Evaluation of ABCA4 RNA Exon Editing and Replacement in Non-Human Primate

M-181

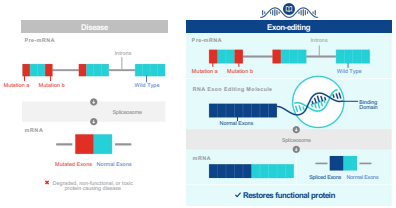
Rebekka Krumbach, Lingtao Peng, Xiaojin Liu, Kelly Becker, Kirk Burkhart, Eric Carlin, Adam Clemens, Akiko Doi, Lai Mung Fong, Kirk Gosik, Jesse Gray, Julia Meredith, Akiko Noma, Brian Olshaw, Anika Pavis, Crystal Shih Byers, Shimyn Slomovic, Madison Stulir, Carrie Wager, Qin Yu, Robert D. Bell, Michael D. Ehlers

Ascidian Therapeutics, Boston, MA 02135, USA

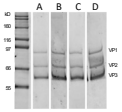
Background

Recessively inherited mutations in ABCA4 are causal in the development of progressive forms of blindness including Stargardt disease 1 and cone-rod dystrophy 3. The 6882 bp coding sequence of ABCA4 is too large to be delivered in its entirety by a single AAV vector. Moreover, with hundreds of disease-causing mutations found throughout the gene, a single base editing approach would not address a significant number of patients. Therefore, we developed a large-scale exon editing solution by delivering a therapeutic RNA construct capable of trans-splicing into endogenous ABCA4 pre-mRNA thereby introducing functional exons. RNA trans-splicing with a single AAV-based construct can address approximately 60% of all patient mutations. Here we report the editing efficiency and tolerability of lead AAV-ABCA4 Exon Editing Molecules following subretinal injection in healthy African Green Monkey non-human primates (NHP).

Exon Editing via pre-mRNA Splicing



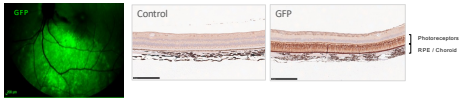
Exon Editors Packaged in AAV8



Sample	Titer (vg/mL)	Percent Full Capsids	Endotoxin (EU/mL)
A	$1x10^{12}$	41.5	<0.5
B	$1x10^{12}$	46.0	<0.5
C	$1x10^{12}$	43.0	<0.5
D	$1x10^{12}$	23.9	<0.5

AAV8 preparations of Exon Editing molecules (coded A-D) were characterized using qPCR of shared regulatory elements to titer, Stunner (Unchained Labs) for measurement of full and empty capsids and Endosafe nexgen-PTS to quantify endotoxin levels. VP1, VP2 and VP3 proteins are visualized on an SDS gel run at $5x10^{10}$ vg/well.

Regulatory Elements Ensure Retinal Expression

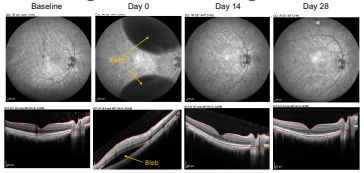


Exon Editor-specific regulatory elements produce robust protein expression in photoreceptors when used to drive GFP expression when dosed subretinally at $1x10^{11}$ AAV8 vg/100µl injection. GFP live image (color fundus) and anti-GFP IHC staining of fixed retinal sections.

All animals treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

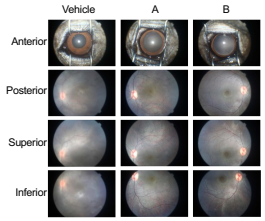
Correspondence
rkrumbach@ascidian-tx.com
rbell@ascidian-tx.com
mehlers@ascidian-tx.com

Preservation of Retinal Architecture Following Subretinal Dosing



Example of an Exon-Editor treatment: cSLO (confocal scanning laser ophthalmoscope) and OCT (optical coherence tomograph) images of baseline, day 0 post subretinal injections, day 14 and day 28. Post injection the superior and inferior blebs are visible. Each injection was 100µl containing $1x10^{11}$ vg. A slight 'shadow' of the bleb area remain visible through day 28.

Color Fundus - Normal Retinal Morphology

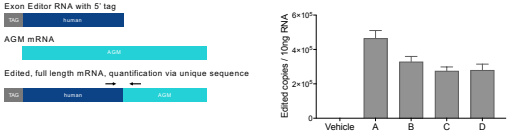


Examples of day 28 color fundus imaging.

