Preclinical Assessment of *In Vivo* Gene Editing Efficiency, Specificity, and Tolerability of EDIT-101, an Investigational CRISPR Treatment for Leber Congenital Amaurosis 10 (LCA10)

P251

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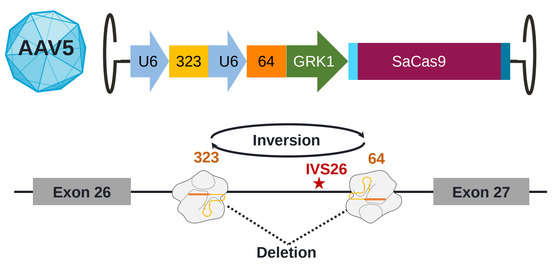
Background

LCA10 and EDIT-101

Leber Congenital Amaurosis Type 10 (LCA10) is an early-onset retinal degeneration disease caused by mutations in the CEP290 gene. It is characterized by infantile-onset of poor vision, nystagmus, and a flat electroretinogram; visual acuity is typically counting fingers or worse.

EDIT-101 is a therapeutic candidate designed to treat LCA10 patients that carry the most prevalent causative CEP290 mutation, c.2991+1655A>G in intron 26, abbreviated here as IVS26. EDIT-101 is an AAV5 vector packaged with DNA encoding the S. aureus Cas9 (SaCas9) protein, along with two guide RNAs. When expressed in photoreceptor cells, the dual gene editing machinery removes or inverts the IVS26 mutation and restores expression of the full length CEP290 protein[1]. We expect this gene editing to improve photoreceptor function and bring clinical benefit to LCA10 patients harboring the IVS26 mutation.

EDIT-101 Schematic



EDIT-101 is an AAV5 vector delivering 3 components. Two guide RNAs, termed gRNA-323 and gRNA-64 under control of a U6 polymerase III promoter and a S. aureus Cas9 expressed via the photoreceptor specific GRK1 (Rhodopsin Kinase) promoter.

Methods

In Vivo Assessment

Human CEP290 IVS26 KI transgenic mice[2] 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 method (UDiTaS™,[3]), whereas expression levels of Cas9 mRNA and gRNA were measured by RT-qPCR in mouse retinas (Panel 1).

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[3] (Panel 5).

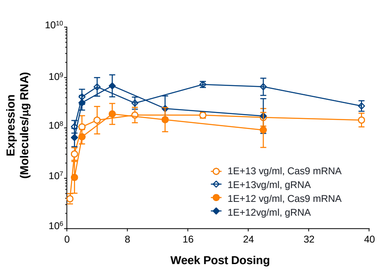
Specificity Assessment

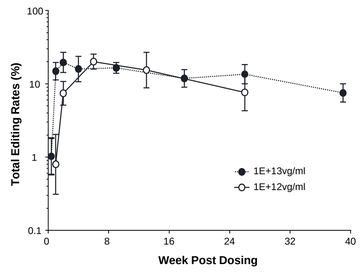
Specificity is a significant aspect of any gene editing therapeutic, as at the cellular level changes to DNA are permanent. A number of factors contribute to the specificity of EDIT-101, that include: limiting the physical distribution of the vector by subretinal injection, selection of the AAV5 serotype that shows tropism for photoreceptors, and the use of a photoreceptor-specific GRK1 promoter to restrict expression of SaCas9.

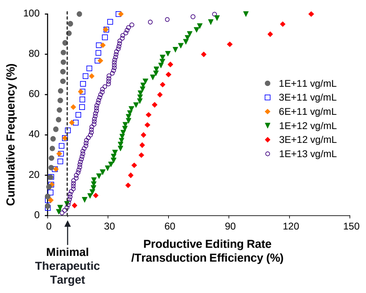
In this study, DNA-editing specificity of Guide 64 and Guide 323 was assessed in two distinct phases: Discovery and Verification. In the Discovery Phase, three orthogonal methods were used to identify candidate off-target sites: *in silico* prediction using CAS-OFFinder[4], detection of cutting in purified genomic DNA using the empirical biochemical assay Digenome-Seq[5], and detection of editing using the empirical cellular assay GUIDE-Seq[6]. Each method produced a set of candidate off-targets that were pooled and brought forward. In the Verification Phase, we assessed EDIT-101 editing at the candidate off-target sites using targeted Next Generation sequencing (NGS) panels. Cell selection is critical, and we used therapeutically relevant human photoreceptor cells: human retinal explants derived from cadavers (as well as 2 human cell lines).

