Bezuclastinib Selectivity to KIT A-Loop Mutations, Minimal Brain Penetration, and Favorable PK Properties In Preclinical Models

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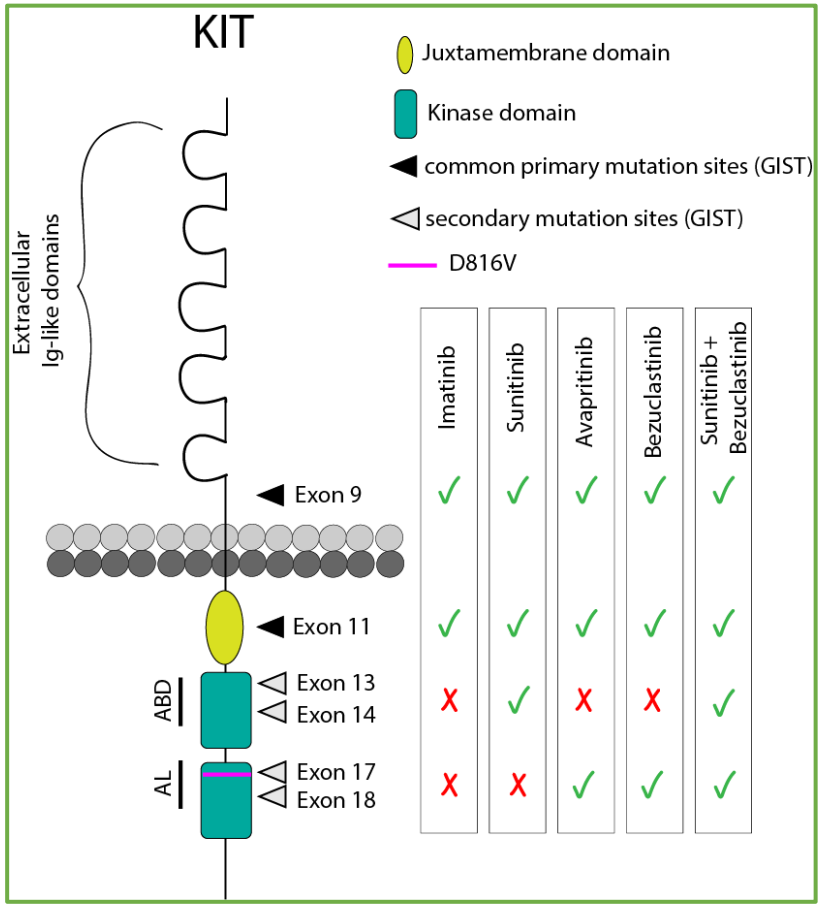
KIT Activation Loop Mutants are Key Targets for Systemic Mastocytosis and Refractory GIST



ABD= ATP-Binding Domain; AL= Activation Loop

- *KIT* mutations serve as driver mutations in up to 80% of gastrointestinal stromal tumors (GIST) and in over 90% of systemic mastocytosis (SM)[1,2]
- In GIST, patients often relapse after front-line imatinib treatment due to secondary mutations in ATP-binding domain (ABD) or Activation Loop (AL)[3]
  - Second-line sunitinib is active against ABD mutations, but not AL mutations
- Inhibitors targeting AL mutations, notably D816V (a common AL mutation in SM), have shown activity in the treatment of advanced SM, but off-target toxicities of available compounds may limit optimal clinical dosing[4, 5]
- Bezuclastinib is a novel type I TKI that was developed as a highly potent and selective inhibitor of KIT D816V

1. Klug LR et al., Nature Reviews Clinical Oncology, 2022:1-14; 2. Shomali W, Gotlib J. Hematology. 2018;2018(1):127-136; 3. Napolitano A, Vincenzi B, British Journal of Cancer. 2019;120(6):577-578; 4. RyDAPT [package insert]. East Hanover, New Jersey: Novartis Pharmaceuticals; 2021; 5. AYVAKIT [package insert]. Cambridge, MA: BluePrint Medicines; 2021.

