POSTER# 906

# Gene Editing Specificity Assessment for EDIT-101, an LCA10 Therapeutic Candidate

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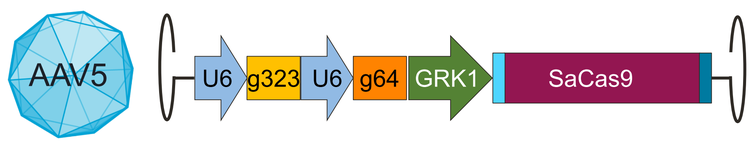
## 1) LCA10 and EDIT-101 Background

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*. We expect this gene-editing to improve photoreceptor function and bring clinical benefit to LCA10 patients harboring the IVS26 mutation.

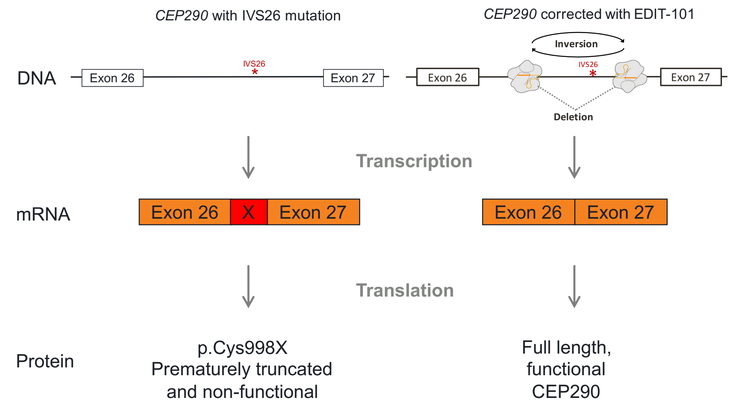
* Maeder, M.L., et al. (2016). 124. Mol. Ther. 24, S51–S52

## 2) EDIT-101 Schematic




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

## 3) Therapeutic Mechanism of Action

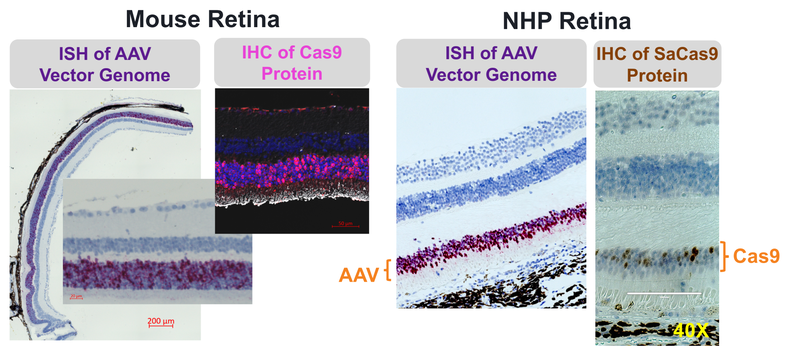



## 4) EDIT-101 Specificity Assessment Introduction

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 sub-retinal 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.

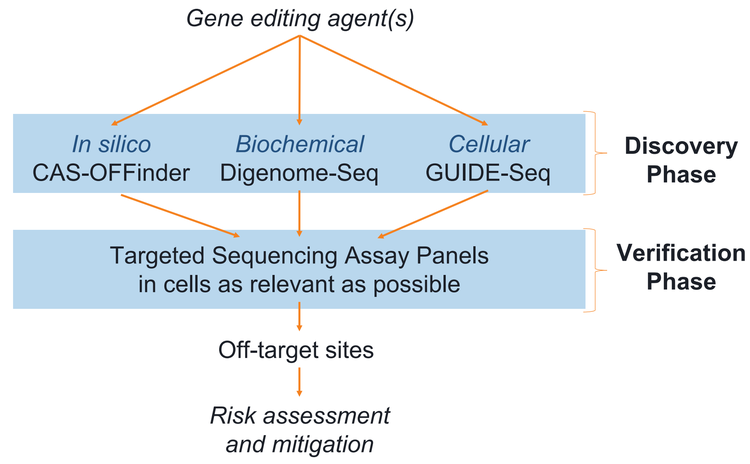
In this study, DNA-editing specificity of Guide 64 and Guide 323 were assessed in two distinct phases: **Discovery** and **Verification**. In the Discovery Phase, three orthogonal methods were used to identify candidate off-target sites: in silico prediction using CAS-OFFinder, detection of DNA cuts using purified genomic DNA with the empirical biochemical assay Digenome-Seq, and detection of editing using the empirical cellular assay GUIDE-Seq. 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 two human cell lines).

