AACR-NCI-EORTC Virtual International Conference on
MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 7-10, 2021

Preclinical data identifies bezuclastinib as a differentiated KIT inhibitor with unique selectivity to KIT D816V and minimal evidence of brain penetration

Anna L. Guarnieri, Ph.D.
Cogent Biosciences, Boulder, CO

Disclosures

Anna L. Guarnieri Ph.D.

I have the following financial relationships to disclose:

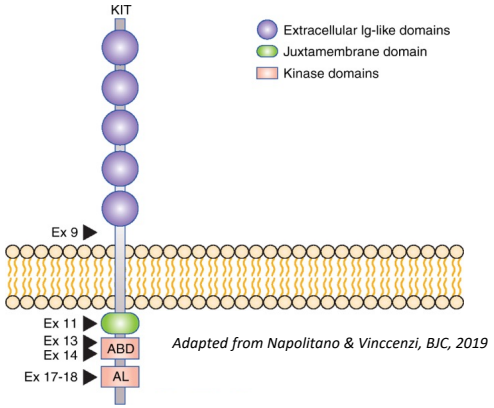
Stockholder in: Cogent Biosciences

Employee of: Cogent Biosciences

I will not discuss off label use in my presentation. I will discuss non-clinical investigative uses of bezuclastinib.

KIT Activation Loop Mutations are Key Therapeutic Targets for SM and Refractory GIST

- KIT mutations are found in 75-80% of gastrointestinal stromal tumors (GIST) and in over 90% of cases of systemic mastocytosis (SM)
- In GIST, primary driver mutations commonly occur in exon 9 and 11, which are covered by front-line treatment with imatinib, however, durable responses are rarely achieved due to secondary mutations in the ATP-binding domain (exon 13/14), or in the activation loop (exon 17/18)
- Second-line sunitinib is active against exon 13/14 mutations, but identifying inhibitors that target exon 17/18 (including D816V) without incurring off-target toxicities related to broad spectrum kinase inhibition has been challenging
- Inhibitors targeting 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
- **Bezuclastinib (CGT9486)** is a novel type I TKI with activity against primary KIT mutations (exons 9 & 11) and activation loop mutations (exons 17 & 18)



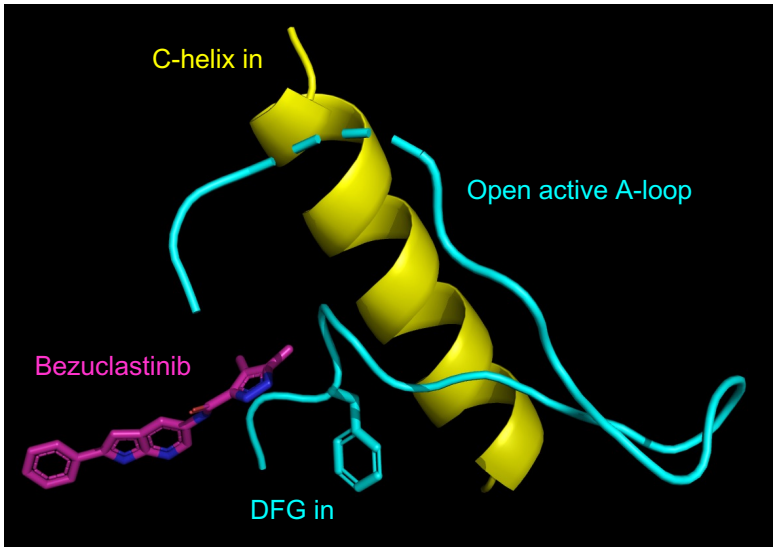
Adapted from Napolitano & Vinccenzi, BJC, 2019

