ABCA4 RNA Exon Editing *in vitro* and *in vivo*

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Disclosures: none

Introduction and Purpose

Ascidian Therapeutics has developed RNA Exon Editor technology to overcome historical barriers to progress in the gene therapy field. Multiple indications, such as Stargardt disease, cannot be addressed readily with gene therapy due to limited AAV packaging capacity, high mutational variance across patients or, in some cases, a narrow therapeutic index of gene expression.

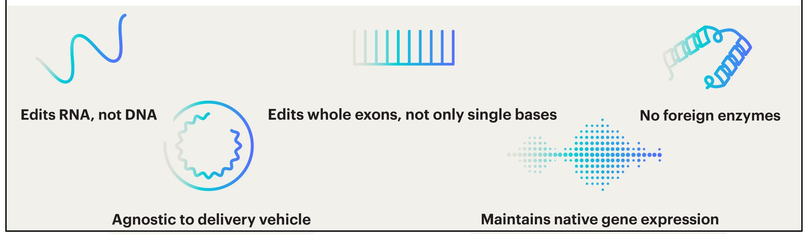
Delivered in DNA form, Exon Editor RNA molecules are designed to replace a substantial portion of a targeted disease gene's pre-mRNA with the correct sequence via RNA trans-splicing, resulting in a full-length, correct mRNA molecule and protein rescue. Since this technology utilizes the host cell's endogenous spliceosome, it avoids the risks associated with exogenous protein expression.

To unlock the full potential of this approach, we generated a high throughput, multiplexed screening platform to design, evaluate, and optimize the RNA editing efficiency of thousands of Exon Editor candidates at a time.

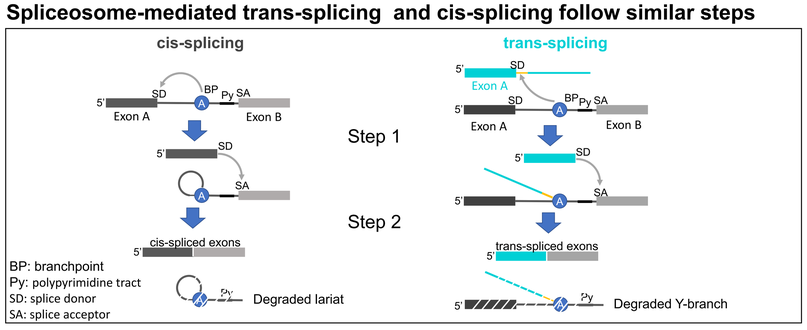
We report on Exon Editors that replace the 5' half of the 7 kb *ABCA4* coding sequence, which includes ~60% of known *ABCA4* mutations with the potential to address >70% of patients. We demonstrate Exon Editing at therapeutically relevant levels in an engineered disease model cell line, Non-Human Primate retina with six-month durability, and human retinal explants. This report highlights the translational viability and potential of this therapeutic strategy.

Exon Editing advantages over conventional gene therapy

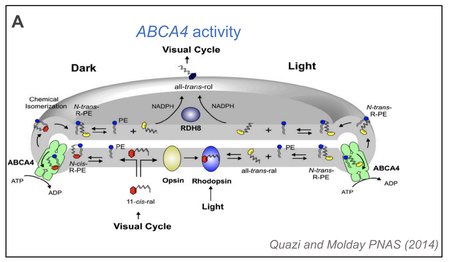
Large Genes	High Mutational Variance	Narrow Therapeutic Index
• Can address genes larger than 4.7kb AAV capsid capacity, replacing kilobase-long stretches of target sequence	• Edits multiple exons at once instead of individual bases, thereby addressing broader patient populations	• Maintains native gene expression levels, enabling correction of genes where over expression is a risk

