A Novel Base Editing Approach to Directly Edit the Causative Mutation in Sickle Cell Disease

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Introduction

Sickle cell disease (SCD) is a severe, hereditary monogenic blood disorder that alters the structure and function of oxygen-carrying hemoglobin in red blood cells. So far, in a clinical setting, gene therapy approaches have focused on either expression of artificial anti-sickling globin or upregulation of fetal hemoglobin. However, direct editing of the sickle causing mutation (Glu6Val) has not been possible at high efficiency without causing double strand DNA breaks. Adenine base editors (ABEs) have been shown to precisely make A-T to G-C base pair conversions with low rates of indels and without double strand DNA breaks (1-2).

We identified ABE variants that efficiently recognized and edited the sickle mutation, converting the sickle-causing valine to an alanine. This conversion generates a naturally occurring form of β-globin, Hb G-Makassar. This variant was previously identified in asymptomatic homozygous individuals that have normal hematologic parameters and no evidence of hemoglobin polymerization or sickling of red blood cells (3-5). *In vitro* data demonstrated that these ABE variants could successfully edit human CD34$^+$ cells harboring the sickle trait and be maintained throughout hematopoiesis, especially erythropoiesis.

We additionally developed a high sensitivity ultra-high-performance liquid chromatography (UPLC) assay to detect and distinguish the Hb G-Makassar β-globin protein from sickle globin (HbS), as well as confirmed the presence of this globin variant by LC-MS. Our strategy achieves high, bi-allelic editing and conversion to the Makassar β-globin variant, reducing HbS globin to a level of <15% and reducing *in vitro* sickling under hypoxia.

This next generation base editing approach provides a promising new modality for treating patients with SCD.

Genome Editing with Adenine Base Editors (ABE)

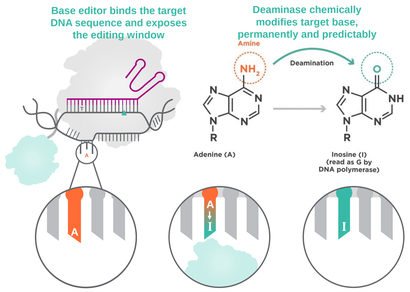

Figure 1. ABE is a fusion protein comprising an evolved TadA* deaminase (teal) connected to CRISPR-Cas enzyme (grey). The base editor binds to a target sequence that is complementary to the guide-RNA (magenta) and exposes a stretch of single-stranded DNA. The deaminase converts the target adenine into inosine (which is read as guanine by DNA polymerases) and the Cas enzyme nicks the opposite strand. The nicked strand is repaired completing the conversion of an A:T to G:C base pair (1-2).

Makassar β-globin Variant Direct Editing SCD Strategy

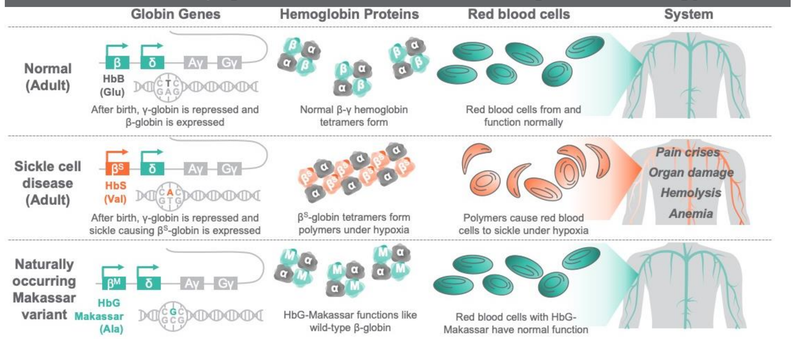

Figure 2. Current base editing technologies cannot yet efficiently convert mutations like those that result from the A:T to T:A transversion in HbS; however, ABE variants were designed to recognize and edit the opposite stranded adenine residue of valine. This results in the conversion of valine to alanine and the production of a naturally occurring variant in human genetics, Hb G-Makassar, which presents with normal hematological parameters and red blood cell morphology (3-5). Furthermore, alanine substitutions at this residue of the β–hemoglobin subunit does not contribute to polymer formation *in vitro* (6-8).

Editing of the Sickle Trait in HbAS and HbSS Cells

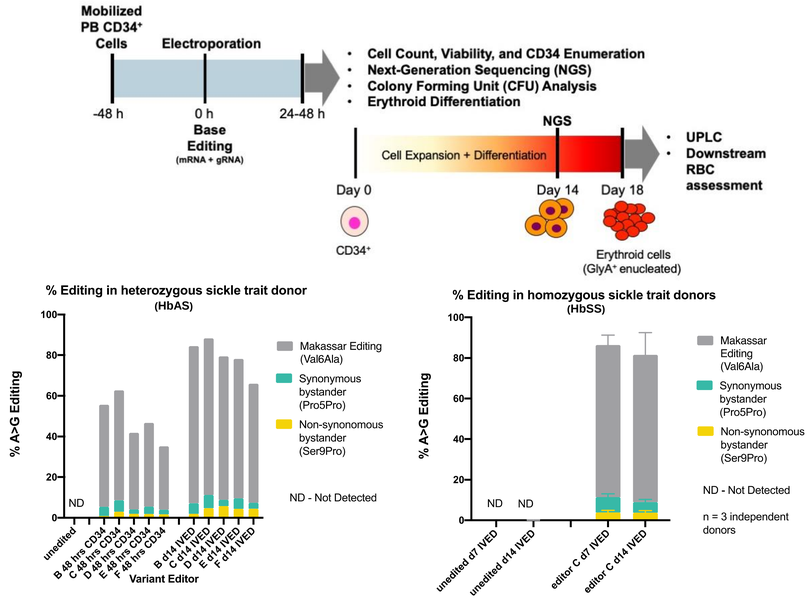

Figure 3. Several ABE variants were chosen to edit mobilized peripheral blood CD34+ hematopoietic stem and progenitor cells (HSPCs) isolated from a heterozygous sickle cell trait donor (HbAS). Editing rates ranging from 40%-60% of the sickle allele at 48 hours post-electroporation with high cell recovery and viability were achieved, with no evidence of any immunophenotypic changes. Subsequent *in vitro* erythroid differentiation (IVED) of edited CD34+ cells confirmed that Makassar editing was retained throughout erythropoiesis.

