EDIT-301: An Experimental Autologous Cell Therapy Comprising Cas12a-RNP Modified mPB-CD34+ Cells for the Potential Treatment of SCD

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Sickle cell disease (SCD) is caused by a single mutation E6V of the β-globin chain, leading to polymerization of sickle hemoglobin (HbS) and formation of HbS fibers. Fetal hemoglobin (HbF) protects against SCD by inhibiting HbS polymerization. Individually with compound heterozygosity for HbS and deletional hereditary persistence of total hemoglobin (HPFH) express approximately 30% HbF with pancellular distribution and exhibit no features of SCD.

The HBG distal CCAAT box region was identified in our previous screen to be one of the most robust HbF expression regions. Several naturally occurring HPFH mutations are also clustered at this region, which led us to optimize Cas12a for its capacity to promote durable and high-levels HbF inducing in CD34+ cells.

Cas12a, a highly specific gene editing enzyme, a variant and gRNA modifications. Editing activity at target editing sites discovered in clonal erythroid cultures, derived from RNP-electroporated CD34+ cells. Lineage reconstitution (flow cytometry), editing (NGS), and HbF expression (HPLC and flow cytometry) analyses were conducted 16-20 weeks post-engraftment.

References

Methods

Edited cells were generated by transfection of healthy donor mobilized CD34+ cells with SPCas9 or Cas12a ribonucleoproteins (RNPs). Targeted Next-Gen Seq (NGS) was conducted for on and off-target editing levels and indel profile analysis. Identification of candidate off-target sites was performed using in silico prediction, Digenome and Guide-seq.

HBF-inducing RNPs were identified in clonal erythroid cultures, derived from RNP-electroporated CD34+ cells. In vivo studies were performed in NBSGW mice or NSG mice infused with mock or edited CD34+ cells. Lineage reconstitution (flow cytometry), editing (NGS), and HbF expression (HPLC and flow cytometry) analyses were conducted 16-20 weeks post-engraftment.

Optimization Enabled Editing Levels >90%

IND-enabling activities are ongoing for EDIT-301: an experimental autologous cell therapy comprising Cas12a-RNP modified mPB-CD34+ cells for the potential treatment of SCD.

Conclusions

Indels (%)

0 10 20 30 40 50 60 70 80 90 100
Mock EDIT-301

In vivo HbF Expression

No off-target editing observed in Cas12a treated CD34+ cells

EDIT-301 – High Polyclonality In Vivo

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Disclosures

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