## 5) EDIT-101 Transduction and Cas9 Expression is Restricted to Photoreceptor cells




In both mice and non-human primates (NHPs), specific transduction of photoreceptor cells after subretinal injection of EDIT-101 was demonstrated using in situ hybridization (ISH) to the minus strand of the genome vector and anti-Cas9 immuno-histochemistry (IHC).

## 6) Approach to Editing Specificity Assessment

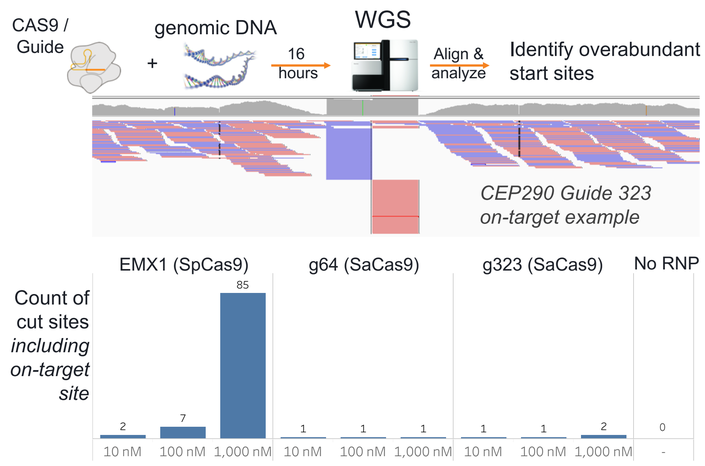



## 7) In Silico Prediction of Off-Target Sites Using CAS-OFFinder

- hg38 reference genome
- PAM used: **NNGRRN**
- Mismatches excluded at the 5' 22nd base
  - Based on Paired Library Screen, BioRxiv doi: 10.1101/269399
- Choose all sites with:
  - <5 mismatches
  - <3 mismatches + 1 bulge

| Mismatches | Bulges (gaps) | Count of sites in the human genome (hg38) Guide 64 | Guide 323 |
|---|---|---|---|
| 0 | 0 | 1 (on-target site) | 1 (on-target site) |
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 4 | 7 |
| 4 | 0 | 23 | 80 |
| 0 | 1 bulge | 0 | 0 |
| 1 | 1 bulge | 0 | 0 |
| 2 | 1 bulge | 0 | 3 |
| TOTAL unique off-target sites | | **27** | **89** |

## 8) Biochemical Discovery of Off-Target Cut Sites for Guides 64 & 323 Using Digenome-Seq



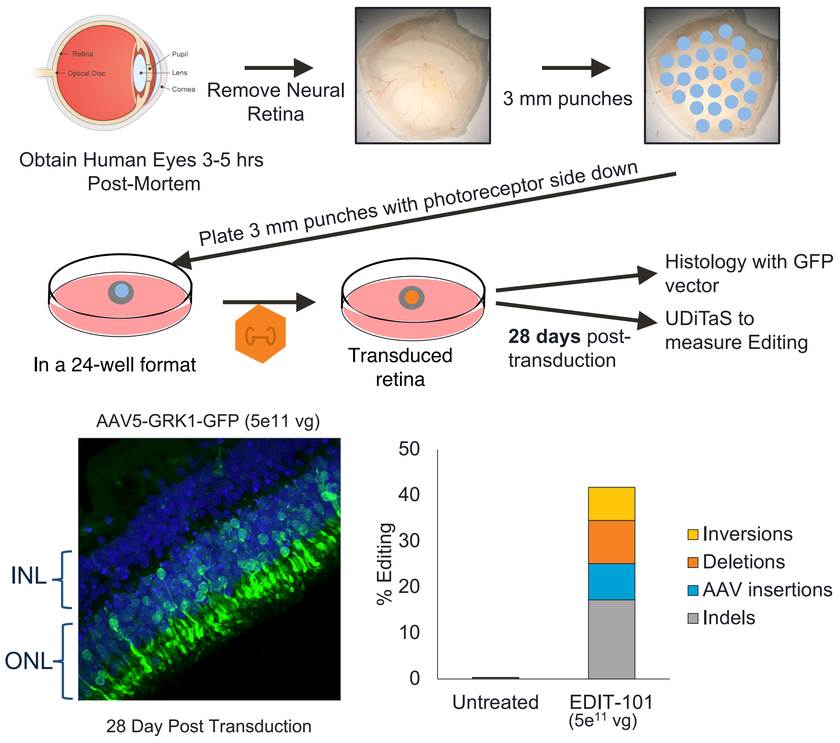

Digenome-Seq results for g64 and g323 at 10 nM, 100 nM and 1 µM RNP. On-target cutting is observed for both sites, while no off-target cutting sites are detected for g64 and only one site at 1 µM for g323.

