The role of *TP53* loss-of-function mutation in the clonal expansion of primary hematopoietic stem or progenitor cells (HSPCs)

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Abstract

To investigate the role of *TP53* loss-of-function mutation in clonal hematopoiesis and leukemogenesis, we established a specific gene-editing approach that allows for the disruption of *TP53* directly in human hematopoietic stem and progenitor cells (HSPCs). To examine the effect of *TP53* alteration in HSPC fitness, we co-cultured $TP53^{KO}$ or $AAVS1^{KO}$ ("safe harbor" locus) HSPCs with unedited control *in vitro* for five weeks. The growth kinetics of $TP53^{KO}$ or $AAVS1^{KO}$ clone was tracked by examining the respective variant allele frequency (VAF) at different time points through Sanger Sequencing. After five weeks co-culture with unedited HSPCs, VAF of $TP53^{KO}$ clone increased while *AAVS1 clone* VAF remain unchanged, which means $TP53^{KO}$ delivered a growth advantage to HSPCs compared to the *AAVS1* controls.

Introduction

- **Clonal hematopoiesis (CH)** is the presence of a genetically distinct population of hematopoietic cells derived from a single mutated HSC without overt hematological malignancies.
- **CH driven by *TP53* loss-of-function mutations** is enriched in cancer survivors, which is associated with the highest risk for therapy-related myeloid neoplasms (t-MNs).
- ***TP53^{mut}* t-MN** is an aggressive and lethal disease, which is refractory to most conventional therapies, leading to a dismal two-year overall survival of only 12.8%.

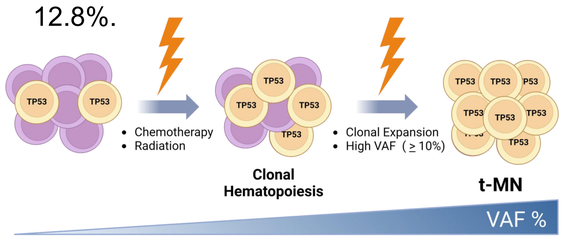


Figure 1: Cell extrinsic and cell intrinsic events significantly affect the development of *TP53* mutant cells into MNs.

Hypothesis

Hypothesis – Loss-of-function of *TP53* grants mutant HSPCs a growth advantage in clonal competition.

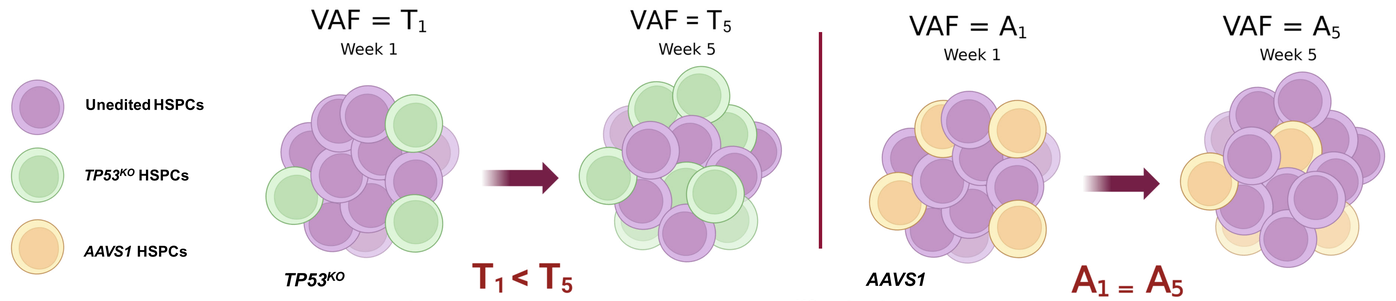


Figure 2: Clonal expansion model with $TP53^{KO}$ HSPC over 5 weeks.

