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BGB-16673, A BTK Degrader, Overcomes On-Target Resistance From BTK Inhibitors And Presents Sustainable Long-Term Tumor Regression In Lymphoma Xenograft Models

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INTRODUCTION

- Bruton tyrosine kinase (BTK) is a key component of the BCR signaling pathway whose chronic activation is critical for cell proliferation and survival in various B cell malignancies[1]. Inhibition of BTK by covalent BTK inhibitors (cBTKis), such as ibrutinib, acalabrutinib, and zanubrutinib, have revolutionized the management of CLL and other B cell malignancies.
- However, frequently acquired BTK resistant mutations at cysteine 481, which abrogate cBTKi binding capacity, and other mutations inducing kinase hyperactivation or kinase independent function, limit long-term clinical benefit.
- Non-covalent BTK inhibitors (e.g., pirtobrutinib) have demonstrated promising efficacy in CLL patients with BTK C481 mutations who progressed on cBTKis[2]. Even so, BTK mutations beyond BTK C481 emerged in some patients[3].
- Agents which could tackle resistance mutations from both covalent and non-covalent BTKis may provide novel treatment options. Moreover, though BTK dependency for certain aggressive lymphomas is well documented, the clinical benefit of approved BTKis seems to be modest and further clinical investigation is warranted. A compound with BTK-targeted degradation may bring additional advantage over BTK inhibition for those aggressive diseases.
- BGB-16673 is an orally available BTK-targeting chimeric degradation activation (BTK-CDAC) compound designed to degrade wildtype BTK and multiple mutant forms. It is currently under investigation in two phase I studies (NCT05006716, NCT05294731).

OBJECTIVE

- Here, we investigated the capability of BGB-16673 to overcome commonly observed on-target mutations from both covalent and non-covalent BTKis in cell lines and mouse xenograft models. Additionally, BTK and downstream phosphorylation events in relevant cell lines were evaluated. We further examined whether BGB-16673 is superior to BTKis in suppressing tumor growth and metastasis.

METHODS

- TMD-8 cells expressing wildtype or mutant BTK were incubated with BTK inhibitors and BGB-16673. Cell viability was measured by CTG assay.
- Western blot was utilized to detect phosphorylation of BTK Y223 and PLCγ2 Y1217.
- Wildtype or mutant BTK-expressing TMD-8 cells were inoculated subcutaneously into NCG mice for *in vivo* efficacy determination.
- Comparisons between groups were performed using unpaired t tests.

CONCLUSION

- BGB-16673 is a potent degrader against tumors expressing wildtype and clinical-relevant BTK mutations. In addition, BGB-16673 exhibits longer duration of response and less metastatic infiltration to the spleen than ibrutinib and pirtobrutinib.

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2. Mato, A. R. et al. Lancet. 2021;397: 892-901.
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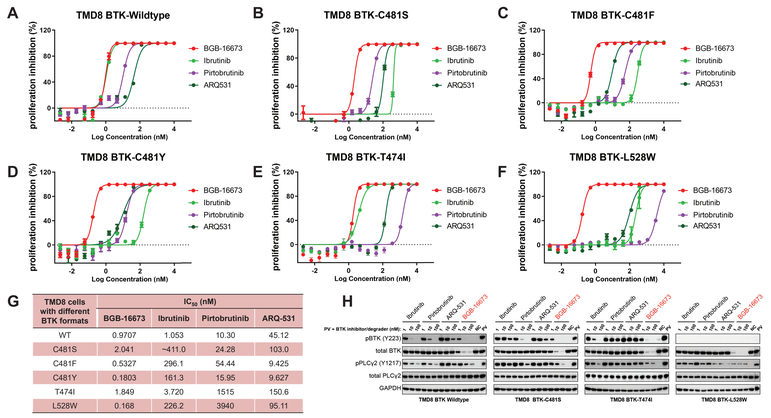
RESULTS

◆ Figure 1. BGB-16673 exhibits high potency on clinically relevant BTK mutants resistant to covalent and non-covalent BTK inhibitors in cancer cells *in vitro*



