BTK-A428D is a cross-resistant mutation to both BTK inhibitors and degraders

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Background

BTK inhibitors have revolutionized the management of chronic lymphocytic leukemia (CLL) and other B cell malignancies. However, acquired resistant mutation of BTK develops. Mutations at Cysteine 481 (C481) result in resistance to covalent BTK inhibitors (BTKi). Non-covalent BTKis, such as pirtobrutinib, can overcome C481 mutations but are susceptible to other BTK mutations like V416L, T474I, and L528W. Recently, a BTK mutation A428D was reported to confer resistance from the BGB-16673 trial and was also modeled to disrupt the binding of BTK to another BTK-degrader, NX-2127. This raises the question of whether A428D insensitivity is compound specific or common to BTK degraders and inhibitors currently in development and market. This study investigates the impact of BTK A428D on the response to approved BTK inhibitors and multiple degraders via preclinical models.

Methods

TMD8 cells overexpressing BTK-wildtype, C481S, T474I, A428D, V416L, M437R mutations were generated by lentivirus transduction and utilized to evaluate their sensitivity to individual BTK degrader or inhibitor via *in vitro* CTG assay. TMD8 BTK-A428D cell lines, which were generated by CRISPR-Cas9 mediated in situ gene editing, were used for BTK degradation examination by ELISA, BCR signaling evaluation by Western blot, and *in vivo* tumor growth inhibition (TGI) assessment via xenograft models.

Results

Among the four tested BTK degraders (BGB-16673, NX-2127, NX-5948, and Abbv Compound 1), BGB-16673 and NX-5948 potently inhibit the growth of TMD8 cells overexpressing BTK-C481S, T474I, V416L, M437R mutations measured by *in vitro* CTG assay. BGB-16673 is more potent than Abbv Compound 1 against BTK-T474I, -V416L, and -M437R overexpressing TMD8 cells. NX-2127 is generally less potent in all the mutations. However, TMD8 cells overexpressing BTK-A428D are resistant to all tested BTK degraders and inhibitors (ibrutinib, acalabrutinib, zanubrutinib, pirtobrutinib). The cross-resistance of A428D mutation is further confirmed *in vitro* using two TMD8 BTK-A428D cell lines generated by *in situ* mutation.

Consistently, the A428D mutation blocks BTK degradation in TMD8 cells. The phosphorylation at PLCγ2(Y1217), an event catalyzed by BTK kinase activity, remains intact after treatment with BTK degraders and inhibitors in TMD8 BTK-A428D cells, suggesting these compounds are also ineffective in inhibiting BTK-A428D downstream signaling. Moreover, no obvious tumor growth inhibition is observed in TMD8 BTK-A428D xenografts treated with any tested degrader or inhibitor, even at relatively higher doses, whereas they all induce tumor regression in TMD8 wildtype xenografts.

Summary/Conclusion

BTK-A428D is a mutation that exhibits cross-resistance to multiple BTK degraders (BGB-16673, NX-2127, NX-5948, Abbv Compound 1) and inhibitors (ibrutinib, acalabrutinib, zanubrutinib, and pirtobrutinib) both *in vitro* and *in vivo*, as supported by evidence from multiple aspects. In the future, BTK-A428D mutated cancer cells may be treated by other targeted therapies.