Zanubrutinib (Zanu) overcomes BTK V416L resistance in B cell lymphoma models

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Abstract Content:

Introduction: The second-generation BTK inhibitors (BTKis), e.g., zanu, acalabrutinib (acala), have further transformed the therapy landscape of chronic lymphocytic leukemia (CLL). However, acquired mutations in BTK (mainly at position C481) results in covalent BTKi resistance and disease progression. Non-covalent BTKi e.g., pirtobrutinib (pirto) can overcome C481 mutations, but is susceptible to other BTK mutations like the recently described kinase impaired V416L and L528W mutations, which both maintain downstream signaling. However, the impact of V416L on BTKi therapy remains not well characterized. Here, we investigated how V416L affects acala, zanu, and pirto response in B cancer cells.

Method: TMD8 BTK V416L cell lines were generated by CRISPR-Cas9 mediated gene editing. Kinase activity and phosphorylation of BTK Y223 were evaluated in V416L and wildtype (WT) TMD8 cells by Western blot. Structural modeling of human BTK was performed. Viability of TMD8 cells with V416L or WT was measured with CellTiter-Glo assay. Tumor growth inhibition (TGI) was measured in V416L and WT xenograft models. BTK occupancy in TMD8 cells was measured via ELISA with biotinylated probes.

Results: Western blot analysis demonstrates that V416L encodes a kinase with lower enzymatic activity compared to WT indicated by reduced phosphorylation of BTK Y223. However, BTK downstream signaling remains intact. Structural modeling predicts V416L leads to clashes in ATP binding, supporting impairment of kinase activity. TMD8 cells expressing V416L were more sensitive to zanu (IC50=0.8 nM) than to acala (IC50=144.4 nM) and pirto (IC50=2074.8 nM) in cell viability assays. Administration of a clinically relevant dose of zanu (20 mpk BID) resulted in significant regression of TMD8 V416L xenografts compared to vehicle ($p<0.0001$), while a clinically relevant dose of acala (4 mpk BID) did not achieve significant TGI. Neither a higher dose of acala (12 mpk BID) nor 50 mpk BID of pirto showed significant efficacy. Computational structural modeling predicts that V416L creates steric clashes with acala and pirto at the binding site, whereas zanu binding remains unaffected. Consistently, a BTK occupancy assay demonstrated that zanu, but not acala, can efficiently bind to V416L. Furthermore, western blot analysis shows efficient BTK downstream signal inhibition in V416L expressing TMD8 cells treated with zanu, whereas downstream signals are maintained in cells treated with acala and pirto.

Conclusion: These data demonstrate that zanu retains potent antitumor activity against TMD8 cells expressing BTK V416L, whereas acala and pirto may be attenuated by steric clashes. Thus, we hypothesize that clonal expansion of V416L is less likely to occur in patients with CLL treated with zanu, whereas it may lead to resistance in patients treated with acala or pirto. This hypothesis should be validated in clinical studies.