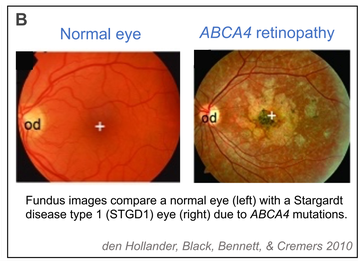


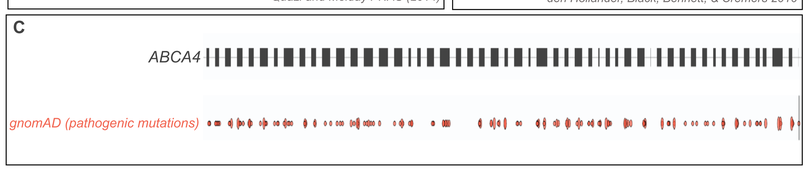
Spliceosome-mediated trans-splicing and cis-splicing follow similar steps



Exon Editing for the treatment of *ABCA4* retinopathies





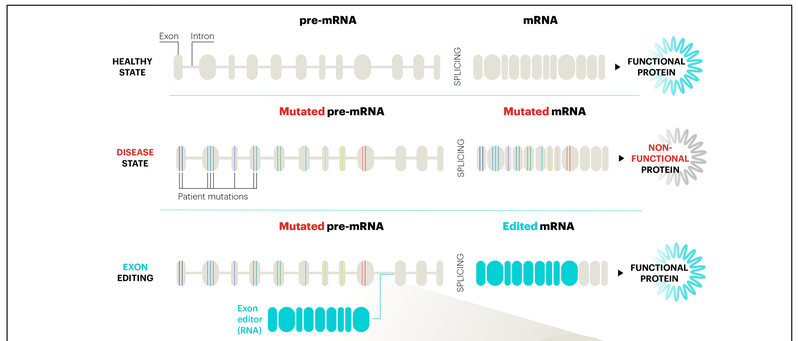


Mutations in *ABCA4* result in several different retinopathies including Stargardt, cone-rod degeneration, fundus flavimaculatus, certain types of retinitis pigmentosa and a possible link to increased risk of age-related macular degeneration. The majority of *ABCA4* mutations are autosomal recessive and account for 95% of Stargardt cases, which is the most common form of juvenile macular dystrophy at a prevalence of 1:8,000 - 1:10,000. The disease commonly presents in the 2nd or 3rd decade of life with progressive bilateral visual loss, in the range of 20/30 - 20/200 visual acuity (VA), with only ~20% of patients maintaining a VA of 20/40 in one eye by age 39.

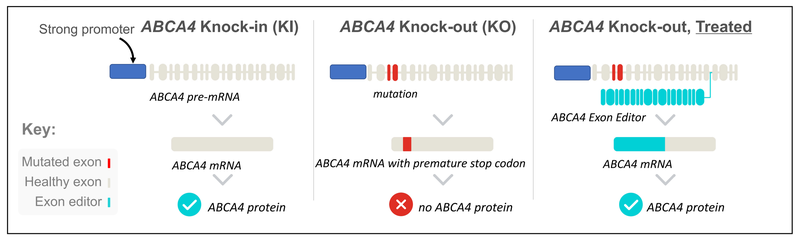
(**A**) Schematic detailing *ABCA4* activity. Loss of ABCA4 protein results in build up of fatty byproducts (lipofuscin) in the macula leading to cellular toxicity. (**B**) The ensuing loss of photoreceptors leads to progressive loss of vision. The STGD1 fundus demonstrates typical features seen in this disease, including a beaten-bronze appearance, macular atrophy, and yellow flecks indicating increased lipofuscin/A2E accumulation. (**C**) The *ABCA4* coding sequence is too large (6.8 kb) for AAV-mediated gene replacement, and >900 unique mutations have been identified, rendering it unamenable to DNA or RNA base-editing.

