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Efficient in vivo editing of CEP290 IVS26 by EDIT-101 as a novel therapeutic for the treatment of Leber Congenital Amaurosis 10

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Introduction

- LCA10 is an early-onset retinal degeneration caused by mutations in the *CEP290* gene. CEP290 localizes to the connecting cilium of photoreceptors and is required for ciliogenesis and the trafficking of proteins from the inner segment to the outer segment.
- The majority of LCA10 patients are homozygous or compound heterozygous for a common intron 26 (IVS26) mutation, c.2991+1655A>G, that creates an aberrant splice site, leading to the inclusion of a cryptic exon of 128 nucleotides, and consequently a mutant, non-functional CEP290 protein.
- In this study, we assessed the kinetics and pharmacodynamics of EDIT-101 (Fig 1), a CRISPR-based medicine, in humanized CEP290 IVS26 knock-in (KI) mice to determine the potential therapeutic dose range.
- Previously, we demonstrated that delivery of SaCas9/gRNA pair can specifically remove the intronic sequence containing the mutation, thus restoring normal CEP290 RNA splicing and protein expression[1] (Fig 2).
- A two-phase Discovery and Verification approach was used for assessing EDIT-101 specificity using multiple orthogonal methods.

Methods

- Human CEP290 IVS26 KI transgenic mice contain the human CEP290 exon 26, intron 26 with the LCA mutation c.2991+1655A>G and exon 27 in the murine CEP290 gene[2].
- Mixed gender transgenic mice, at 6–12 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 from Day 3 to Month 9. Fresh mouse neural retina samples were collected for genomic DNA and RNA extraction. On-target CEP290 gene editing was determined by the Uni-directional Targeted Sequencing deep sequencing method (UDiTaS™ [5]), whereas expression levels of Cas9 mRNA and gRNA were measured by RT-qPCR in mouse retinas (Fig 4 and Fig 5).
- Human retinal punches were transduced with EDIT-101 (5E13 vg/mL) and cultured for 28 days, untreated punches served as controls. Genomic DNA and RNA were isolated, pooled across punches, and the on-target total and productive (deletions and inversions) editing at the IVS26 locus was measured by UDiTaS[5,6,7] (Fig 7).
- Off-targets for guides A and B were characterized using multiple orthogonal approaches in a “Discovery Phase” and “Verification Phase” (Fig 3 and Fig 8).

FIGURE 1. Schematic of EDIT-101

Human U6 promoter; hGRK1, human G protein-coupled receptor kinase 1 promoter; Sa, *Staphylococcus aureus*; Cas9, CRISPR-associated protein 9.

FIGURE 2. EDIT-101 editing strategy

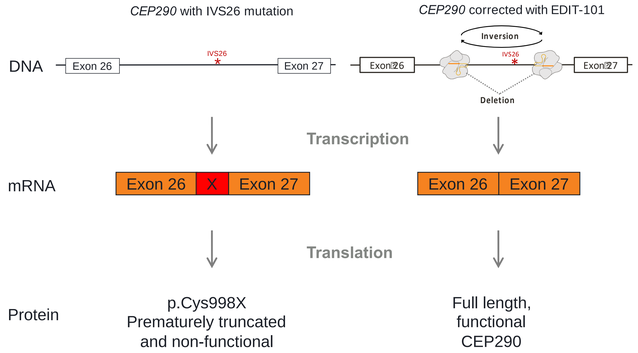
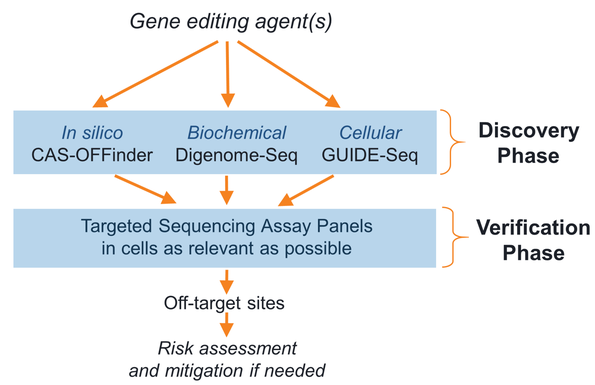
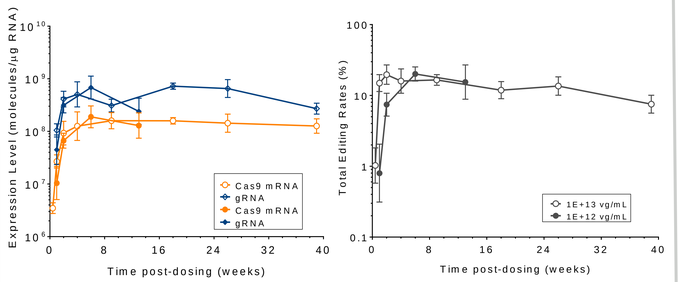


