A Mutation-Independent CRISPR/Cas9-Based ‘Knockout and Replace’ Strategy to Treat Rhodopsin-Associated Autosomal Dominant Retinitis Pigmentosa (RHO-adRP)

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The speaker and the co-authors are employees and stockholders of Editas Medicine.
Autosomal Dominant Retinitis Pigmentosa (adRP)

- An inherited autosomal dominant retinal disease leading to blindness in later life
- Symptoms:
  - Decreased night vision (nyctalopia)
  - Loss of peripheral vision (tunnel vision), and eventually significant decline in central vision
- No approved treatments
Rhodopsin-Associated adRP (RHO-adRP)

**Rhodopsin (RHO)**
- A light-sensitive receptor protein involved in visual phototransduction in rods
- Located in the outer segments of rods
- Approximately 30% (US and UK) of adRP caused by *RHO* dominant mutations
- Prevalence: 7,500 patients in US and 12,100 patients in EU and UK
- >150 mutations identified in the *RHO* gene cause RHO-adRP
- Dominant mutations in the *RHO* gene are toxic for the rods: progressive loss of rods followed by loss of cones

**Photoreceptor structure**

- Rods: light-sensitive cells
- Cones: color-sensitive cells
- Rods and cones have outer segments with light-absorbing pigments and inner segments with phototransduction machinery

**Retinal Layers**
- Nerve fiber layer (NFL)
- Ganglion cell layer (GCL)
- Inner plexiform layer (IPL)
- Inner nuclear layer (INL)
- Outer plexiform layer (OPL)
- Outer nuclear layer (ONL)
- Inner/outer segments of photoreceptors (IS/OS)
- Retinal pigment epithelium (RPE)
- Choroid

**Normal vs. End-stage RP**
- Normal retina with intact layers
- End-stage RP showing degeneration and loss of photoreceptor layers

EDIT-103: Dual AAV-Based “Knockout and Replace” Therapeutic Strategy

• Agnostic to any RHO mutation – thus will knockout any dominant gain-of-function rhodopsin mutant
• **Step 1:** Both mutant and normal endogenous *RHO* will be knocked out in the treated area
• **Step 2:** Exogenous normal *RHO* (resistant to editing) will replace endogenous *RHO*
• One-time subretinal administration aimed to restore/prevent vision loss

**Details:**
- gRNA is on the vector carrying *RHO*-replace thus assuring knockout takes place only in photoreceptor cells that express *RHO*-replace
- The *RHO* promoter for Cas9 and *RHO*-replace restricts therapeutic activity to rod photoreceptors

The RHO Promoter Restricts Gene Expression to Rod Photoreceptors in the Mouse Eyes

AAV: adeno-associated virus; GFP: green fluorescent protein; INL: inner nuclear layer; IS/OS: inner/outer segments of photoreceptors; ONL: outer nuclear layer; OPL: outer plexiform layer; RHO: rhodopsin
EDIT-103 is Highly Specific: No Detectable Off-Target Editing

NO OFF-TARGET EDITING AT OFF-TARGET CANDIDATES
(rhAmpSeq verification in human retina explants transduced with EDIT-103)
EDIT-103 in Humanized \( mRho^{hRHO/+} \) Mice: Demonstrates Rapid and Stable Gene Editing

**Editing**

- Dose escalation
- Time course

**hRHO mRNA replacement**

- >25% of rods are edited
- Editing rates increase in a dose-dependent manner
- Editing plateau at ~6 weeks post-injection at doses \( \geq 3E12 \) vg/ml

~8

EDIT-103 in Non-Human Primates (NHPs): Approximately 100% Editing in Transduced Photoreceptors

EDIT-103 (KO and Replace)

- **RHO promoter** → **SaCas9**
- **U6 → U6** → **RHO promoter** → **coRHO**

Knockout (KO only)

- **RHO promoter** → **SaCas9**
- **U6 → U6** → **Stuffer**

AAV: adeno-associated virus; coRHO: codon-optimized rhodopsin; gRNA: guide ribonucleic acid; KO: knockout; NHP: non-human primates; OS: oculus sinister; RHO: rhodopsin; SaCas9: *Staphylococcus aureus* CRISPR-associated protein 9; SD: standard deviation.

