HIGHLY EFFICIENT CRISPR/CAS9 MEDIATED GENE EDITING IN LONG-TERM ENGRAFTING HUMAN HEMATOPOIETIC STEM PROGENITOR CELLS

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Introduction

- Gene-modified CD34$^+$ hematopoietic stem progenitor cell (HSPC) transplantation is curative for select hematologic diseases
- Electroporation of human HSPCs with Cas9 ribonucleoprotein complex (RNP) supports effective gene editing *ex vivo*

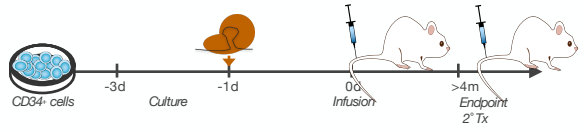
Goals of Study

- Evaluate reproducibility of Cas9 RNP gene editing across human CD34$^+$ cells donors (*HBB, AAVS1, CXCR4* loci)
- Compare engraftment of human CD34$^+$ cells electroporated with D10A nickase RNPs targeting *HBB*

Summary

- Cas9 RNP supports efficient, reproducible gene editing in human CD34$^+$ cells across multiple donors and experiments
- Gene-edited human long-term repopulating CD34$^+$ cells retain engraftment capability and multipotency *in vivo*

In vivo Experimental Design



- CD34$^+$ cells were cultured for 2 days, electroporated with RNPs targeting *HBB*, and transplanted into immunodeficient (NSG) mice
- ≥4 months after transplantation, human blood reconstitution of NSG mice and gene editing in human blood cells were evaluated

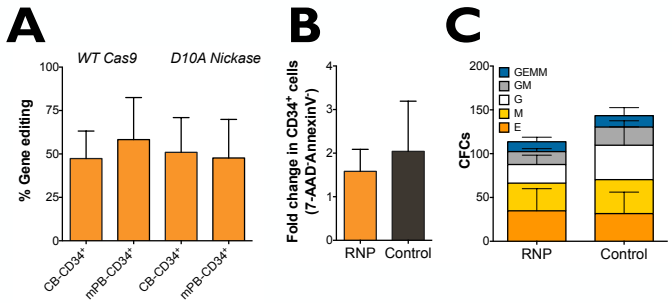


FIGURE 1. Cas9 RNP treated human CD34$^+$ cells maintain viability and hematopoietic activity *ex vivo*. A. Gene editing (determined by sequencing) in cord blood (CB) and adult mobilized peripheral blood (mPB) CD34$^+$ cells after electroporation with wild-type (WT) Cas9 or D10A paired nickases targeting *HBB* (n=20 donors, 15 experiments). **B.** Fold change of viable RNP-treated or donor matched untreated control CD34$^+$ cells. **C.** Hematopoietic activity in RNP-treated and control CD34$^+$ cells (colony forming cell [CFC] assays). GEMM: Granulocyte Erythroid Macrophage Monocyte. Statistics: Mean + S.D. For panels B, C, P-values were calculated using a paired t-test (all P-values n.s.).

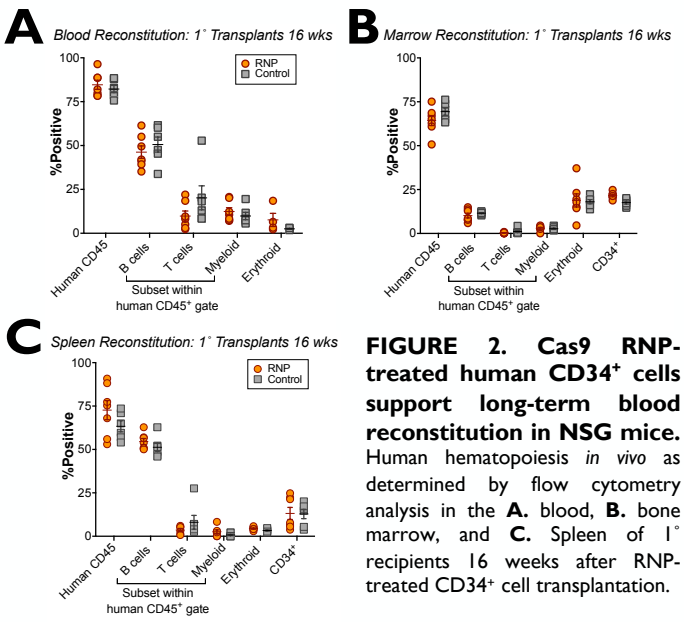


FIGURE 2. Cas9 RNP-treated human CD34$^+$ cells support long-term blood reconstitution in NSG mice. Human hematopoiesis *in vivo* as determined by flow cytometry analysis in the **A.** blood, **B.** bone marrow, and **C.** Spleen of 1° recipients 16 weeks after RNP-treated CD34$^+$ cell transplantation.

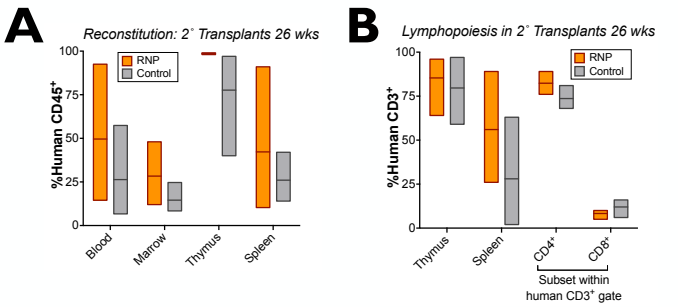


FIGURE 3. Cas9 RNP-treated human CD34$^+$ cells repopulate hematopoiesis in secondary recipient mice. A. Reconstitution of human blood in hematopoietic organs and **B.** T cell lymphopoiesis in the thymus and spleen of 2° transplant recipient mice.

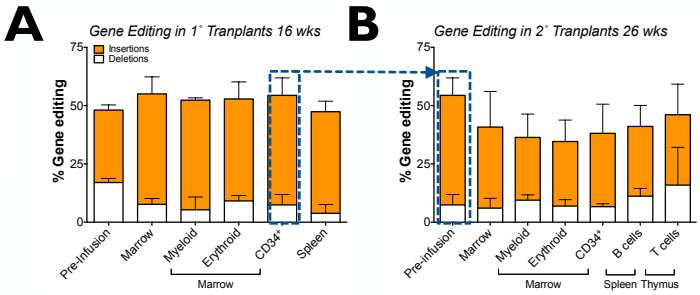


FIGURE 4. Gene-edited human CD34$^+$ cells differentiate into gene-edited myeloid, erythroid, and lymphoid progeny *in vivo*. Gene editing levels detected in the human cells that repopulated the indicated hematopoietic organs and human blood subsets in 1° (NSG) and 2° (NSG-SGM3) mice.

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