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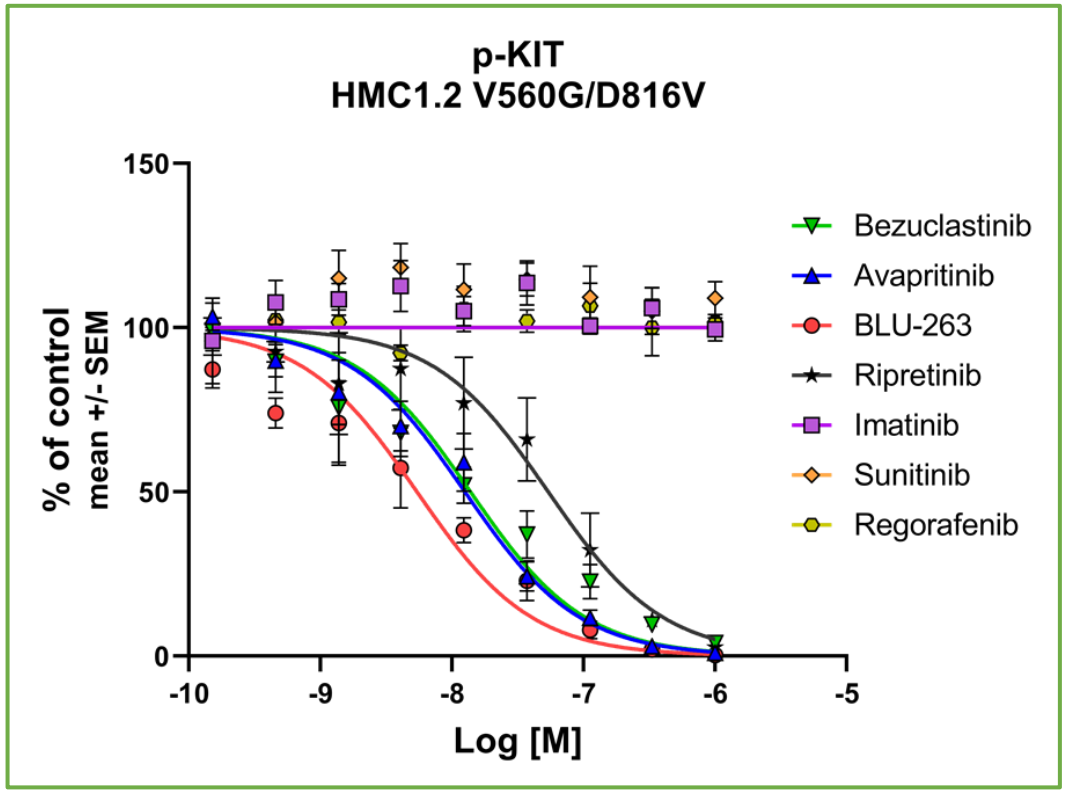
Bezuclastinib is a Potent Inhibitor of KIT D816V, an Activation Loop Mutation



HMC1.2 human mast cells (V560G/D816V) were treated with inhibitors for 1 hour followed by analysis for phosphorylated c-KIT ELISA (R&D Systems)

Compound	Cell IC_{50} (nM)
	KIT V560G/D816V (HMC 1.2)
Bezuclastinib	**14**
Avapritinib	13
BLU-263	6
Ripretinib	54
Imatinib	>1000
Sunitinib	>1000
Regorafenib	>1000

IC_{50} values from ELISA in (A) in nM are represented for bezuclastinib and other KIT inhibitors

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Bezuclastinib Demonstrates Superior Selectivity Against Closely Related Kinases

- Bezuclastinib demonstrates no activity on closely related kinases, unlike other KIT inhibitors
- Inhibition of these closely related kinases have been linked to off-target toxicities, such as edema and pleural effusions[1,2]

Compound	Cell IC_{50} (nM)				
	PDGFRα	PDGFRß	CSF1R	FLT3	KDR
Bezuclastinib	**>10,000**	**>10,000**	**>10,000**	**>1000**	**>1000**
Avapritinib	53	10	249	305	>1000
BLU-263	21	6	161	345	>1000
Ripretinib	20	34	312	534	110
Imatinib	75	247	1027	>1000	>1000
Sunitinib	23	14	313	1	4
Regorafenib	138	1180	473	237	101

