The development of CRISPR-based medicines for the treatment of ocular diseases

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ASGCT 2021
Moving Genome Editing to the Clinic: From Technology to Therapeutics

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Editas Medicine’s powerful engine

Differentiated platform: the *only* company with multiple proprietary CRISPR editing systems

Unparalleled IP: broadest and deepest CRISPR IP portfolio

Ability to develop widest range of transformational genomic medicines for serious diseases
**Preclinical development of gene editing experimental medicine EDIT-101**

**Introduction:** Leber congenital amaurosis type 10 (LCA10) disease

**Guide RNA selection:** Editing with lead guide RNA combination

**Specificity assessment:** On-target and off-target editing in relevant tissues

**Safety and tolerability:** Mouse and NHP study to evaluate efficacy, safety, and tolerability of EDIT-101

**Conclusion:** Clinical development of EDIT-101
LCA10 is caused by loss-of-function mutations in the CEP290 protein

- The CEP290 gene encodes a 2479 amino acid, 290 kDa protein that localizes to the photoreceptor connecting cilium.
- Required for the protein trafficking critical to outer segment regeneration and phototransduction.
- Restoring CEP290 protein expression in surviving foveal photoreceptors may improve vision in patients with LCA10.

Gene repairs at c.2991+1655A>G mutation of CEP290 to full functional protein

LCA10 disease

Gene editing therapeutic concept

**DNA**

<table>
<thead>
<tr>
<th>Exon 26</th>
<th>Exon 27</th>
</tr>
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<tbody>
<tr>
<td>IVS26</td>
<td></td>
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<tr>
<td>*</td>
<td>G</td>
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**mRNA**

<table>
<thead>
<tr>
<th>Exon 26</th>
<th>Exon 27</th>
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</table>

**Protein**

- p.Cys998X prematurely truncated and non-functional CEP290

**Gene editing therapeutic concept**

- **Inversion**
  - IVS26
  - G

- **Deletion**
  - Exon 26
  - Exon 27

- Full-length, functional CEP290
Editing causes inversions, deletions, and indels


**Inversion**

**Deletion**

Exon 26

Exon 27

indels

Large inversions

18

Large deletions

40

Small indels

17
Targeted deletions and inversions correct splicing

Correct splicing as determined by GFP expression

**U2OS Cell Line**

- **WT**
- **IVS26 mutant**
- **Deletion**
- **Inversion**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Relative GFP expression</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>19.0 ± 0.5</td>
</tr>
<tr>
<td>IVS26 mutant</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td>Deletion</td>
<td>17.0 ± 0.4</td>
</tr>
<tr>
<td>Inversion</td>
<td>18.0 ± 0.6</td>
</tr>
</tbody>
</table>

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**Ex26/27, exon 26/27; SA, splice acceptor; SD, splice donor; WT, wild type**

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Editing corrects CEP290 splicing thereby restoring mRNA and protein expression

**CEP290 mRNA expression**

Relative CEP290 expression (normalized to ACTB)

- WT Mutant
- WT Mutant
- Edited (g323 + g64)

<table>
<thead>
<tr>
<th>Relative CEP290 expression</th>
<th>Ctrl</th>
<th>Edited (g323 + g64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>0.0003</td>
<td>0.0005</td>
</tr>
<tr>
<td>0.0002</td>
<td>0.0004</td>
<td>0.0006</td>
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<td>0.0003</td>
<td>0.0005</td>
<td>0.0007</td>
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<tr>
<td>0.0004</td>
<td>0.0006</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

**p-values:**
- p=0.07
- p=0.01
- p=0.03

**CEP290 protein expression**

- Ctrl
- Edited (g323 + g64)

- CEP290
- GAPDH

Ctrl, control

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Comprehensive specificity assessment

**Discovery**

- **In silico modeling**
  Predict where an enzyme can cut

- **Digenome-Seq**
  Find where an enzyme cuts naked DNA

- **GUIDE-Seq**
  Find where an enzyme cuts DNA in context of a cell

**Verification**

- **Targeted sequencing in relevant cells by NGS**
  Measures effect of enzyme activity on “discovered” sites

- **Risk assessment and mitigation as needed**
  Off-target

To identify gRNAs without measurable off-target cut
Guide RNAs g323 and g64 demonstrated highly specific on-target cutting in a biochemical assay.

