Bruton Tyrosine Kinase (BTK) Protein Degrader BGB-16673 Is Less Apt To Cause, And Able To Overcome Variable BTK Resistance Mutations Compared To Other BTK Inhibitors

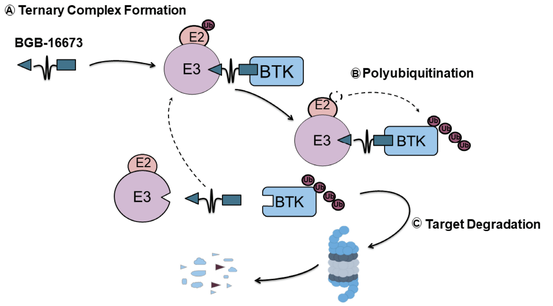
Xiaoyu Feng[1], Yangyang Wang[1], Tao Long[1], Lanyue Bai[1], Xiaojiao Yang[1], Ailing Yang[1], Xiangmei Chen[1], Oscar Puig[1], Yangbo Yue[1,*], Zhirong Shen[1,*]

[1]Translational Discovery, Research and Medicine, BeiGene (Beijing) Co., Ltd, Beijing, China. *Correspondence author

INTRODUCTION

- Bruton tyrosine kinase (BTK) plays a critical role in the B-cell antigen receptor signaling pathway and the pathogenesis of multiple B-cell malignancies [1].
- Irreversible covalent BTK inhibitors (BTKi) have been instrumental in the management of B-cell malignancies [2], but mutations in BTK can lead to BTKi resistance and limit therapeutic effectiveness due to reduction binding capacity to BTK, scaffold function leading to BTK kinase activity independent NF-κB activation and/or kinase hyperactivation [3,4,5].
- Although on-target resistant mutations emerging from covalent BTKi have been described, less is known about resistance mutations caused by reversible non-covalent BTKi, and very little is known regarding resistance to BTK degraders.
- BGB-16673 is an orally available chimeric degradation activating compound (CDAC) with demonstrated preclinical BTK degradation activity (**Figure 1**). It is under the evaluation in two clinical phase I trials (NCT05006716 and NCT05294731) [6].

Figure 1. CDAC mechanism of action



Abbreviations: BTK, bruton tyrosine kinase; CDAC, chimeric degradation activating compound; E2, ubiquitin-conjugating enzymes; E3, ubiquitin ligases; Ub, ubiquitin.

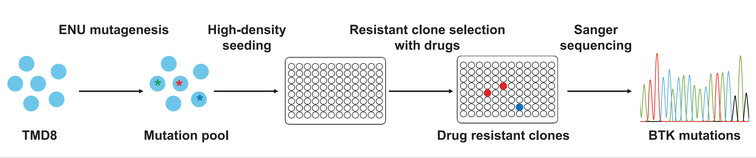
OBJECTIVES

- To characterize the profile and tendency of BGB-16673 to cause on-target BTK resistance mutations as compared with non-covalent BTKi.
- To determine its ability to overcome clinically emergent BTK mutations and compare this activity to other covalent and non-covalent BTKi.

METHODS

- ENU mutagenesis screen (**Figure 2**)
  - BTKi-sensitive TMD8 lymphoma cells were treated with N-ethyl-N-nitrosourea (ENU, a highly potent mutagen) for 18h. Mutant cells were treated with serial doses of either BGB-16673, compound-ED (an E3 ligase dead control with the same warhead as BGB-16673; binds BTK reversibly, but cannot degrade it), or pirtobrutinib (a non-covalent BTKi).
  - BTK cDNA from resistant clones was sequenced to identify and calculate the frequency of mutations.

