Crenolanib is a unique drug that binds specifically to certain types of cancer-related enzymes, known as tyrosine kinases (RTKs). These enzymes are involved in the growth and spread of tumors. Crenolanib is particularly active against some mutant forms of RTKs, which are found in various types of cancer, including glioma and leukemia.

### Crenolanib BESYLATE (CP-868,596-26)

- Benznimidoazole tyrosine kinase inhibitor (TKI) known to preferentially bind to class III receptor tyrosine kinases (RTKs) at nanomolar concentrations (Table 1).
- 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.

### COMPLEX 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).²

### 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 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.

### CRENOLANIB HAS A GREATER AFFINITY FOR PHOSPHORYLATED KINASES

Analysis of crenolanib's affinity for kinases ABL1 and ABL(T315I) demonstrated that the molecule exhibits the characteristic mechanism of a type I inhibitor. Crenolanib's binding constants for phosphorylated ABL1 and ABL(T315I) were 7- and 15-fold lower than its binding constants for non-phosphorylated ABL1 and ABL(T315I), respectively. Though crenolanib is not active against ABL1, the molecule's significantly greater affinity for the phosphorylated kinase suggests that crenolanib may be a type I TKI.

### 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 shown in Figure 2, crenolanib has a Kd of 0.63nM 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.

### 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.

### REFERENCES