## 9) Cellular Discovery of Off-Targets for Guides 64 & 323 Using GUIDE-Seq

| gRNA | Cell Line | Exp No. | On-target Signal (Unique Read Counts) | Estimated relative detection limit (95% conf) | Off-target integration sites identified |
|---|---|---|---|---|---|
| 64 | U-2 OS | 1 | 173 | 3.43% | **0** |
| | | 2 | 7,810 | 0.08% | **0** |
| | | 3 | 4,092 | 0.15% | **0** |
| | ARPE-19 | 1 | 1,434 | 0.42% | **0** |
| | | 2 | 2,242 | 0.27% | **0** |
| | | 3 | 3,300 | 0.18% | **0** |
| | SH-SY5Y | 1 | 5,047 | 0.12% | **0** |
| | Fibroblasts | 1 | 559 | 1.07% | **0** |
| 323 | U-2 OS | 1 | 913 | 0.66% | **0** |
| | | 2 | 12,818 | 0.05% | **0** |
| | | 3 | 6,034 | 0.10% | **0** |
| | ARPE-19 | 1 | 2,499 | 0.24% | **0** |
| | | 2 | 4,239 | 0.14% | **0** |
| | | 3 | 4,783 | 0.13% | **0** |
| | SH-SY5Y | 1 | 6,715 | 0.09% | **0** |
| | Fibroblasts | 1 | 318 | 1.88% | **0** |

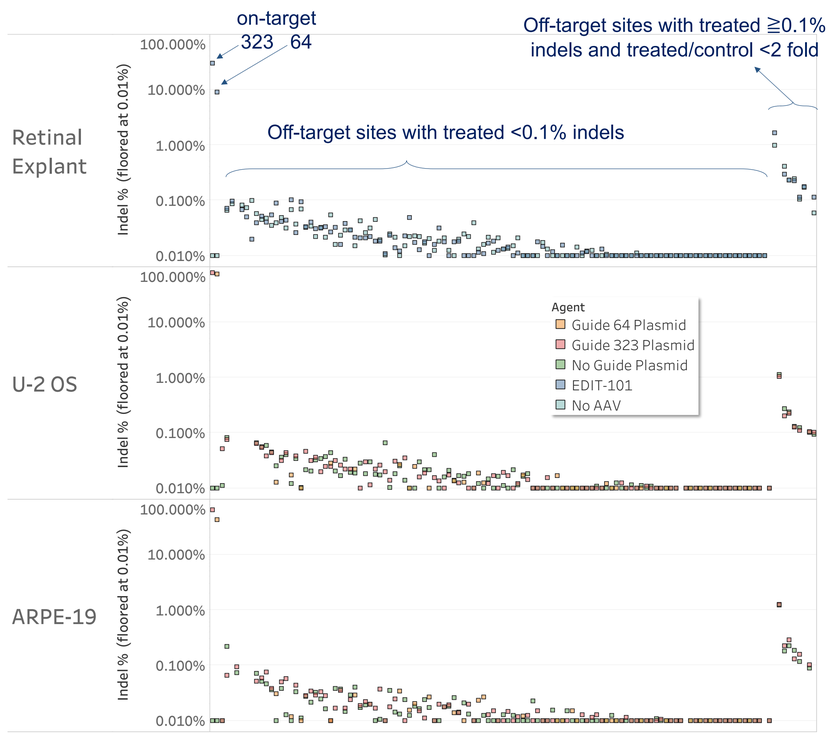
GUIDE-Seq results for g64 and g323. Plasmids were nucleofected into the given cell line. No off-target sites were found in any of the samples.

## 10) 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. Four weeks after transduction, the tissue was harvested. An AAV5 GRK1-GFP vector shows photoceptor-specific label, and editing measured using UDiTaS. The percent editing results are from an average of 25 EDIT-101 punches and 2 untreated punches.

## 11) NGS Panels Show No Off-Target Editing in Human 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. Five of 117 candidate sites were not amenable to NGS.

## 12) Summary of orthogonal specificity methods

| 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 |
| | 323 | LLoD ~0.1% - 2% varies by cell line |
| 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 | |

## 13) Conclusions

EDIT-101 is a novel gene editing clinical candidate for LCA10 patients that removes or inverts the IVS26 mutation and restores expression of the full length CEP290 protein. We expect EDIT-101 will improve photoreceptor function and bring clinical benefit to LCA10 patients.

- 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 highly relevant tissue – human retinal explants – at over 100 candidate sites.
- This specificity framework can be broadly applied to gene editing therapeutics.