Complementary Base Editing Approaches for the Treatment of Sickle Cell Disease and β-thalassemia

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Introduction

Sickle cell disease (SCD) and β-thalassemia are disorders of beta globin production and function that lead to severe anemia and significant disease complications across a multitude of organ systems. Autologous transplantation of hematopoietic stem cells engineered to upregulate fetal hemoglobin (HbF) or correct the beta globin gene have the potential to reduce disease burden in patients with beta hemoglobinopathies.

Base editing is a recently-developed technology that enables a potential new class of precision genetic medicines that target a single base in the genome without the introduction of double-stranded DNA breaks (Gaudelli, et al). Previously SCD and β-thalassemia patients were identified that harbored natural genetic variants in the gamma globin gene promotor which caused beneficial hereditary persistence of fetal hemoglobin (HPFH). Using Adenine Base Editors (ABEs), we recreated these naturally occurring HPFH variants by installing base edits in the promotor region of HBG1 and HBG2 (Forget, et al). *In vitro* and *in vivo* data demonstrated that ABEs could successfully edit human CD34+ cells, leading to long term engraftment and retention of editing.

In parallel studies, ABEs were screened that directly target the causative Glu6Val mutation in SCD patients. Since current base editing technologies cannot yet convert mutations like those that result from the A-T transversion in sickle beta globin, ABE variants were designed to recognize and edit the opposite stranded adenine residue of valine. This results in the conversion of valine to alanine and the production of a naturally occurring, asymptomatic, non-polymerizing variant known as Hb G-Makassar (Ahmad, et al). SCD patient fibroblasts and sickle cell trait CD34+ cells edited with ABEs achieved 40%-70% conversion of the target adenine, a level of editing which holds the promise of curing the disease.

These next generation editing approaches provide a promising new modality for treating patients with β-thalassemia and SCD.

Base Editing for SCD and β-Thalassemia

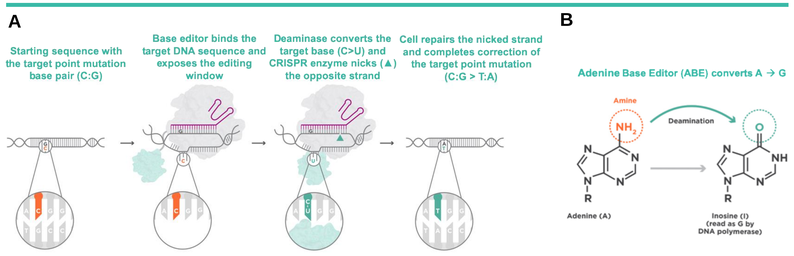

Figure 1. (A) Base editors are comprised of two components: the CRISPR protein and the deaminase, which are fused together to form a single protein. When introduced into a cell, the CRISPR protein targets the desired genomic location based on the sequence of the guide RNA; the Base editor then makes the desired edit (deamination) to a target base in the editing window. (B) ABEs convert an adenine to inosine. Inosine is then replaced by guanine through DNA replication. This enables A-T to G-G editing. Our strategies for SCD and β-thalassemia both utilize ABEs to generate the desired mutation.

Hereditary Persistence of Fetal Hemoglobin (HPFH)

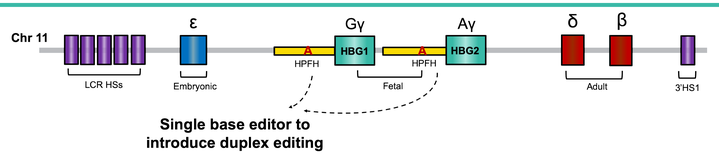

Figure 2. β-thalassemia and SCD patients who have a natural HPFH mutation are asymptomatic or experience a much milder form of their disease. HPFH is the result of naturally occurring single point mutations in the regulatory region of the HbF genes (HBG1 and HBG2). These mutations lead to an increase in the expression of fetal hemoglobin by preventing the binding of one or more γ-globin repressor proteins (e.g. BCL11A).

Base Editor Screening in Human CD34+ Cells

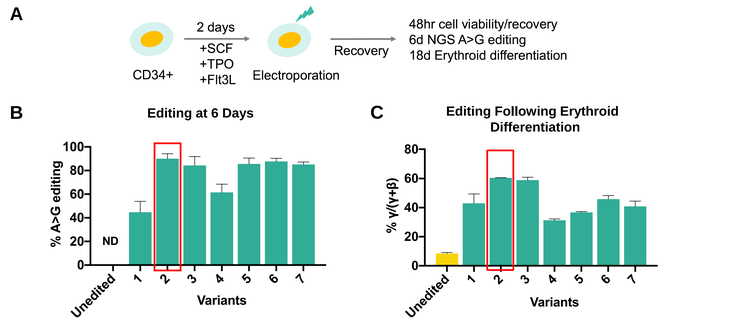

Figure 3. ABEs (delivered as mRNA) were screened in human CD34+ cells according to the diagram (A) for % gene editing (B) or for upregulation of fetal hemoglobin as determined by UPLC (C). A lead editor was identified for HPFH that resulted in high editing efficiency, γ-globin expression, and viability.

