts reported for other type I TKIs and is far outside the range of 100- to 1000-fold affinity shifts reported for type II TKIs.

### Crenolanib is Active Against Constitutively Active Mutations

Crenolanib has high activity against constitutively active mutations in class III RTKs and is more active against these mutations than other known TKIs.

### References

5. Crenolanib KINOMEScan assay, DiscoveRx, 2011.

### CONCLUSIONS

- Crenolanib is a unique chemotype, mutant-specific type I TKI.
- Crenolanib’s greater affinity for phosphorylated receptors makes it an ideal candidate for targeting indications that are driven by constitutively-active mutations of class III receptors.

### Appendix

[Image of binding constants for several known TKIs for three constitutively active mutations of FLT3 and two constitutively active mutations of KIT. Crenolanib has the greatest sensitivity for the FLT3 mutations of any TKI tested and is more sensitive for the KIT mutations than the majority of the TKIs tested.]

Table 3. The Kd of crenolanib for phosphorylated ABL1 is 7-fold lower than the Kd for non-phosphorylated ABL1. The Kd of crenolanib for phosphorylated ABL(T315I) is 15-fold lower than the Kd for non-phosphorylated ABL(T315I).5

### Figures

- Figure 1. Selectivity profile of crenolanib. Crenolanib is highly specific for class III RTKs.
- Figure 2. Mechanism of the KINOMEScan competition binding assay. The assay combines a DNA-tagged kinase, an immobilized ligand and a test compound to determine the binding constant of the test compound for the kinase.5
- Figure 3. Type I TKIs (shown here as PD166326) prefer to bind to kinase in the active conformation (a) with the DFG motif out. Type II TKIs (shown here as imatinib) prefer to bind to kinases in inactive conformation (b) with the DFG motif out.