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

editas MEDICINE

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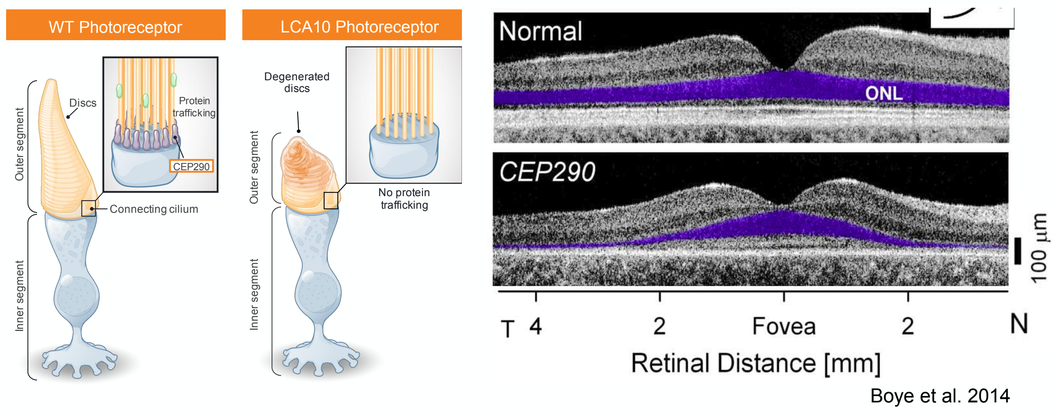
Introduction

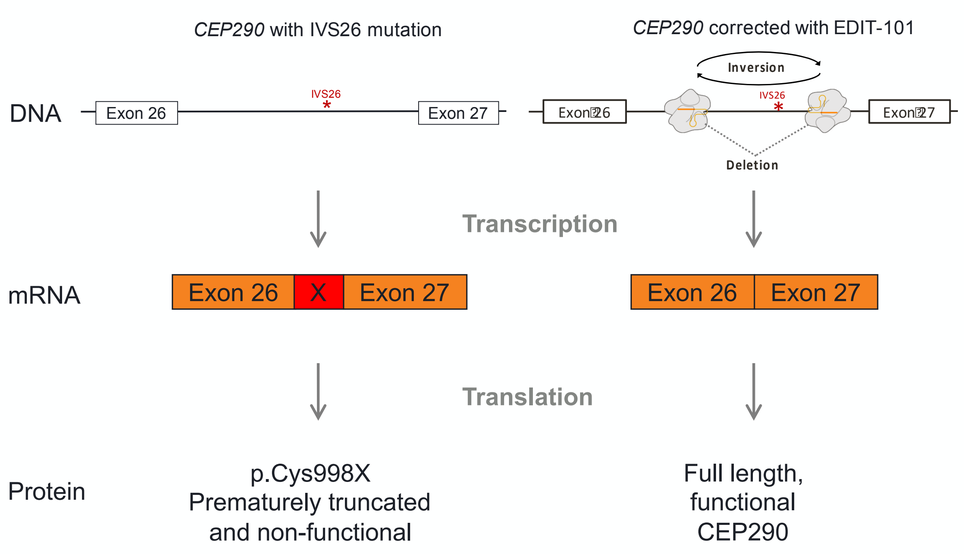
For ocular diseases with well-defined genetic defects, such as Leber Congenital Amaurosis (LCA), CRISPR-based genome editing represents a novel therapeutic modality for previously unaddressable disease targets. LCA10 is an early-onset retinal degeneration caused by mutations in the *CEP290* gene. LCA10 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.

- In normal photoreceptor cells, CEP290 protein is located in the connecting cilium, where it is required for protein trafficking that supporting outer segments development and vision.
- In CEP290-associated LCA10 patients, despite severe vision loss and rapid loss of rods, central nonfunctional foveal cones and intracranial visual pathways remain structural intact.



Boye et al. 2014

- 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 128 nucleotides, and consequently a mutant, non-functional CEP290 protein.
- Our therapeutic strategy is to use a SaCas9/gRNA pair to specifically remove the intronic sequences flanking the mutation, thus restore normal CEP290 RNA splicing and protein expression.



