Stereopure oligonucleotides produce potent and durable activity in the eye supporting their development for inherited retinal diseases

TIDES: Oligonucleotides and Peptide Therapeutics

Michael Byrne, PhD

Sept 18, 2020

WAVE™ LIFE SCIENCES

Forward-looking statements

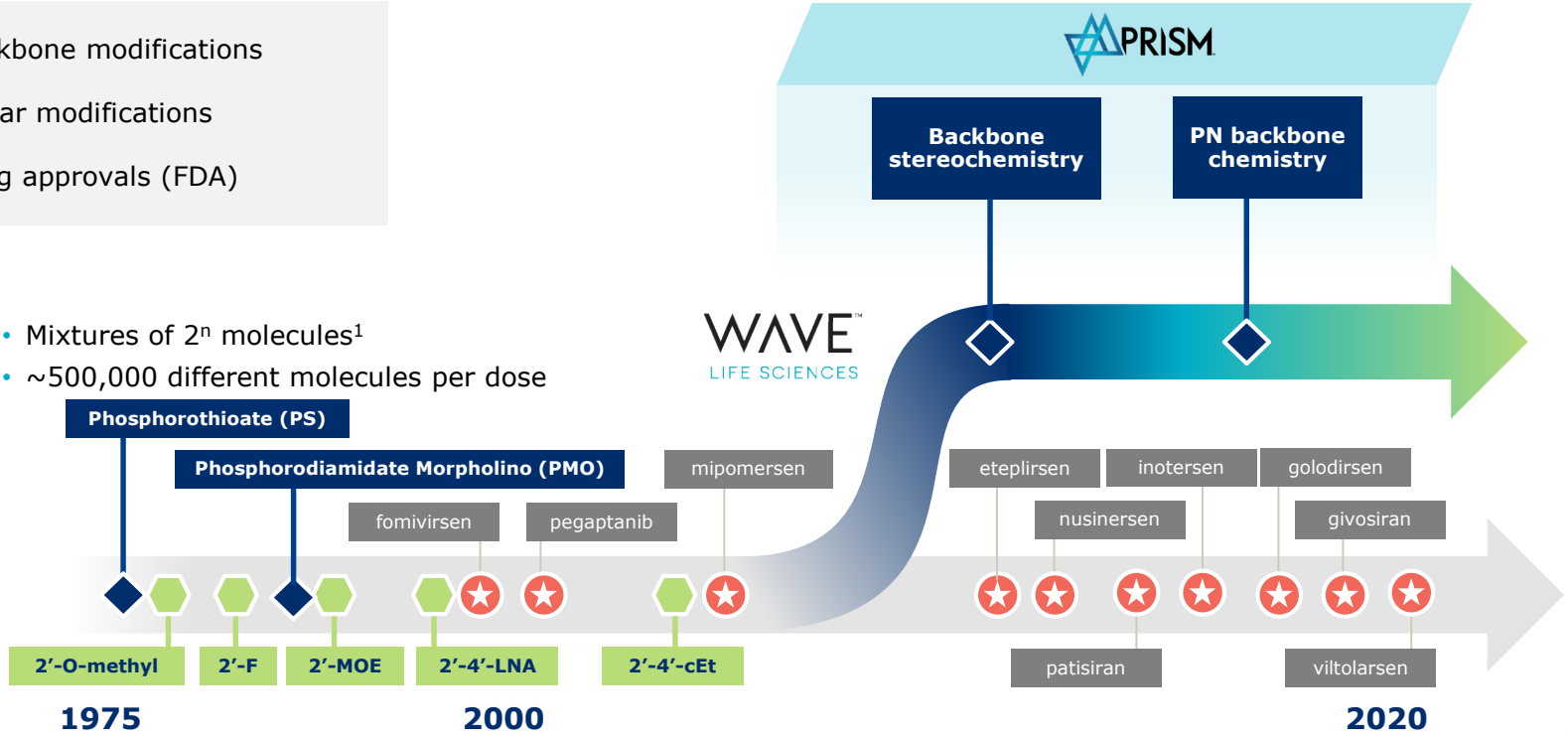
This document contains forward-looking statements. All statements other than statements of historical facts contained in this document, including statements regarding possible or assumed future results of operations, preclinical and clinical studies, business strategies, research and development plans, collaborations and partnerships, regulatory activities and timing thereof, competitive position, potential growth opportunities, use of proceeds and the effects of competition are forward-looking statements. These statements involve known and unknown risks, uncertainties and other important factors that may cause the actual results, performance or achievements of Wave Life Sciences Ltd. (the "Company") to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. In some cases, you can identify forward-looking statements by terms such as "may," "will," "should," "expect," "plan," "aim," "anticipate," "could," "intend," "target," "project," "contemplate," "believe," "estimate," "predict," "potential" or "continue" or the negative of these terms or other similar expressions. The forward-looking statements in this presentation are only predictions. The Company has based these forward-looking statements largely on its current expectations and projections about future events and financial trends that it believes may affect the Company's business, financial condition and results of operations. These forward-looking statements speak only as of the date of this presentation and are subject to a number of risks, uncertainties and assumptions, including those listed under Risk Factors in the Company's Form 10-K and other filings with the SEC, some of which cannot be predicted or quantified and some of which are beyond the Company's control. The events and circumstances reflected in the Company's forward-looking statements may not be achieved or occur, and actual results could differ materially from those projected in the forward-looking statements. Moreover, the Company operates in a dynamic industry and economy. New risk factors and uncertainties may emerge from time to time, and it is not possible for management to predict all risk factors and uncertainties that the Company may face. Except as required by applicable law, the Company does not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