Methods

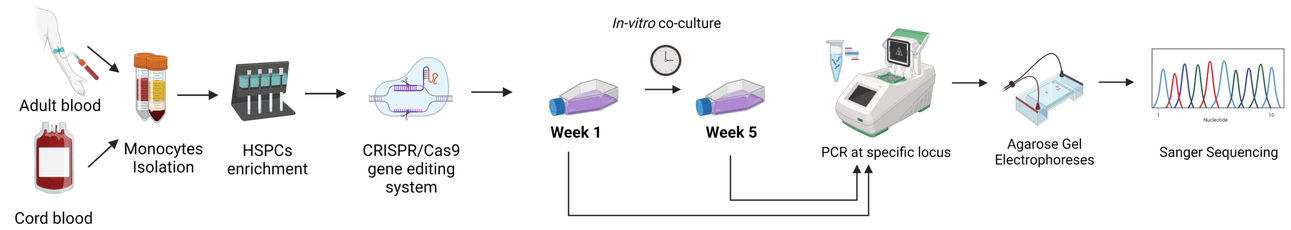
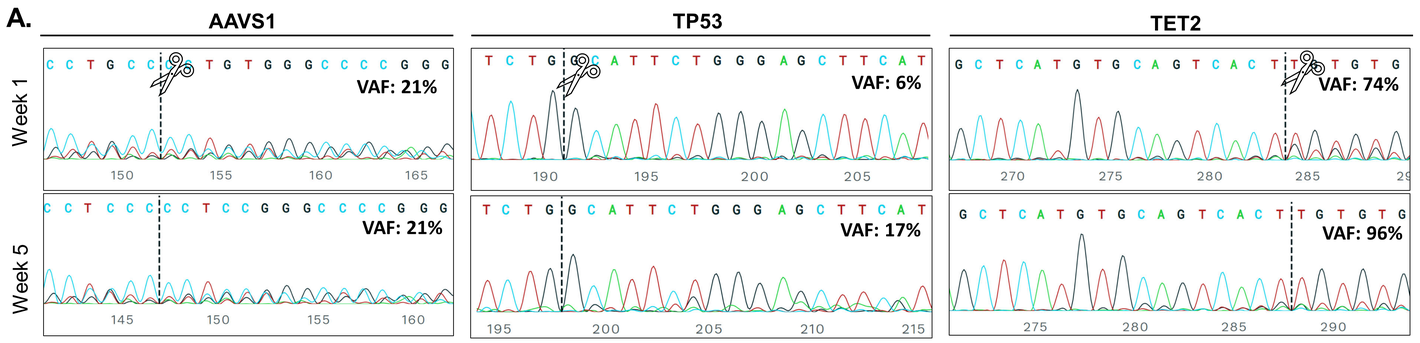


Figure 3: Work-flow of $CD34^{+}$ HSPC purification, CRISPR/Cas9 genome editing, and VAF examination

Results



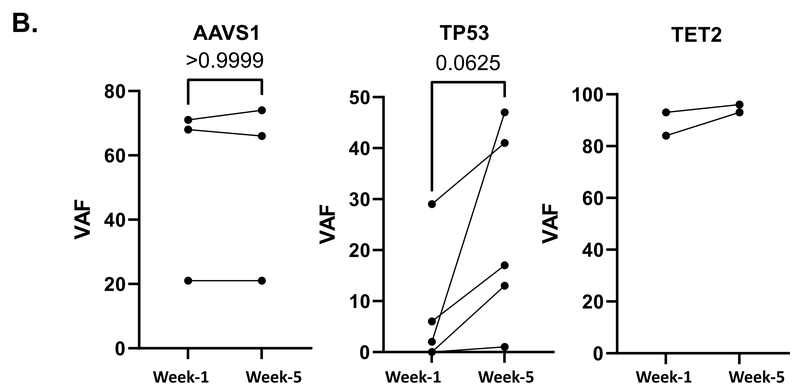


Figure 4 – *$TP53^{KO}$* HSPCs showed increased VAF over 5 weeks co-culture with unedited HSPCs. A. VAF call from Sanger sequencing at the cutting site at 1st vs. 5th week. Vertical dotted lines represent the cutting site. Contains mixed base calls. B. Summary plot of VAF changes over 1st vs 5th week. Minimal increase in AAVS1 group (p = 0.99, n = 3, Wilcoxon paired T test); substantial VAF increase in *TP53*-edited group (p =0.0625, n = 4, Wilcoxon paired T test); TET2 knock-out are performed at the same time as a positive control.

Conclusions

During the period from first week to fifth week, we observed increased *TP53* VAF along with time, while the VAF from AAVS1 control remained within a relatively narrow range (Figure 4). We conclude from this observation that $TP53^{KO}$ clone expanded and competed over the unedited clone *in vitro* co-culture while the *AAVS1* clone didn't exhibit a growth advantage versus the unedited clone. This data showed increased HSPC fitness delivered by *TP53* loss-of-function compared to AAVS1 controls.

Future Directions

- Employ *in vivo* xenograft model to validate the growth advantage granted by *TP53* loss *in vitro.*
- Investigate which genotoxic stressor accelerates the clonal expansion driven by *TP53* loss.
- Explore prevention therapies that target the *TP53* mutant clone.

Acknowledgements

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References

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- Model figures created with BioRender.