Results

1. Rapid Onset and Stable CEP290 Gene Editing by EDIT-101 in HuCEP290 IVS26 KI Mice







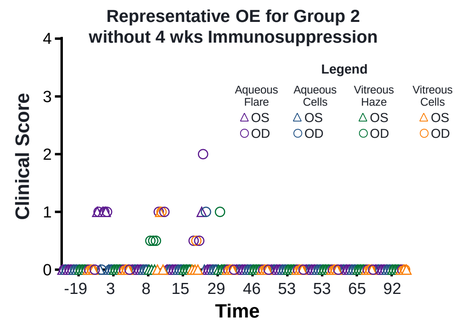
Vector Dose (vg/mL)	Productive Editing Rate in EDIT-101 Treated Mice	Productive Editing Rate in Surrogate Treated NHPs
1.00E+11	5.5±4.1	3.5±5.5
3.00E+11	14.8±10.6	
6.00E+11	15.7±11.5	
1.00E+12	44.2±20.3	27.9±20.7
3.00E+12	60.8±30.2	
1.00E+13	25.8±13.5	

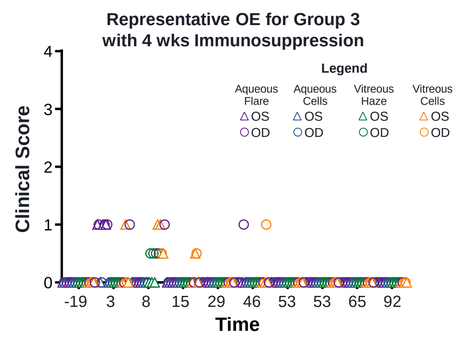
- Cas9 mRNA and gRNA expression peaked at 2 weeks post-injection and remained stable through 40 weeks.
- Total CEP290 gene editing peaked at 2 weeks for 1.00E+13 vg/ml and at 6 weeks for 1.00E+12 vg/ml post-injection and was maintained through 40 weeks.
- Editing levels were similar at the two doses, and time to peak was shorter at the higher dose.
- EDIT-101 achieved target therapeutic threshold of 10% of productive CEP290 edits in photoreceptors[3,4] in a dose-dependent manner.

2. Ocular Tolerability Study of EDIT-101 Following Subretinal Injection in Cynomolgus Macaques

Group	Treatment		Vector Dose vg/ml (100 ul/eye)	Methyl-Prednisolone (day-1 to Week 4)	Study Duration (weeks)
	OS	OD			
Group1	Vehicle	Vehicle	-	-	6
Group2	EDIT101	Vehicle	1E+12	-	13
Group 3	EDIT101	Vehicle	1E+12	✓	13
Group 4	VIR067	VIR067	7E+11	✓	6
Endpoint Analysis					
Tolerability and safety*	Ophthalmic exam and intraocular pressure measurements (OE/IOP) Electroretinogram (ERG): Groups 2 and 3 Histopathology (H&E)				
Immunogenicity Evaluations*	Antibody (ADA) and T-cell (ELISPOT) responses to SaCas9 and AAV5 capsid				
Analyses/Activity	Distribution of AAV vector genome by In situ hybridization (ISH) Expression of SaCas9 protein by Immunohistochemistry (IHC) On-target CEP290 gene editing by next generation sequencing: Groups 1 and 4				