WAVE LIFE SCIENCES

PRISM has unlocked novel and proprietary advances in oligonucleotide design

- Backbone modifications
- Sugar modifications
- Drug approvals (FDA)

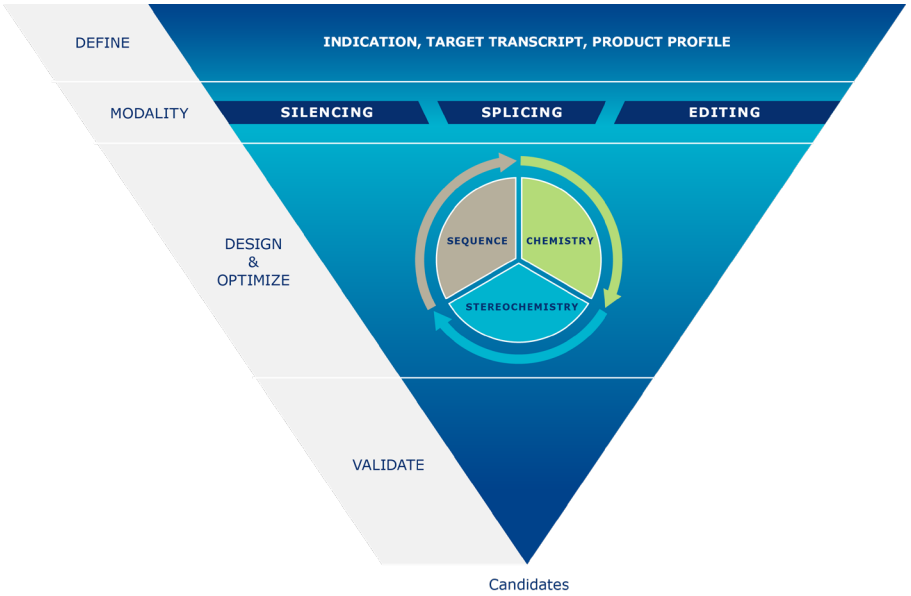
- Mixtures of 2^n molecules[1]
- ~500,000 different molecules per dose



WAVE LIFE SCIENCES

[1]n=number of chiral centers

Foundational value



- PRISM has the capability to address a wide variety of genetic diseases
- Each oligo is optimized based on our ability to control:
  - **Sequence**
  - **Chemistry**
  - **Stereochemistry**
- Manufacturing
- We continue to innovate with each new program, and these innovations have resulted in exciting preclinical results

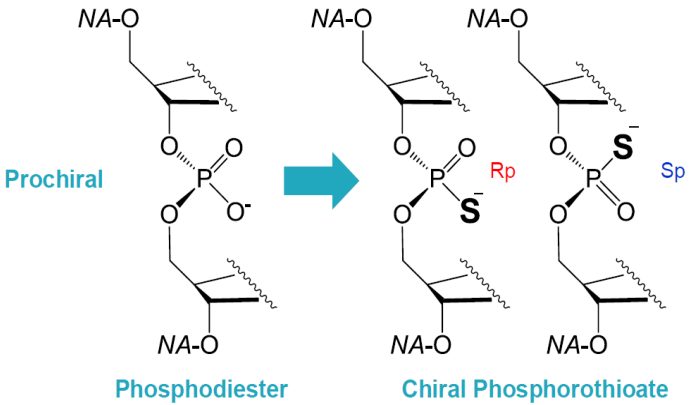
Continuous Learning

Platform improves with each iterative analysis of *in vitro* and *in vivo* Outcomes and predictive modeling

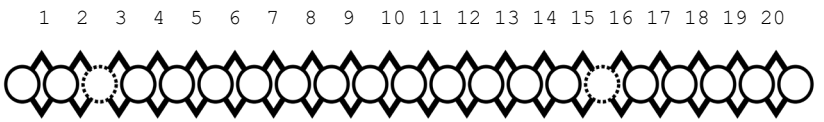
Stereopure oligonucleotides

Phosphorothioate (PS) modifications introduce chiral centers

An enormous number of permutations exist (2^n), often resulting in over 500,000 different molecules in every dose



