# The second generation BTK inhibitor Zanubrutinib (Zanu) demonstrates robust efficacy in both TP53 wildtype and mutated B cancer cells in preclinical studies

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## Background

TP53 mutations and del(17)(p13.1), reported inferior prognostic biomarkers in CLL/SLL and other B lymphomas, are associated with lower ORR, shorter PFS, and poor OS to chemotherapies. The chemoresistance of del(17)(p13.1) is also associated with TP53 deletion or mutation, implying TP53's role as a tumor suppressor. CLL/SLL patients with TP53 alterations are thus considered high-risk. Over the past decade, BTK inhibitors have revolutionized the treatment of CLL/SLL patients, including for those with TP53 mutations and del(17)(p13.1). While TP53-mutated patients respond well to BTK inhibitors, the reported efficacy varies across trials, making it unclear how TP53 mutations influence BTK inhibitor response. This study investigates the impact of TP53 knockout or mutation on the response of B lymphoma cells to BTK inhibitors. The efficacy of second-generation BTK inhibitors Zanubrutinib (Zanu) and Acalabrutinib (Acala) is also compared in TP53 knockout and mutant B lymphoma cells and animal models.

## Methods

A lentivirus pool consisting of 29 TP53 hotspot mutations was transduced into TMD8 TP53- knockout cells (hereafter referred the mutants-expressing cells as TMD8$^{TP53\ mutants}$) for in vitro CTG assay and next generation sequencing after Zanu treatment. TMD8 cell lines with TP53-knockout (KO) or TP53-R248Q in situ mutation were generated by CRISPR-Cas9 mediated gene editing. The viability of TMD8 wildtype, TP53-KO, -R248Q mutation cancer cells was measured by CTG assay *in vitro*. TMD8 wildtype, TP53-KO, -R248Q cells were inoculated subcutaneously into NOG mice for *in vivo* efficacy evaluation.

## Results

In vitro CTG assay of TMD8$^{TP53\ mutants}$ demonstrates similar potency to Zanu as wildtype cells, indicating loss of TP53 function does not affect cellular sensitivity to Zanu. Next generation sequencing of TMD8$^{TP53\ mutants}$ after *in vitro* treatment of Zanu for 7 days at its clinically relevant concentrations also shows no evident selection pressure on specific TP53 mutations. The observation is further validated by TMD8 TP53-KO and -R248Q (one of the most prevalent mutation in CLL patients) cell lines. TMD8 TP53-KO and -R248Q cells exhibit more aggressive growth *in vitro* and *in vivo* compared with wildtype cells, but Zanu remains equally potent to cells with or without TP53 functionality. Intriguingly, TMD8 TP53-KO and -R248Q cells are more sensitive to Zanu than to Acala, as similarly observed in wildtype cells. Consistently, Zanu demonstrates better efficacy than Acala in TMD8 TP53-wildtype, -KO, and -R248Q xenografts at their clinically relevant doses. In addition to TP53-R248Q mutation, Zanu also induces deeper and more durable tumor regression than Acala in a TP53-I232F mutated CLL PDX model.

**Summary/Conclusion**

These results indicate that Zanu remains effective in TMD8 cells with TP53 KO or hotspot mutations and has better efficacy than Acala in both TP53-wildtype, -KO, and -R248Q cell lines and xenografts.