Method

Exon Editing approach

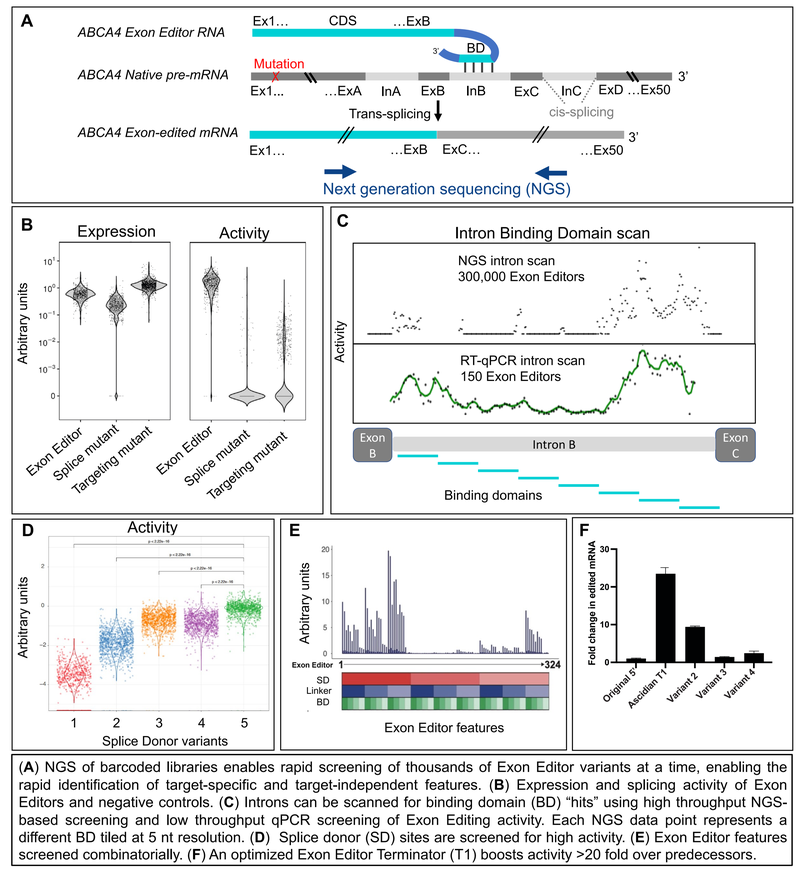


Engineered *ABCA4* protein knock-out (KO) cells for Exon Editor analysis



Results

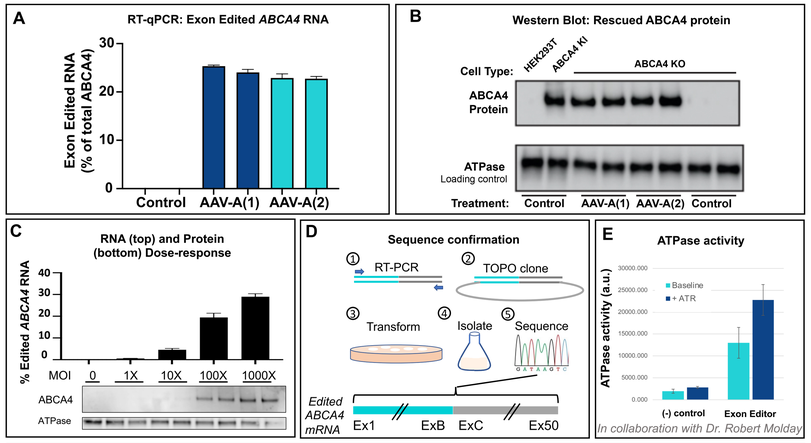
Next Generation Sequencing enables rapid screening



(**A**) NGS of barcoded libraries enables rapid screening of thousands of Exon Editor variants at a time, enabling the rapid identification of target-specific and target-independent features. (**B**) Expression and splicing activity of Exon Editors and negative controls. (**C**) Introns can be scanned for binding domain (BD) "hits" using high throughput NGS-based screening and low throughput qPCR screening of Exon Editing activity. Each NGS data point represents a different BD tiled at 5 nt resolution. (**D**) Splice donor (SD) sites are screened for high activity. (**E**) Exon Editor features screened combinatorially. (**F**) An optimized Exon Editor Terminator (T1) boosts activity >20 fold over predecessors.

