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.

FIGURE 2. Genome editing in human mobilized peripheral blood CD34+ cells after electroporation of Cas9 and gRNA plasmid DNA.

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