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Potent HbF Induction Following ssODN-mediated Repair of Cas9-induced DSB at the HBG Promoter in CD34+ HSPC

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Fetal Hemoglobin expression in adulthood prevents the onset of SCD symptoms

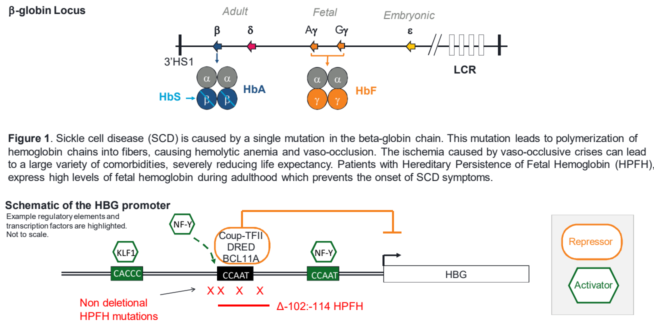


Figure 1. Sickle cell disease (SCD) is caused by a single mutation in the beta-globin chain. This mutation leads to polymerization of hemoglobin chains into fibers, causing hemolytic anemia and vaso-occlusion. The ischemia caused by vaso-occlusive crises can lead to a large variety of comorbidities, severely reducing life expectancy. Patients with Hereditary Persistence of Fetal Hemoglobin (HPFH), express high levels of fetal hemoglobin during adulthood which prevents the onset of SCD symptoms.

Figure 2. Multiple HPFH mutations are clustered around the distal CCAATbox at the HBG1/2 promoters, likely overlapping with the binding domains of repressive regulatory elements. Several transcription factors or complexes (including CoupTF-II, DRED or BCL11a) have been described to bind that domain and to participate in HBG repression during adulthood. The existence of deletional HPFH mutations at this site demonstrate the theoretical feasibility of a Crispr-editing approach targeting that domain to promote the expression of HbF in patients with SCD.

Objective

- Development of a Crispr-mediated gene editing approach targeting the HBG distal CCAAT-box that promotes a potent fetal globin expression in the erythroid progeny of edited human hematopoietic stem and progenitor cells (mPB CD34+)

Identification of RNPs disrupting the HBG distal CCAAT

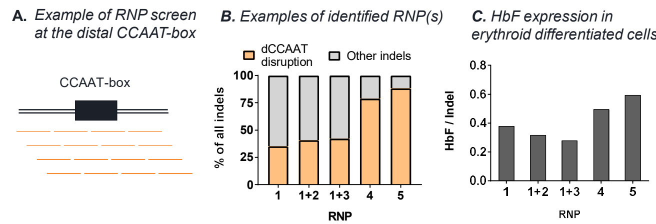
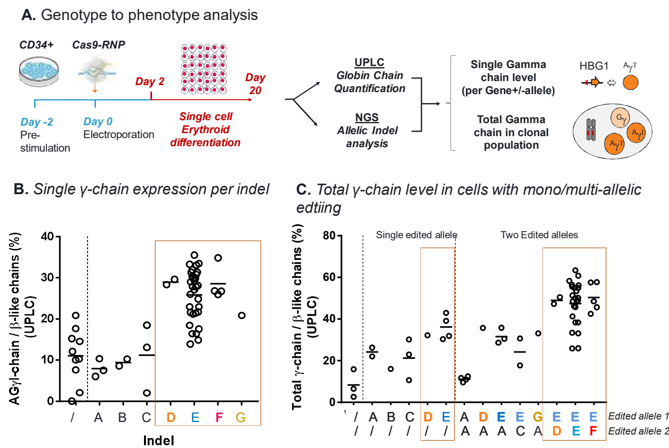


Figure 3. A. Example of screening of RNP (or RNP pairs) targeting the distal CCAAT box. B. Several RNP or RNP pairs generating medium to high disruption of the CCAATbox were identified. C. HbF quantification in the erythroid progeny of edited CD34+ cells confirmed the capacity of identified RNPs to promote HbF expression.

Quantification of γ-chain expression mediated by CCAAT-box disrupting indels



D. HbF-inducing indels

	Sequence	
A	CTTGCCTTGA-CAATAGCCTTGACAA	Low HbF
B	CTTGCCTTGA--AATAGCCTTGACAA	
C	CTTGCCTTGA---ATAGCCTTGACAA	
D	CTTGCCTTGA----TAGCCTTGACAA	High HbF
E	CTTGCCTTGA-------------CAA	
F	CTTG------------------ACAA	
G	CTTGCCTTG-CCAATAGCCTTGACAA	

Figure 4. A. Indels disrupting the distal CCAATbox may differ in their capacity to induce HbF expression. To identify which indels lead to high HbF expression, treated CD34+ cells were individually differentiated in erythroid cells. NGS analysis was performed to detect indels on each HBG allele and globin chains were quantified by UPLC. Aγ-chains expressed from both chromosome could be differentiated thanks to asymptomatic mutations of the Aγ protein. **B.** Levels of single γ-chain expression for a given indel detected on the corresponding allele. Several indels inducing ~30% γ-chain expression were identified (indels D-G), while other indels yielded low γ-chain expression (A-C). **C.** Total γ-chain level in clonal erythroid population. Highest level of γ-chain expression were observed in cells with indels D-G. Cells with 2 alleles bearing those mutations reached up to 60% of gamma over total beta-like chains. **D.** Sequences of the characterized indels.

