Introduction

The A→T mutation in the first exon of the β-globin gene is the defining SCD mutation leading to all pathophysiological consequences. Currently, hematopoietic stem cell transplantation is the only cure for SCD. Unfortunately, most SCD patients don’t have a matched donor. Novel approaches to treat SCD are still needed.

Ex vivo viral vector based gene transfer and BCL11a knock-down via short-gene expression are under evaluation. However, HDR-mediated gene correction is more appealing, permitting the correct endogenous gene expression at its natural regulatory locus. Gene correction would also result in reduction of HbS concentration due to the introduced Indels, which could affect the propensity for sickling as demonstrated by the delay in gelation which is dependent on the 30% power of deoxyhemoglobin S concentration. Thus small changes in HbS concentration can theoretically have profound consequences.

Correction of the SCD mutation has been shown to be feasible. However, reported correction rates remain too low to reach the required target of 20% cellular correction, either double or single allele correction. With the successful demonstration of efficient “hotspot” mutations in CYBB gene in X-Linked CGD, we report here the result of the efficient correction of another mutation, the SCD mutation, using a highly efficient CRISPR (Cas9 and sgRNA) system with MaxCyte’s oligo, employing MaxCyte’s commercially/clinically validated cGMP/Regulatory compliant and closed platform technology.

Materials and Methods

Oligo and gRNA. Two gRNA targeting either SCD mutation (gRNA 1) or wild type (gRNA 2) were designed to target the same site at the SCD mutation. Transfection was performed to A.) integrate HindIII recognition sequence or converse wild type into SCD mutation with oligo 2 in SCD patient B cells, and B.) integrate HindIII recognition sequence or converse wild type into SCD mutation with oligo 1 in healthy cells.

Target Correction. ~30% HDR of Integration of HindIII recognition sequence was achieved. Since the only difference in health HSC from SCD HSC is one nucleotide difference, we expect the same efficiency could be achieved when gRNA targeting SCD mutation is used for SCD patient HSC.

MaxCyte Delivery Overview Platform

- High viability and loading efficiency
- Consistent product quality and function
- Scalable [5 x10⁵ up to 2 x10¹¹ cells per run in <30 min]
- Customized biology [enhanced potency]
- ISO9001:2008 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
- Clinical / Commercial Validation
- 15+ licenses for clinical development
- 1 - commercially marketed therapy
- Broad IP (US & ROW patents / applications)

Summary

- Therapeutic level efficiency of gene correction of monogenic mutation is feasible as demonstrated in this study and previous study using HSC from X-Linked CGD patient.
- Efficient correction of healthy HSC into SCD mutation (~30% biallelic conversion rate)
- Efficient correction of SCD B-LCL into healthy cell genotype (~30% biallelic correction rate)
- The gRNA is specific
- The conversion or correction rate is much higher than that hypothetically required for significantly benefiting SCD patients

Scientific Rational

Cell Engineering

- Target Correction: ~30% in vitro & ~20% long-term engraftment (with single allele correction)
- HDR in HSC

Product Concept and Manufacture Procedure. MaxCyte’s clinically and commercially validated Flow Electroporation™ technology is used for delivery of CRISPR and Oligo for gene correction in HSC and patient B cells. In this developmental study, HDR of integration of HindIII recognition 6-nucleotide sequence, or correction of SCD mutation into wild type genotype, or converse of wild type genotype into SCD mutation is performed. The detection of the HDR efficiency is performed by HindIII digestion following oligo annealing, and sequencing of the PCR-amplified genomic DNA post correction. Based on published studies, the target is set up with single allelic gene correction of 30% in vitro and 20% in vivo engraftment.

Mutation in SCD. SCD is a monogenic mutation in 6th Codon of gene in β-globin gene (HBB) from A in WT to T in SCD, resulting in Gln in WT to Val in SCD. Correcting SCD mutation T to wild type A with ≥ 20% in SCD patient HSC will be significantly benefit SCD patient.

Materials

- gRNA purchased from Genomic Engineering Center, University of California, San Diego
- mRNA generated in-house or purchased from IDT
- mRNA by in house IVT (Amination Kit)

Cell Engineering

HDR in SCD patient B-LCL Cells. SCD patient B-LCL cells were transfected for integration of HindIII recognition sequence. The amplified genomic DNA by PCR was digested with HindIII and analyzed by agarose gel. The density ratio of digested bands to total of parental band and the digested bands was used as integration rate.

Expected safety is the integration of HindIII recognition sequence in SCD mutation site in SCD patient B cells.

Specification of gRNA. gRNA targeting to SCD mutation was applied to both SCD patient and healthy cells to integrate HindIII recognition sequence. HDR efficiency of Integration of HindIII recognition sequence was analyzed by agarose gel. HDR efficiency is much higher in SCD patient B cells than that in healthy B cells.

HDR in health HSC. HDR targeting to WT type sequence corresponding to the SCD mutation site was used to integrate HindIII recognition sequence in HSC from healthy donor. HDR of Integration of HindIII recognition sequence was achieved. Since the only difference in healthy HSC from SCD HSC is one nucleotide difference, we expect the same efficiency could be achieved when gRNA targeting SCD mutation is used for SCD patient HSC.