Activity of KIT inhibitors on KIT exon mutations

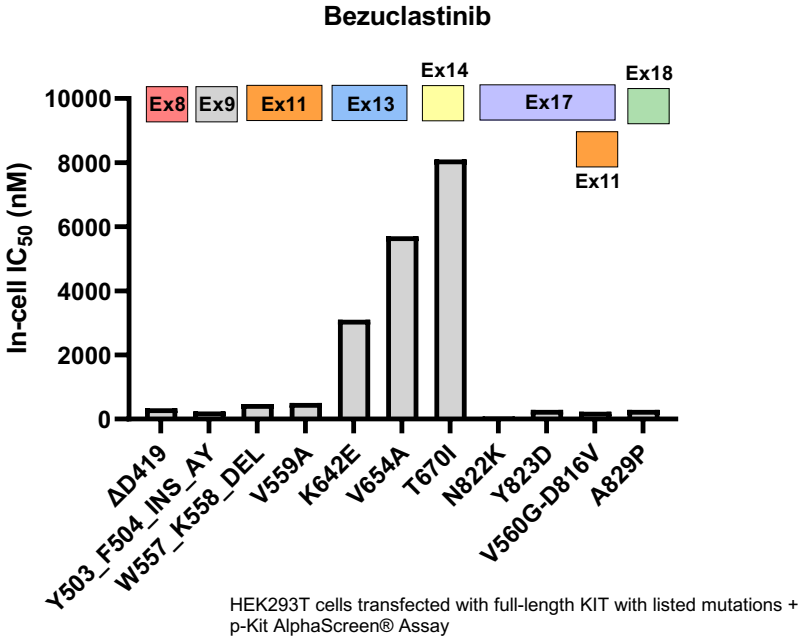
	Imatinib[a]	Sunitinib[a]	Regorafenib[a]	Ripretinib[b]	Avapritinib[c]	Bezuclastinib[d]
Exon 9/11						
Exon 13/14						
Exon 17/18 (Activation loop)						
D816V (Activation loop)						

***Ref*. a**. *Serrano et al, BJC, 2019;* **b**. *Smith et al, Cancer Cell, 2019;* **c.** *Evans et al, Sci Transl Med, 2017,* **d.** *Plexxikon, data on file*

Bezuclastinib was Designed as a Potent and Selective KIT Mutant Inhibitor



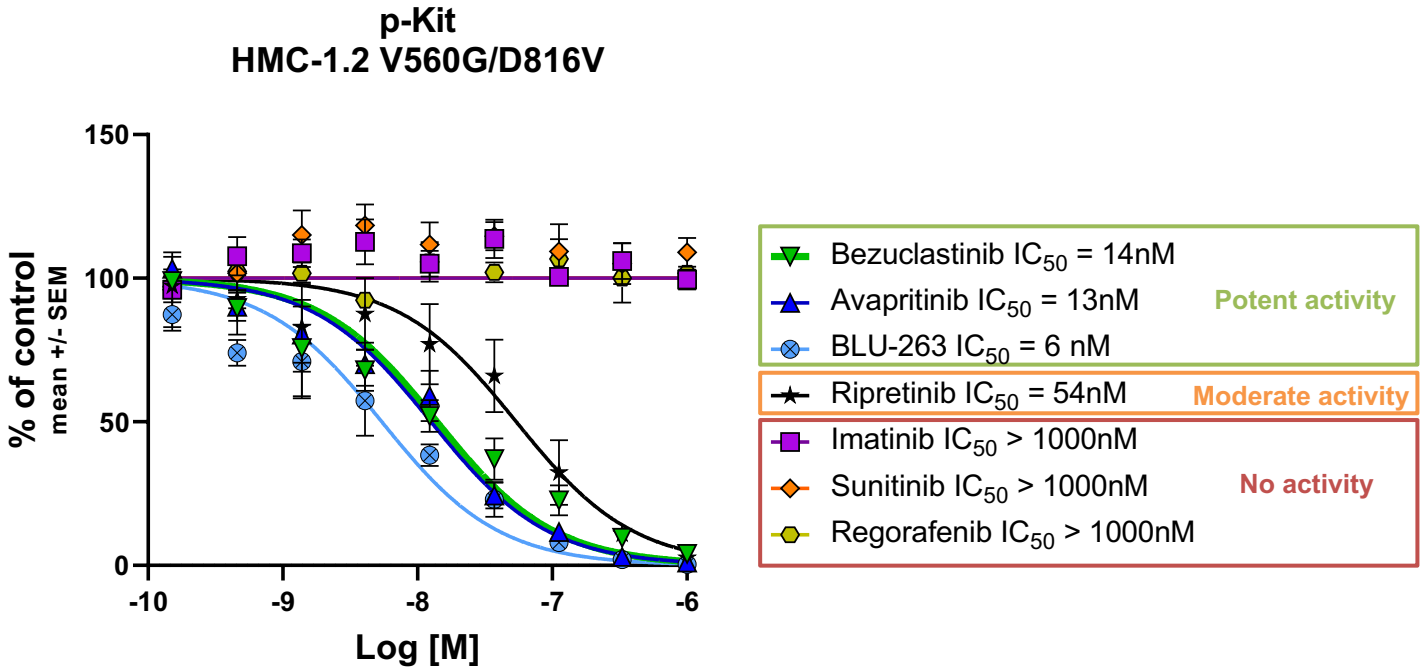
cKit-D816V crystal structure with Bezuclastinib (PDB#7GHK)



