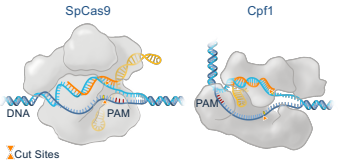
Expanding CRISPR Genome Editing Strategies in Hematopoietic Stem and Progenitor Cells for the Treatment of Hematologic Diseases

Aditi Chalishazar, Carrie Margulies, Geetha Nichanametla, John Labella, Katherine Loveluck, Ramya Viswanathan, Jack Heath, Edouard deDreuzy, KaiHsin Chang, Hari Jayaram, John Zuris, Vic Myer, Charlie Albright, Cecilia Cotta-Ramusino, Jennifer Gori

Editas Medicine, 11 Hurley Street, Cambridge, MA 02141

Introduction

Current *ex vivo* genomic modifications use lentivirus-mediated gene transfer and nuclease-directed non-homologous end joining (NHEJ). To expand the types of genomic modifications possible, we tested the potential for homology directed repair (HDR) mediated targeted integration and Cfp1-directed edits in human mobilized peripheral blood (mPB) CD34⁺ cells. Targeted integration may be useful for diseases such as Sickle Cell Disease and β-Thalassemia, and other targets where normal transcriptional regulation is important. Cpf1-directed editing expands the number of genomic sites accessible for gene therapy.



HDR Experimental Method

Study Goal: Establish baseline HDR at *HBB* locus in viable mPB CD34⁺ cells

Experimental Method

Day	Step
-3	Thaw and culture CD34⁺ cells
0	*S. Pyogenes* (Sp) Cas9 RNP electroporation + AAV6 transduction
2	Viability (AO/PI), GFP (flow cytometry)
7	Viability and integrated GFP expression On-target integration (ddPCR) On-target editing (sequencing)
14	On-target integration in GFP⁺ GEMMs

CRISPR/Cas9 Homology Directed Repair Proof of Concept

FIGURE 1. ddPCR assay for targeted integration measurement

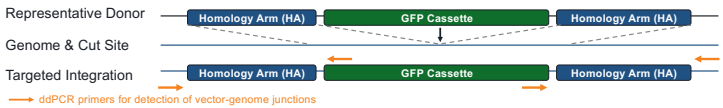
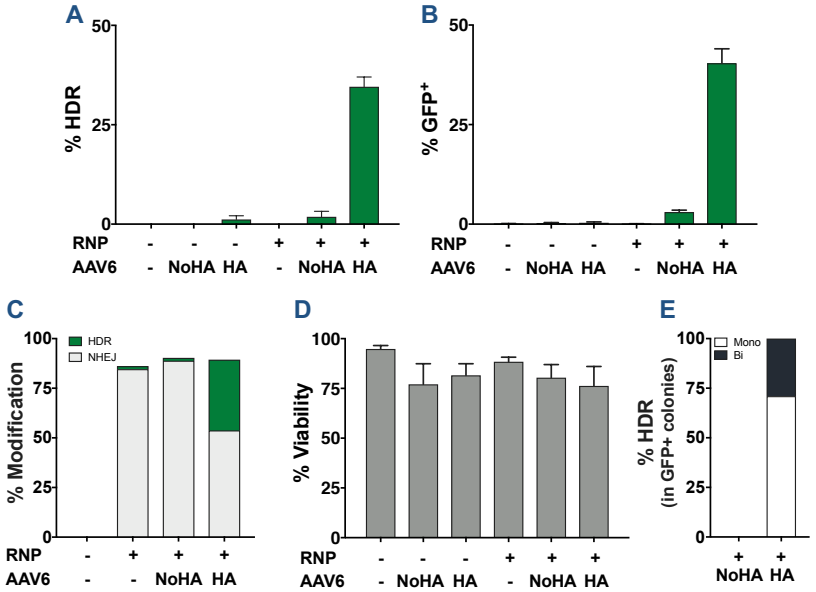


FIGURE 2. SpCas9 RNP and AAV6 donor with *HBB* homology arms cause targeted integration through HDR



A. On-target integration (% HDR) at *HBB* locus in CD34⁺ cells electroporated with Cas9 RNP ± AAV6 (± homology arms or no homology arms, HA or NoHA). Mean ± S.D. of the percentages of on-target integration detected at 5' vector-genome junctions. **B.** Integrated GFP expression in live CD34⁺ cells 7 days after electroporation. **C**. On-target integration (% HDR) or indels (% NHEJ) by sequencing. **D.** Viability in CD34⁺ cells 48 hours after electroporation. **E.** Percentage of monoallelic (mono) and biallelic (bi) on-target integration (% HDR) by ddPCR analysis of GFP⁺ clones (CFU-GEMMs). For A-D, Mean values ± S.D. from 4 experiments with different CD34⁺ cell donors are shown.

CRISPR/Cpf1 Directed Editing Proof of Concept

Study Goal: Evaluate *Acidaminococcus BV3L6* (As)Cpf1 at multiple target sites

Experimental Method: Thaw and culture CD34⁺ cells → Electroporate with RNP → On-target sequencing

FIGURE 3. Engineered Cpf1 variants expand PAM targeting space

Variant	PAM	Expected genome frequency (nt)
SpCas9	NGG	1 per 16
SaCas9	NNGRRT	1 per 64
SaCas9 KKH	NNNRRT	1 per 16
AsCpf1 WT	TTTV	1 per 85
AsCpf1 RR	TYCV/CCCC	1 per 42
AsCpf1 RVR	TATV	1 per 85
FnCpf1	TTN	1 per 16

FIGURE 4. Cpf1 edits certain target sites more efficiently than Cas9

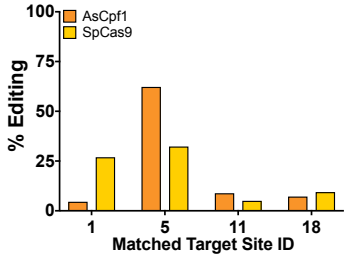


FIGURE 5. PAM variants can increase editing at HPFH target site

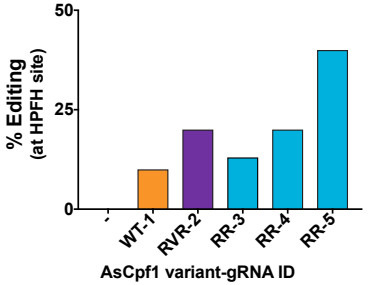
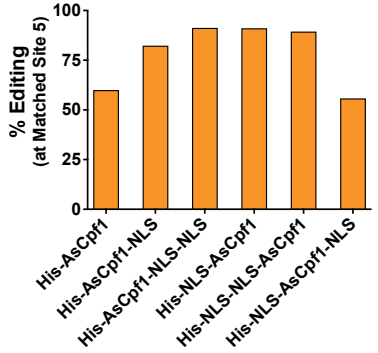


FIGURE 6. NLS variants can increase editing at target site



Conclusions

- Efficient and reproducible HDR in CD34⁺ cells by Cas9 with minimal impact on cell viability (~80% viability)
- 30% biallelic and 70% monoallelic targeted integration in Cas9 HDR-modified clones
- First report of efficient Cpf1 directed editing in CD34⁺ cells
- AsCpf1 edits certain sites more efficiently than SpCas9 in CD34⁺ cells