~100% editing (within the transduced area) in NHP

Volume: 100 µl
AAV ratio: 1:1
Time point: 13 weeks
Mean (±SD) is presented
*p<0.05, ***p<0.001

### Editing (%) in rods (within the transduced area)

- **Vehicle**
- **KO**
- **EDIT-103**
- **EDIT-103**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (±SD)</th>
<th><em>p-value</em></th>
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<tbody>
<tr>
<td>Vehicle</td>
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<tr>
<td>KO 3E12 (vg/ml)</td>
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<td>EDIT-103 3E12 (vg/ml)</td>
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<tr>
<td>EDIT-103 6E12 (vg/ml)</td>
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Volume: 100 µl
AAV ratio: 1:1
Time point: 13 weeks
Mean (±SD) is presented
*p<0.05, ***p<0.001

**Bleb**

Macula
Parafovea
Fovea

Optic disc
EDIT-103 in NHPs: Nearly Complete Knockdown of the Endogenous RHO and Over 30% RHO Protein Replacement

**RHO mRNA**

- 80% and 90% knockdown of endogenous NHP RHO mRNA levels were achieved at doses of 3E12 and 6E12 vg/ml, respectively. This resulted in 90% and 100% of RHO protein knockdown.

**RHO Protein**

- RHO replacement mRNA levels increased with dose and resulted in >30% of RHO protein levels.

**Volume:** 100 µl

**AAV ratio:** 1:1

**Time point:** 13 weeks

Mean (±SD) is presented:

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
RHO Protein Expression and Retina Morphology was Preserved in EDIT-103-Treated NHP Retinas Compared with KO-Treated Retinas

- AAV transduction in the treated groups reveals positive Cas9 genome staining
- Nearly absent RHO protein and lack of OS (yellow arrow) in the KO group
- Preservation of RHO protein, improved photoreceptor organization, and improved IS/OS morphology in the EDIT-103-treated groups

AAV: adeno-associated virus; H&E: Hematoxylin and Eosin; IS/OS: inner/outer segments of photoreceptors; KO: knockout; NHP: non-human primate; ONL: outer nuclear layer; RHO: rhodopsin

AAV ratio: 1:1; Injection volume: 100 μL; Time point: 13 weeks; Scale bars, 50 μm
Retina Function Preserved in the EDIT-103-Treated NHP Eyes Compared to the KO Only Treated Eyes

KO of endogenous RHO significantly reduced a- and b-wave amplitudes

EDIT-103 dosing preserved a- and b-wave amplitudes

ERG: electroretinogram; GCL: ganglion cell layer; INL: inner nuclear layer; IPL: inner plexiform layer; IS/OS: inner/outer segments of photoreceptors; KO: knockout; NFL: nerve fiber layer; ONL: outer nuclear layer; OPL: outer plexiform layer; RHO: rhodopsin; RPE: retinal pigment epithelium; SD: standard deviation.
EDIT-103 is a **one-time, high efficacy, mutation-agnostic gene medicine** to permanently suppress the toxic gain-of-function associated with RHO-adRP

**Ex vivo:** EDIT-103 shows high specificity in human retinal explants

**In vivo:**

- **mRho^{hRHO/+}** mouse:
  - EDIT-103 achieved **rapid** and **stable** gene editing:
    - Editing plateau at 6 weeks and is sustained until end of study (13 weeks)
    - > 25% gene editing at doses ≥ 3E12 vg/ml
  
- **NHP**:
  - EDIT-103 achieved nearly **100%** editing
  - >30% RHO replacement protein levels
  - Morphological and functional photoreceptor preservation
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* Alphabetical order according to last names
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