HBG Precise Repair by ssODN/RNP co-delivery

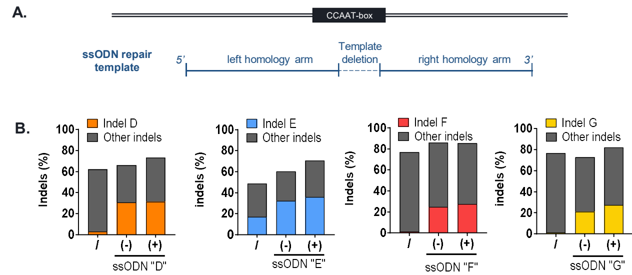


Figure 5. A. To increase total Gamma chain expression, ssODN templates encoding indels identified to promote high HbF expression were designed and co-delivered with RNP targeting the distal CCAAT-box. **B.** Sequencing analysis demonstrated that the frequency of the encoded indel amongst edited alleles was increased up to 40% following ssODN co-delivery.

Increased HBF expression using ssODNs encoding high-HbF inducing indels

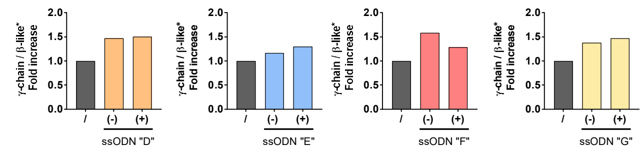


Figure 6. The co-delivery of ssODN template encoding high-HbF inducing indels increased the level of γ-chain induction by up to 1.6 fold.
*γ-chain/total β-like (background subtracted, range:5-8%)

RNP and ssODN co-delivery induces ~40% HBF in the progeny of CD34 cells

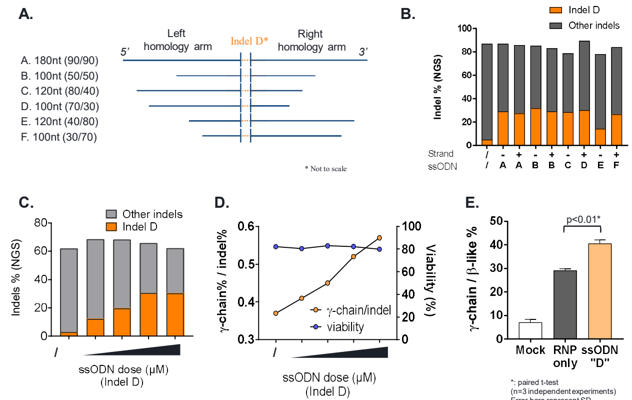


Figure 7. A. ssODN with homology arms of different length, strand and symmetry were designed. **B.** Optimal gene correction efficiency was observed with homology arms as short as 50nt. **C-D.** The relative dose of RNP (not shown) and of ssODN were optimized to achieve high levels of gene correction and HbF expression. Increasing dose of ssODN yielded higher levels of gene correction (C), resulting in higher levels of γ-globin induction without affecting viability (D). **E.** Optimized conditions reproducibly promoted ~40% HbF induction when co-delivering an ssODN versus ~30% when delivering RNP only..

Summary

- Several RNPs were identified with up to ~90% of indels disrupting the HBG distal CCAAT-box
- Genotype to phenotype analysis allowed the quantification of γ-globin expression induced by multiple distal CCAAT-box disrupting indels. High-HbF inducing indels were identified and encoded on ssODNs donor templates to promote the precise repair of Crispr-mediated DSB.
- The design of the donor ssODN and the electroporation conditions were optimized allowing to achieve high levels of gene correction in mPB-CD34 cells. Precise repair of the HBG distal CCAAT-box by co-delivery of RNP and an ssODN donor template yielded a potent HbF-expression in the erythroid progeny of HSPC.
- Engraftment studies in humanized mouse model will determine whether the ssODN-mediated directed repair can occur efficiently in long term hematopoietic stem cell resulting in long-term expression of therapeutically relevant levels of HbF

Author Disclosures : ED, JH, AB, DR, AD, EM, TW, FH, KG, SS, MS, AC, DT, FT, GG, TT, GG, ST, CW, CCR, CA, HJ, and KHC are employees and shareholders of Editas Medicine. JG is a shareholder of Editas Medicine.

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