Figure 2. Procedure of the ENU mutagenesis screen

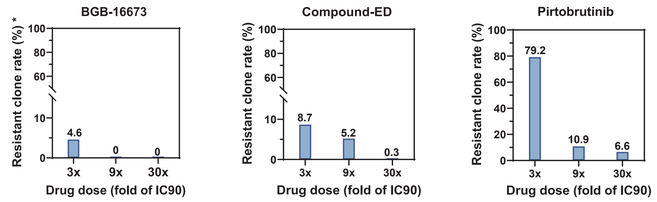


- Clinically emergent BTK resistance mutation test
  - The ability of BGB-16673 to overcome BTK mutations was evaluated with the CellTiter-Glo Luminescent cell viability assay in both TMD8 and OCI-LY10 lymphoma cell lines where various known clinically emergent BTK resistance mutants were overexpressed.
  - Cells were treated with multiple doses of BGB-16673, Compound-ED or pirtobrutinib. IC50s were calculated from the dose response curves.
  - A homogeneous time resolved fluorescence assay and western blot were conducted to evaluate BTK mutants' degradation by BGB-16673 in the TMD8 cell line.

RESULTS

- In ENU mutagenesis screens, fewer resistant clones and lower BTK mutation frequency were seen with BTK CDAC treatment as compared to non-covalent BTKi treatment (**Figure 3 and Table 1**).

Figure 3. Resistance clone rates in the ENU mutagenesis screens



**Resistant clone rate is defined by the proportion of the number of emerged resistant clones to the number of seeded wells.*
Abbreviations: IC, inhibition concentration.

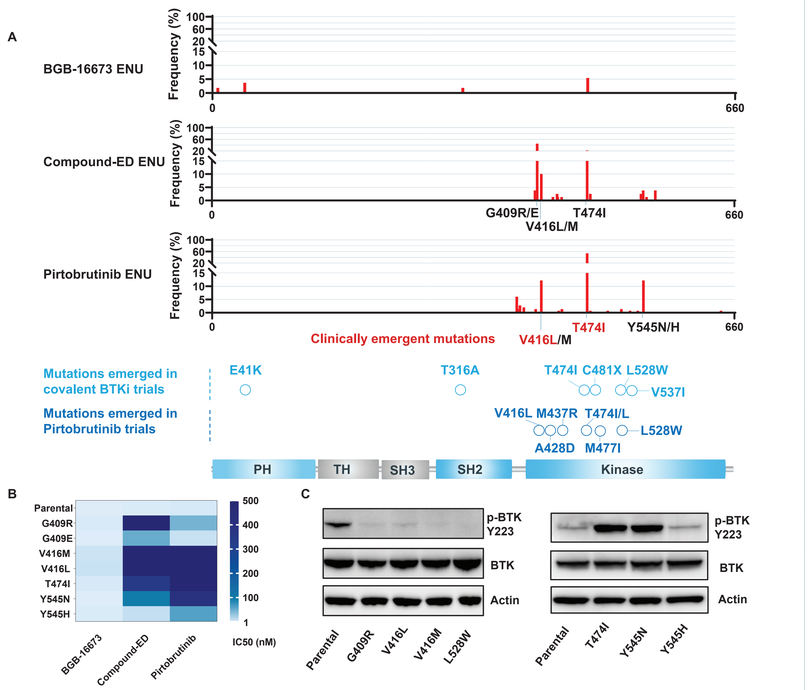
Table 1. BTK mutation frequencies in the ENU mutagenesis screens

Compound	BGB-16673 (BTK CDAC)	Compound-ED (E3 dead non-covalent control)	Pirtobrutinib (non-covalent inhibitor)
BTK mutation Frequency **	12.7% (7 out of 55)	100% (80 out of 80)	100% (144 out of 144)

***BTK mutation frequency is defined by the proportion of the number of resistant clones harboring BTK mutations to the number of resistant clones been sequenced.*

- BGB-16673 appears to have a unique BTK resistance mutation profile vs. pirtobrutinib. Much fewer BTK mutants appeared in BGB-16673 resistant clones.
  - No BTK mutations with frequency >10% were observed in the BGB-16673 arm. In comparison, more clones resistant to pirtobrutinib had mutations in BTK, many of which arose in the kinase domain. Among the pirtobrutinib clinically derived BTK mutations, V416L and T474I were identified as high frequent mutations, and one L528W clone was detected (**Figure 4A**).