Previously, we demonstrated that CEP290 gRNAs induce targeted deletion leading to normal CEP290 mRNA and protein expression in LCA10 patient-derived fibroblasts. Productive edits include sequence inversions and deletions that correct the LCA10-associated splicing defect of CEP290. GUIDE-Seq followed by next generation targeted sequencing did not detect any off-target cutting by CEP290 gRNAs in a number of human cells. Finally, the surrogate NHP vector achieved targeted CEP290 gene editing in macular photoreceptor cells following a single subretinal injection in cynomolgus macaques. (Maeder et al 2017)

In this study, we determined the kinetics and the dose response of EDIT-101 in the transgene Cas9/gRNA expression and on-target CEP290 editing in human CEP290 IVS26 knock-in (KI) mice to support First-in-Human trials.

Methods

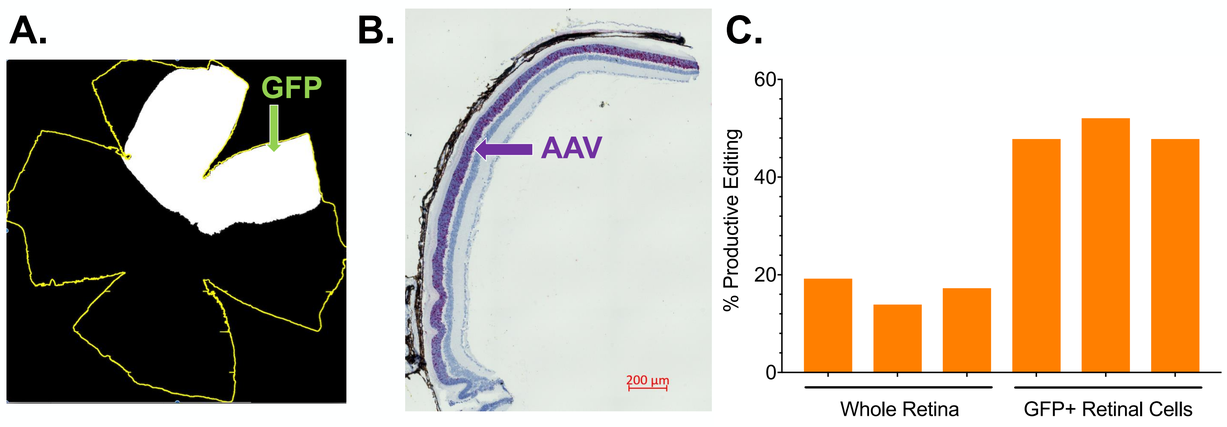
EDIT-101, which is an rAAV5 vector expressing SaCas9 and human CEP290-specific gRNAs, was produced by transient plasmid transfection of HEK293 cells, and processed with final sterilize filtration.

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 heterozygous for the HuCEP290 IVS26 mutation, thus the % of CEP290 gene editing is the same as the % of cells edited.

Mixed gender of HuCEP290 IVS26 KI mice, at 6 – 8 weeks of age, were treated in both eyes with a single subretinal injection of either vehicle or escalating doses of EDIT-101. Animals were sacrificed at specified time points at Day 3 – 6 months. Fresh neuroretinal samples were collected for genomic DNA and RNA extraction. On-target CEP290 gene editing was determined by UDiTaS™ 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.