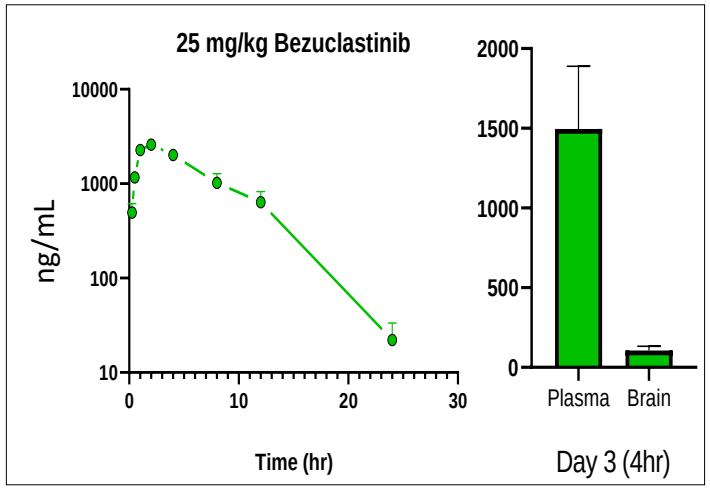
The table displays IC_{50} values (nM) for the closely related kinase assays. Color key displays where the fold change of these values vs. on-target KIT activity falls. On-target KIT activity was calculated with the following information for each KIT inhibitor: Bezuclastinib (KIT D816V = 14nM), Avapritinib (KIT D816V = 13nM), BLU-263 (KIT D816V = 6nM, Ripretinib (KIT D816V = 54nM), Imatinib (KIT V560G, HMC.1.1 cells = 10.7nM[3]), Sunitinib (KIT ΔJMD/T670I GIST T1 5R cells = 8.8nM), and Regorafenib (KIT K642E = 20nM[4])

Key: Fold change from on-target KIT activity

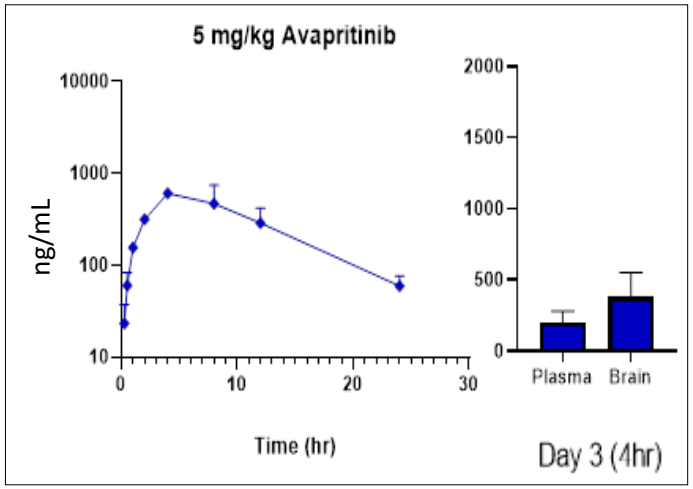
Color key displays where the fold change of these values vs. on-target KIT activity falls. On-target KIT activity was based on potency presented in prior slide.

1. Giles FJ et al, Leukemia. 2009;23(10):1698-1707; 2. Liu S, Kurzrock R. Seminars in Oncology. 2015;42(6):863-875 3. Smith BD et al., Cancer Cell. 2019;35(5):738-751;
4. Wilhelm S et al,Molecular Cancer Therapeutics. 2007;6(11_Supplement): B260-B260;

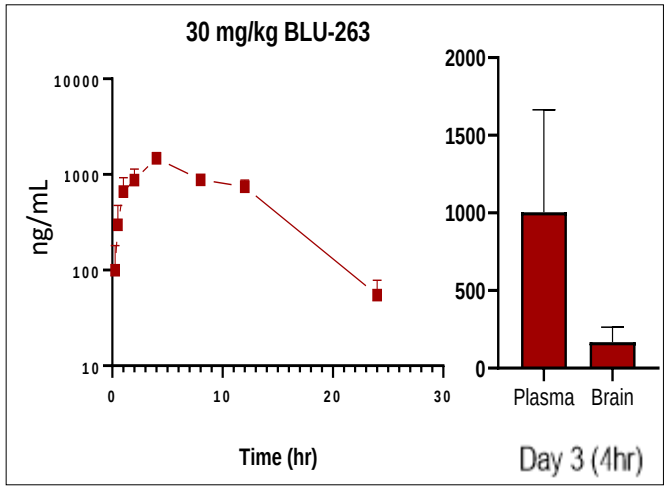
Bezuclastinib Demonstrates Minimal Brain Penetration



$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
7	2592 ± 364	21509 ± 2558	0.07



$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
13	672 ± 102	6980 ± 1156	2.0



