



Clonal Interdependency between Wild-type and Mutant p53 AML Abrogates BCL-2 Inhibitor-Mediated Apoptosis

Atreyu Balasundaram

Baylor University, College of Arts and Sciences, Honors College

Background

- Acute myeloid leukemia (AML) is the most common acute leukemia in adults with heterogenous genetic background.
- Around 5-10% of AML patients have abnormal p53 mutant expression which results in arrest of normal myeloid differentiation and proliferation.
- TP53 mutations account for 5-10% of newly diagnosed AML, with the worst survival outcome. Interestingly, the survival outcome did not change by the extent of variant allele frequencies (Fig. 1), suggesting that small proportional mutant p53 clones influence the survival outcome.⁽¹⁾
- TP53 mutations consist of two different forms of alterations; missense and truncation mutations. One of the most common missense mutations is R248Q mutation.⁽¹⁾
- Venetoclax (VEN), a potent and selective Bcl-2 inhibitor that triggers apoptotic pathway of AML has been approved by the FDA in combination with hypomethylating agent (HMA), however, mutant p53 AML cells are more resistant to VEN and p53 mutation is one of the major cause of resistance to VEN/HMA.⁽²⁾

Methods

- We used AML MOLM-13 cell line for all experiments. The wild-type (WT) cells and mutants were transfected with GFP and BFP respectively.
- First, we treated WT-GFP, KO-BFP, and 248-BFP cells alone and then mixed in an 8:2 (WT: Mutant) ratio with 100 nM of VEN over 12 days (Fig. 2)
- Concurrently we used the supernatant of these and cultured fresh cells with them to record levels of cells death (Fig. 3)
- We used Annexin V/ PI staining to analyze the growth/death of cells using flow cytometry methods in intervals of 3 days