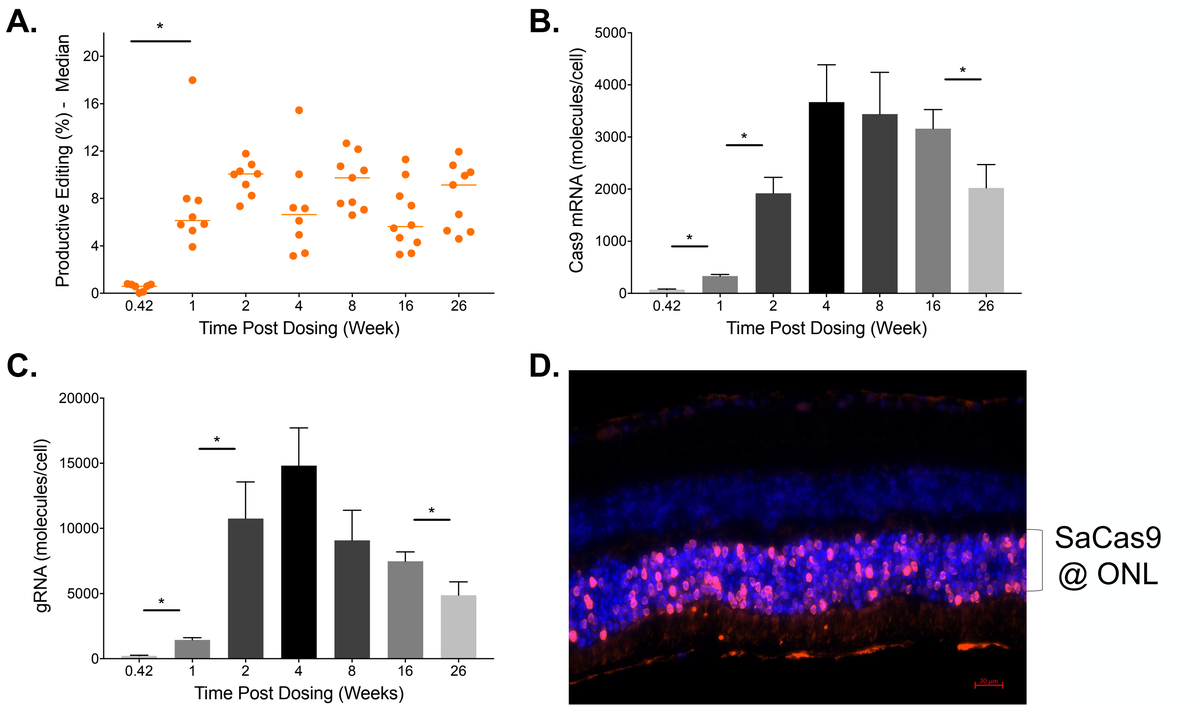
Results

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



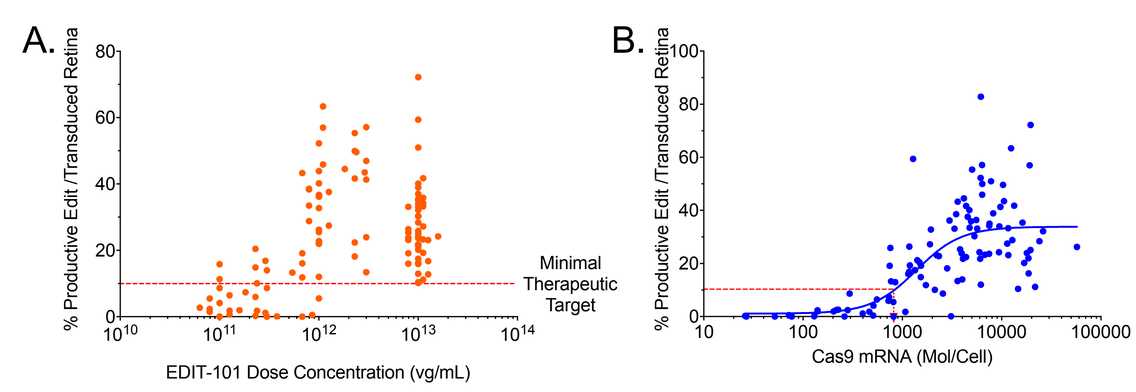
HuCEP290 IVS26 KI mice were treated with AAV5-GRK1-GFP either alone or with EDIT-101 by subretinal injection. Six weeks later, GFP+ area was quantified in flat-mounted retinas which showed that approximately 30% of the neuroretina were positive for GFP expression (A), which is consistent with the ISH of AAV vector genome (B). Productive HuCEP290 editing rates were determined in genomic DNAs isolated from either total neuroretinal cells, or sorted GFP+ photoreceptor cells (C).

Rapid Onset and Stable CEP290 Gene Editing by EDIT-101 in Mice



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 photoreceptor 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/gRNA 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: (1) Boye, S. E. et al. 2014. *PLoS One*, 9: e92928. (2) Maeder, M. et al. 2017. *Mol Ther,* 25(5S1): 353. (3) Garanto, A. et al, 2013. *PLoS One*, 8: e79369. (4) Geller, A. M., and P. A. Sieving. 1993. *Vision Res*, 33: 1509-24. (5) Geller, A. M., P. A. Sieving, and D. G. Green. 1992. *J Opt Soc Am A*, 9: 472-7.