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Impact of TP53 aberrations on the transformation of human hematopoietic stem and progenitor cells (HSPCs)

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Background/Aims: Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy derived from transformed HSPCs. AML patients with TP53 aberrations face an exceedingly poor prognosis, even when treated with intensive regimens or novel strategies. To improve their outcome, deeper insight into underlying pathogenetic mechanisms is needed. Therefore, we aim to investigate if particular TP53 aberrations - mutations, deletions and a combination thereof - representing mono- and bi-allelic states, have a different impact on HSPC function and influence malignant transformation.

Results: Using an innovative CRISPR-Cas9 genome-editing approach combined with flow cytometry, allowed us to successfully enrich for human HSPCs comprising hot spot mutations (TP53 p.R175H and p.R273H) and gene knock-outs in a mono- and bi-allelic manner. Successful editing was confirmed on DNA and RNA levels via PCR and Sanger sequencing and p53 protein expression was demonstrated by Western blot analysis. In cell cycle analysis, bi-allelic TP53 aberrant HSPCs showed increased proliferation. These aberrations also form significantly more total colonies in methylcellulose assays with a propensity for erythroid differentiation. Furthermore, they revealed serial replating capacity indicating enhanced self-renewal.

Conclusion: We could successfully generate and characterize mono- and bi-allelic TP53 alterations in healthy, primary human HSPC using a CRISPR-Cas9-mediated knock-in strategy. We showed that bi-allelic mutant TP53 in HSPCs resulted in increased proliferation and enhanced self-renewal potential. These data clearly demonstrate the impact of TP53 aberrations on HSPCs and confirm the loss of function (LOF) phenotype. Current work focuses on the impact of these aberrations on genomic instability.