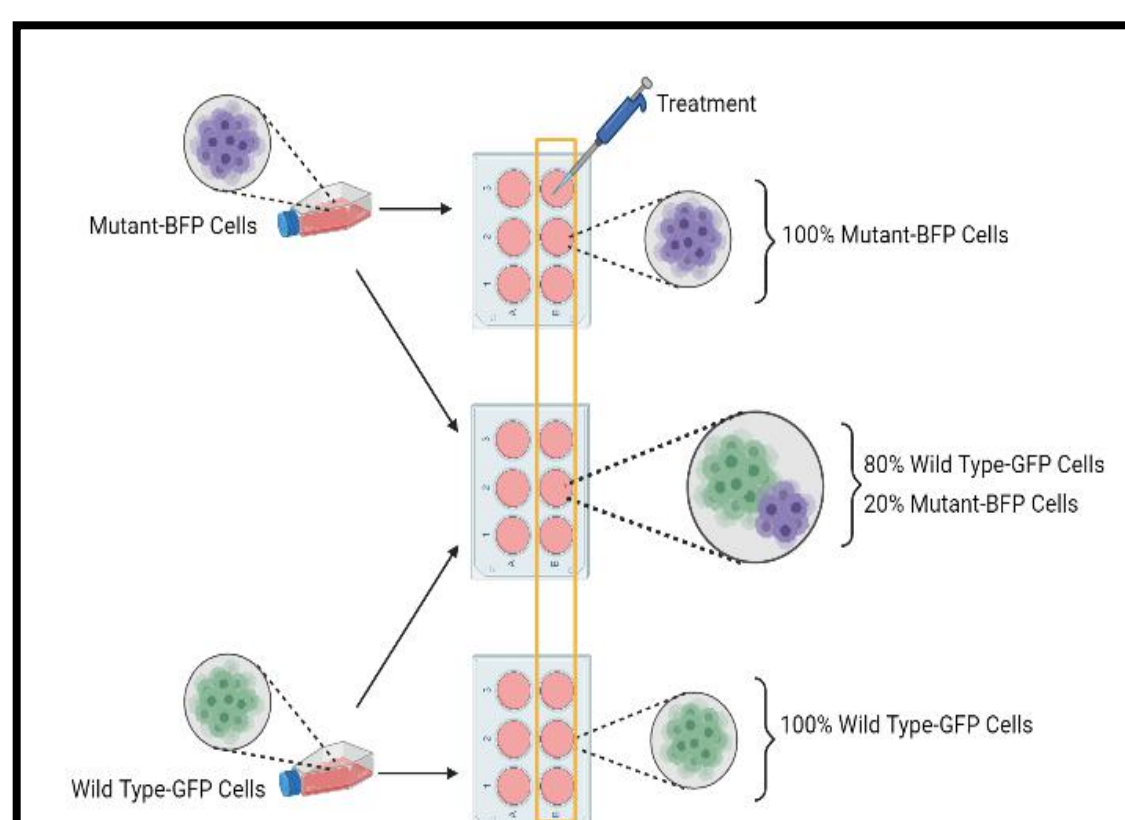


Fig. 2 Illustration of the experimental setup for the original treatment of the mutants (KO and R248Q) and the Wild Type cells. Treatment was 100nM of Venetoclax and control was an identical treatment of DMSO.