HEK293T cells transfected with full-length KIT with listed mutations + p-Kit AlphaScreen® Assay

- Structural insight was used to develop **bezuclastinib** as a potent and selective type I TKI with activity against DFG-in and open active A-loop mutations

Bezuclastinib is a Potent Inhibitor of KIT Activation Loop Mutants, Including D816V



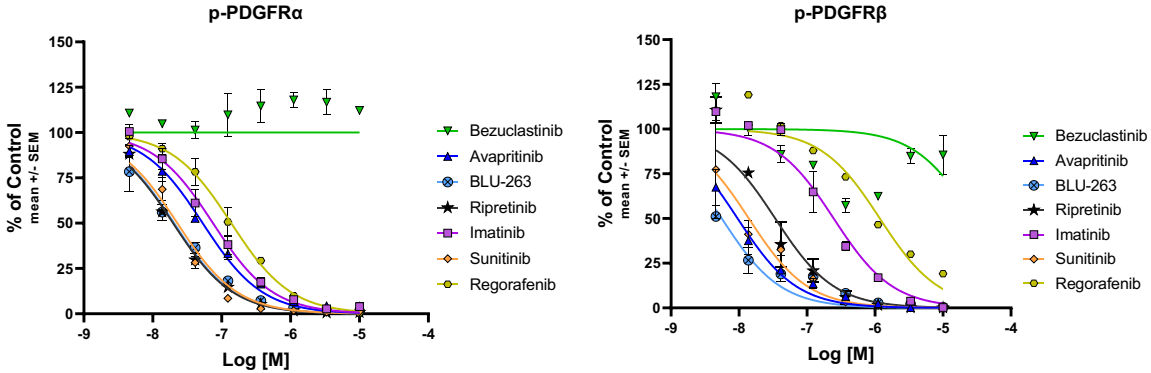
- HMC-1.2 human mast cells were treated with indicated inhibitors for 1 hour (n = 3 biological replicates)
- Readout is phosphorylated c-Kit (Human Phospho c-Kit ELISA, R&D Systems)

Bezuclastinib Demonstrates Best-in-Class Potential with Selectivity Against Related Kinases

Other selectivity data

- In a broad screen of 71 ion channels, receptors, transporters, and enzymes, no assays showed inhibition greater than 30% when screened at 10 µM

In-cell selectivity data



Summary of clinically relevant KIT V560G/D816V mutations vs. known off targets

	HMC1.2 KIT V560G/D816V	H1703 pPDGFRα	NIH3T3 p-PDGFRβ	THP-1 p-CSF1R
	Cellular IC50 (nM)			
Bezuclastinib	14	> 10,000	> 10,000	> 10,000
Avapritinib	13	53	10	249
BLU-263	6	21	6	312
Ripretinib	54	20	34	312
Imatinib	>1000	75	247	1027
Sunitinib	>1000	23	14	313
Regorafenib	>1000	138	1180	473

