Envisioning a Gene Editing Approach to Treat Inherited Blindness

Morgan Maeder
Why Gene Editing in the Eye?

Benefits of targeting inherited retinal diseases

Significant unmet medical need
- Vision loss has huge negative impact on quality of life
- Most inherited retinal disease patients have no therapeutic options

Proven delivery
Gene therapy using viral vectors has demonstrated success in the clinic

Confined, immune-privileged location
- Limited immune response
- Any negative response easily detected and likely confined to the eye

Clear, non-invasive end points
- Visual acuity and other imaging measurements well characterized
- Built-in case-control: treated eye compared to untreated eye
RPE65 Validates Retinal Gene Therapy

Clinical trials for a broad range of retinopathies

Clinical trials of gene therapy for inherited and acquired retinopathies

AAV-Mediated Retinal Gene Therapy

On-going field of research to optimize current approach

Sub-retinal delivery of appropriate AAV serotype for target cell encoding cDNA for gene augmentation

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Potential solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not all cell types are as easily transduced as RPE</td>
<td>Testing and optimization of non-AAV2 serotypes, modification and evolution of AAV</td>
</tr>
<tr>
<td>Sub-retinal injections are difficult and associated with risk</td>
<td>Evolution of AAV serotypes that can transduce the retina via intravitreal injection</td>
</tr>
<tr>
<td>Several disease-causing genes (ex. ABCA4, USH2A, CEP290) exceed the packaging limit of AAV</td>
<td>Lentivirus, Trans-splicing AAV, Gene Editing</td>
</tr>
<tr>
<td>Dominant diseases are difficult to address</td>
<td>Gene Editing</td>
</tr>
</tbody>
</table>

Hiroyuki Miyoshi et al. PNAS 1997
Leber Congenital Amaurosis

LCA10 caused by mutations in the CEP290 gene

- Group of heterogeneous inherited retinal dystrophies
- Early onset: infancy/early childhood
- CEP290 coding sequence = 7.5kb

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Target: Residual Photoreceptors

- Early loss of rod photoreceptors
- Survival of the central island of cone photoreceptors
- Normal intracranial visual pathways

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CEP290-Associated LCA10
Molecular mechanism of IVS26 mutation

Wild-type CEP290

DNA

Exon 26          Intron 26          Exon 27

Transcription and splicing

mRNA

Exon 26       Exon 27

Correct CEP290 protein

IVS26 mutant CEP290 (c.2991 + 1655 A>G)

DNA

Exon 26          Intron 26          Exon 27

Transcription and splicing

mRNA

Exon 26       128bp       Exon 27

Cys998X

Exon 26       Exon 27

Correct CEP290 protein
CEP290 Single gRNA Gene Editing Approach

Single gRNA induces NHEJ to delete splice mutation

Gene editing with Cas9 and single gRNA targeted close to mutation

DNA

Exon 26

AATTGTGAGT

Exon 27

Edited DNA

Exon 26

Indels delete IVS26 mutation

Exon 27

Exon 26

Exon 27

Exon 26

Exon 27

mRNA

Exon 26

Exon 27

Correct CEP290 protein
CEP290 Single gRNA Gene Editing Approach

Single gRNAs induce targeted indels
CEP290 Single gRNA Gene Editing Approach

Single gRNA-induced indels fail to efficiently delete IVS26 mutation

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CEP290 Dual gRNA Gene Editing Approach

A targeted deletion approach to excise mutation

Gene editing with S. aureus Cas9 and two gRNAs flanking the mutation

DNA

Exon 26

AATTGTGA GT

Exon 27

NHEJ

Edited DNA

Exon 26

Exon 27

Exon 26

Exon 27

Exon 26

Exon 27

Transcription and splicing

mRNA

Exon 26

Exon 27

Correct CEP290 protein

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CEP290 Dual gRNA Gene Editing Approach

A targeted deletion approach to excise mutation

Exon 26

128bp
cryptic exon

IVS26
mutation

Alu
Repeat

Exon 27

1000 bp upstream of Alu
41 NNGRRT PAMs

31 gRNAs (various lengths) tested

1000 bp downstream of mutation
29 NNGRRT PAMs

20 gRNAs (various lengths) tested

37 gRNA pairs tested

7 gRNA pairs selected
gRNA Pairs Induce Targeted Deletion

Targeted deletion quantification in primary patient fibroblasts by ddPCR

Quantification of Targeted Deletion in IVS26 Fibroblasts Following Transfection with Cas9 and gRNAs

%Deletion

Guide RNA Pairs

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<thead>
<tr>
<th>323+11</th>
<th>323+64</th>
<th>490+496</th>
<th>490+502</th>
<th>490+504</th>
<th>492+502</th>
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<td>15</td>
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*** P < 0.001
**** P < 0.0001
Targeted Deletion Corrects Splicing

Increased WT and decreased mut CEP290 mRNA measured by qRT-PCR

Quantification of WT and Mutant CEP290 Transcripts by qRT-PCR in IVS26 Fibroblasts