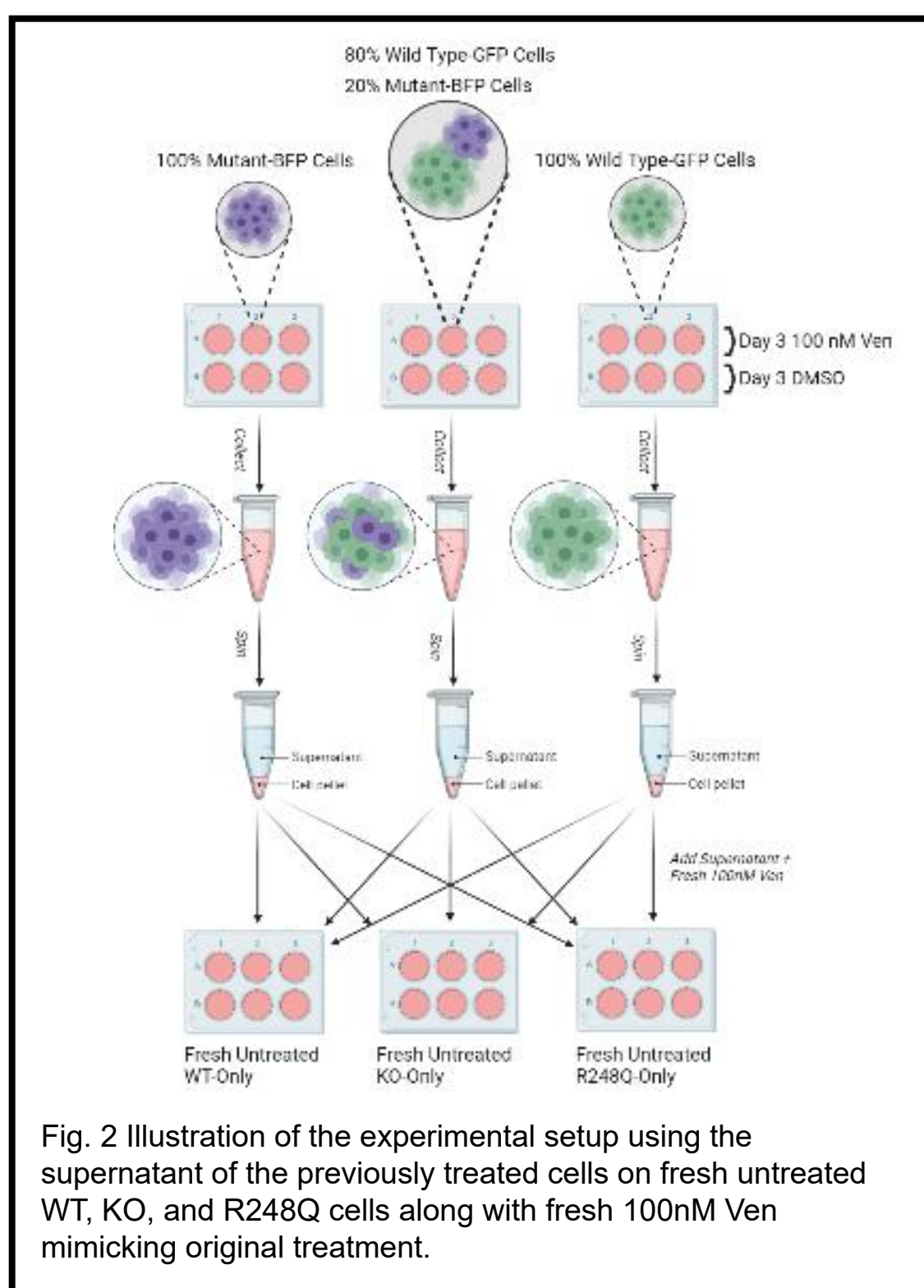


Fig. 2 Illustration of the experimental setup using the supernatant of the previously treated cells on fresh untreated WT, KO, and R248Q cells along with fresh 100nM Ven mimicking original treatment.

Results

- After 3 days of treatment the WT, KO, and R248Q cells cultured alone had worse survival ($p < 0.005$) than those in the co-cultures (Fig. 3,4)
- By day 6 the difference in survival we had observed on day 3 was less significant ($p > 0.05$)
- The WT-cells cultured with WT-KO and WT-248 supernatant showed lower levels of Annexin-V, a marker of cell death, than those cultured in the WT-Only supernatant.
- Similarly, KO and 248 cells cultured with the co-culture supernatant showed lower levels of cell death when compared to being cultured in KO-only and 248-only supernatant, respectively. (Fig. 5)

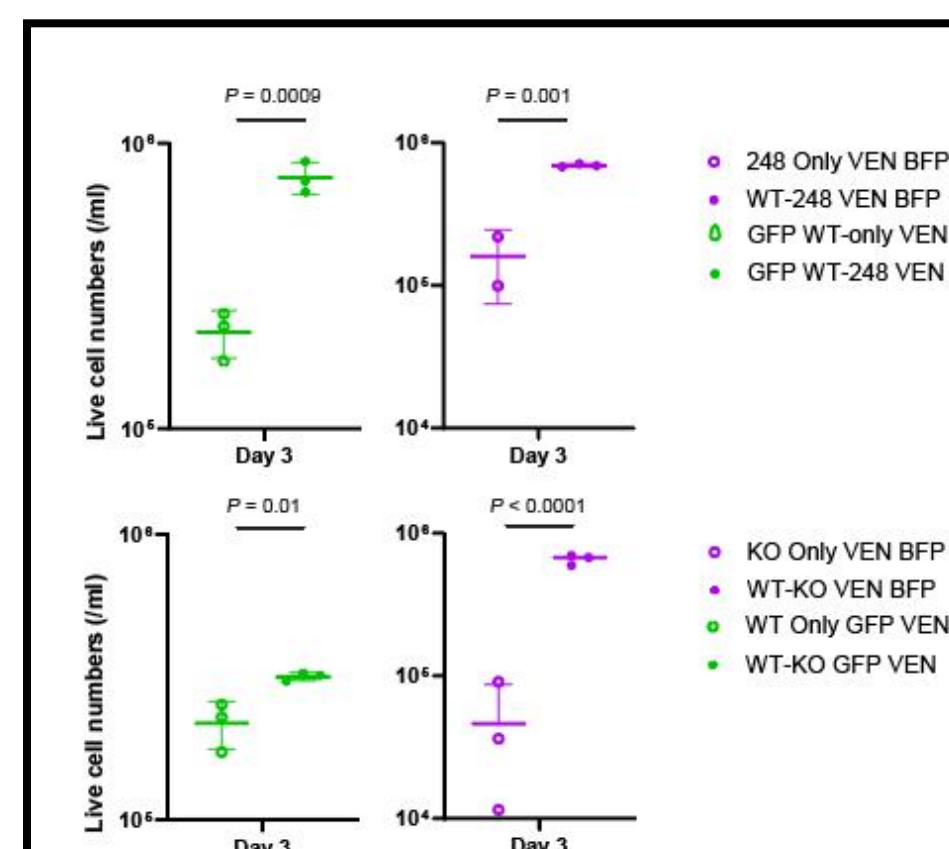


Fig 3. Scatter dot plot showing the significant difference by Day 3 in the number of live cells under 100 nM of Venetoclax in the co-cultures versus the single cell type cultures