Stereorandom ASO



2^{20} = 1,048,576 diastereoisomers

Stereopure ASO

1 diastereoisomer

Nucleotide

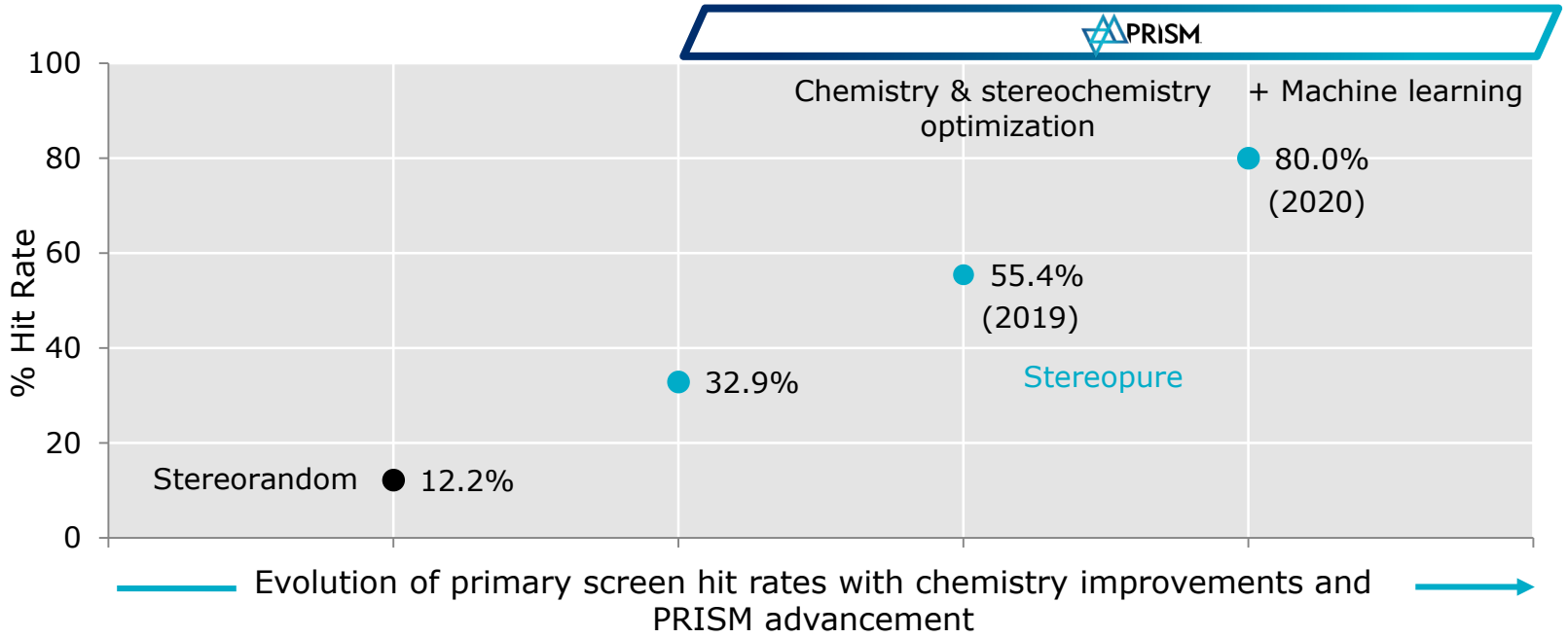
Stereorandom

Rp

Sp

PRISM platform advancements

Primary screen hit rates in neurons far above industry standard hit rates



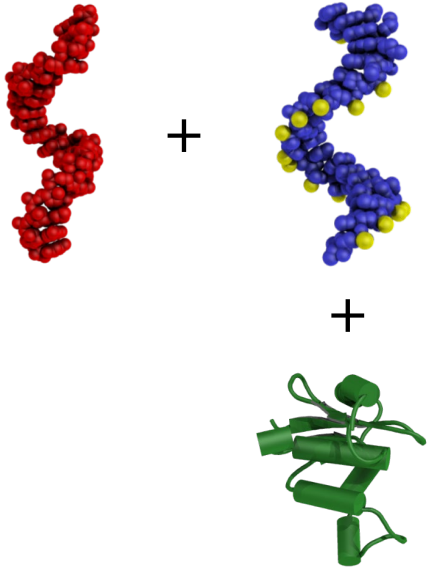
All screens used iPSC-derived neurons; Data pipeline for improved standardization. Hit rate = % of oligonucleotides with target knockdown greater than 50%. Each screen contains >100 oligonucleotides.

PRISM enables optimal placement of backbone stereochemistry

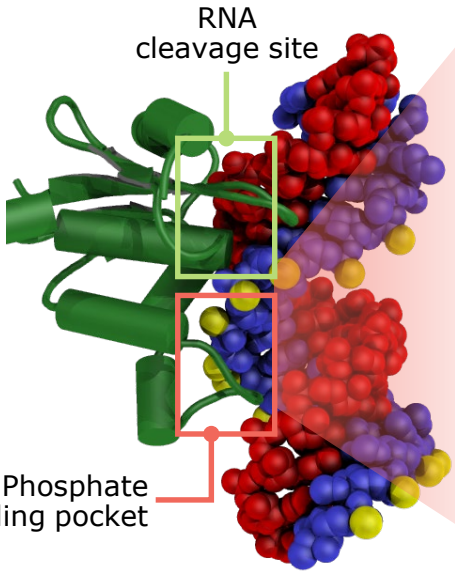
Crystal structure confirms phosphate-binding pocket of RNase H binds 3′-SSR-5′ motif in stereopure oligonucleotide – supports design strategy for Wave oligonucleotides

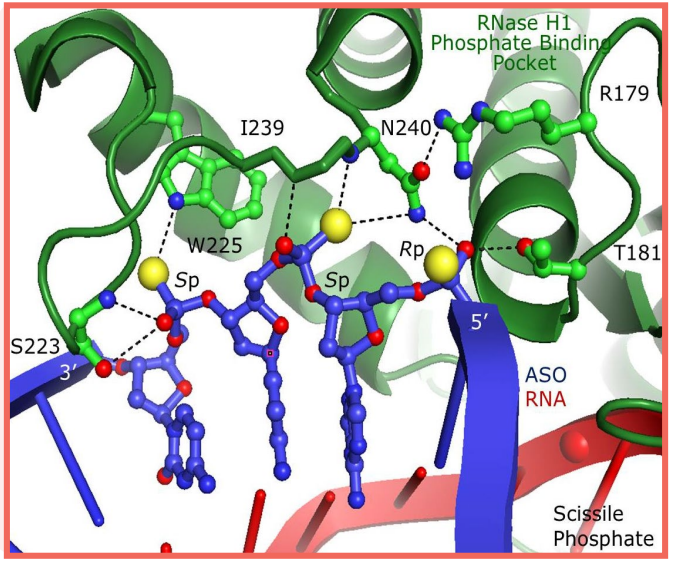
Target RNA

Stereopure Oligonucleotide (C9orf72 compound)