We were also able to achieve high level of editing in non-mobilized whole blood CD34+ cells isolated from several independent homozygous SCD patients (HbSS) with >75% A>G Makassar editing following IVED.

High Bi-Allelic Makassar Editing of HbSS Cells

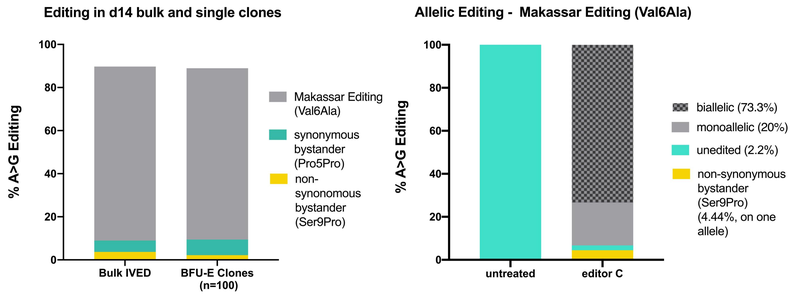

Figure 4. To assess the allelic editing frequencies in HbSS samples with our strategy, we conducted NGS on bulk erythroid in vitro cultures and individual BFU-E colonies plated in methylcellulose a day after electroporation. Bulk cells and BFU-E colonies were harvested 14 days later. At high bulk Makassar editing levels (~80%), nearly 75% of cells were bi-allelically edited, with very few cells (2.2%) completely unedited.

Detection of the Hb G-Makassar β-globin Variant

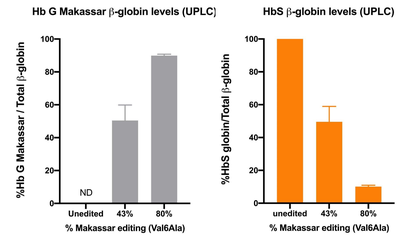

Figure 5. As successful resolution of Hb G-Makassar from HbS proteins has not been previously achieved, we developed a high sensitivity ultra-high-performance liquid chromatography (UPLC) assay to detect and distinguish the Hb G-Makassar globin protein from HbS in IVED cells. The Hb G-Makassar variant was further confirmed by liquid chromatography mass spectrometry (LC-MS). The level of Makassar globin levels correlated closely with the percent A>G Makassar editing. Conversion of HbS to Hb G-Makassar at levels >80% could be obtained in edited cells, corresponding to a reduction of HbS levels to <15%.

Modeling of β6 Substitutions

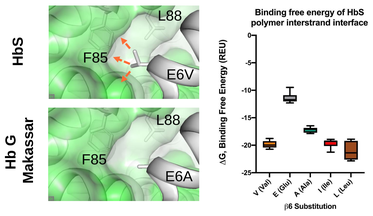

Figure 6. Molecular modeling and calculation of the binding free energy (ΔG, REU: Rosetta Energy Unit) (by RosettaCommons) of β6 substitutions at the inter-β-strand interface of deoxy-HbS between β6-Valine and a hydrophobic pocket that is only exposed in the deoxy state. This modeling agrees with the observations that wildtype Glu (E) does not polymerize (mean ΔΔG = +8 REU) and Leu (L) or Ile (I) substitutions enhance polymerization (9). Ala (A) at this position is predicted to cause a significant loss in binding free energy at the polymer interface (mean ΔΔG = +2.6 REU). Components of the ΔΔG arise from loss of buried hydrophobic area and attractive van der Waals energy.

Makassar β-globin Variant Reduces Sickling *in vitro*

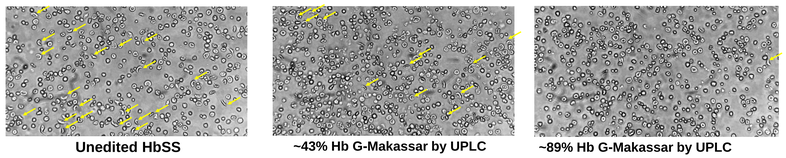

Figure 7. Under hypoxic conditions (2% O_2), reduction of sickling was observed in IVED HbSS cells with higher levels of bi-allelic editing and conversion of HbS to Hb G-Makassar.

Conclusions

Our Hb G-Makassar direct editing strategy demonstrated high editing efficiency of single bases of CD34$^+$ cells after electroporation, which is sustained through erythroid differentiation in both heterozygous and homozygous sickle trait cells. Furthermore, we were also able to resolve and confirm installation of Hb G-Makassar β-globin variant by a novel UPLC method and LC-MS. We demonstrate that at high editing efficiencies, high bi-allelic editing can be achieved with reduction of HbS globin levels to <15% and a reduction of *in vitro* sickling of edited cells exposed to hypoxic conditions. Coupled with autologous stem cell transplantation, the direct editing of the causative sickle cell mutation to the naturally occurring and asymptomatic Hb G-Makassar is a promising new treatment paradigm for patients with SCD.

References and Disclosures

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