

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*

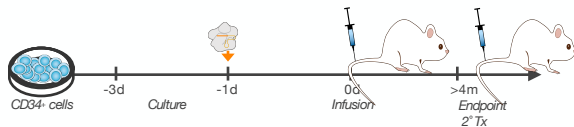
Goals of Study

- Evaluate reproducibility of Cas9 RNP gene editing across human CD34⁺ cells donors (*HBB*, *AAVSI*, *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

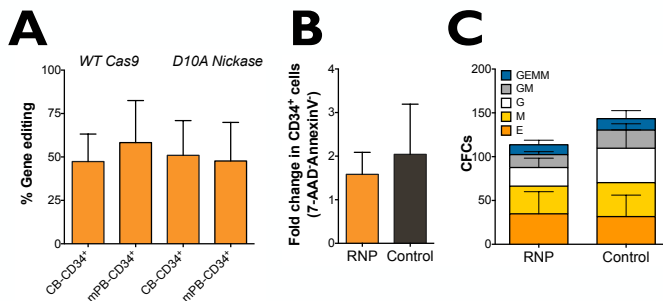


FIGURE 1. Cas9 RNP treated human CD34⁺ cells maintain viability and hematopoietic activity *ex vivo*. **A.** Gene editing (determined by sequencing) in cord blood (CB) and adult mobilized peripheral blood (mPB) CD34⁺ cells after electroporation with wild-type (WT) Cas9 or D10A paired nickases targeting *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.).

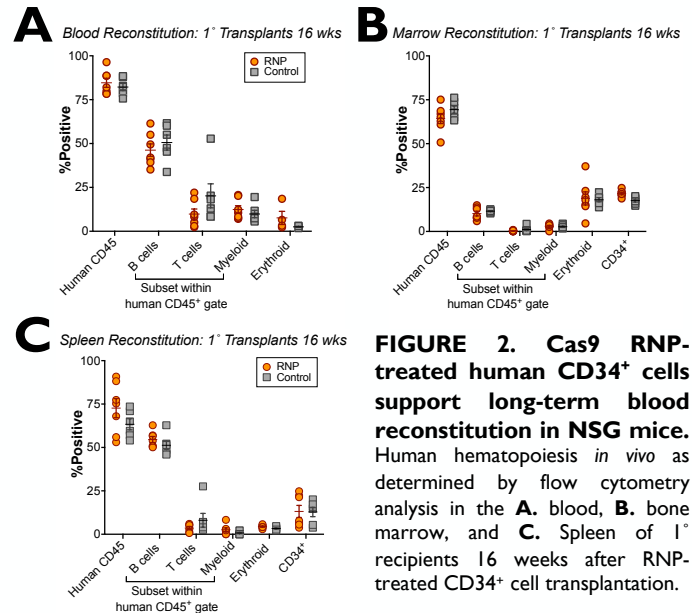


FIGURE 2. Cas9 RNP-treated human CD34⁺ cells support long-term blood reconstitution in NSG mice. Human hematopoiesis *in vivo* as determined by flow cytometry analysis in the **A.** blood, **B.** bone marrow, and **C.** Spleen of 1^o recipients 16 weeks after RNP-treated CD34⁺ cell transplantation.

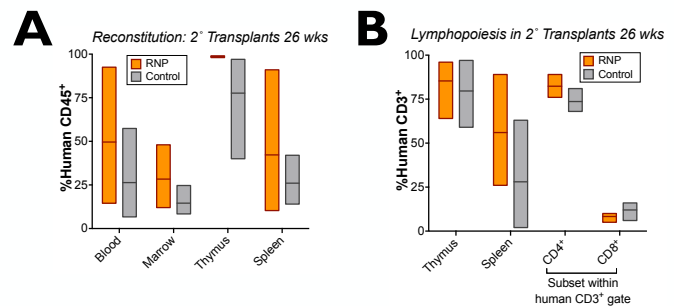


FIGURE 3. Cas9 RNP-treated human CD34⁺ cells repopulate hematopoiesis in secondary recipient mice. **A.** Reconstitution of human blood in hematopoietic organs and **B.** T cell lymphopoiesis in the thymus and spleen of 2^o transplant recipient mice.

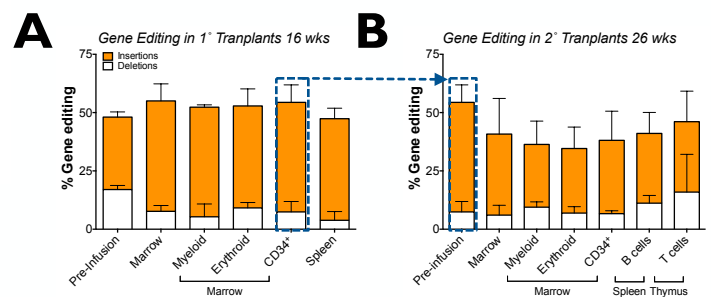


FIGURE 4. Gene-edited human CD34⁺ cells differentiate into gene-edited myeloid, erythroid, and lymphoid progeny *in vivo*. Gene editing levels detected in the human cells that repopulated the indicated hematopoietic organs and human blood subsets in **A.** 1^o (NSG) and **B.** 2^o (NSG-SGM3) mice.

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