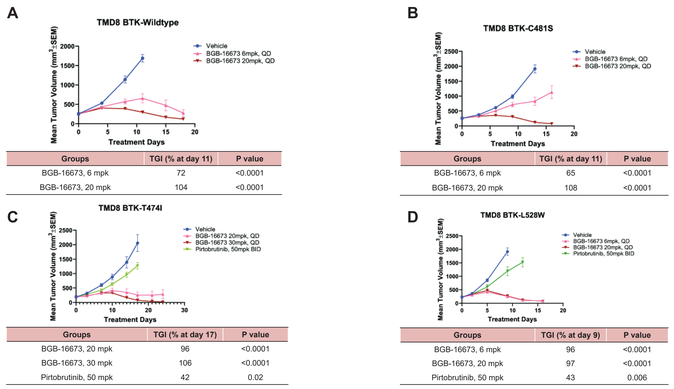
G

TMD8 cells with different BTK formats	IC_{50} (nM)			
	BGB-16673	Ibrutinib	Pirtobrutinib	ARQ-531
WT	0.9707	1.053	10.30	45.12
C481S	2.041	~411.0	24.28	103.0
C481F	0.5327	296.1	54.44	9.425
C481Y	0.1803	161.3	15.95	9.627
T474I	1.849	3.720	1515	150.6
L528W	0.168	226.2	3940	95.11

H

(A-F) TMD8 cells expressing wildtype or mutant BTK were incubated with different concentrations of BGB-16673 and BTK inhibitors for 5 days, cell viability was measured by CTG assay. Relative proliferation inhibition was shown. (G) IC_{50} of BGB-16673 and BTK inhibitors from panels A-F. BTK C481 substitutions were frequently observed in CLL patients progressed from covalent BTK inhibitors (e.g., Ibrutinib), BTK T474I and L528W mutations were reported in pirtobrutinib progressed patients. BGB-16673 presents single-digit nM IC_{50} in TMD8 cells harboring these mutations. (H) Wildtype and mutant BTK-expressing TMD8 cells were treated with BGB-16673 and BTK inhibitors for 24 hours, followed by pervanadate (PV) treatment for 20 minutes. Cell pellets were harvested for western blot. Phosphorylation of BTK Y223 and PLCγ2 Y1217 were examined. At 10 nM, BGB-16673 efficiently degrades wildtype and mutant BTK proteins in cell.

◆ Figure 2. BGB-16673 drives complete tumor regression of lymphoma xenograft models expressing wildtype or BTK mutations resistant to covalent and non-covalent inhibitors



Groups	TGI (% at day 11)	P value
BGB-16673, 6 mpk	72	<0.0001
BGB-16673, 20 mpk	104	<0.0001

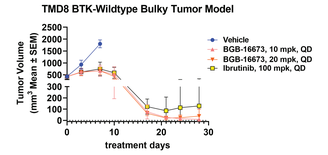
Groups	TGI (% at day 11)	P value
BGB-16673, 6 mpk	65	<0.0001
BGB-16673, 20 mpk	108	<0.0001

Groups	TGI (% at day 17)	P value
BGB-16673, 20 mpk	96	<0.0001
BGB-16673, 30 mpk	106	<0.0001
Pirtobrutinib, 50 mpk	42	0.02

Groups	TGI (% at day 9)	P value
BGB-16673, 6 mpk	96	<0.0001
BGB-16673, 20 mpk	97	<0.0001
Pirtobrutinib, 50 mpk	43	0.006

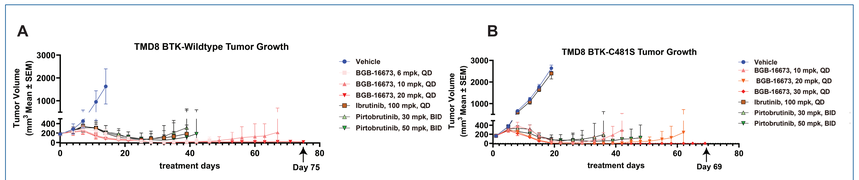
(A-D) Wildtype or mutant BTK-expressing TMD8 cells were injected into 6-8 weeks old female NCG mice subcutaneously. Animals were randomly grouped into 8 mice per group according to tumor volume and body weight. BGB-16673 was administered once daily, pirtobrutinib was administered twice daily, tumor volume was recorded twice weekly. Mean tumor volume ± SEM after treatment was shown. Tumor growth inhibition (TGI) was presented in table. In therapeutic xenograft models, BGB-16673 shows significant dose-dependent anti-tumor activity although different doses are required for complete tumor regression.