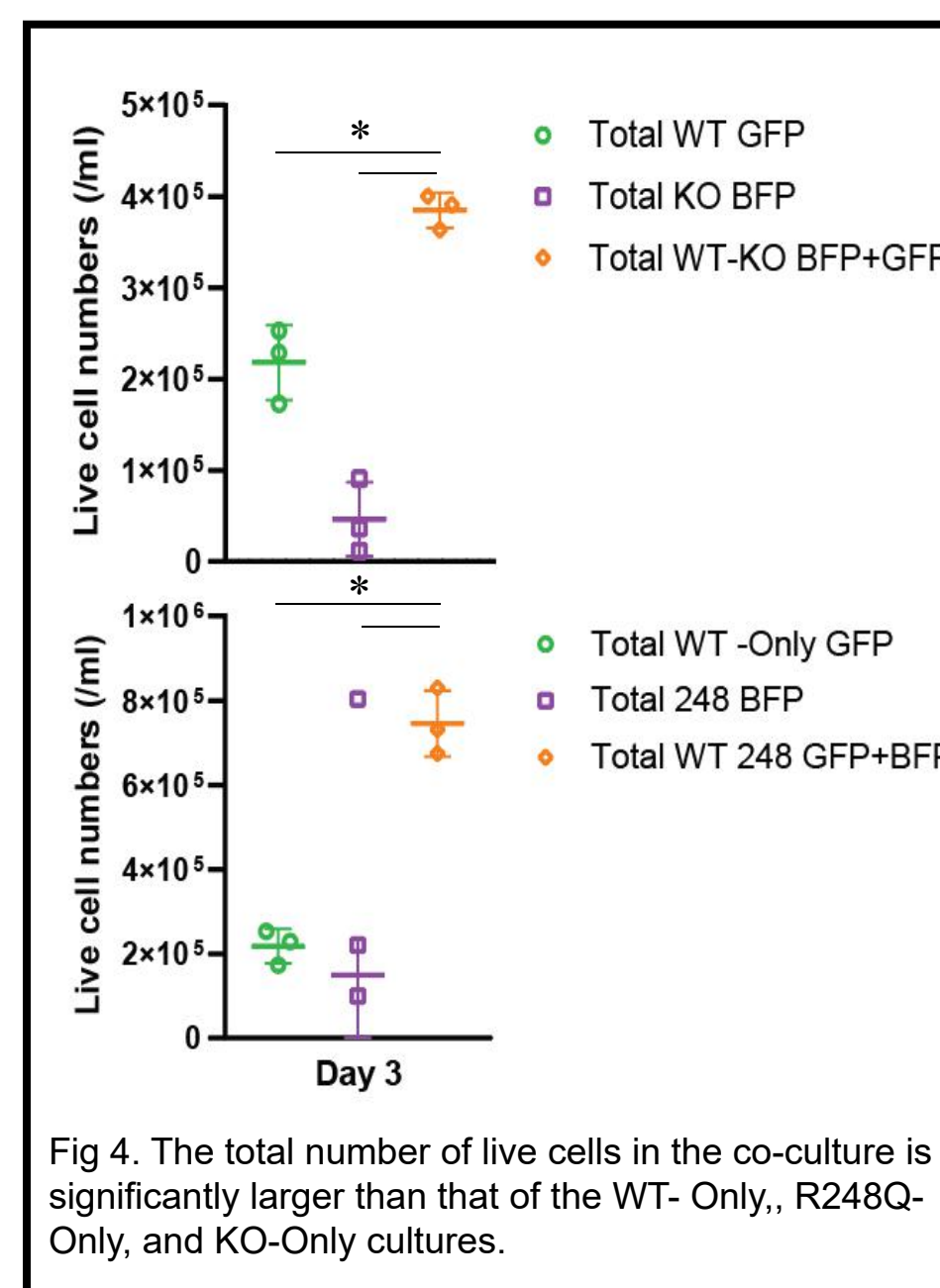


Fig 4. The total number of live cells in the co-culture is significantly larger than that of the WT- Only, R248Q-Only, and KO-Only cultures.

