Zanubrutinib (Zanu) demonstrates robust efficacy in both TP53 wildtype and mutated B cancer cells in preclinical studies

Haitao Wang[1*], Hao Wang[1*], Yang Shi[1], Yiwen Wang[1], Weiwei Song[1], Shuang Peng[1], Sijia Zhai[1], Ziyu Jia[1], Peng Chi[1], Yongchun Tong[1], Teiko Sumiyoshi[3], Lanjun Xu[1], Wei Jin[1$], Zhirong Shen[2$]

1. BeiGene (Beijing) Co., Ltd., Beijing 102206, China; 2. BeiGene (Shanghai) Co., Ltd., Shanghai 200131, China;3. BeiGene USA Inc., San Mateo, CA USA

* Equal contribution; $ Correspondence

INTRODUCTION

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.

METHOD

A lentivirus pool consisting of 29 TP53 hotspot mutations was transduced into TMD8 TP53-knockout (KO) 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-KO or TP53-R248Q *in situ* mutation were generated by CRISPR-Cas9 mediated gene editing.

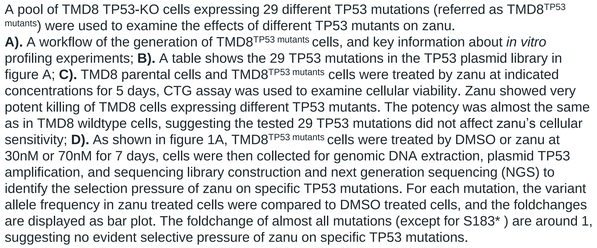
The viability of TMD8 wildtype, TP53-KO, -R248Q mutation cancer cells was measured by CTG assay *in vitro*.

TMD8 wildtype, TP53-KO, -R248Q cells were inoculated subcutaneously into NOG mice for *in vivo* efficacy evaluation.

RESULTS

Figure 1. TMD8 cells expressing TP53 hotspot mutations are still sensitive to Zanu

A. Workflow of *in vitro* profiling



B. The 29 TP53 mutations in the library

Mutation site	Mutation site	Mutation site	Mutation site
R175H	R273H	G293Rfs*13	P278S
R248W	Y220C	H179P	Q165*
C242Afs*5	A84G	I195F	R273C
R248Q	C238S	I255F	R273L
G245S	C275F	K132E	R306*
H179R	E286G	L194P	S183*
I195T	E286V	M237I	S241F
R196*			

Note: * nonsense mutation; fs*N means a frameshift leading to nonsense mutation after translation of N extra amino acids.

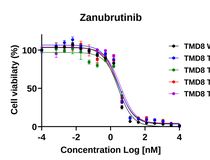
C. CTG assay of TMD8$^{TP53\ mutants}$ cells

Note: TMD8$^{TP53\ mutants}$ are TMD8-TP53 KO cells expressing the 29 TP53 mutation libraries

D. *In vitro* profiling suggested no evident selective pressure of zanu on specific TP53 mutations

A pool of TMD8 TP53-KO cells expressing 29 different TP53 mutations (referred as TMD8$^{TP53\ mutants}$) were used to examine the effects of different TP53 mutants on zanu.
A). A workflow of the generation of TMD8$^{TP53\ mutants}$ cells, and key information about *in vitro* profiling experiments; **B).** A table shows the 29 TP53 mutations in the TP53 plasmid library in figure A; **C).** TMD8 parental cells and TMD8$^{TP53\ mutants}$ cells were treated by zanu at indicated concentrations for 5 days, CTG assay was used to examine cellular viability. Zanu showed very potent killing of TMD8 cells expressing different TP53 mutants. The potency was almost the same as in TMD8 wildtype cells, suggesting the tested 29 TP53 mutations did not affect zanu's cellular sensitivity; **D).** As shown in figure 1A, TMD8$^{TP53\ mutants}$ cells were treated by DMSO or zanu at 30nM or 70nM for 7 days, cells were then collected for genomic DNA extraction, plasmid TP53 amplification, and sequencing library construction and next generation sequencing (NGS) to identify the selection pressure of zanu on specific TP53 mutations. For each mutation, the variant allele frequency in zanu treated cells were compared to DMSO treated cells, and the foldchanges are displayed as bar plot. The foldchange of almost all mutations (except for S183*) are around 1, suggesting no evident selective pressure of zanu on specific TP53 mutations.

Figure 2. Zanu has similar potency in TP53-KO and -R248Q cells as in wildtype cells

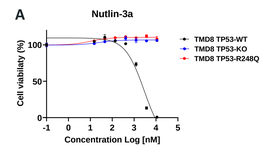


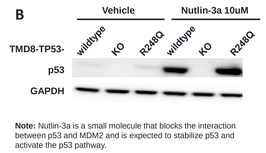
B

Cell lines	Zanubrutinib IC50 (nM)
TMD8 WT	2.86
TMD8 TP53-KO-24#	3.24
TMD8 TP53-KO-36#	3.4
TMD8 TP53-R248Q-6#	3.3
TMD8 TP53-R248Q-38#	3.73

TP53-KO (knock out) and –R248Q mutation (the most dominant TP53 mutation in CLL patients) were chosen to confirm the discovery from *in vitro* profiling. To do this, two TMD8 TP53-KO cell lines and two TP53-R248Q mutation cell lines generated by CRISPR-Cas9 mediated *in situ* mutation were treated by zanu to examine their effect on zanu sensitivity. **A).** The TP53-KO cells and TP53-R248Q cells showed similar potency as TMD8 wildtype cells, further confirming TP53 mutation does not affect zanu sensitivity on TMD8 cancer cells; **B).** A table shows the half maximum inhibition concentration (IC50) of zanu in different cell lines from figure A.

