Bezuclastinib is a Differentiated KIT Inhibitor that Exhibits Unique Selectivity to KIT A-loop Mutations, Minimal Brain Penetration, and Favorable Pharmacokinetic Properties in Preclinical Models

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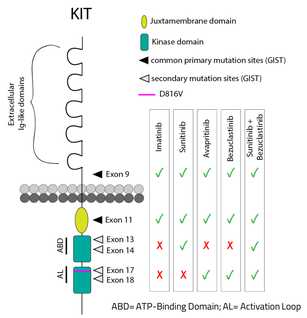
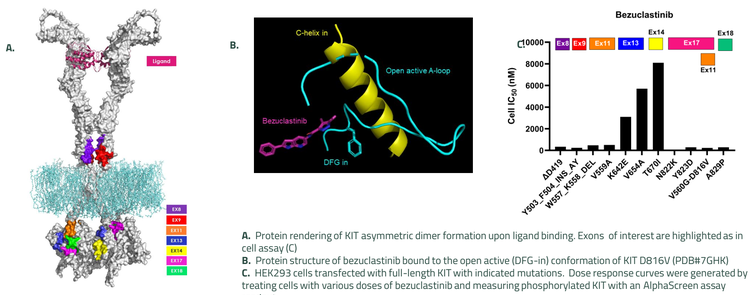
Background

Figure 1. KIT activation loop mutants are key targets for systemic mastocytosis and refractory GIST



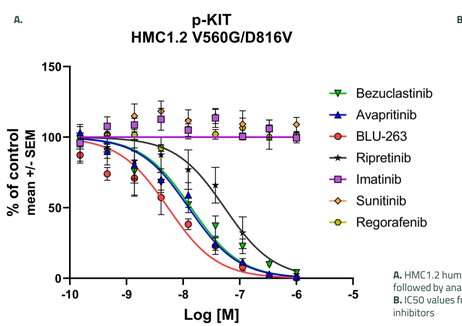
- Kit mutations serve as driver mutations in up to 80% of gastrointestinal stromal tumors (GIST) and in over 90% of systemic mastocytosis (SM)[1,2]
- In GIST, patients often relapse after front-line imatinib treatment due to secondary mutations in ATP-binding domain (ABD) or Activation Loop (AL)[3]
- 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**

Fig. 2 Bezuclastinib is a novel type I TKI with activity against activation loop Kit mutations



A. Protein rendering of KIT asymmetric dimer formation upon ligand binding. Exons of interest are highlighted as in cell assay (C)
B. Protein structure of bezuclastinib bound to the open active (DFG-in) conformation of KIT D816V (PDB#7GHK)
C. HEK293 cells transfected with full-length KIT with indicated mutations. Dose response curves were generated by treating cells with various doses of bezuclastinib and measuring phosphorylated KIT with an AlphaScreen assay readout

Fig. 3 Bezuclastinib is a potent inhibitor of KIT D816V, an activation loop mutation



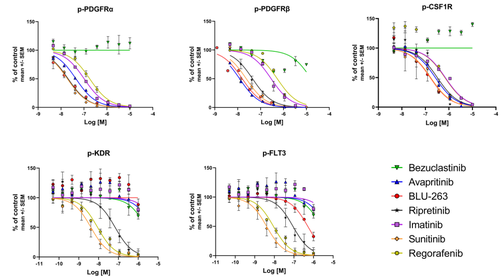
B.

Compound	Cell IC50 (nM) KIT V560G/D816V (HMC 1.2)
Bezuclastinib	**14**
Avapritinib	13
BLU-263	6
Ripretinib	54
Imatinib	>1000
Sunitinib	>1000
Regorafenib	>1000

A. 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)
B. IC50 values from ELISA in (A) in nM are represented for bezuclastinib and other KIT inhibitors

Results

Figure 4. Bezuclastinib demonstrates superior selectivity against closely related kinases



Off-target assays were completed in the cell lines listed here by phosphor ELISA assays (R&D Systems) against the target listed. Cell lines and conditions: PDGFRa in H1703 cells, PDGF-AA stimulated; PDGFRb in NIH3T3 cells, PDGF-BB stimulated; CSF1R in THP-1 cells, M-CSF1R stimulated; KDR in engineered HEK293 cells, VEGF stimulated, FLT-3 in engineered HEK293 cells, FLT-3 ligand stimulated. *n*=3 biological replicates +/- SEM displayed.