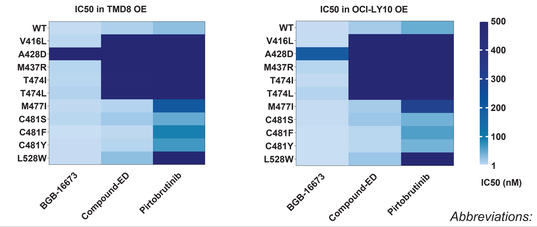
Figure 4. BTK mutation spectrums in the ENU mutagenesis screens



A, BTK cDNA sequencing analysis; B, Heatmap of average IC50 in multiple ENU clones with the same BTK mutation; C, Western blot analysis of high frequent and ENU mutagenesis screen/clinic overlapped BTK mutations.

- BGB-16673 was more potent against BTK mutations derived from compound-ED and pirtobrutinib screens (**Figure 4B**).
- BTK kinase activities of BTK mutations that were both frequently observed in the ENU screen and known to be clinically emergent displayed significant variability (**Figure 4C**).
- BGB-16673 could overcome all BTK resistance mutations from both covalent and non-covalent BTKi trials, except A428D which was tested to be resistant to all BTKi (**Figure 5**).

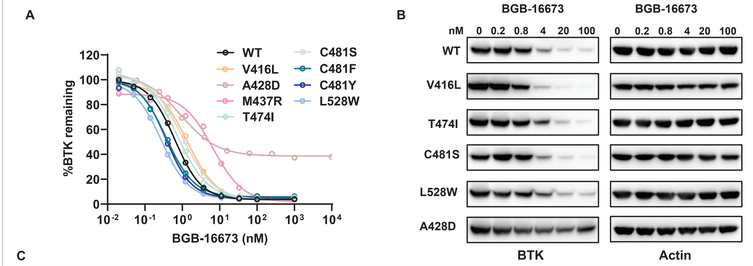
Figure 5. Heatmap of average IC50 in the BTK mutant overexpression cell lines



Abbreviations: OE, overexpression

- BGB-16673 could degrade BTK in the presence of clinically relevant resistance mutations (V416L, M437R, T474I, C481S, C481F, C481Y, and L528W) (**Figure 6**).

Figure 6. Degradation of the BTK mutants by BGB-16673



BGB-16673 treatment time	Degradation	WT	V416L	A428D	M437R	T474I	C481S	C481F	C481Y	L528W
24h	DC50 (nM)	0.7	1.4	4.1	6.8	0.8	1.1	0.4	0.4	0.3
	Dmax (%)	96	97	62	98	95	96	94	95	96

A ,BTK degradation was evaluated in TMD8 overexpression cell lines by HTRF assay; B, Further verified by western blot analysis; C, Summary of BTK degradation data from HTRF assay; Abbreviations: DC, degradation constant; Dmax, maximum degradation

CONCLUSIONS

- Relative to other BTKi, the BTK CDAC BGB-16673 is less apt to cause on-target resistance mutations, demonstrated a unique on-target resistance mutation profile. Further studies exploring the dominant BGB-16673 resistance mechanisms not reliant on BTK are in progress.
- BGB-16673 could overcome a wide variety of BTK resistance mutations derived from both ENU mutagenesis screens and relapsed patients.
- These findings suggest that BGB-16673 is a promising novel BTK degrader that could benefit patients who develop BTKi on-target resistance mutations.

ACKNOWLEDGMENTS

- We sincerely thank **Jinhong Ren** and **Shifan Ma** (BeiGene Molecular Science department) for the valuable inputs on the structure predictions of BTK protein and drug interactions, **Lisa McGraw** (Medical Writing, Translational Discovery, Research and Medicine, BeiGene USA, Inc) for the fantastic support on manuscript development, and **Jason Paik** (Medical lead of BGB-16673 clinical trial) for insightful scientific discussions and manuscript review,

DISCLOSURE

- All authors have no conflicts of interest to disclose.

CONTACT INFORMATION

- yangbo.yue@beigene.com; zhirong.shen@beigene.com

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Presented at the 2nd Bermuda Translational Summit on Hematologic Malignancies (DAVA) July 26-30, 2023, Bermuda
Previously presented at EHA 2023 Hybrid Congress; June 8-15, 2023; Frankfurt, Germany