Figure 3. TP53 is functional in TMD8 cells

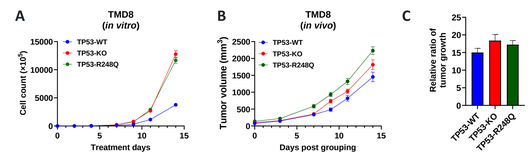




Note: Nutlin-3a is a small molecule that blocks the interaction between p53 and MDM2 and is expected to stabilize p53 and activate the p53 pathway.

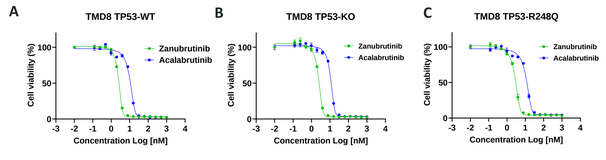
TP53's functionality in TMD8 cells were confirmed by Nutlin-3a, a MDM2 inhibitor to stabilize p53 (the protein encoded by TP53 gene) and activate p53 pathway in cells. **A).** TMD8 wildtype, TP53-KO, and TP53-R248Q cells were treated by nutlin-3a at different concentrations for 3 days and cell viability was examined by CTG assay. TMD8 cells expressing wildtype p53 showed dose-dependent sensitivity to nutlin-3a, while the cell death was blocked by TP53-KO or –R248Q mutations, suggesting TP53 is functional in TMD8 cells; **B).** Cell were treated by nutlin-3a at indicated concentrations for 24 hours, cellular lysates were used for western blot. Nutlin-3a efficiently stabilizes p53 protein, confirming p53-MDM2 signaling in TMD8 cells.

Figure 4. TP53 knockout or R248Q mutation exhibits more aggressive growth *in vitro* and *in vivo*



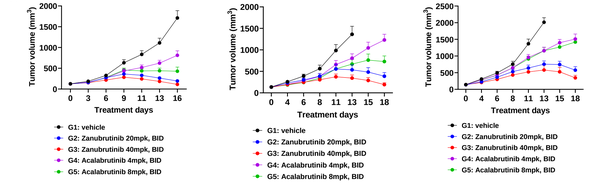
The impact of TP53-KO or –R248Q mutation on TMD8 cells were examined *in vitro* and *in vivo*. **A).** TMD8 cells without TP53 (TP53-KO) or with TP53-R248Q mutation grow faster than wildtype cells, indicating more aggressive growth of TP53 mutants. **B).** TP53-KO and –R248Q mutated cancer cells also showed more aggressive growth *in vivo*; **C).** The relative growth ratio of tumor by comparing tumor volume on day 14 to day 0 in figure B further supports the more aggressive growth of TP53 mutated TMD8 cancer cells.

Figure 5. Zanu is more potent than Acala in TP53-WT, -KO, and -R248Q mutated TMD8 cancer cells *in vitro*



Zanu and acala's cellular toxicity was also evaluated in TMD8 TP53 wildtype and mutated cells. **A).** Zanu is more potent than acala in inhibiting the growth of wildtype cells; **B-C).** In TMD8 TP53-KO, and –R248Q cells, which showed more aggressive growth, zanu is also more potent than acala, suggesting zanu has higher potency than acala in TMD8 cells with or without TP53 functionality.

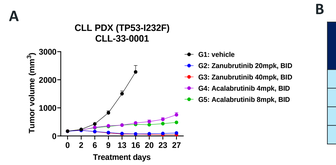
Figure 6. Zanu demonstrates better efficacy than Acala in TMD8 TP53-WT, -KO, and -R248Q xenografts at their clinically relevant doses



Note: the exposure of 20mpk BID of zanu in mouse is close to 160mg BID in human; the exposure of 4mpk BID acala in mouse is close to 100mg BID in human.

TMD8 TP53-WT, TP53-KO, -R248Q cells were inoculated subcutaneously into NOG mice for *in vivo* efficacy evaluation. **A).** Zanu at its clinically relevant dose 20mpk BID showed better tumor growth inhibition than the clinically relevant dose of acala (4mpk BID) and its higher dose (8 mpk BID) in wildtype tumors; **B-C).** Zanu maintained its better efficacy than acala at clinically relevant doses in more aggressive tumors harboring TP53-KO or –R248Q mutations.

Figure 7. Zanu induces deeper and more durable tumor regression than acala in a TP53-I232F mutated CLL PDX model



B

Groups	TGI (%) Day16
G2: Zanubrutinib, 20mpk, BID	104.6
G3: Zanubrutinib, 40mpk, BID	104.8
G4: Acalabrutinib, 4mpk, BID	86.1
G5: Acalabrutinib, 8mpk, BID	88.6

A). A CLL patient derived xenograft (PDX) model CLL-33-0001, which expresses TP53-I232F mutation, was inoculated into NOD-SCID mouse for efficacy. Zanu at 20mpk BID drove complete and durable regression of PDX and presented better efficacy than acala. **B).** A table shows tumor growth inhibition (TGI) on day 16.

CONCLUSIONS

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.

ACKNOWLEDGEMENT

We thank Dr. Weilong Zhang and Dr. Hongmei Jing from Beijing 3rd hospital for providing us the TP53 mutation plasmid library.