Title:

BGB-16673 Differentiates from Other BTK Degraders Through Potent Intrinsic BTK Inhibition That Limits E3 Ligase Loss–Driven Resistance

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Background:

BTK-targeting protein degraders comprise three components: a BTK binder that engages the BTK kinase domain, an E3 ligase binder that recruits cereblon (CRBN) a part of E3 ligase, and a linker. By bringing BTK and E3 ligase together, the degrader induces ubiquitination and proteasomal degradation of BTK.

As a therapeutic modality, BTK degraders may have advantages over conventional small-molecule inhibitors, including event-driven other than occupancy-driven target disruption, the potential to overcome resistance mutations from BTK inhibitors, and suppression of scaffold functions. However, the degrader activity is highly dependent on the integrity of the E3 ligase–proteasome pathway. It makes intuitive sense that loss of function mutations of E3 ligase is a potential resistance mechanism to BTK degraders. Notably, mutations in CRBN E3 ligase complex have been reported in multiple myeloma treated with lenalidomide, a CRBN-binding agent, implying that similar acquired resistance mechanisms could emerge during BTK degrader treatment.

Because BTK degraders bind to BTK in its kinase pocket, there is a potential to inhibit BTK kinase function as well. How intrinsic kinase inhibition activity of BTK degraders influences their antitumor activity remains less understood. Here, we demonstrate that, among BTK degraders with comparable degradation potency, differences in intrinsic BTK inhibition impact the ability to overcome E3 ligase-proteasome pathway mediated resistance.

Aims

To investigate differences in intrinsic BTK inhibition among BTK degraders and determine how these differences influence their anti-tumor activity, we compared two late-stage BTK degraders, BGB-16673 and NX-5948, in preclinical studies.

Methods

CRISPR-Cas9 was used to generate BTK-C481S and CRBN knockout (KO) cell lines. Cytotoxicity was assessed via CellTiter-Glo (CTG) assay. BTK degradation and phosphorylation were quantified using ELISA and HTRF assays, respectively. TMD-8 and OCI-LY10 cells (wild-type, BTK mutant, or CRBN KO) were implanted subcutaneously into NCG mice for *in vivo* efficacy studies.

Results

BGB-16673 induced rapid and potent BTK degradation in vitro (DC_{50}=0.57nM), confirming its activity as a highly potent BTK degrader. NX-5948 exhibited comparable BTK degradation potency. To isolate the contribution of intrinsic kinase inhibition from degradation, CRBN-dependent degradation was pharmacologically blocked with lenalidomide. Under these conditions, suppression of BTK-Y223 phosphorylation primarily reflected direct kinase inhibition. NX-5948 showed minimal inhibition of BTK-Y223 phosphorylation in the presence of lenalidomide, whereas BGB-16673 maintained potent inhibition (IC_{50}=0.8nM). These findings suggest that E3 ligase impairment may represent a potential resistance mechanism for NX-5948 but not for BGB-16673.

Consistently, CRBN KO conferred resistance to NX-5948 but not to BGB-16673 in B lymphoma cells. In addition, NX-5948 lost inhibitory activity against BTK-C481S when degradation was blocked, indicating limited capacity to suppress BTK mutant signaling once E3 ligase is compromised.

BGB-16673 presented potent antiproliferative activity across a broader panel of BTK mutations, including mutations near the BTK–E3 ligase interface that conferred resistance to NX-5948. Furthermore, a spontaneous resistance screen also identified multiple NX-5948-resistant clones harboring CRBN loss or truncating alterations that remained sensitive to BGB-16673. In xenograft models, the antitumor activity of NX-5948 was abrogated by either CRBN loss or the BTK-M477P mutation, whereas BGB-16673 retained significant efficacy under both conditions, further confirming ubiquitin E3-proteasome loss of function mutation is a resistance mechanism to NX-5948 but not BGB-16673.

Conclusions

BTK degraders can differ substantially from each other. Under similar degradation potency, BTK degraders with stronger intrinsic kinase inhibition may be less susceptible to develop acquired resistance driven by E3 ligase–proteasome pathway impairment, potentially translating to more durable clinical response. Mechanistically, the requirement for both BTK and E3 ligase binding creates resistance nodes beyond those of BTK inhibitors, which can be mitigated by degraders with dual mechanisms—degradation and strong intrinsic BTK inhibition. Clinical data is warranted to confirm the clinical impact of these mechanistic differences.

###Abstract format and requirements

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