3. Ocular Exams (OE) with and without immunosuppression

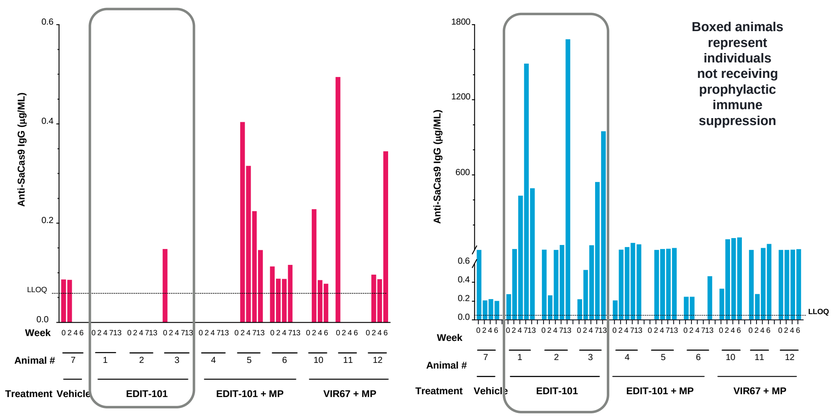




- Ophthalmic Examination scoring was based on Modified SUN, Hackett-McDonald & Spot Uveitis Scoring Systems.
- Delayed mild ocular inflammation was observed in non-immunosuppressed NHPs.
  - Resolved following local or systemic steroid treatment.
- Prophylactic treatment with systemic steroids effectively prevented vector-related ocular inflammation.

4. Immunogenicity Evaluations for presence of Anti-SaCas9 and Anti-AAV5 Capsid

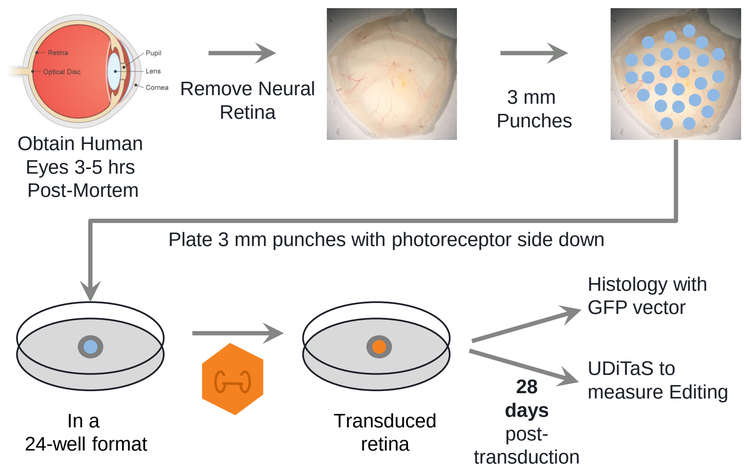
Anti-SaCas9 Ab ELISA | **Anti-AAV5 Capsid Ab ELISA**

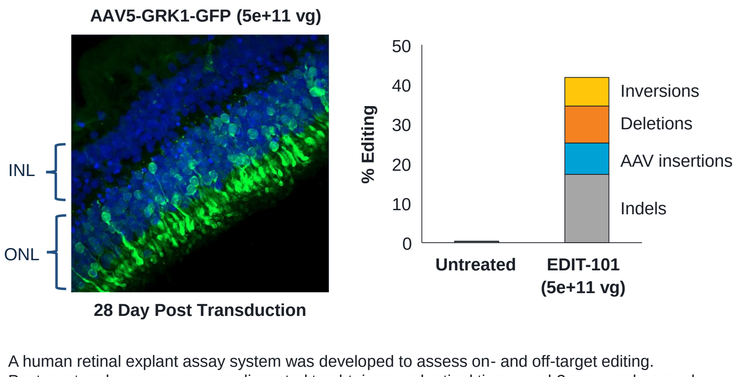


Low levels of Anti-SaCas9 Ab not correlating with delayed ocular inflammation observed in non-immunosuppressed animals (EDIT-101).