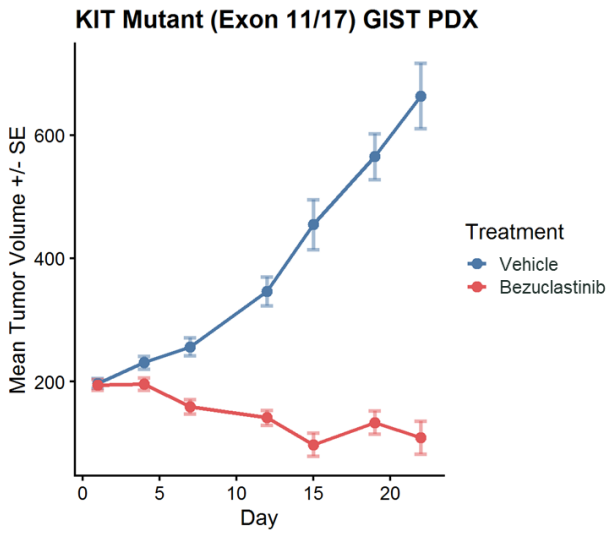
Phosphorylated kinases were measured by ELISA (CST PathScan® Phospho Sandwich ELISA), n= 3 biological replicates

- Inhibition of these closely related kinases have been linked to off-target toxicities, such as edema and pleural effusions[1,2]

1. *Giles et al, Leukemia, 2009*; **2**. *Liu and Kurzrock, Seminars in Oncology, 2015*

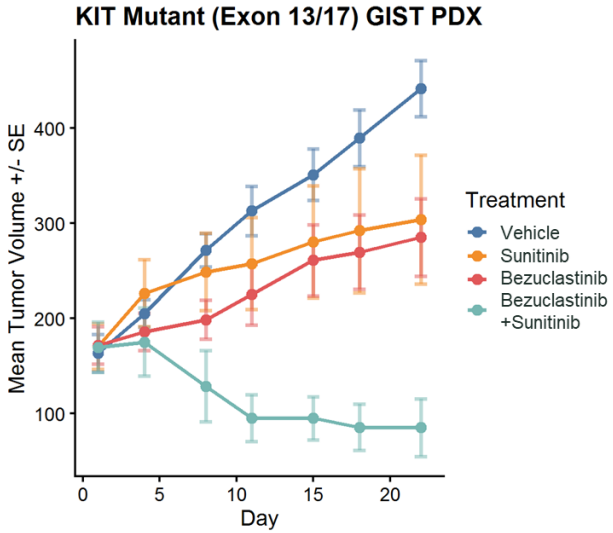
Dual-conformation KIT Inhibition Drives Tumor Regression in Heterogeneous GIST Patient-Derived Xenograft Models

Ex11 (W557_K558del), Ex17 (Y823D)



Gebreyohannes et. al, *Clin Exp Med*, 2019

Ex13 (K642E), Ex17 (N822K)



Activity of Sunitinib and Bezuclastinib on KIT exon mutations as single therapy and in combination