FIGURE 3. Approach to editing specificity



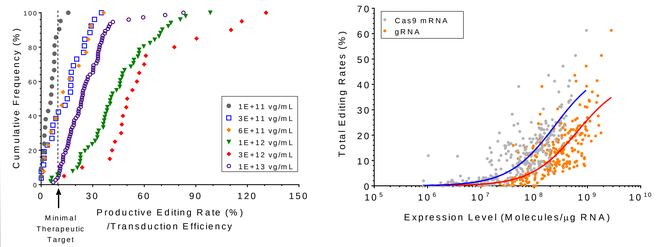
Results

FIGURE 4. Rapid onset and stable CEP290 gene editing by EDIT-101 in HuCEP290 IVS26 KI mice



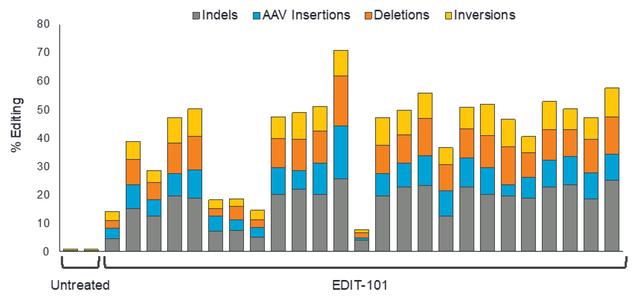
- Cas9 mRNA and gRNA expression peaked at 2 weeks post-injection and remained stable through 40 weeks.
- Total CEP290 gene editing peaked at 6 weeks post-injection and was maintained through 40 weeks.
- Editing levels were similar at the two doses, and time to peak was shorter at the higher dose.

FIGURE 5. Productive editing exceeds therapeutic threshold and correlates with Cas9 mRNA and gRNA levels



- EDIT-101 achieved target therapeutic threshold of 10% of productive CEP290 edits in photoreceptors[3,4] in a dose-dependent manner.
- Total CEP290 gene editing efficiency by EDIT-101 correlated with expression levels of SaCas9 mRNA and gRNA using a nonlinear regression model.

FIGURE 6. Targeted CEP290 editing achieved in human retinal explants treated with EDIT-101



- Total editing achieved in human retinal explants (n=25) was 41.7% ± 15.9%, of which 16.6% ± 6.5% being productive at 28 days post-treatment with EDIT-101 at 5E13 vg/mL.

FIGURE 7. No off-target sites were detected for gRNA A and gRNA B

Study	Method	Guide	Result
In silico selection	CAS-Off Finder <5 mismatches and <3 mismatches plus a bulge	A	27 sites selected
		B	89 sites selected
Digenome-Seq	RNP (at 10 nM, 100 nM, and 1000 nM) cuts human genomic DNA; whole genome sequencing (WGS) used to identify cut sites	A	no off-targets detected
		B	1 off-target detected at 1000 nM only
GUIDE-Seq	1) Plasmid transfection 4 cells lines: U-2 OS, ARPE-19, SHSY5Y, fibroblasts	A	No sites identified in any cell line LLoD ~0.1% - 1% varies by cell line
	2) RNP nucleofection in human T cells	B	
Targeted Sequencing	Plasmid transfection 2 cells lines: U-2 OS, ARPE-19	A	112 of 117 had no detectable editing; LLoD ≤0.1%* for 106 assays
		B	
	Human retinal explants transduced with EDIT-101	A + B	5 sites were refractory to NGS

* In the verification phase, no editing was measured for the gRNA B off-target site identified in Digenome-Seq (chr14:38144570-38144571). The lower limit of detection is 0.1%.

Conclusions

1. Subretinal delivery of EDIT-101 has demonstrated efficient transduction of mouse neural retina and achieved predictive therapeutic levels of targeted CEP290 gene editing in HuCEP290 IVS26 KI mice.
2. Human retinal explants transduced with EDIT-101 showed productive editing.
3. Specificity for both guides was characterized using multiple orthogonal approaches, and did not detect any off-target editing.

In summary, the results support the clinical development of EDIT-101 for the treatment of patients with LCA10-IVS26.

References: (1) Maeder, M. et al. 2017. *Mol Ther,* 25(5S1): 353. (2) Garanto, A. et al, 2013. *PLoS One*, 8: e79369. (3) Geller, A. M., and P. A. Sieving. 1993. *Vision Res*, 33: 1509-24. (4) Geller, A. M., P. A. Sieving, and D. G. Green. 1992. *J Opt Soc Am A*, 9: 472-7. (5) Giannoukos, G., et al. 2018, *BMC Genomics* 19:212. (6) Shengdar, Q.T., et al. 2015, *Nat Biotechnol* 33:187. (7) Kim, D., et al. 2015, *Nature Methods* 12:237.

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