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 (BTKi)

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Background:
BTK plays a critical role in the B-cell antigen receptor signaling pathway and the pathogenesis of multiple B-cell malignancies. Covalent, irreversible BTKi have been instrumental in the management of B-cell malignancies, but mutations in BTK that affect binding capacity, exhibit scaffold functionality, or cause kinase hyperactivation can lead to BTKi resistance and limit therapeutic options. Though on-target resistant mutations emerging from covalent BTKi have been well-characterized, little is known about resistance mutations for non-covalent, reversible BTKi or BTK degraders such as BGB-16673, an orally available chimeric degradation activating compounds (CDAC) currently in Phase I (NCT05006716, NCT05294731) with demonstrated preclinical BTK degradation activity.

Aims:
To characterize the profile and tendency of BGB-16673 to cause on-target BTK resistance mutations in comparison to non-covalent BTKi and to determine its ability to overcome identified BTK mutations in comparison to other covalent and non-covalent BTKi.

Methods:
BTKi-sensitive TMD8 cells were treated with ethyl-N-nitrosourea (ENU, a highly potent mutagen) for 18h. Mutant cells were then treated with serial doses of either BGB-16673, Compound-ED (an E3 ligase dead control with the same warhead as BGB-16673; reversibly binds to BTK, 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.

The ability of BGB-16673 to overcome BTK mutations was evaluated with cell viability assay in both TMD8 and OCI-LY10 BTK mutant overexpression cell lines. Cells were treated with multiple doses of BGB-16673 or BTKi (Compound-ED, pirtobrutinib, ibrutinib, and acalabrutinib). IC50s were calculated from the dose response curves. A Homogeneous Time Resolved Fluorescence (HTRF) assay was conducted to evaluate BTK mutants' degradation by BGB-16673 in a TMD8 cell line and further verified by Western blot.

Results:
Treatment of ENU-mutated cells with BGB-16673 resulted in fewer resistant clones with BTK mutations (12.7%; 7/55). In contrast, 100% of resistant clones treated with Compound-ED (80/80) and 100% of resistant clones treated with the pirtobrutinib (144/144) contained BTK mutations with most concentrated within the BTK kinase domain. Three mutations (V416L, T474I and

L528W) reported in relapsed or refractory CLL patients who acquired resistance to pirtobrutinib were also observed in the pirtobrutinib screen.

BGB-16673 degraded BTK protein even in the presence of clinically relevant resistance mutations (V416L, M437R, T474I, C481S, C481F, C481Y, and L528W), except A428D. Consistent with this observation, cell growth inhibition and BTK protein degradation were positively correlated in BGB-16673 treated cells.

Summary/Conclusion:

Relative to other BTKi, the BTK CDAC BGB-16673 is less likely to cause on-target resistant mutations, demonstrated a unique on-target resistant mutation profile, and could overcome a wide variety of BTK resistance mutations. These findings suggest that BGB-16673 is a promising next-generation BTK inhibitor that could benefit patients who developed BTKi on-target resistant mutations.