**Digeneome-Seq Assay**
- gRNA + Cas9
- Genomic DNA
- Whole genome sequencing

**Sequence analysis (g323 on-target example)**
- Enriched fragments on either side of cut site

**Graph**
- Number of cut sites including on-target site
  - g323 (SaCas9): ~100× on:off-target window
  - g64 (SaCas9): >100× on:off-target window
  - Positive control (Emx1 [SpCas9]): 272
  - Negative control (No RNP): 0

RNP, ribonucleoprotein; SaCas9, Staphylococcus aureus Caspase 9; SpCas9, Streptococcus pyogenes Caspase 9.
EDIT-101: an AAV5 vector with two gRNAs and DNA encoding Cas9 injected sub-retinally

DNA packaged in AAV5 vector with tropism for photoreceptors

Localized subretinal injection

Two specific guide RNAs (g323 and g64) direct the SaCas9 protein to the c.2991+1655A>G mutation site

GRK1 promoter restricts SaCas9 protein expression to photoreceptor cells

SaCas9 protein cuts the DNA at either end of the c.2991+1655A>G mutation only in photoreceptor cells

Localized delivery, AAV5 tropism, specific guide RNAs, and restricted Cas9 expression facilitate targeted editing by EDIT-101 in photoreceptor cells
Human eyes 3–5 hours postmortem

Remove neural retina

3 mm punches

Plate 3 mm punches with photoreceptor-side down in 24-well format

EDIT-101

EDITing and specificity analysis in human retinal explant model

Morphology: Histology with GFP vector

Editing: UDiTaS to measure editing

Specificity: Specificity verification panel

Harvest 28 days post-transduction
Confirmation of editing and quantitation in human retinal explant model

28 days post-transduction

AAV5-GRK1-GFP (5e11 vg)

EDIT-101 (5e11 vg)

INL, inner nuclear layer; ONL, outer nuclear layer

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Specificity verification in retinal explants confirmed no off-target sites

The presence of on-target sites together with the absence of off-target sites confirmed that the guide RNAs were highly active and specific to the human CEP290 target sequence.
Efficient transduction and editing of mouse retina by subretinal delivery of EDIT-101

*HuCEP290-IVS26 KI mice*

Over 80% of productive editing was achieved in the transduced photoreceptors
Efficient transduction of photoreceptor cells with EDIT-101 in HuCEP290-IVS26 KI mice

ISH of AAV vector genome

IHC of Cas9 protein

Counter-stained with rhodopsin

IHC of Cas9 in the area of AAV ISH showed essentially all photoreceptors in the bleb region were transduced

IHC, immunohistochemistry; ISH, in situ hybridization
Both gRNA and Cas9 mRNA were highly expressed in the mouse retina

Adapted from Nature Medicine, Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10, 25, 2019;229–233, Maeder ML, et al, © The Author(s), under exclusive license to Springer Nature America, Inc., with permission of Springer. From: Fig. 2 | In vivo editing in HuCEP290 IVS26 knock-in mice
EDIT-101 demonstrated rapid and stable gene editing in HuCEP290-IVS26 KI mice

Total editing rates with EDIT-101 were maintained over 40 weeks post-injection

The optimal dose of EDIT-101 in the LCA10 mouse model appeared to be $\sim 10^{12}$ vg/mL

EDIT-101 achieved productive editing rates in a dose-dependent manner in an LCA10 mouse model
Retinal structural differences between mice and NHPs

**Monkey retina**
- **Macula**
- **Photoreceptors**: 25–30% of cells
- **Most foveal photoreceptors are cones**
- **8 mm retinal punch covering most of the bleb used for analysis but only photoreceptors (25–30%) express GRK1**

**Mouse retina**
- **No macula**
- **Photoreceptors**: 85–90% of cells
- **97% of photoreceptors are rods**
- **Entire retina collected for analysis but only 30% of neural retina transduced with 1 µL AAV5**

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IF, immunofluorescence; IPL, inner plexiform layer; OPL, outer plexiform layer; POS, photoreceptor outer segments
EDIT-101 had a similar dose response in mice and NHPs

At maximally tolerated doses, >50% editing is observed in NHPs and was not distinguishable from placebo.

EDIT-101 was well tolerated in NHPs and was not distinguishable from placebo.
BRILLIANCE: A Phase 1/2 open-label study of EDIT-101 in adult and pediatric patients

**Objective:**
To evaluate the safety, tolerability, and efficacy of EDIT-101 in patients with LCA10

**Inclusion criteria**
- Adult (≥18 years) or pediatric (3–17 years) patients
- LCA10 caused by c.2991+1655A>G mutation in the CEP290 gene
- BCVA 0.4 logMAR (20/50 Snellen equivalent)
- Failed mobility course at maximum level of difficulty

**Phase 1/2, open-label, single ascending dose study (NCT03872479)**

**Primary outcomes: Safety**
- Adverse events
- Dose-limiting toxicities

**Key secondary outcomes:**
- Maximum tolerated dose
- Visual navigation (Δ Mobility course score)
- BCVA (Δ LogMAR)
- Δ Macula thickness
- Pupillometry and microperimetry
- Light and contrast sensitivity
- Δ Color vision score
- Quality of life

**Adult doses:**
- Low dose, 6x10^{11} (n=2)
- Middle dose, 1.1x10^{12} (n=4)
- High dose, 3x10^{12} (n=4)

**Pediatric doses:**
- Middle dose (n=4)
- High dose (n=4)

**Follow up visits:**
- Month 0, 3, 6, 9, 12

“Progress Toward a Clinical Trial of CRISPR/Cas9-Mediated Genome Editing for CEP290-Associated Retinal Degeneration” © 2021 Editas Medicine
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