Introduction

The c.358G>A mutation of β-globin gene causes sickle cell disease (SCD). Allogeneic hematopoietic stem cell (HSC) transplantation can cure SCD, but most lack a suitable donor. For patients with transfusion-dependent β-thalassemia or beta-negative thalassemia, the use of gene therapy to correct β-globin gene mutations would add an alternative strategy and permit endogenous gene expression at its natural regulatory locus and the beneficial reduction of the pathogenic α-thalassemia trait. CRISPR-mediated genome editing has established the therapeutic potential for genetic repair in ≥200 donor-recipient pairs. We previously reported efficient correction of a monogenic “repair” mutation in the CyBB gene in X-linked chronic granulomatous disease (CGD) patient HSCs with a robust, scalable, and regulatory compliant process (Sci Transl Med 2017) that now apply to SCD.

In initial studies using a 8-bp full-length (HLA-C) retrovirus from SCD patient and healthy donors' CD34+ HSCs, we developed a SCD correction specific guide RNA, and a normal 6-bp specific guide RNA (control). The converse guide differed by only one nucleotide from the SCD mutation specific guide, where each guide could be used together with a single-stranded DNA donor to effectively alter the wild type to SCD and the SCD to wild type, respectively (ASGCT 2017).

At first, we optimized homology directed repair (HDR) at the SCD locus by integrating a HindIII enzyme site. We observed efficient site-specific insertion of the HindIII marker in the BGL2 as evidenced by HindIII digestion of the PCR products (~50%), fingerprinting, Sanger sequencing (~35% HDR and ~55% Indel). The optimized process was applied to correct SCD CD34+ HSCs to achieve similar biologic HDR rates for HindIII site insertion as well as gene correction from the SCD mutation to the normal (6-bp correction sequence to ~35%, correction 20%). Interestingly, this correction was maintained during erythroid differentiation in culture. Among erythroblasts differentiated from corrected SCD CD34+ cells in vitro, wild type adult Hb protein levels were above 50% as assessed by both reverse phase HPLC and Hb electrophoresis, and sickle Hb production decreased from 100% to 25% after correction.

In summary, based on these in vitro correction rates confirmed by targeted sequencing, wild type adult Hb protein expression, and substantially decreased sickle Hb amounts, we are starting to evaluate engraftment of corrected SCD patient HSCs in immunodeficient mice. The high rate of engraftment in immunodeficient mice of similarly corrected HSCs observed in our published CSG study puts these results observed in for in vitro correction of SCD within the therapeutic window of removing SCD.

Scientific Rational

Materials and Methods

Materials

- gRNA & sequencing service were purchased from Generalis Engineering Center, Washington University in St. Louis.
- Olga-processed from ET.
- rCD8+T in mouse T (American type).

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Correction in SCD HSC

Gene correction of SCD HSCs: Phenocyt-mutated HSCs from three donors were processed by the MaxCyte GT system. Phenocyt SCD HSCs were maintained in vitro for up to 6 days for follow-up analysis. The viability is presented in the top left graph; cell proliferation is presented in the top right graph; HbW designation is presented in the bottom left graph; and the Hb electrophoresis in the bottom right graph. Consistent integration of HindIII recognition site are within the three donor's observed.

Consistency of Correction: Phenocyt-mutated HSCs from a single patient were processed using the MaxCyte GT system with multiple correction conditions. Corrected SCD HSCs were non-differentiated and differentiated into erythroid cells, viability ≥70%. Correction rates were maintained throughout differentiation. Gene editing efficiency by deep sequencing is shown with high consistency.

SCD Correction Scheme

SCD Correction Scheme

MaxCyte Delivery Platform Overview

Product and Manufacturing Procedure. MaxCyte clinically and commercially developed and marketed the first viral free extracellular (target) delivery technology. The initial development focused on the development and manufacturing of a commercial-scale delivery system. A proof of principle study, the delivery of a therapeutic adenovirus into human primary erythroid cells, demonstrated proof of concept and demonstrated the ability to deliver a therapeutic adenovirus into human primary erythroid cells. A Phase I trial was designed to assess the safety and feasibility of delivering a therapeutic adenovirus to human primary erythroid cells. The Phase I clinical trial results were published in 2011 and demonstrated that the delivery system was safe and feasible in human primary erythroid cells.

- In-vitro differentiation into red cells
- Healthy β-globin expression
- 30% or more, long-term engraftment (with single allele correction)

MaxCyte GT GMP-compliant Non-viral CRISPR-mediated Process Correcting the Sickle Cell Disease Mutation in SCD Patient CD34+ Cells Achieves 60% Wild Type Adult Hemoglobin Expression in Differentiated Erythrocytes.

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- High viability and loading efficiency
- Consistent product quality and function
- Scalable (2 to 5 in seconds up to 2 x 10^9 cells in ~20 min)
- ISO0001:2008 certified Quality Systems
- Rapid, automated platform with single-use disposable technology
- No human or animal-derived materials employed
- Regulatory Support: Master File with US FDA and Health Canada
- CE-marked
- Local Commercial Validation
- >5x increases for clinical development
- 2 commercial partners
- 50+ partnered programs

Summary

- Therapeutically relevant levels of SCD mutation correction is achieved using a non-viral cell approach.
- The gene correction procedure results in consistent gene correction efficiency.
- The achieved level of gene correction lead to ≥440% HbA expression.
- The correction rate is believed to be therapeutically beneficial for SCD patients.