Cas9-mediated genome editing in hematopoietic stem/progenitor cells

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Hypothesis

- **Nonviral methods of Cas9/gRNA delivery reduce CD34⁺ HSPC viability, survival, multipotency**
- **Optimization of culture and delivery conditions to maintain CD34⁺ cell survival and ex vivo hematopoietic potential will facilitate development of clinically beneficial levels of targeted gene modification in HSPCs**

Outline

- Design/test ***S. aureus*** **and** ***S. pyogenes*** **gRNAs** in 293T, K562, CD34⁺ cells
- Optimize conditions and Cas9 components to **maintain HSCs**
- **Compare DNA, RNA, and RNP** delivery in human CD34⁺ cells
- **Evaluate editing at target loci** (T7E1 assay on locus PCR products), **HSC phenotype, function, viability**

Summary

- ***Cas9/gRNA DNA delivery***

>20% genome multiplex genome editing while maintaining HSC viability and hematopoietic potential

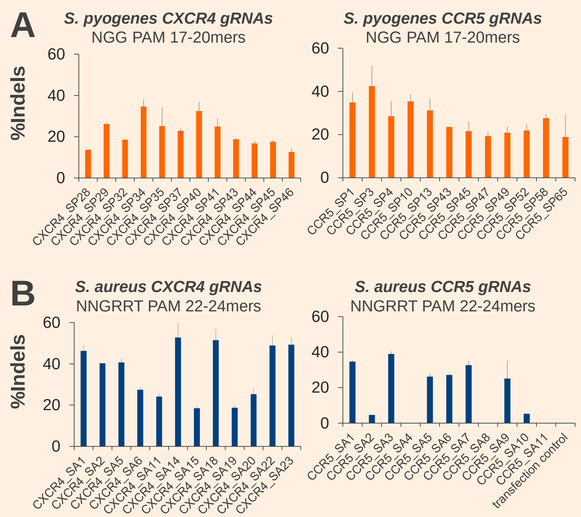
- ***Cas9 mRNA/gRNA delivery***

>25% editing while maintaining HSC viability and hematopoietic potential

- ***Cas9/gRNA RNP delivery***

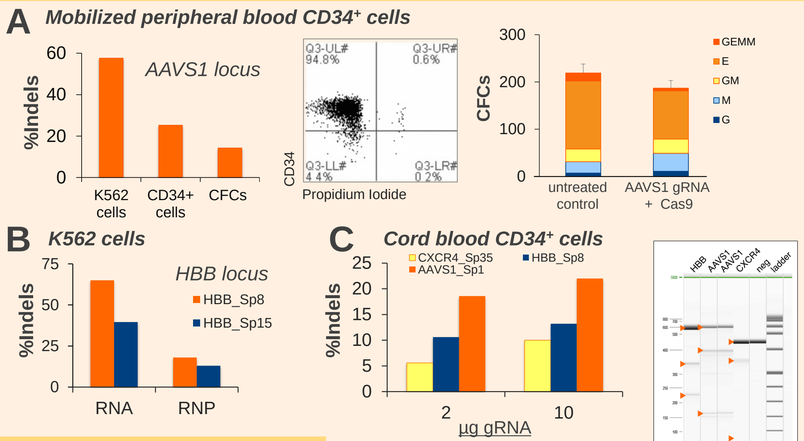
20% editing in K562 cells, HSCs require further development

FIGURE 1. Screening of *S. pyogenes* and *S. aureus* CXCR4 and CCR5 gRNAs.