RNase H





ASO/RNA duplex

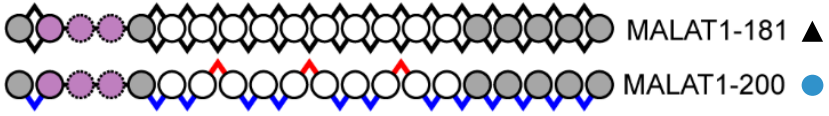
Yellow spheres represent ‘S’ atoms

WAVE LIFE SCIENCES

1.3 Å resolution

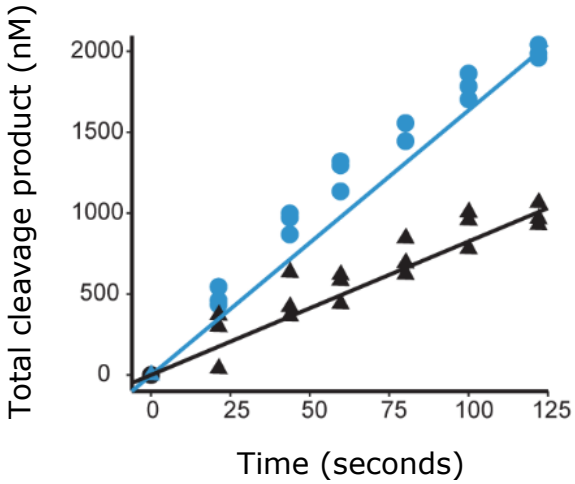
Precision RNase H-mediated RNA degradation

Stereopure oligonucleotide is more potent *in vitro*

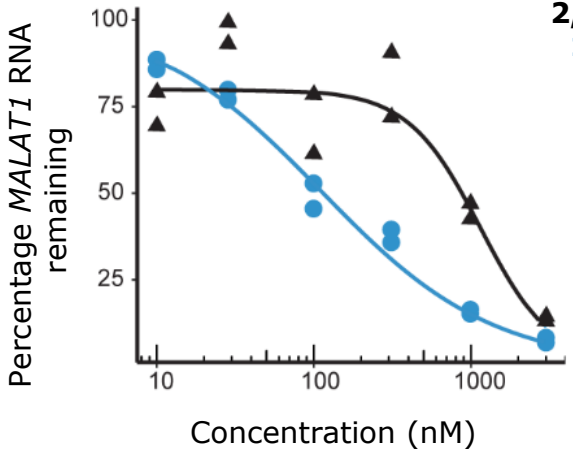


Backbone
- Stereorandom
- Rp
- Sp
- Phosphodiester

RNase H cell-free assay



***In vitro* dose-response**
iCell neurons



IC_{50}:
2,864 nM ▲
120 nM ●

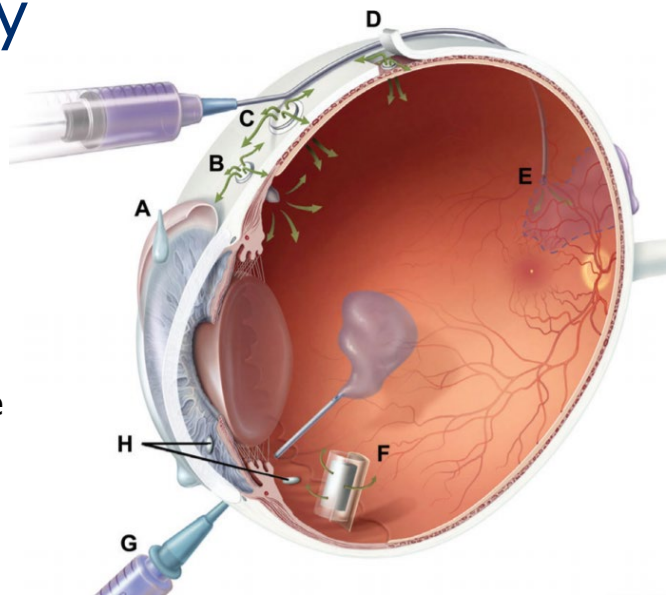
(Left) Time-dependent activity of RNase H1 in vitro on heteroduplexes formed between a complementary *Malat1* RNA and the stereopure (MALAT1-200) or stereorandom (MALAT1-181) oligonucleotide. V_0 were calculated from the slopes of the lines. N=3 per time point. (Right) Relative expression of *MALAT1* in iCell neurons after treatment with increasing concentrations of stereorandom or stereopure oligonucleotide. IC_{50}s were calculated from the best-fit curves. N=2.

V_0, initial velocity; IC_{50}, half-maximal inhibitory concentration.

Oligonucleotides in Ophthalmology

Stereopure oligonucleotides can:

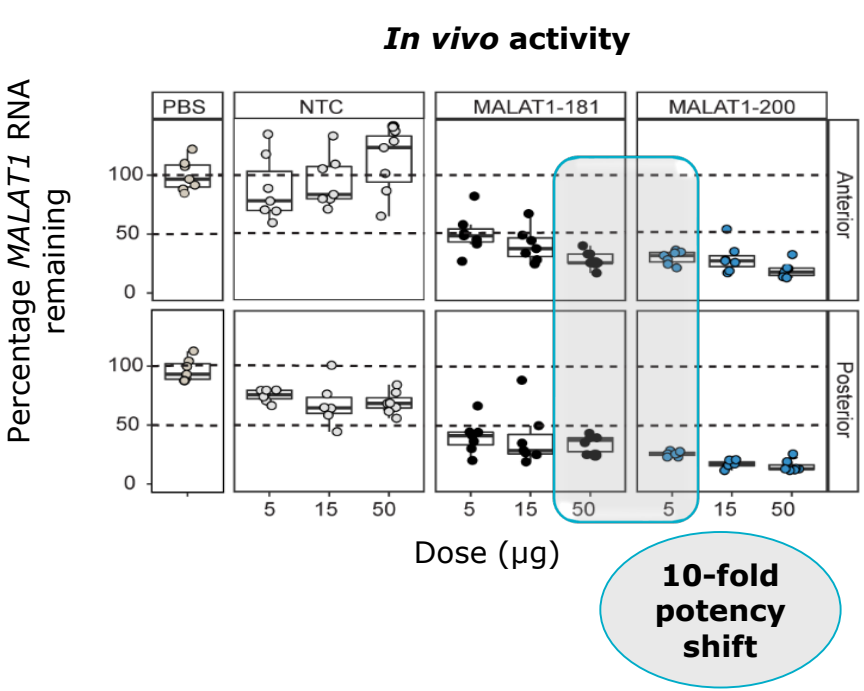
- be administered by intravitreal (IVT) injection during an office visit; targeting twice-per-year dosing
- open novel strategies in both dominant and recessive IRDs; potential for potent and durable effect with low immune response
- distribute in the vitreous to the central and peripheral retina
- penetrate all retinal layers and the RPE layer without aid of a delivery vehicle

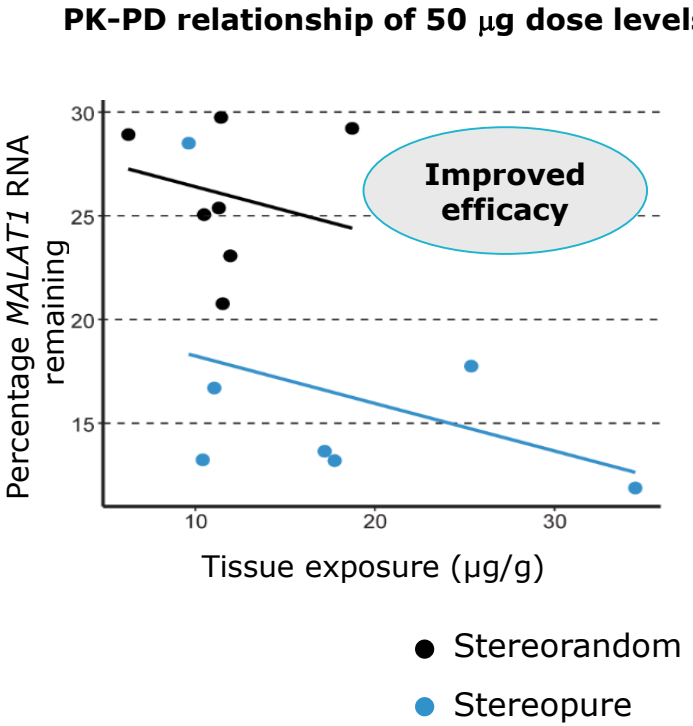


Intravitreal injection

Sources: Daiger S, et al. *Clin Genet*. 2013;84:132-141. Wong CH, et al. *Biostatistics*. 2018; DOI: 10.1093/biostatistics/kxx069. Athanasiou D, et al. *Prog Retin Eye Res*. 2018;62:1–23. Daiger S, et al. *Cold Spring Harb Perspect Med*. 2015;5:a017129. Verbakel S, et al. *Prog Retin Eye Res*. 2018:66:157-186.; Short, B.G.; *Toxicology Pathology*, Jan 2008.

Potency benefits translate *in vivo* in mouse eye 1-week post injection



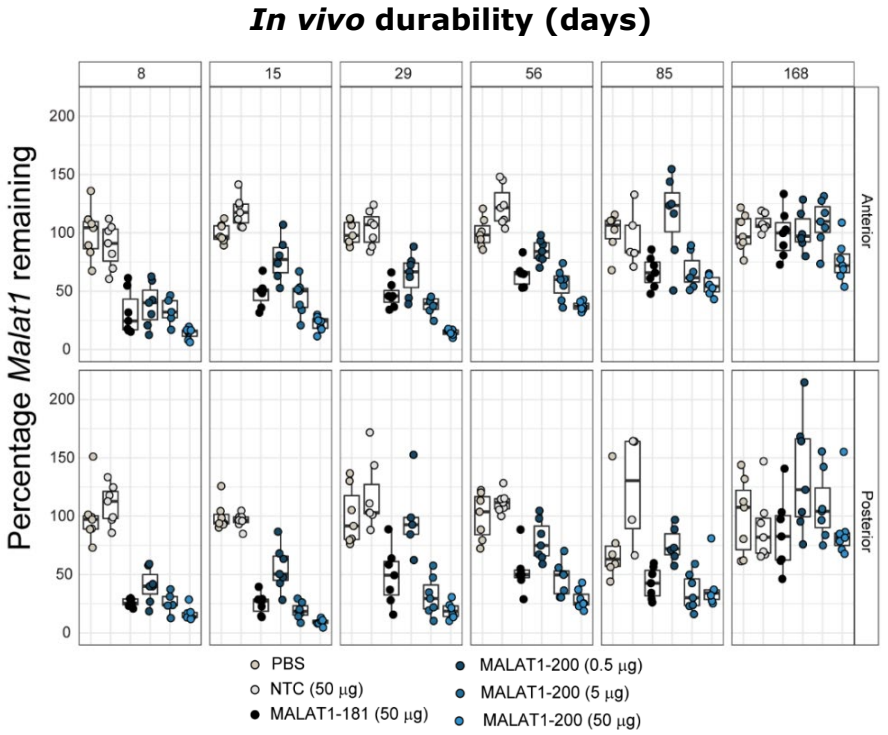


(Left) *In vivo* expression of *Malat1* in anterior (top) or posterior (bottom) compartments of mouse eyes one 1-week post-single IVT injection of PBS or the indicated doses of NTC, stereorandom (MALAT1-181), or stereopure (MALAT1-200) oligonucleotide. N≥6. (Right) PK-PD relationships for 50 ug doses of each oligonucleotide are shown. The percentage of *Malat1* remaining is plotted with respect to the concentration of oligonucleotide detected in the tissue. N=7. NTC, nontargeting control; PBS, phosphate-buffered saline; IVT, intravitreal

WAVE LIFE SCIENCES

Stereopure oligonucleotide is more durable *in vivo* in mouse eye

- 50 µg **stereorandom**: *Malat1* recovered to ~50% levels by **29 days**
- 50 µg **stereopure**: *Malat1* recovered by **day 85**
- 5 µg **stereopure**: *Malat1* recovered by **day 56** in the anterior and **day 85** in the posterior
- **5 µg and 50 µg doses of stereopure oligonucleotide led to more durable knockdown than 50 µg of stereorandom**

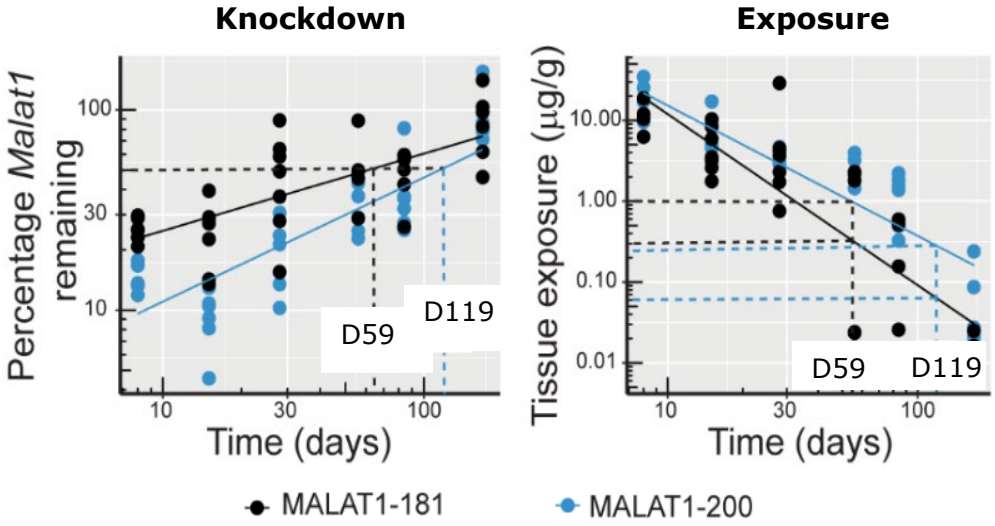


Percentage of remaining *Malat1* RNA after treatment at days 8, 15, 29, 56, 85, and 168 post-IVT injection. From left to right within each time point, data for treatment with PBS (beige), 50 µg NTC (gray), 50 µg stereorandom MALAT1-181 (black) or stereopure MALAT1-200 at 0.5 µg (dark blue), 5 µg (blue), and 50 µg (light blue) are shown. N=7

WAVE LIFE SCIENCES

Stereopure oligonucleotide yields greater knockdown *in vivo* in mouse eye, with better tissue exposure

- 50 µg **stereorandom** rises above 50% *Malat1* knockdown threshold at **day 59**
- 50 µg **stereopure** reaches 50% threshold at **day 119**
- **Stereopure** has greater tissue exposure than **stereorandom**
  - 4X at **day 59**
  - 6.5X at **day 119**

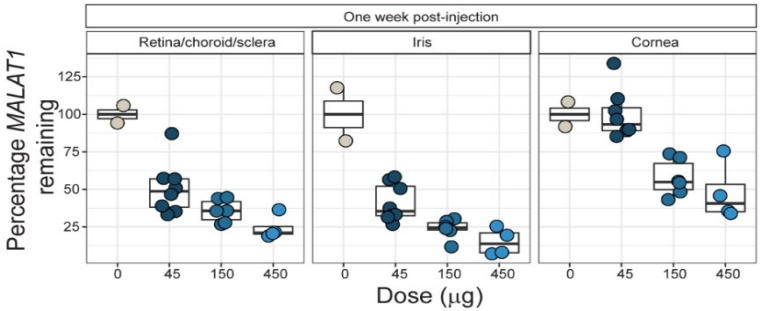


(Left) graph shows: the percentage *Malat1* remaining over time. Days 59 (black) and 119 (blue), when MALAT1-181- or MALAT1-200-treated samples return to 50% expression, respectively, are indicated by dotted lines. (Right) graph shows tissue exposure over time, with days 59 and 119 indicated by dotted lines. Data for stereorandom (black) and stereopure (blue) oligonucleotides are shown for all time points, days 8-168.

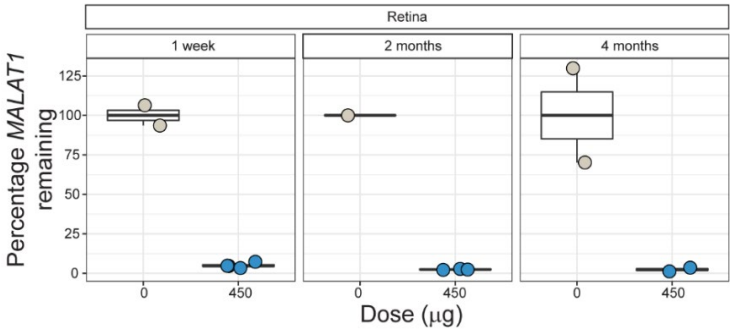
WAVE LIFE SCIENCES

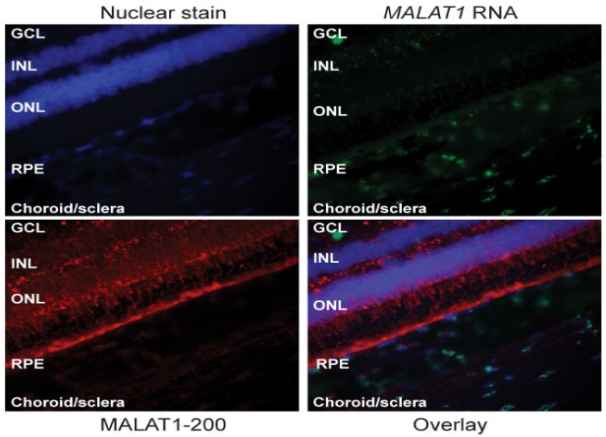
Stereopure oligonucleotide induces potent and durable activity in the NHP eye

Dose response (single dose)



Duration (single dose)

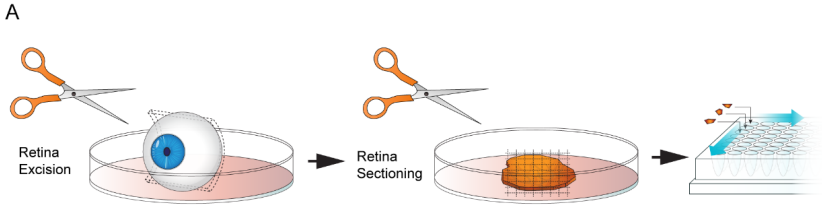


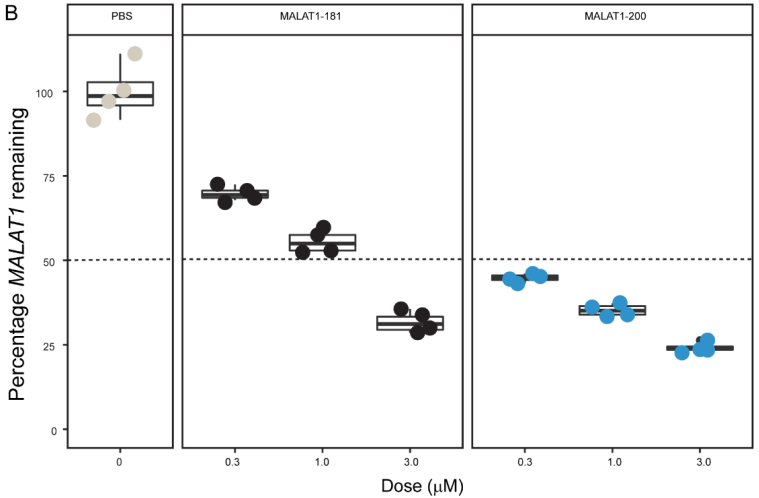


- **Stereopure** oligonucleotide was detected throughout the retina (GCL, INL, ONL, and RPE) 4 months after 450 µg injection
- *MALAT1* was detected at very low levels in the INL, GCL and ONL

(Left) Percentage *MALAT1* RNA expression at 1 week after a single IVT injection of PBS (beige, 0 µg) or 45, 150 or 450 µg stereopure oligonucleotide (blue, MALAT1-200) in NHP retina/sclera/choroid, iris, and cornea. N=2 (PBS), N=7 (oligonucleotide). (Right) *MALAT1* expression at 1 week, 2 months, and 4 months after IVT injection of PBS (beige, 0 µg) or 450 µg stereopure oligonucleotide (blue, MALAT1-200) in NHP retina. N=2 (PBS), N=3 (oligonucleotide).

Stereopure oligonucleotide shows efficacy and potency benefit in human retinal tissue *ex vivo*