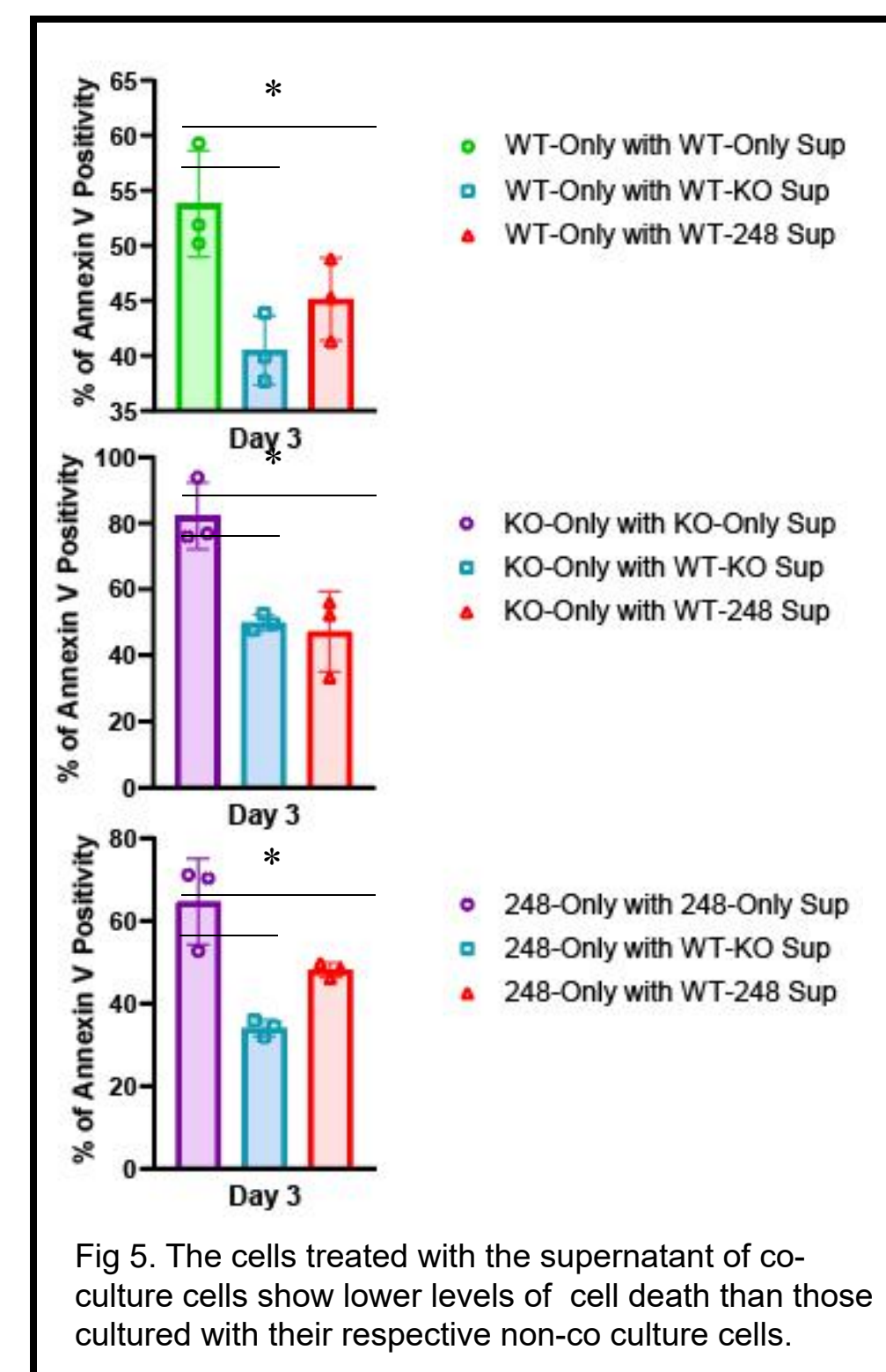


Fig 5. The cells treated with the supernatant of co-culture cells show lower levels of cell death than those cultured with their respective non-co culture cells.

Conclusions

- Our observations of the cocultures supports our hypothesis that mutants confer some drug resistance to WT cells and that there are communication molecules in the supernatant that a role in cell-cell communication between the mutant and WT cells.
- In the clinical perspective, it indicates the consequences of administering non-lethal doses of the drug on treatment success and relapse.
- Future studies could look toward using RNA sequencing of the cells treated to determine if there are changes to major apoptotic pathways.