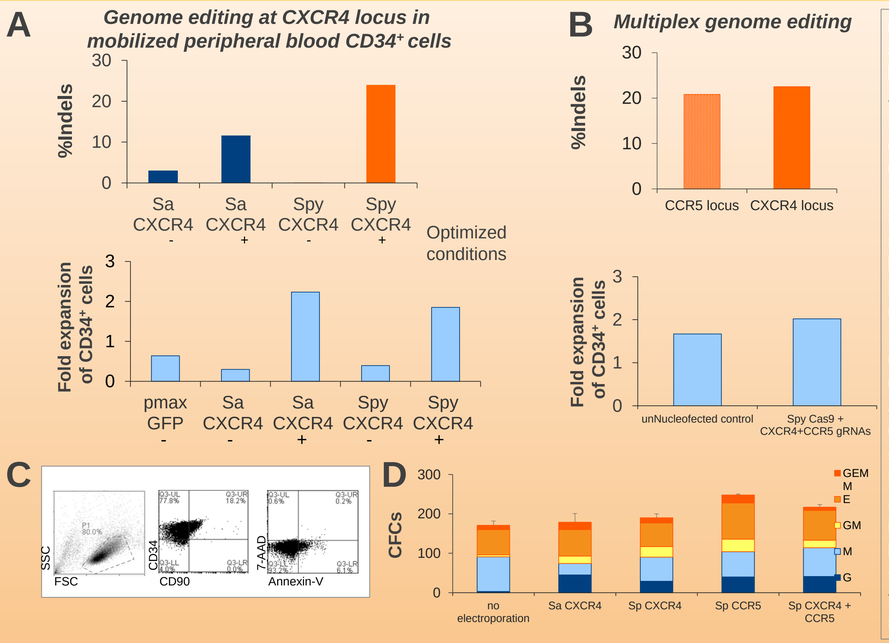
(A) *S. pyogenes* Cas9 with gRNAs targeting loci with different PAMs. % insertion/deletion (non-homologous end joining) on the Y axis represents on-target cleavage rates as measured by T7E1 assay. **(B)** *S. aureus* Cas9 with gRNAs targeting loci with different PAMs. % insertion/deletion (indels, non-homologous end joining) on the Y axis represents on-target cleavage rates as measured by T7E1 assay. Transfection into HEK293T cells using Lipofectamine 3000 in 24-well format with 750 ng/well Cas9) plasmid (CMV promoter) and 250 ng/well of gRNA (U6 promoter) constructs.

FIGURE 3. Human CD34⁺ cells electroporated with *S. pyogenes* Cas9 mRNA and gRNA maintain viability and multipotency and have sustained genome editing.



(A) Percentage of indels detected at *AAVS1* locus 72 hours after electroporation of *S. pyogenes* Cas9 and AAVS1 gRNA in K562 cells, mobilized peripheral blood CD34⁺ cells, and the progeny of edited CD34⁺ cells (CFCs). *Middle panel:* Representative flow cytometry analysis of viable (propidium iodide negative) CD34⁺ cells. *Right panel*: Hematopoietic colony forming potential of unedited and edited hematopoietic progeny. **(B)** Genome editing at the *HBB* locus in K562 cells after *S. pyogenes* Cas9 mRNA or Cas9 ribonucleoprotein co-delivered with single HBB gRNAs (HB_Sp8 or HBB_Sp15). **(C)** Percentage of indels detected at the indicated target genetic loci after delivery of Cas9 mRNA and gRNA to cord blood CD34⁺ cells. *Right:* Representative gel showing cleavage at the indicated loci (T7E1 analysis). **(D)** Cell viability in CB CD34⁺ cells 48 hours after delivery of Cas9 mRNA and indicated gRNAs as determined by co-staining with 7-AAD and Annexin V and flow cyotometry analysis. **(E)** CFC analysis and representative colonies generated from edited CB CD34⁺ cells.

FIGURE 2. Genome editing in human mobilized peripheral blood CD34⁺ cells after electroporation of CAS9 and gRNA plasmid DNA.



(A) Percentage of indels detected at CXCR4 locus 72 hours after electroporation of *S. aureus* or *S. pyogenes* Cas9 and paired gRNAs (CXCR4_Sp35 or CXCR4_Sa18) in human CD34⁺ cells +/- optimized cell culture conditions. *Bottom:* Fold expansion of live CD34⁺ cells 72 hours after electroporation. **(B)** Multiplex genome editing in CD34⁺ cells after co-delivery of *S. pyogenes* Cas9, CXCR4_Sp35, and CCR5_Sp43 gRNA plasmids. *Bottom:* Fold expansion of live CD34⁺ cells 72 hours after electroporation. **(C)** Representative flow cytometry analysis of CD34⁺ cells cultured in optimized conditions at 72 hours after Cas9/gRNA plasmid delivery. **(D)** Ex vivo colony forming potential of DNA electroporated CD34⁺ cells cultured in optimal conditions.