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TITLE: The role of *TP53* loss in the fitness of hematopoietic stem cells.

Along with aging, mutations accumulate in hematopoietic stem cells (HSCs) through exposure to environmental mutagens. Some of these somatic mutations provide a growth advantage and increased fitness of mutant HSCs compared to normal HSCs, which give rise to its clonal expansion. The presence of genetically distinct populations of hematopoietic cells from a single mutated HSC is referred to as clonal hematopoiesis (CH).

While the overall risk of overt *de novo* hematopoietic malignancy is low in the population with CH, certain CH mutations are associated with increased risk for therapy-related myeloid neoplasms (t-MN). In particular, mutations in *TP53* deliver the highest hazardous ratio (HR) of secondary leukemia among all the CH-related mutations. Moreover, $TP53^{mut}$ t-MN is an aggressive and lethal disease which is refractory to most conventional therapies. While it is important to monitor for signs of potential leukemia in cancer survivors with *TP53* mutation detected in blood, there is currently no preventative therapy available.

In order to develop a novel therapy that targets $TP53^{mut}$ HSPCs, we established a model with human primary hematopoietic stem and progenitor cells (HSPCs) and studied whether $TP53^{mut}$ HSPCs possess a growth advantage compared to the control. We employed CRISPR/Cas9 genome editing technology to knock-out *TP53* in primary HSPCs. To examine the effect of TP53 alteration in HSPC fitness, we co-cultured $TP53^{KO}$ and $AAVS1^{KO}$ (“safe harbor” locus) HSPCs with wild-type (WT) HSPCs in vitro for five weeks. The growth kinetics of $TP53^{KO}$ or $AAVS1^{KO}$ clones was tracked by examining the respective variant allele frequency (VAF) at different time points through Sanger Sequencing. Preliminary results showed VAF of *TP53* increased along with time, which directly shows $TP53^{KO}$ cells have a growth advantage and outcompeted the WT controls.