Total clinical scores
Vehicle: 17
Exon Editor A: 0
Exon Editor B: 1

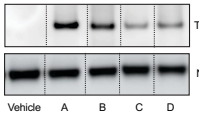
Note pupil in Vehicle eye not dilated to the same extent as examples A and B

Efficient Exon Editing in the NHP Retina



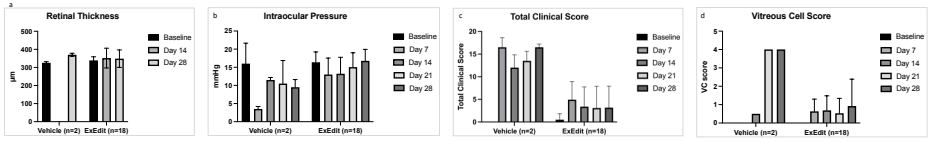
In the Exon Editor-treated retina chimeric mRNA was detected with quantitative RT-PCR amplifying the junction between the human coding sequence of the Exon Editor and the AGM RNA sequence. An example for each Exon Editor (A, B, C and D) is shown. Sequencing the edited full-length mRNA confirmed the precise edited sequence.

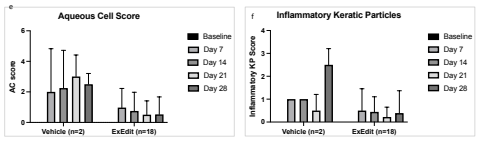
Exon Editing Produces Full-Length Protein



The full-length exon edited mRNA produces a tagged full-length ABCA4 protein. This was detected in the membrane extracts of the retina by western blot of the tag.
An example for each Exon Editor (A, B, C and D) is shown.

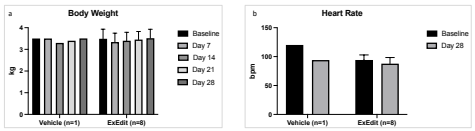
Ocular Health was Maintained Following Exon Editor Administration





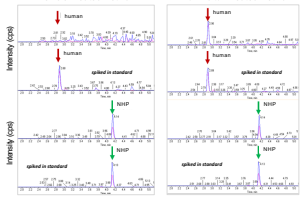
Ophthalmic health was monitored throughout the study. In general, ophthalmic health was maintained after treatment with AAV8 subretinally-delivered Exon Editors (Data shown as mean of Vehicle and mean of Exon Editors A, B, C and D ± S.D.).
Retinal thickness (a) remained normal throughout the study.
Tonometry (b) showed intraocular pressure was reduced on day 7 in vehicle-treated eyes and stabilized at later timepoints. Intraocular pressure remained in a healthy range in Exon Editor-treated eyes.
Ocular inflammation was assessed by slit lamp biomicroscopy and fundoscopy (c-f). Individual eyes treated with Exon Editors showed some signs of mild to moderate inflammation, but not consistently for a certain Editor or AAV preparation, suggesting that these observations were likely due to the procedure in an individual eye.

General Health was Maintained Following Exon Editor Administration



All animals on the study remained in good health. Body weight (a) and heart rate (b) measurements did not reveal any clinical signs indicative of adverse systemic effects caused by dosing of Exon Editors. Values present mean ± S.D. for n = 8 animals treated with Exon Editors.
Besides these measures cage side observations were performed daily, food consumption was monitored, and no changes were found (not shown).

Mass Spectrometry Confirms Specificity of Protein



Unique peptides distinguishing the fully NHP ABCA4 and the chimeric, edited ABCA4 were identified by coupling immunoaffinity enrichment with MRM-MS. The identities of the peptides were confirmed by spiking in standards.
In the vehicle treated retina only NHP peptide was detected.
In the Exon Editor treated retinal tissue both the human and NHP peptides were detected. This confirms the full-length exon edited mRNA is translated.

Conclusions

Exon-editing and replacement demonstrated efficiency and tolerability in vivo in African Green Monkey non-human primates (NHP) following subretinal injection, the first trans-splicing report at clinically relevant efficiencies in the NHP retina. All subretinal delivered AAV-mediated exon editing molecules were well-tolerated. This report highlights the potential of RNA exon editing to treat ABCA4-related retinopathies, and other diseases for which replacement of multiple contiguous exons may provide a novel treatment strategy. Additional studies are ongoing to advance this technology toward the clinic.

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