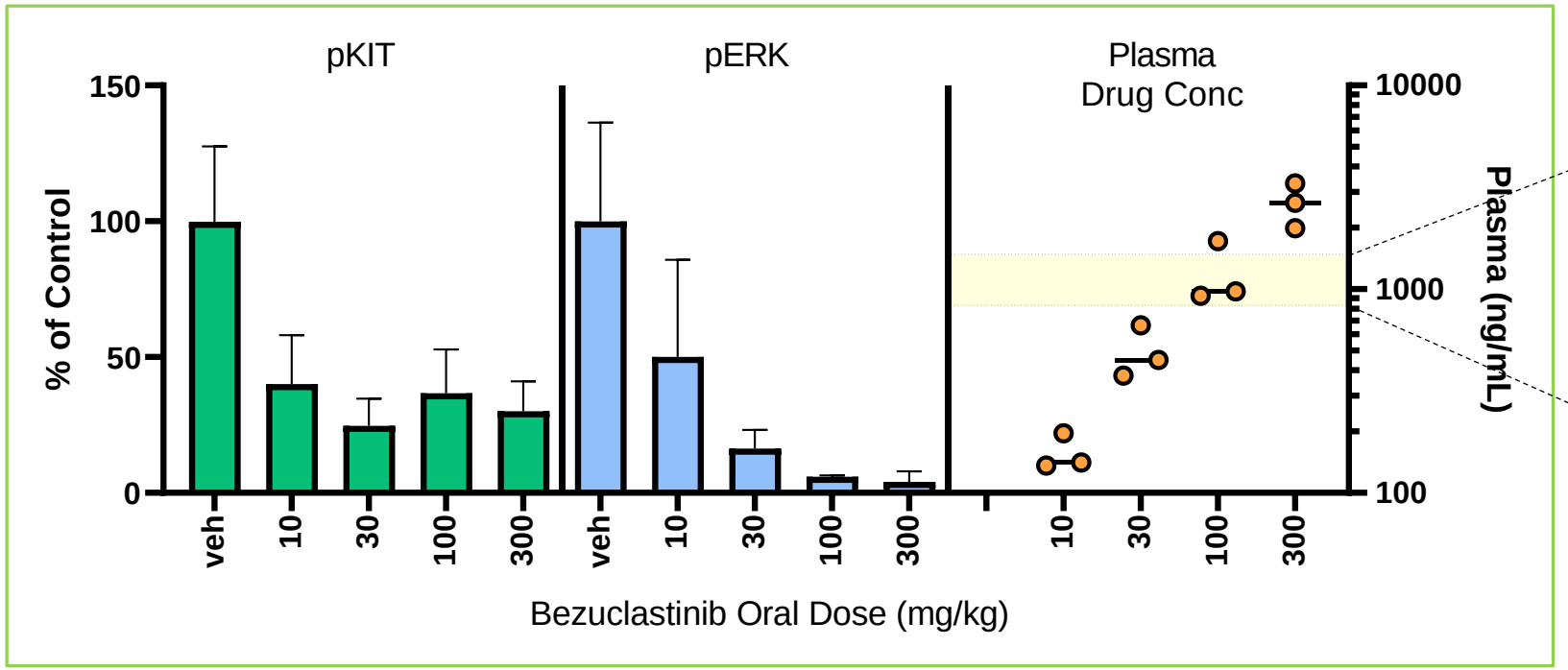
$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
4	1470 ± 222	16200 ± 414	0.18

- Bezuclastinib shows minimal brain penetration with brain to plasma ratio of 0.07 compared to 2.0 for avapritinib
- The absence of brain penetration is a preferred feature for a KIT A-Loop inhibitor given the CNS-related adverse events that have been observed in this targeted class[1,2]
- In a separate neurobehavioral (CNS) safety pharmacology study, rats were treated with oral doses of 0, 5, 25, or 100 mg/kg of bezuclastinib. No effect on behavioral endpoints were observed in this study, or in repeat dose toxicology studies (Data on File)

To assess brain distribution, male Sprague Dawley rats were administered 25 mg/kg bezuclastinib SDD, 5 mg/kg avapritinib, or 30 mg/kg BLU-263 by oral gavage. Dose levels were selected to correlate with clinical exposures reported in human clinical studies. Plasma samples were collected after a single dose and assessed for drug concentration by LC-MS/MS. Animals were dose administered for 2 additional days and plasma/brain harvested 4 hr post final dose. This repeat-dose administration – rather than single dose- allowed for a proper survey of steady state brain levels.

