Development of a Subretinally Delivered CEP290-Specific CRISPR Medicine for the Treatment of Leber Congenital Amaurosis 10 (LCA10)

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Introduction

For ocular diseases with well-defined genetic defects, such as Leber Congenital Amaurosis (LCA), CRISPR-based genome therapy represents a novel therapeutic modality for previously unaddressable disease targets. LCA10 is an early-onset retinal degeneration caused by mutations in the CEP290 gene. LCA10 is not amenable to AAV-mediated gene replacement therapy because the large size of the CEP290 cDNA (~7.5kb) exceeds the packaging capacity of AAV. For ocular diseases with well-defined genetic defects, such as Leber Congenital Amaurosis (LCA), CRISPR-based genome therapy represents a novel therapeutic modality for previously unaddressable disease targets. LCA10 is an early-onset retinal degeneration caused by mutations in the CEP290 gene. LCA10 is not amenable to AAV-mediated gene replacement therapy because the large size of the CEP290 cDNA (~7.5kb) exceeds the packaging capacity of AAV.

The majority of LCA10 patients are homozygous or compound heterozygous for a common intron 26 (IVS26) mutation that creates an aberrant splice site, leading to the misincorporation of a cryptic exon of 126 nucleotides, and consequently a mutant, non-functional CEP290 protein. Our therapeutic strategy is to use a SaCas9/gRNA pair to specifically remove the intrinsic sequences flanking the mutation, thus restore normal CEP290 mRNA splicing and protein expression.

Methods

EDIT-101, which is an rAAV5 vector expressing SaCas9 and human CEP290-specific gRNAs, was produced by transient plasmid transfection of HEK293 cells, and processed with final sterile filtration. Human CEP290 IVS26 KI mouse contains the human CEP290 exon 26, intron 26 with the LCA mutation c.2991+1655A>G and exon 27 in the murine CEP290 gene through homologous recombination (Garanto, Duijkers, and Collin 2015). All mice used in these studies were inbred C57BL/6J, and were housed in a pathogen-free environment. Mice were sacrificed at specified time points at Day 3 – 6 months. Fresh retinal samples were collected for genomic DNA and RNA extraction. On-target CEP290 gene editing was determined by UDIaSTM deep sequencing method. The expression levels of Cas9 mRNA and gRNA were measured by RT-QPCR. Mouse eye cups were also fixed for immunohistochemistry of Cas9 protein and in-situ hybridization (ISH) of AAV vectors.

Results

Efficient Editing of Mouse Retina by Subretinal Delivery of EDIT-101

In EDIT-101-treated HuCEP290 KI mice, the productive edits were detectable as early as on Day 3, and increased significantly by Week 1 (p<0.0001 vs Day 3). The editing rates were maintained through 6 months (A). The levels of SaCas9 mRNA/gRNA increased significantly by 2 weeks post dosing and then reduced significantly by 6 months (p<0.05) (B & C), without impacting the editing rate. The Cas9 protein was detected exclusively in photoreceptors cells (D).

Dose Response in CEP290 Gene Editing and CRISPR Expression

Given the stable gene editing efficiency over time, we pooled the productive gene editing data across different time points and normalized to the transduction efficiency of the total retina. (A) The dose range of EDIT-101 to achieve target therapeutic threshold of 10% of productive CEP290 edits in photoreceptor cells (Geller and Sieving 1993; Geller, Sieving and Green 1992). (B) The correlation of productive CEP290 editing efficiency with the expression levels of SaCas9 mRNA.

Conclusions

Subretinal delivery of EDIT-101 has demonstrated efficient transduction of mouse neuroretina and achieved predictive therapeutic levels of targeted CEP290 gene editing in HuCEP290 IVS26 KI mice. The onset of CEP290 gene editing is rapid, correlates with the levels of Cas9/sgRNA and lasting, while the expression of CRISPR reduces over time. The results provide strong support for clinical development of EDIT-101 for treatment of LCA10. If successful, this in vivo CRISPR approach may have broad application to other inherited retinal degenerations with significant unmet medical needs.

References: