A Large-Scale Exon Editing Solution for Treating Genetic and Complex Disorders

Robert D. Bell, Ph.D.

ASGCT 2022
May 16 – 19, 2022
Washington, D.C.

ascidian

Disclosures

- Robert Bell is an employee and shareholder of Ascidian Therapeutics
- All animals treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research

Gene therapy promises cures for many diseases, but has limitations

Limited cargo capacity of AAV

Genes with coding sequences >4.7 kb cannot be treated by traditional gene replacement

Diverse spectrum of patient mutations

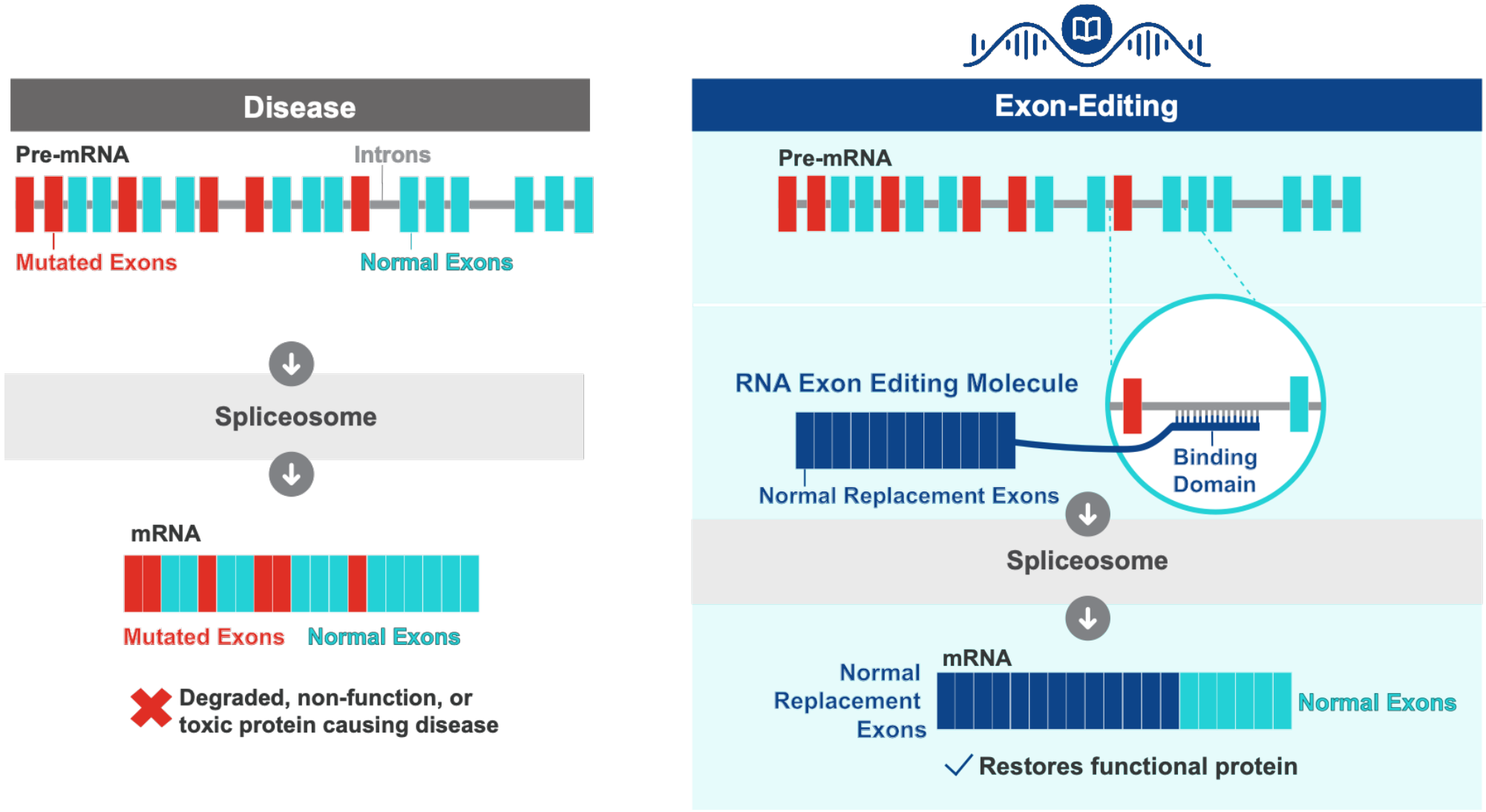
Base editing cannot address all patients

Difficulty controlling gene expression in different cell types

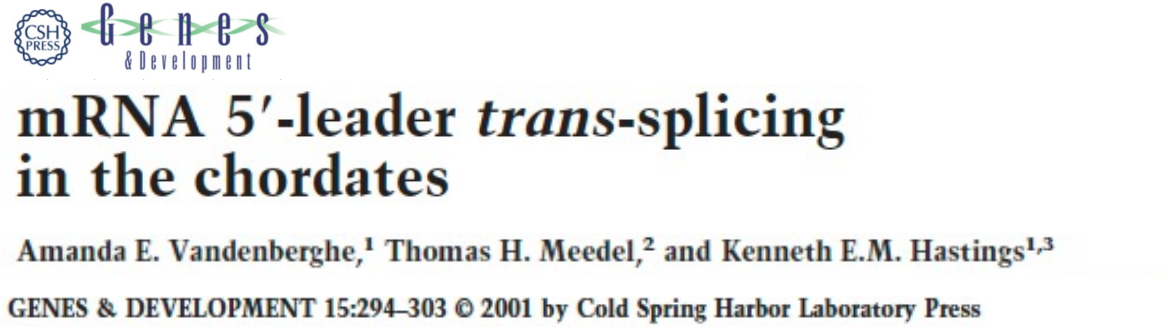
Safety risks of over- and ectopic-expression

Exon editing via RNA trans-splicing has the potential to overcome these limitations

Exon editing via pre-mRNA splicing replaces mutated exons



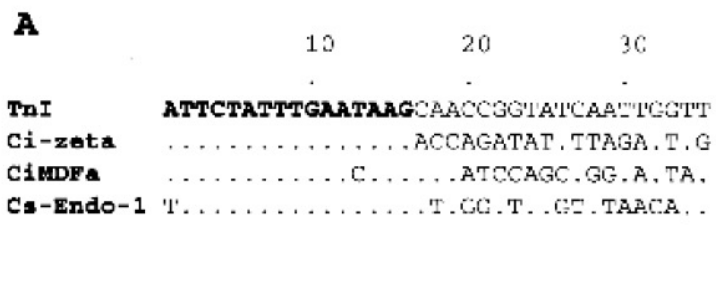
Discovery of RNA trans-splicing occurring in *Ciona intestinalis* Ascidians

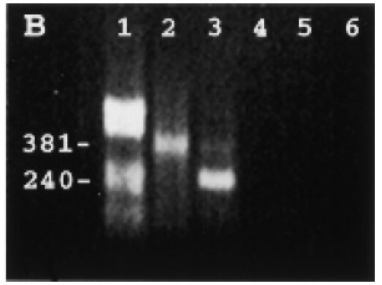

Genes & Development

mRNA 5′-leader *trans*-splicing in the chordates

Amanda E. Vandenberghe,[1] Thomas H. Meedel,[2] and Kenneth E.M. Hastings[1,3]

GENES & DEVELOPMENT 15:294–303 © 2001 by Cold Spring Harbor Laboratory Press





"SL trans-splicing in ascidians raises the possibility of ancestral SL trans-splicing in vertebrate evolution."

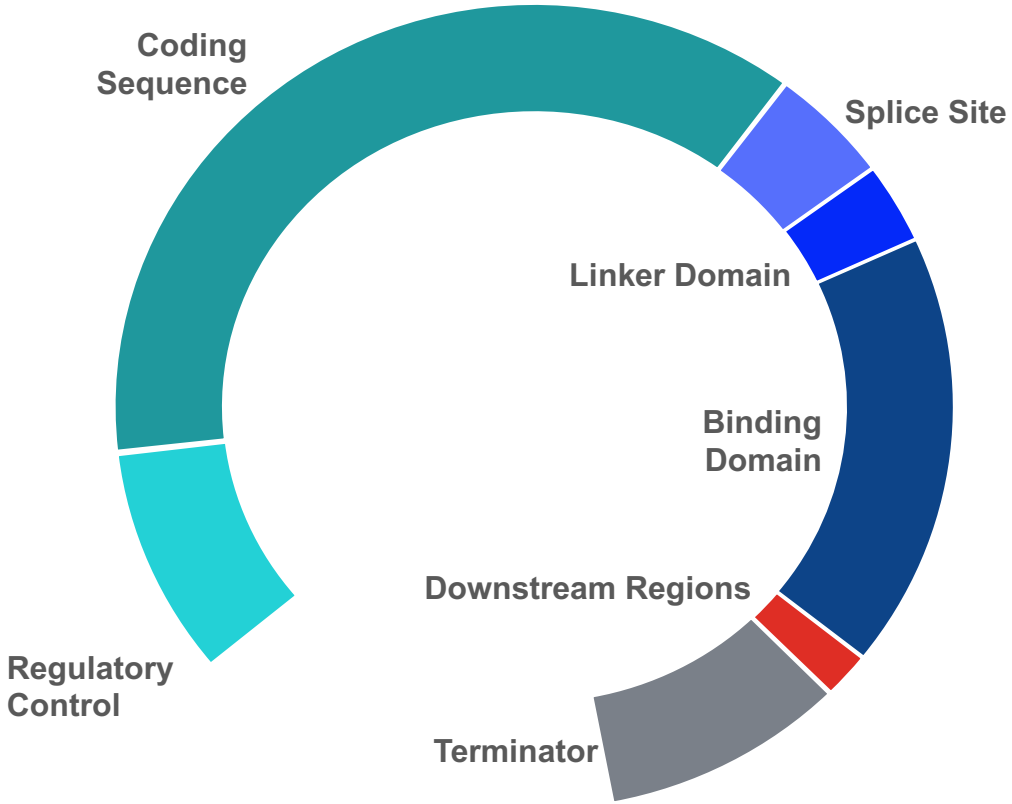
Disease area attempts	Gene	Date(s) of report
Duchenne muscular dystrophy	*DMD*	2007, 2010
Dystrophic epidermolysis bullosa	*COL7A1, K14*	2007, 2013
Huntington's disease	*HTT*	2012, 2017
Cystic fibrosis	*CFTR*	2001, 2002, 2007
Spinal muscular atrophy	*SMN2*	2003, 2013, 2014
Dysferlinopathies / Titinopathies	*DYSF / TTN*	2005
Retinitis pigmentosa	*RHO*	2008
X-linked hyper-IgM	*CD40L*	2004

Previous Challenges

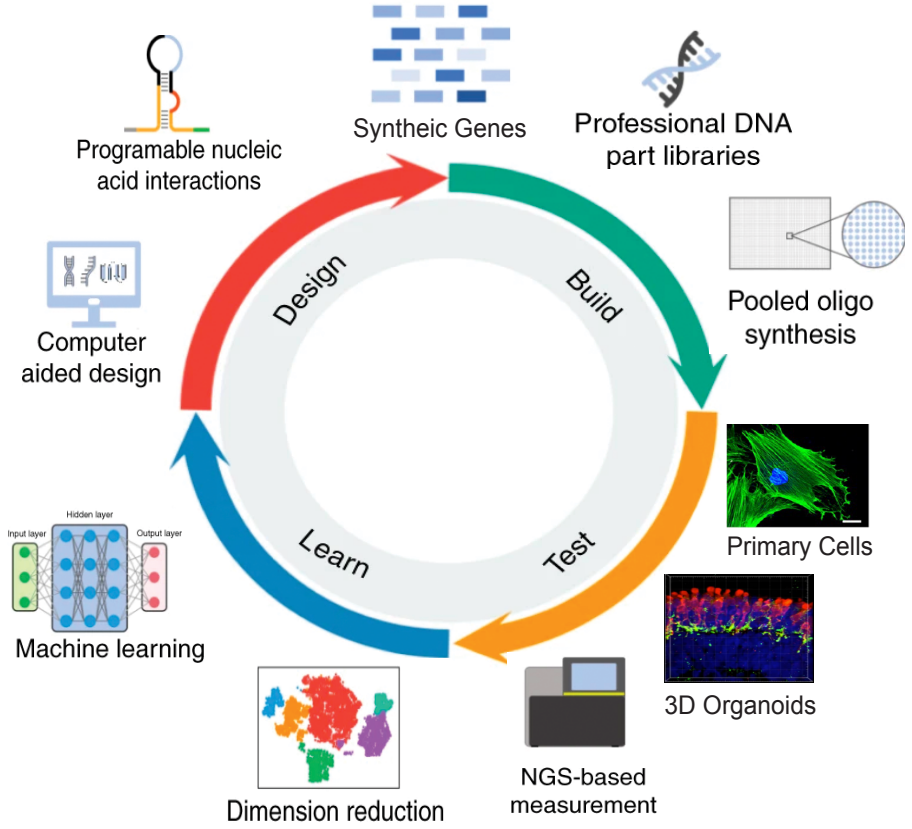
- Testing in non-native models (e.g. co-transfecting mini-genes)
- Efficiency challenges
- Limited *in vivo* studies
- Lack of translational delivery strategies

Advances in synthetic biology, computation sciences and RNA design allows Ascidian to discover therapeutic RNA exon editing molecules

RNA Exon Editing Molecules



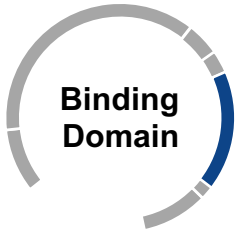
Enabled by modern synthetic biology

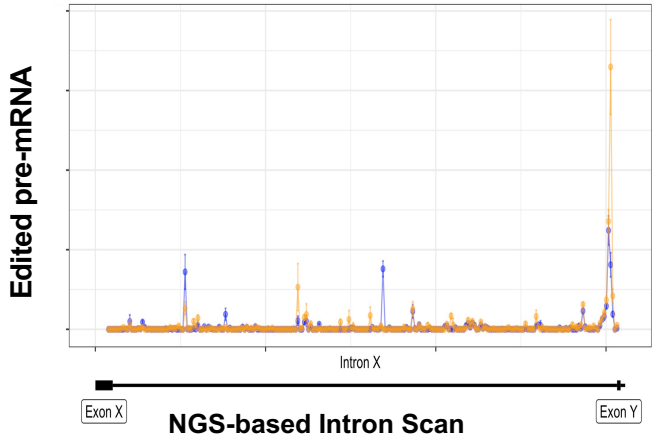


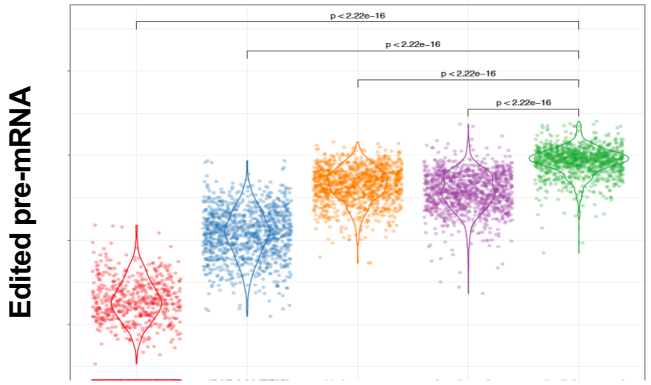
Ascidian considers RNA structural biology and RNA-protein interactions to ensure high efficiency designs

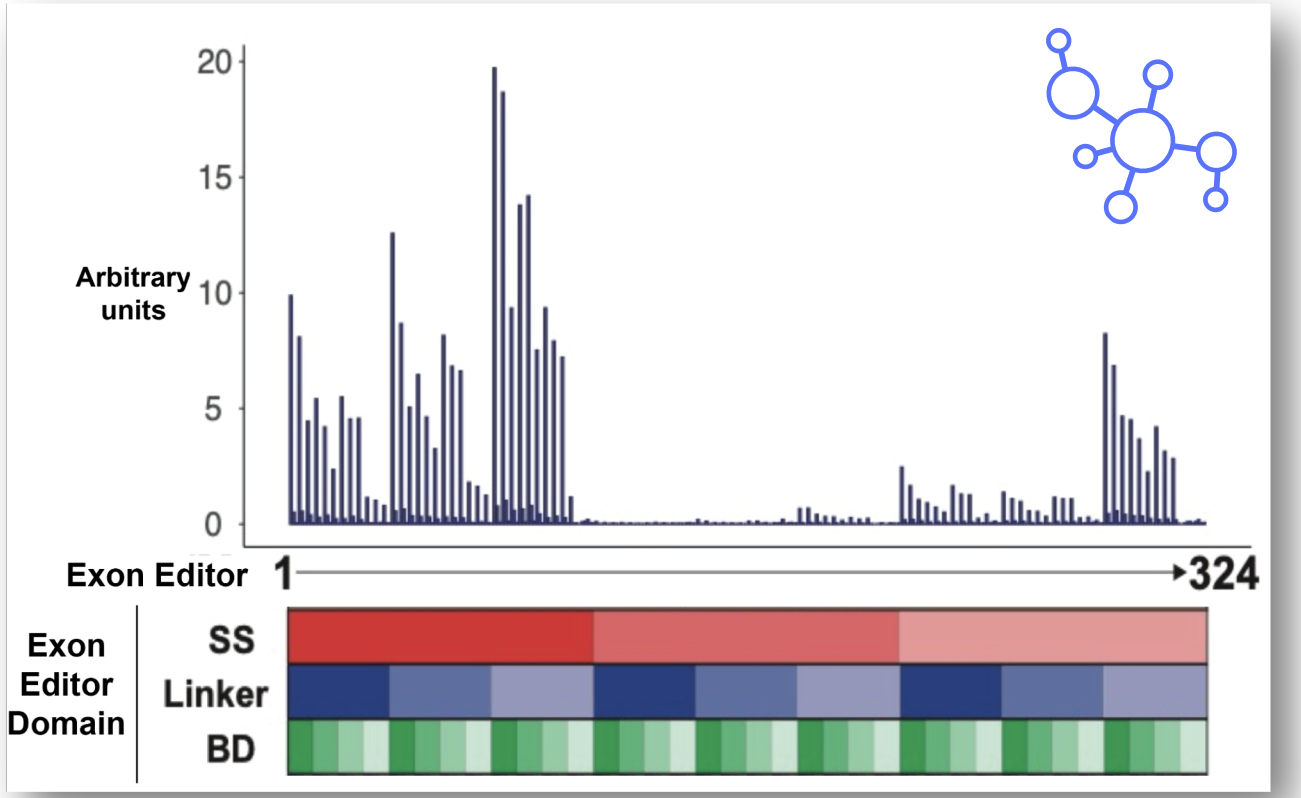
Adapted from Meng, F., Ellis, T. The second decade of synthetic biology: 2010–2020. *Nat Commun* (2020) and Capowski et al. Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. *Development* (2019).

Optimized elements of the exon editor are combined



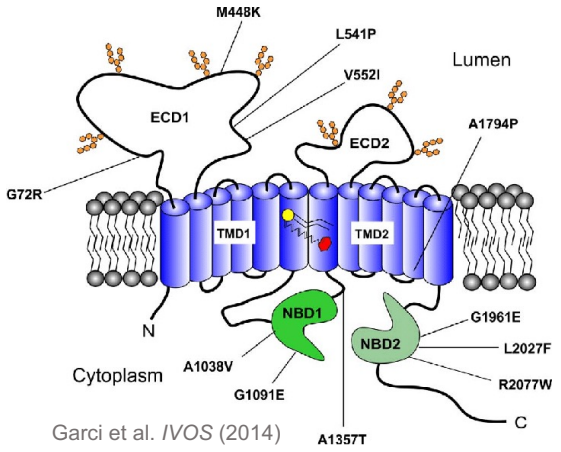




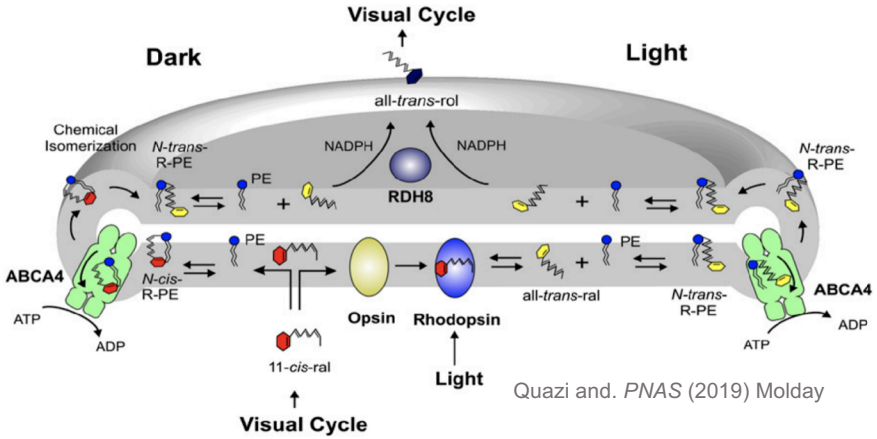


High throughput optimizations are repeated for each element, and then tested combinatorically to develop high efficiency RNA exon editing sequences

ABCA4-related retinopathies have no treatment, high mutational variance, and cannot be addressed with conventional gene therapy

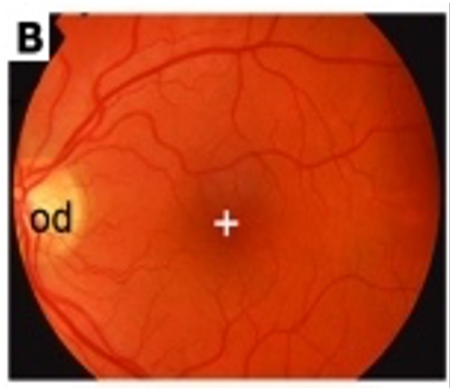


Garci et al. *IVOS* (2014)

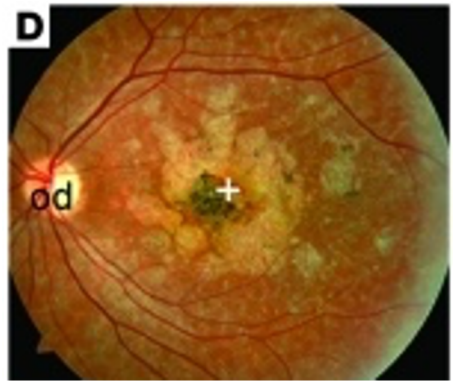


Quazi and. *PNAS* (2019) Molday

Normal Eye



ABCA4 Retinopathy: Stargardt's

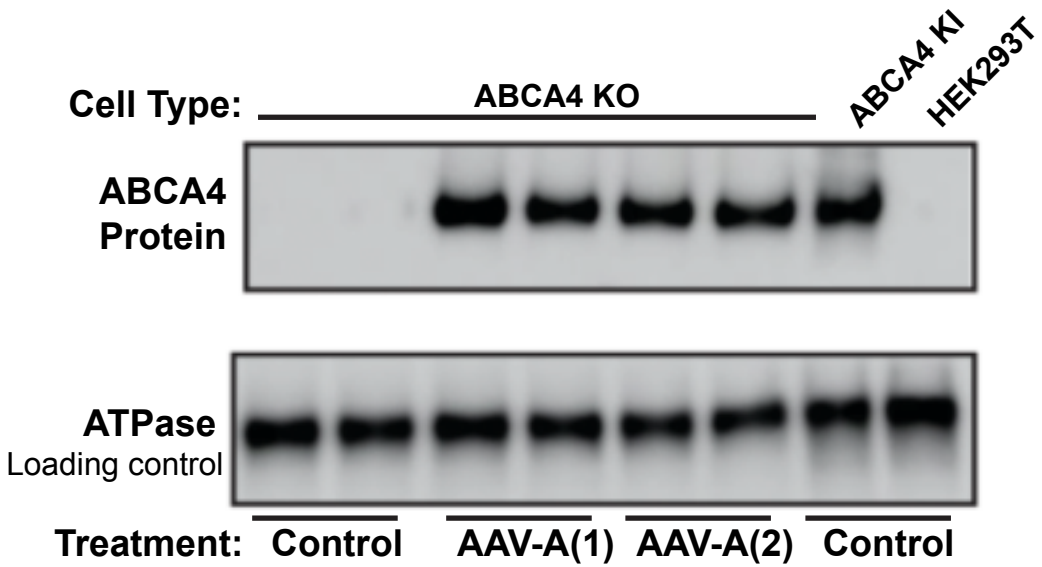


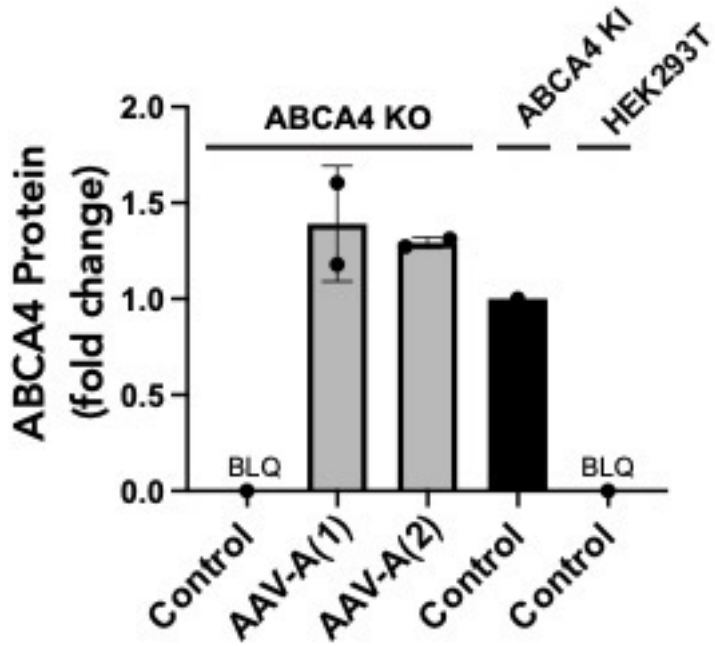
den Hollander, Black, Bennett, & Cremers 2010

- ABCA4 retinopathies are autosomal recessively inherited retinal diseases caused by mutations in the ATP-Binding Cassette sub-family A type 4 (ABCA4) gene
- Loss of ABCA4 results in build up of fatty byproducts (lipofuscin) in the macula leading to cellular toxicity and a progressive loss of vision
- ABCA4 is too large (6.8 kb) for AAV-mediated gene replacement and upwards of 900 unique mutations have been identified
- A single exon editing molecule can correct upwards of 60% of known pathogenic mutations found in the ABCA4 gene

ABCA4 therapeutic candidate restores wild-type protein expression in knock-out cells *in vitro*

AAV-A(1) and AAV-A(2) are identical exon editor constructs packaged using unique AAV-ITR plasmid backbone configurations





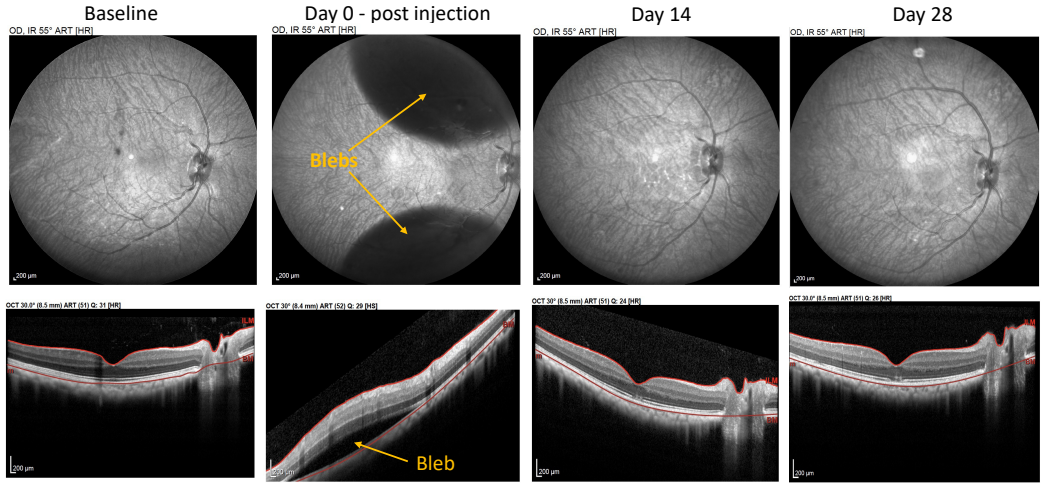
AAV delivered exon editors restore protein levels in vitro in ABCA4 knockout (KO)

BLQ: Below limits of quantitation

Robust non-human primate retinal transduction using AAV8 and ABCA4 exon editor regulatory elements

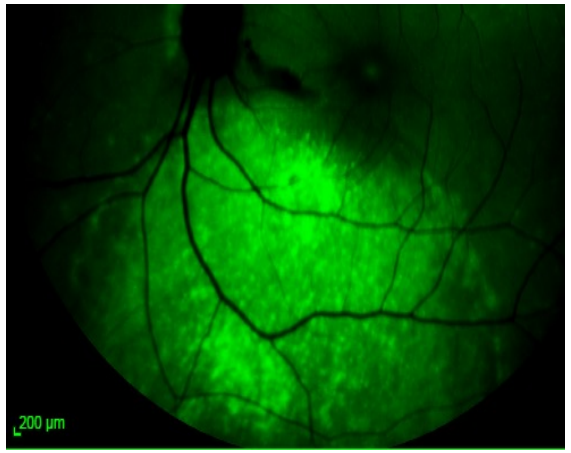
- Several independent reports find 10 - 25% restoration of ABCA4 expression in knock-out mice reduces age-related lipofuscin and A2E accumulation
- Initial assessment of *in vivo* efficiency and safety of exon editing via RNA trans-splicing performed in NHP (n=15) via subretinal injection

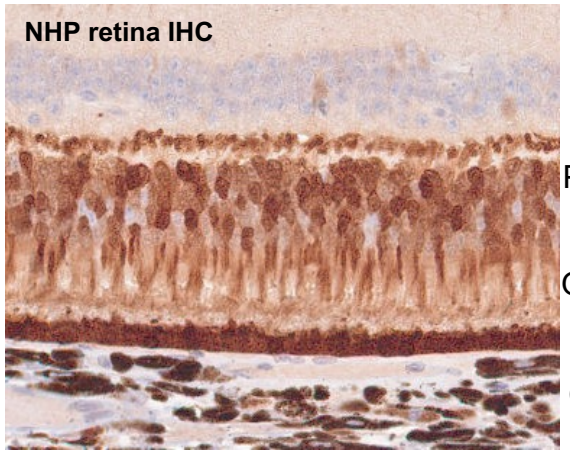
NHP OCT Imaging Following AAV Dosing



Dose: 1E11 vg in 100 uL per bleb
Weekly ocular imaging and functional assessment
Necropsy: 1 month

Transduction (GFP) in NHP retina validates capsid and regulatory elements

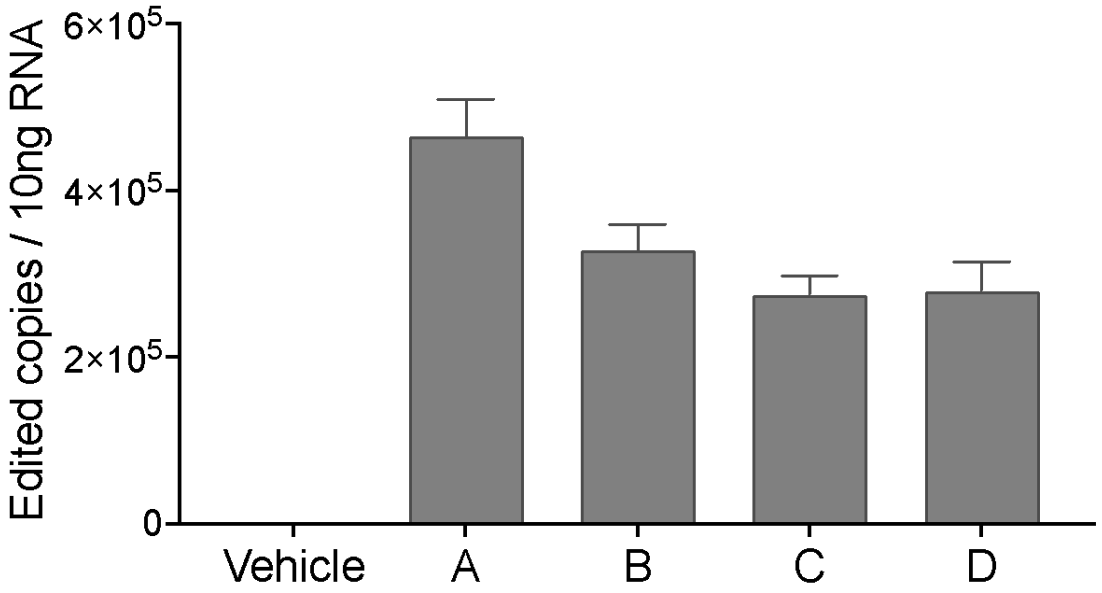




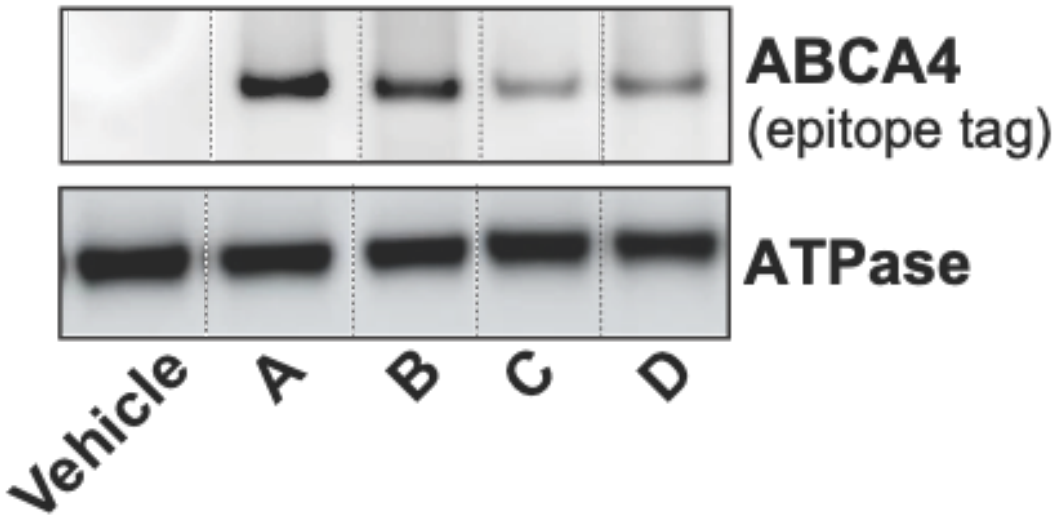
All animals treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research

Successful *in vivo* RNA exon editing and full-length protein production shown in NHP retinal samples

RT-qPCR analysis of human-NHP ABCA4 RNA *in vivo*

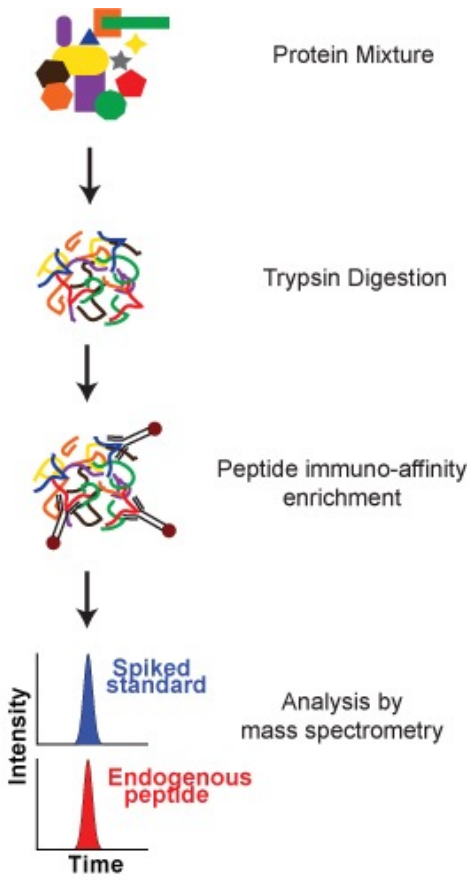


Representative ABCA4 western blotting demonstrating full length trans-spliced protein in retina samples

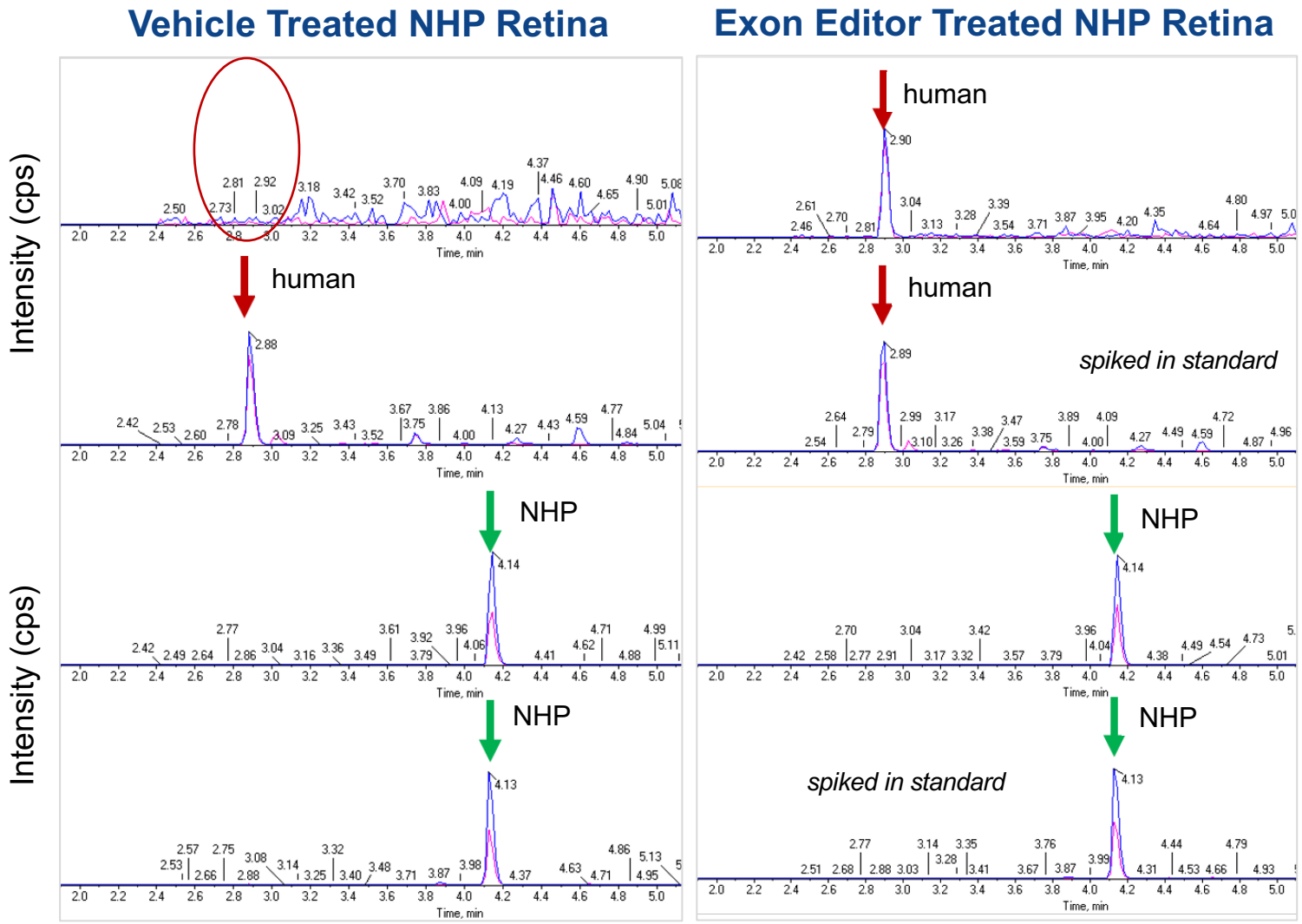


Dose: 1E11 vg in 100 uL per bleb
Weekly ocular imaging and functional assessment
Necropsy: 1 month

Further mass spec evidence confirms protein specificity



Zhao (2011) J. Vis. Exp.



Summary

- Ascidian technology enables precise post-transcriptional editing and replacement of multiple disease-causing exons
- This is the first reported demonstration of potentially therapeutically relevant RNA trans-splicing for any gene target in non-human primates
- These results have warranted continued development of RNA-based exon editing for ABCA4-related retinopathies
- Ascidian is developing a pipeline of programs across ophthalmology, neurology, neuro-muscular, and other rare diseases
- Exon editing and replacement potentially addresses constraints of gene therapies:

Large Genes	High Mutational Variation	Goldilocks Genes
• Replace only damaged exons, not whole gene • Addresses genes larger than 4.7kb AAV capsid capacity	• Edit whole exons at once, instead of individual bases • Address broader populations with each candidate	• Maintain native gene expression circuitry • Correct genes where over or off target expression is high risk

Acknowledgements

Rebekka Krumbach
Kelly Becker
Lai Mung Fong
Kirk Burkhart
Anika Pavis
Xiaojin Liu
Lingtao Peng
Akiko Doi
Kirk GosiK

John Bachmann
Bennett Bremer
Eric Carlin
Adam Clemens
Tayla Cormier
Conor Delaney
Yevgenia Khodor
Savita Matapurkar
Julia Meredith
Akiko Noma

Brian Olshaw
Madison Stulir
Dennis Wu
Jenny Yan
Qin Yu
Laura Barrio Real
Scott Dooley
Gabrielle Hillman
Krishna Jawaharial Fisher
Linda Liu
Bruce C. Schnepp

Jasmine Shong
Philip R. Johnson
James Faulkner
Jesse Gray
Crystal Shih Byers
Shimyn Slomovic
Daniel Rosan
Carrie Wager
Michael D. Ehlers

Thank You and Questions

Additional Ascidian Presentations at ASGCT 2022:

Abstract 300: Evaluation of ABCA4 RNA Exon Editing and Replacement in Non-Human Primate
Rebekka Krumbach, Ph.D.

Abstract 829:Optimization of Pre-mRNA Exon Editing for Efficient Rescue of Protein Expression
Kirk Burkhart, Ph.D.