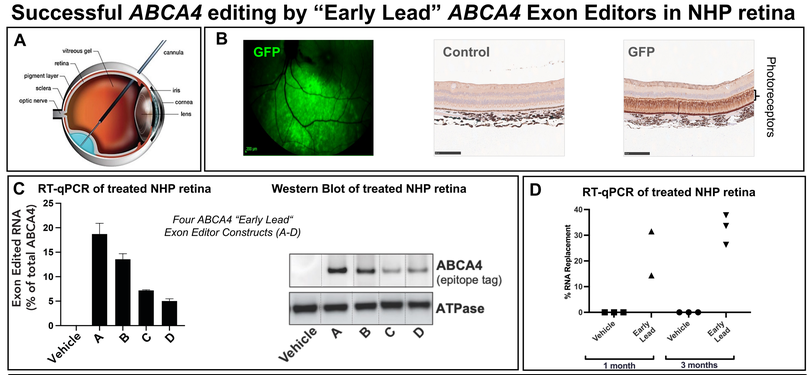
Results continued

Editing activity by *ABCA4* Exon Editors in *ABCA4* KO cells



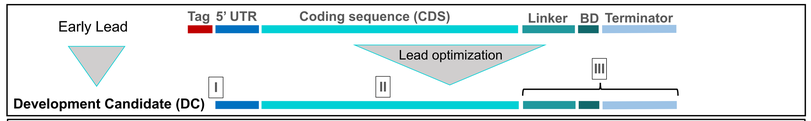
(**A**) RT-qPCR of KO cells transduced with *ABCA4* Exon Editors. (**B**) Western blot of protein from transduced KO cells shows full ABCA4 protein rescue. (**C**) Dose-response Exon Editing activity in transduced KO cells at a series of multiplicity of infection (MOI). (**D**) Sequencing confirmed full-length edited *ABCA4* RNA isolated from treated KO cells. (**E**) ABCA4 protein translated from Exon Edited *ABCA4* mRNA in treated KO cells shows expected baseline ATPase activity and an increase upon addition of all trans-Retinal (ATR).

Successful *ABCA4* editing by "Early Lead" *ABCA4* Exon Editors in NHP retina



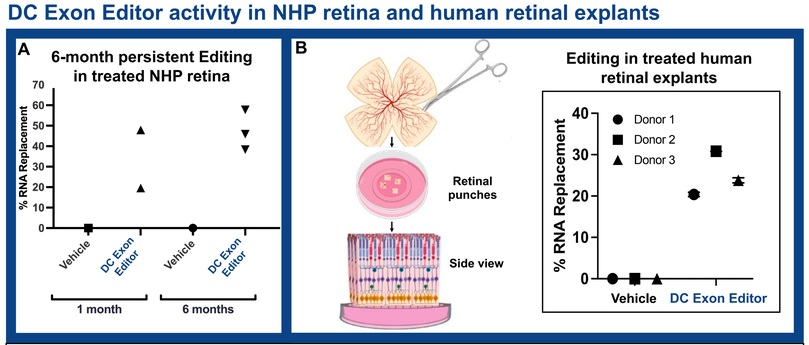
(**A**) Schematic of subretinal delivery of AAV8 GFP or "Early Lead" *ABCA4* Exon Editors. (**B**) Capsid and regulatory elements demonstrate robust targeting and GFP expression in photoreceptors. (**C**) RT-qPCR analysis (left) detects successful *ABCA4* RNA Exon Editing and Western blot analysis (right)) confirms corresponding edited ABCA4 protein – by four different "Early Lead" Exon Editors (labeled as A, B, C, and D). Na/K ATPase serves as a protein loading control. (**D**) NHP study evaluating a Lead *ABCA4* Exon Editor demonstrates robust and persistent *ABCA4* RNA exon editing out to three months.

Lead Optimization from "Early Lead" to Development Candidate (DC)



Lead Optimization from an Early Lead Exon Editor to the Development Candidate (DC). In addition to the removal of an epitope tag [I] and to sequence optimization [II], features [III] were added to improve editing efficiency and de-risk.

DC Exon Editor activity in NHP retina and human retinal explants



(**A**) Development Candidate (DC) Exon Editor demonstrates persistent and therapeutically relevant *ABCA4* RNA Exon Editing levels at 6 months post-treatment. (**B**) Human retinal explants transduced with the DC show high levels of *ABCA4* RNA Exon Editing.

Conclusions

We have demonstrated persistent, therapeutically relevant levels of *ABCA4* editing in human retinal explants and in NHP retina. 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.

All animals were treated in accordance with ARVO standards. We would also like to acknowledge the Human donors of the retinal explants.