1. RyDAPT [package insert]. East Hanover, New Jersey: Novartis Pharmaceuticals; 2021; 2. AYVAKIT [package insert]. Cambridge, MA: BluePrint Medicines; 2021

Bezuclastinib Inhibits KIT D816V and Downstream Signaling in vivo at Concentrations Below Previously Observed Clinical Exposures



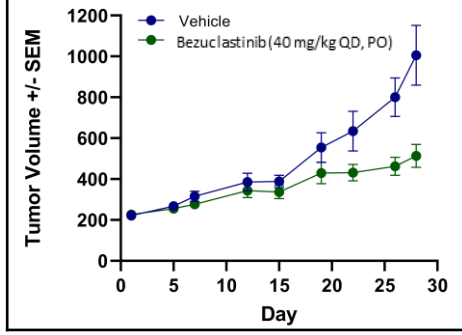
Bezuclastinib Clinical Cmax Range:
C1D15
250-1000 mg
monotherapy

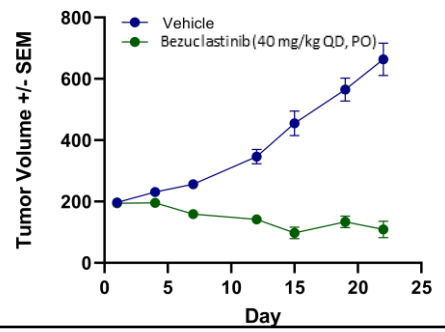
- HMC1.2 tumor-bearing nu/nu NCr female mice were treated with a single oral dose of bezuclastinib formulated as a spray-dry dispersion (Inotiv, Boulder, CO). Tumor and plasma were collected 4 hr post dose administration then assessed for drug concentration in plasma by LC/MS-MS, pKIT in tumor by ELISA (R&D Systems), and pERK normalized to GAPDH by immunoblot analysis. Phospho-protein data are expressed as a percent of vehicle control and represent n=3-6 individual samples.

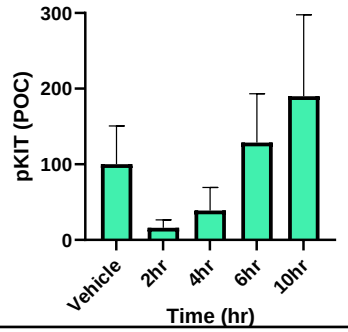
Guarnieri et al, AACR Annual Meeting 2022.

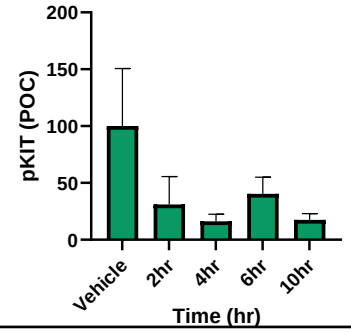
Clinically Achievable Exposures Represented in Nonclinical Models Demonstrate Significant Biological Activity

Model	Tumor growth inhibition GIST011 [KIT-Δ557-8/Y823D]		pKIT PK/PD HMC1.2 (KIT-D560G/D816V)	
Dose	4 mg/kg	40 mg/kg	10 mg/kg	30 mg/kg
AUC	**1310 ng·hr/mL**	**11293 ng·hr/mL**	**2168 ng·hr/mL**	**8342 ng·hr/mL**









GIST011 tumor-bearing NOD SCID female mice were randomized at a starting tumor volume of ~200mm^3 and treated with a single daily oral dose of Bezuclastinib (Crown Bio, San Diego, CA). Tumor volumes were determined three times weekly using the formula V=L*(W)^2/2.

HMC1.2 tumor-bearing nu/nu NCr female mice were treated with a single oral dose of Bezuclastinib formulated as a spray-dry dispersion (Inotiv, Boulder, CO). Tumor and plasma were collected at predetermined time points and assessed as described above (Figure 4).

Clinical AUC (ng·hr/mL) = **18,500 ng·hr/mL**[1]
(C1D15: 250 mg QD monotherapy)

1. Gebreyohannes YK et al., Clinical and Experimental Medicine. 2019;19(2):201-210

Guarnieri et al, AACR Annual Meeting 2022.

Summary

- Bezuclastinib:
  - A potent and selective inhibitor of KIT A-Loop mutations, with no activity demonstrated against closely related kinases
  - Shows minimal brain exposure and no evidence of CNS-related activity in nonclinical safety pharmacology studies
  - Exhibits time- and dose-dependent inhibition of pKIT and downstream signaling at plasma concentrations relevant to the exposures expected in ongoing clinical trials of bezuclastinib, supporting the potential for therapeutic activity in these patients
  - Currently under clinical investigation for Advanced SM (**APEX**, NCT04996875), NonAdvanced SM (**SUMMIT**, NCT05186753), and GIST (**PEAK**, NCT05208047)
  - Initial clinical data from a subset of patients from the APEX trial will be shared at the European Hematology Association meeting at the afternoon poster session on June 10, 2022 (Abstract P1049)