References

- Prochazka et. al. Haematologica, 2019 Volume 104(3):516-523
- Wei et al. Blood, May 2021, Volume 137, Number 20
- Images Created with BioRender.com

Acknowledgements

Dr. Michael Andreeff, Department of Leukemia
Dr. Yuki Nishida, Department of Leukemia
Tallie Patsilevas, Department of Leukemia
Molecular Hematology and Therapy Lab,
MD Anderson Cancer Research Center

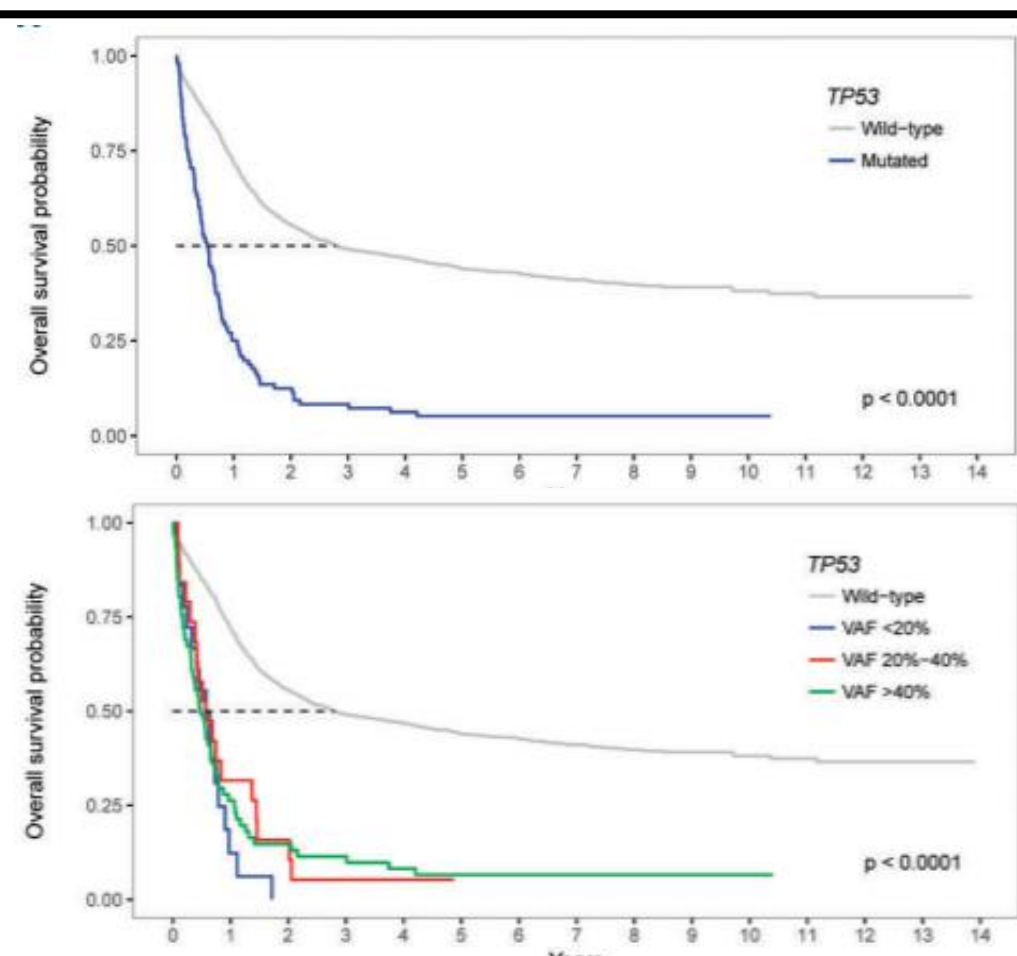


Fig. 1 Kaplan-Meier analysis of overall survival in 1,537 patients with acute myeloid leukemia stratified by TP53 mutational status. (A) Overall survival: TP53 wild-type patients versus TP53-mutated patients. (B) Overall survival: TP53 wild-type patients versus patients in the three groups with the defined variant allele frequencies of mutated TP53 (Prochazka et. al, 2019).

Hypothesis

We hypothesize that the existence of small mutant p53 clones impacts on sensitivity to Venetoclax of wild-type p53 AML cells.