◆ Figure 3. BGB-16673 drives complete regression of large lymphoma xenograft model



TMD8 cells were implanted to 6-8 weeks old female NCG mice. Animals were randomly grouped into 8 mice per group when tumor volume reached ~450 mm³. BGB-16673 and ibrutinib was administered once daily. BGB-16673 tended to have deeper tumor growth inhibition than ibrutinib in bulky tumors. Oral administration of BGB-16673 can drive complete regression of large tumors, which mimics bulky tumors in clinic.

◆ Figure 4. BGB-16673 presents longer duration of response than BTK inhibitors in BTK wildtype and C481S mutant-expressing lymphoma xenograft models



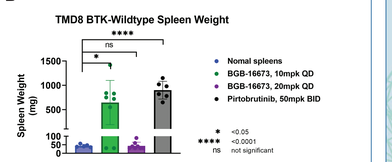
(A) TMD8 cells were implanted to 6-8 weeks old female NCG mice. Animals were randomly grouped into 8 mice per group when tumor volume reached ~150 mm³. Drugs were orally administered as indicated. Tumor volume was examined twice weekly. Mice were sacrificed if primary tumor volume reached ~2000 mm³ or relapsed tumor volume reached 1000 mm³ during treatment. Mean tumor volume ± SEM was shown. Plotting was ended if any mice in the group was sacrificed. BGB-16673 has a longer duration of response. (B) Similar experiment was performed with TMD8 BTK-C481S cancer cells. At a dose of 20-30 mpk, BGB-16673 maintained a longer duration of response.

◆ Figure 5. BGB-16673 has better survival rate and less tumor infiltration in spleens than BTK inhibitors in long-term treatment in BTK wildtype and C481S mutant-expressing lymphoma xenograft models

A

Groups	TMD8 BTK-Wildtype				
	TGI (% at Day 12)	P value (Day 12 vs pirtobrutinib 50 mpk)	Survival rate (Day 70) Survived / Total	Day 70 CR/survived	Splenomegaly mice (Day 70 Spl./survived)
BGB-16673, 6 mpk	99	0.2847	1/8	1/1	1/1
BGB-16673, 10 mpk	100	0.1289	8/8	8/8	6/8
BGB-16673, 20 mpk	101	0.0596	8/8	8/8	1/8
Ibrutinib, 100 mpk	99	0.2491	1/8	0/1	1/1
Pirtobrutinib, 30 mpk	94	0.5730	1/8	1/1	1/1
Pirtobrutinib, 50 mpk	97	--	6/8	5/6	6/6

B



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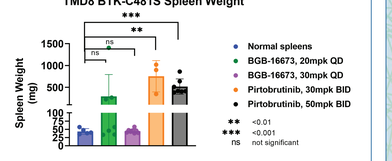
Groups	TMD8 BTK-C481S				
	TGI (% at Day 14)	P value (Day 14 vs pirtobrutinib 50 mpk)	Survival rate (Day 69 Survived / Total)	Day 69 CR/survived	Splenomegaly mice (Day 69 Spl./survived)
BGB-16673, 10 mpk	104	0.5235	0/8	0	NA
BGB-16673, 20 mpk	106	0.0697	7/8	5/7	3/7
BGB-16673, 30 mpk	106	0.0223	8/8	8/8	2/8
Ibrutinib, 100 mpk	10	<0.0001	0/8	0	NA
Pirtobrutinib, 30 mpk	103	0.5764	3/8	2/3	3/3
Pirtobrutinib, 50 mpk	103	--	7/8	5/7	7/7

D



(A) A table summarizing tumor growth inhibition (TGI) on day 12, as well as survival rate (survived mice/total mice per group), the ratio of complete regression (CR) mice versus survived mice, and the ratio of splenomegaly mice versus survived mice on day 70 in a long-term treatment experiment. On day 70, mice that received 10 mpk and 20 mpk BGB-16673 had a survival rate of 100%, while other treatments tended to have lower survival rates. (B) In the same experiment as in A, on day 70, spleen weights from survived mice in each group (if ≥ 3) were examined and compared with normal spleens. Mice treated with 20 mpk BGB-16673 did not show obvious spleen enlargement compared with other groups, indicating less metastatic tumor infiltration. (C-D) A long-term treatment experiment with TMD8 BTK-C481S xenograft model. Mice that received 30 mpk BGB-16673 had a survival rate of 100%. Spleen weights also suggested less spleen infiltration in mice treated with 20 mpk and 30 mpk BGB-16673.