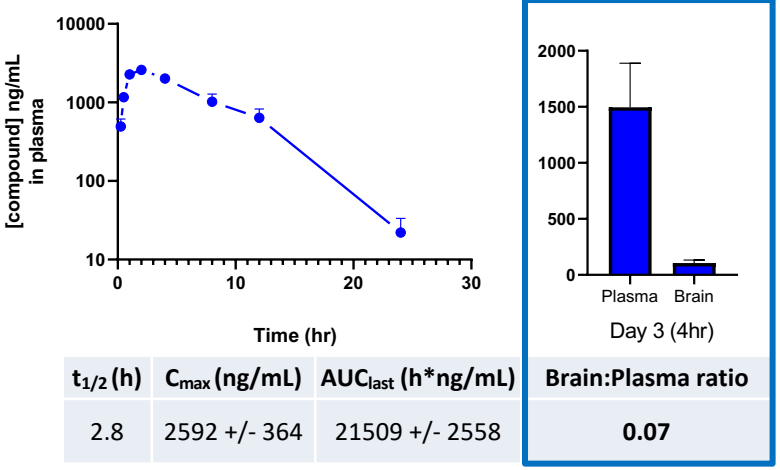
	Sunitinib[a]	Bezuclastinib[b]	Sunitinib + Bezuclastinib
Exon 9/11			
Exon 13/14			
Exon 17/18 (Activation loop)			
D816V (Activation loop)			

Ref. **a.** *Serrano et al, BJC, 2019* **b.** *Plexxikon, data on file*

Preclinical Data Demonstrates Minimal Brain Penetration with Bezuclastinib vs. Another KIT A-Loop Mutant Inhibitor

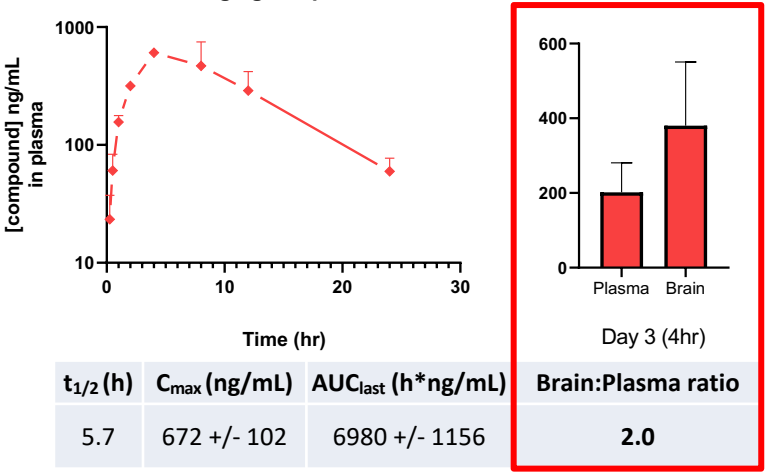
Tissue distribution in rats: plasma vs. brain

25 mg/kg Bezuclastinib



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

5 mg/kg Avapritinib



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

- Selected doses for bezuclastinib and avapritinib closely correlate with clinical exposures in humans for GIST
- Study design includes repeat-dose administration- rather than single dose- which allows for better estimation of exposure in the 'deep' compartment of the brain.
- 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.

Conclusions

- Bezuclastinib is a potent and selective inhibitor of KIT A-Loop mutations, with no activity demonstrated against closely related kinases
  - Other KIT mutant inhibitors demonstrate activity against PDGFRa and PDGFRb
- In vivo results in GIST PDX models show significant tumor growth inhibition
- Bezuclastinib shows minimal brain exposure and no evidence of CNS-related activity in nonclinical safety pharmacology studies
- This selectivity and nonclinical safety profile supports the potential for a best-in-class KIT mutant inhibitor
- Bezuclastinib is currently under clinical investigation in Advanced SM (**APEX***)with additional clinical studies planned in non-advanced SM (**SUMMIT**) and imatinib-resistant GIST

*cogentclinicaltrials.com

AACR-NCI-EORTC Virtual International Conference on
MOLECULAR TARGETS AND CANCER THERAPEUTICS
October 7-10, 2021

Additional abstract authors:

- Mark Chicarelli, Cogent Biosciences
- Yeyun Zhou, Cogent Biosciences
- LouAnn Cable, Cogent Biosciences
- Francis Sullivan, Cogent Biosciences
- Howard Ball, Cogent Biosciences
- Shannon Winski, Cogent Biosciences
- John Robinson, Cogent Biosciences

For questions contact:
Anna.Guarnieri@cogentbio.com