Table 1. Activity against closely related kinases relative to KIT activity

Compound	Cell IC50 (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

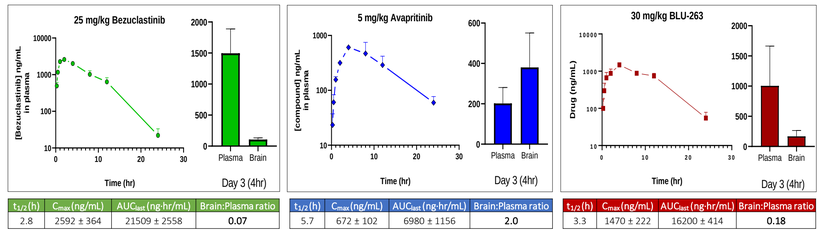
The table displays IC_{50} values (nM) for the closely related kinase assays displayed in Figure 4. Color key displays where the fold change of these values vs. on-target KIT activity falls. On-target KIT activity was calculated with the following information for each KIT inhibitor: Bezuclastinib (KIT D816V = 14nM, Figure 3), Avapritinib (KIT D816V = 13nM, Figure 3), BLU-263 (KIT D816V = 6nM, Figure 3), Ripretinib (KIT D816V = 54nM, Figure 3), Imatinib (KIT V560G, HMC.1.1 cells = 10.7nM[6]), Sunitinib (KIT ΔJMD/T670I GIST T1 5R cells = 8.8nM), and Regorafenib (KIT K642E = 20nM[7])

Key: Fold change from on-target KIT activity

≤ 10x	10x-30x	30x-50x	50x-100x	> 100x

- 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[8,9]

Figure 5 . Bezuclastinib demonstrates minimal brain penetration



$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
2.8	2592 ± 364	21509 ± 2558	0.07

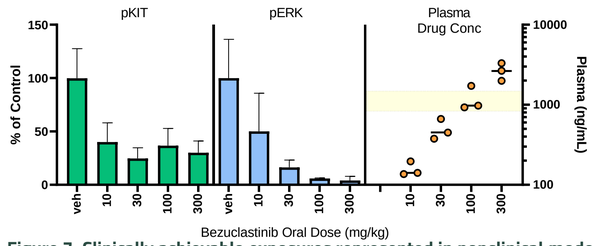
$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
5.7	672 ± 102	6980 ± 1156	2.0

$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{last} (ng·hr/mL)	Brain:Plasma ratio
3.3	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[4, 5]
- 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.

Figure 6. Bezuclastinib inhibits KIT D816V and downstream signaling *in vivo* at concentrations below previously observed clinical exposures



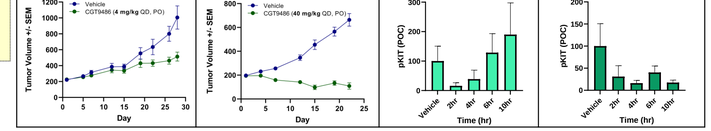
HMC1.2 tumor-bearing nu/nu NCr female mice were treated with a single oral dose of Bezuclastinib formulated as a spray-dry dispersion (Inotiv, Boulder, CO). Tumor and plasma were collected 4 hr post dose administration then assessed for drug concentration in plasma by LC/MS-MS, pKIT in tumor by ELISA (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.

Bezuclastinib Clinical Cmax Range:
C1D15
250-1000 mg monotherapy

Figure 7. 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 (mg/kg)	4 mg/kg	40 mg/kg	10 mg/kg	30 mg/kg
AUC (ng·hr/mL)	**1310 ng·hr/mL**	**11293 ng·hr/mL**	**2168 ng·hr/mL**	**8342 ng·hr/mL**

Clinical AUC (ng·hr/mL) = **18,500 ng·hr/mL**[10] (C1D15: 250 mg QD monotherapy)



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).

Conclusions

- Bezuclastinib is a potent and selective inhibitor of KIT A-Loop mutations, with no activity demonstrated against closely related kinases
- Bezuclastinib shows minimal brain exposure and no evidence of CNS-related activity in nonclinical safety pharmacology studies
- Bezuclastinib 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
- This selectivity and nonclinical safety profile supports the potential for a best-in-class KIT mutant inhibitor
- Bezuclastinib is currently under clinical investigation for Advanced SM (**APEX**, NCT04996875), NonAdvanced SM (**SUMMIT**, NCT05186753), and GIST (**PEAK**, NCT05208047)

References

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*All animal studies were conducted in accordance with The Guide for the Care & Use of Laboratory Animals and IACUC approved policies and procedures at the facilities where they were performed.

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