Erythroid Differentiated CD34+ Cells Express γ-Globin

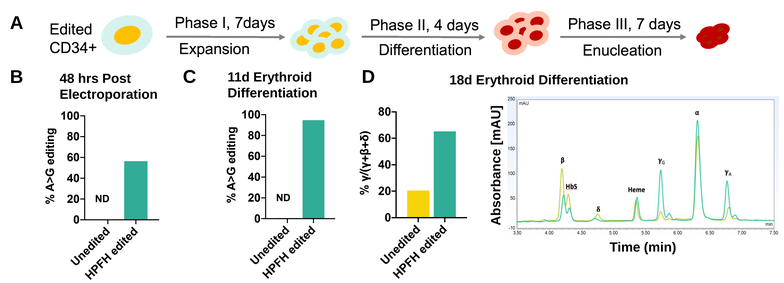

Figure 4. Edited SCD trait CD34+ cells were differentiated to erythroid cells as shown in (A). Editing rates at HBG1/2 promoters were detectable at 48hrs post electroporation (B) and continued to rise during erythroid differentiation (C). Generating the naturally occurring HPFH mutations significantly upregulated γ-globin after erythroid differentiation, as measured by UPLC (D). Similar results were observed in non-SCD trait donors.

Retention of Editing in Hematopoietic Stem Cells *In Vivo*

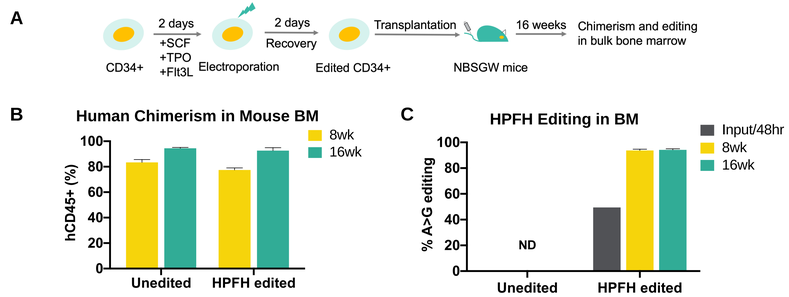

Figure 5. CD34+ cells from a healthy donor were edited and injected into NBSGW mice after 2 days recovery (A). High chimerism was achieved at a similar level to unedited samples as measured by hCD45+ (B). Over 90% of editing was achieved and retained after engraftment in NBSGW mice after 16 weeks (C).

Hemoglobin G Makassar (Hb G-Makassar)

HbB	**CTC (Glu)**
HbS	**CAC (Val)**
HbG-Mksr	**CGC (Ala)**

Figure 6. Correcting the Glu6Val mutation of SCD has been a recent goal of genetic therapies designed for the SCD population. Current base editing technologies cannot yet convert mutations like those that result from the A-T transversion in sickle beta globin; however, ABE variants have been designed to recognize and edit the opposite stranded adenine residue of valine. This results in the conversion of valine to alanine and the production of a naturally occurring variant known as Hb G-Makassar. Beta globin with alanine at this position does not contribute to polymer formation, and individuals with Hb G-Makassar present with normal hematological parameters and red blood cell morphology.

Editing Efficiency in Fibroblasts or SCD Trait CD34+ Cells

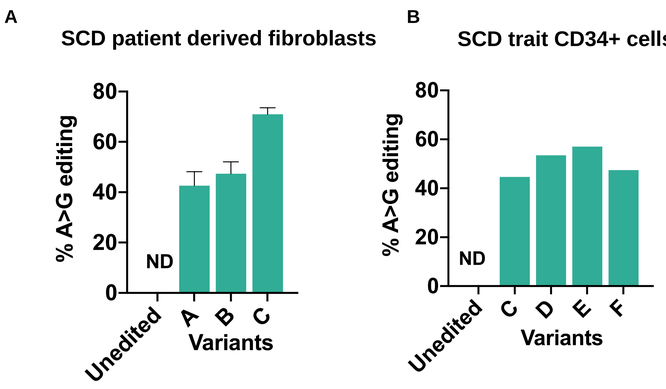

Figure 7. ABEs were screened in SCD patient derived fibroblasts to identify a base editor that demonstrated high level editing of the sickle cell point mutation to the Makassar variant (A). Additional ABE variants, based on variant C, were screened for editing efficiency in sickle trait CD34+ cells. All variants achieved and retained high editing rates from 48 hours post-electroporation through erythroid differentiation (B).

Conclusions

Both HPFH and Hb G-Makassar strategies demonstrated high editing efficiency of single bases without creating double-stranded DNA breaks following electroporation and erythroid differentiation. Furthermore, our HPFH approach achieved high γ-globin levels and long-term editing retention *in vivo*. We are advancing both HPFH and Hb G-Makassar in parallel and are continuing to examine *in vitro* and *in vivo* efficacy in both healthy cells and patient samples. Base editors, particularly ABEs, are a promising gene editing option to introduce clinically validated, naturally occurring mutations for the treatment of these devastating diseases.

References

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