Anti-AAV5 capsid Ab response detected in both EDIT-101 and VIR067-treated NHPs, most robust in non-immunosuppressed animals (EDIT-101).

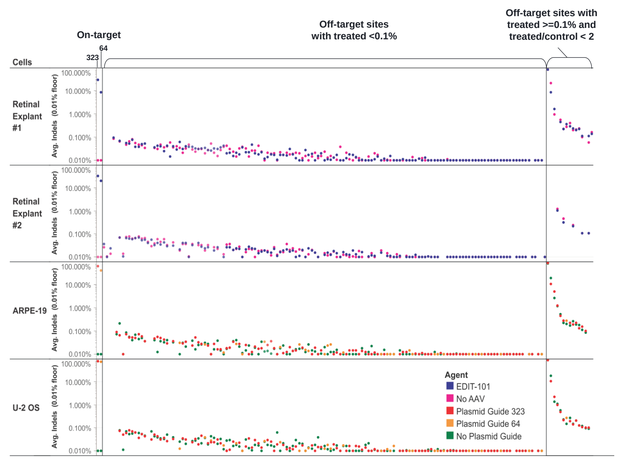
5. Human Retinal Explants as a Clinically Relevant Model for EDIT-101 On- and Off-target Editing





A human retinal explant assay system was developed to assess on- and off-target editing. Post-mortem human eyes were dissected to obtain neural retinal tissue and 3mm punches made (~40 punches per eye). Punches were cultured on membranes in 24-well plates and transduced with 10 µl AAV virus. 28 days after transduction the tissue was harvested. An AAV5 GRK1-GFP vector shows photoceptor specific labelling, and editing measured using UDiTaS. The percent editing results are from an average of 25 EDIT-101 punches and 2 untreated punches.

6. NGS Panels Show No Off-Target Editing in Retinal Explants and Cell Lines



Targeted NGS panel across 106 candidate off-target sites in Retinal Explants transduced with and without EDIT-101. High on-target editing is observed for both guides. For 109 candidate sites no detectable editing is observed with 0.1% limit of editing detection. For 6 sites editing is above 0.1% in the treated samples but control samples are within 2 fold and represent assay background. U-2 OS and ARPE-19 nucleofected with plasmids also detect no off-target sites across the panel. 5 (of 117) candidate site were not amenable to NGS.

Summary Specificity

Study	Guide	Result
In silico selection	64	27 sites selected
	323	89 sites selected
Digenome-Seq	64	No off-targets detected
	323	1 off-target detected at 1000 nM only
GUIDE-Seq	64	No sites identified in any cell line LLoD ~0.1% - 2% varies by cell line
	323	
Targeted Sequencing	64	112 of 117 had no detectable editing; LLoD ≤0.1% for 106 assays. 5 sites were refractory to NGS. The Digenome g323 1 µM site was ≤0.1% in all samples.
	323	
	64 + 323	

Conclusions

- 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.
- Subretinal dosing of EDIT-101 and surrogate VIR067 were well-tolerated in NHP.
- Neither pre-existing nor induced SaCas9- and AAV5-specific immunity impacted the pharmacological activity of the vector.
- EDIT-101 transduction and SaCas9 expression is restricted to photoreceptor cells via subretinal injection, leveraging AAV5 tropism for photoreceptor cells, and utilizing the GRK1 photoreceptor specific promoter.
- EDIT-101 is a highly specific gene editing agent and no off-target editing was verified in human retinal explants at over 100 candidate sites.
- In summary, these results support the clinical development of EDIT-101 for the treatment of patients with LCA10-IVS26.

References

[1]Maeder, M.L., et al. (2016). 124. Mol. Ther. 24, S51–S52
[2]Garanto, A. et al, 2013. *PLoS One*, 8: e79369
[3]Giannoukos, G., et al. 2018, *BMC Genomics* 19:212.
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[6]Shengdar, Q.T., et al. 2015, *Nat Biotechnol* 33:187

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