**** P < 0.0001
Targeted Deletion Corrects Splicing

Increased WT CEP290 protein expression by Western
Specificity Analysis of Candidate gRNAs
Combining GUIDE-Seq and amplicon sequencing to assess specificity

GUIDE-Seq in multiple human cell lines

Computational identification of closely matched sites

TTGCACGTACGTAAACAGGATGG
TTGGAGCTACGTAAACAGGATGG
TTGCAGAACGTAAACAGGATGG
TTGCACGTACGTAAACAGGATGG
TTGCACGTACGTAAACAGGATGG
TAGCACGTACGTAAACAGGCTG

Panel of sites analyzed by targeted NGS
## Specificity Analysis of Candidate gRNAs

Combining GUIDE-Seq and amplicon sequencing to assess specificity

<table>
<thead>
<tr>
<th>Target Site</th>
<th>On-target editing rate (% indels)</th>
<th>Off-Target Site</th>
<th>Off-Target Location</th>
<th>Off-target editing rate (% indels)</th>
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<td>No off-targets identified</td>
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<td>11</td>
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<td>Chr2:10678496 Intron, NOL10</td>
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<td></td>
<td>Chr1:247853709 intergenic</td>
<td>&lt;0.01</td>
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<td></td>
<td>Chr2:2526357 intergenic</td>
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</table>
Is There a Benefit to a Single gRNA Approach?

Single gRNA frees up additional space in the AAV

Continued technology optimization

Additional elements that could be added to free space in AAV:

- Alternative promoters driving Cas9 expression
- Longer intron sequences downstream of promoter to increase Cas9 expression
- Additional gRNA for Cas9 inactivation
Cas9 Engineering to enable a single gRNA approach

Engineered Cas9s with altered PAM recognition broaden targeting range

LETTER

Engineered CRISPR–Cas9 nucleases with altered PAM specificities

Benjamin P. Kleinstiver¹,²,³, Michelle S. Prew¹,², Shengdar Q. Tsai¹,²,³, Ved V. Topkar¹,², Nhu T. Nguyen¹,², Zongli Zheng¹,³,⁴, Andrew P. W. Gonzales⁵,⁶,⁷, Zhuyun Li⁶, Randall T. Peterson⁵,⁶,⁷, Jing–Ruey Joanna Yeh⁵,⁸, Martin J. Aryee¹,³,⁹ & J. Keith Joung¹,²,³

LETTERS

Broadening the targeting range of Staphylococcus aureus CRISPR–Cas9 by modifying PAM recognition

Benjamin P Kleinstiver¹–⁴, Michelle S Prew¹–³, Shengdar Q Tsai¹–⁴, Nhu T Nguyen¹–³, Ved V Topkar¹–³, Zongli Zheng⁵ & J Keith Joung¹–⁴
Engineering Alternate PAM Recognition

Cas9 Engineering could enable a single gRNA approach

WT Cas9

Engineered Cas9 Variant

No PAMs close enough to mutation

Novel PAM immediately adjacent to mutation

Project lead by Barrett Steinberg
Engineering Alternate PAM Recognition

Cas9 Engineering could enable a single gRNA approach

In vitro cleavage assay of novel Cas9 variants at IVS26 splice donor

<table>
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<tr>
<th>Cas9:</th>
<th>DNA Target:</th>
<th>WT</th>
<th>Ctrl</th>
<th>V1</th>
<th>CEP</th>
<th>V2</th>
<th>Ctrl</th>
<th>V2</th>
<th>CEP</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>Ctrl</td>
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</tr>
</tbody>
</table>

Project lead by Barrett Steinberg
What Can We Do with Extra Space in the AAV?

Single gRNA approach allows for inclusion of Cas9-inactivating gRNA

Long term transgene expression is ideal for standard gene therapy, but gene editing may benefit from a “hit and run” approach.

Project lead by Ari Friedland
Self-Inactivating AAV-Cas9

Inclusion of Cas9-targeting gRNA decreases Cas9 expression

Initial experiment performed with 2 AAVs in HEK293 cells

Western blotting shows knockdown of Cas9 protein

T7E1 shows no effect on on-target editing efficiency

Project lead by Ari Friedland
Gene Editing Therapeutic for CEP290-LCA
Subretinal AAV delivers gene editing components for deletion of IVS26 mutation

On-going *in vivo* experiments to understand:
1. Level of gene editing in photoreceptors
2. Specificity
3. Tolerability
Acknowledgments

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McKensie Collins
Ken Simon

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Reshica Baral
Pankhuri Singhal

Protein Engineering:
Barrett Steinberg
Derek Cerchione

Sequencing & Bioinformatics:
Gregory Gotta
Hari Jayaram
Eugenio Marco
Luis Barrera
Georgia Giannoukos
Dawn Ciulla
Tongyao Wang

And the entire Editas Team!
ddPCR Calibration

Observed
Expected

% deletion

no del 11.11% del 22.22% del 33.33% del 44.44% del 55.55% del 66.66% del 77.77% del 88.88% del 100% del
0.0 12.1 21.8 31.9 40.1 54.4 62.1 73.9 84.3 100.0

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