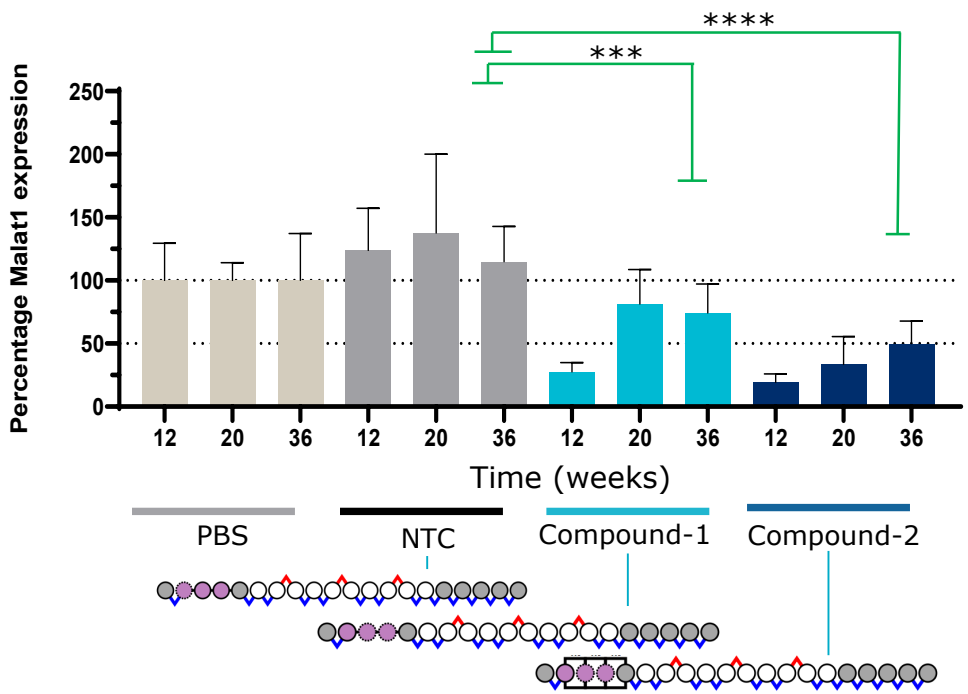


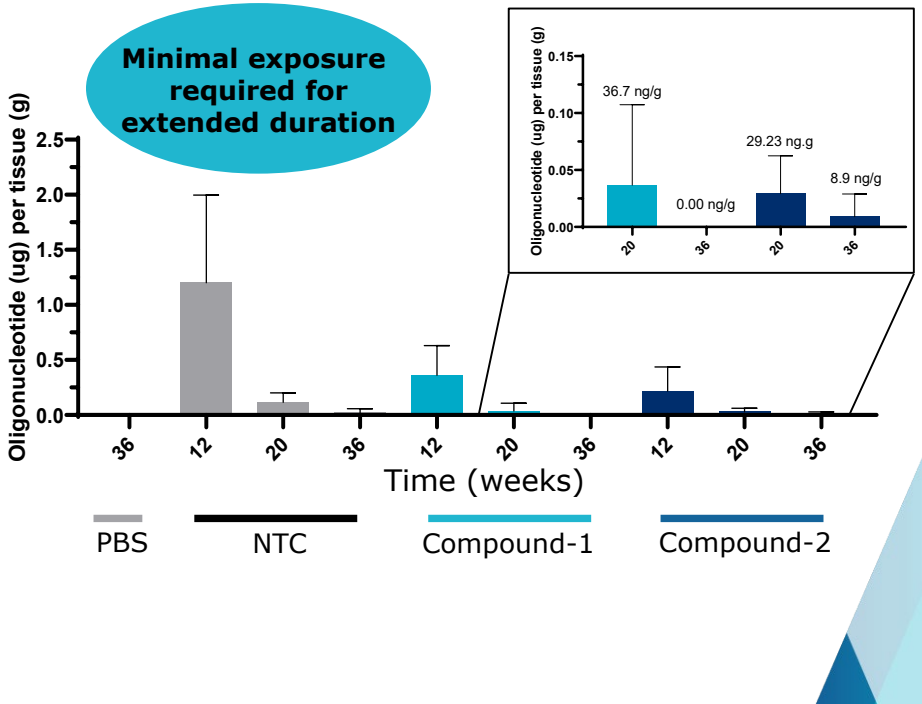
- **Stereopure** oligonucleotide was more active than **stereorandom** at 0.3 and 1 μM, with larger decrease *MALAT1* RNA expression (P<0.05)
- **0.3 μM stereopure** was more active than **1 μM stereorandom** (P<0.05)

Percentage *MALAT1* expression 48 hours after treatment with PBS, stereorandom (MALAT1-181) or stereopure oligonucleotide (MALAT1-200) in human retina tissue *ex vivo*. n=4. PBS, Phosphate-buffered saline

Stereopure oligonucleotides durably deplete *Malat1* for 9 months *in vivo* in mice

~50% *MALAT1* knockdown at 9 months in the posterior of the eye



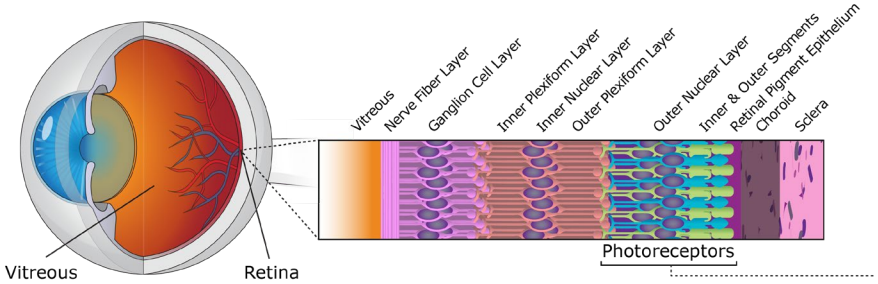


Compound or PBS (1 x 50 μg IVT) was delivered to C57BL6 mice. Relative percentage of *Malat1* RNA in the posterior of the eye (retina, choroid, sclera) to PBS-treated mice is shown at 12-, 20- and 36-weeks post-single injection. Statistics: *Malat1* levels are significantly different from NTC at 36 weeks ***, P<0.001 (Compound 1) **** P<0.0001 (Compound 2). PBS, phosphate buffered saline; NTC, non-targeting control; Compounds-1 and-2 are stereopure *MALAT1*-targeting antisense oligonucleotide.

WAVE LIFE SCIENCES

Autosomal dominant retinitis pigmentosa (adRP) associated with Rhodopsin P23H mutation

- Retinitis pigmentosa (RP) is a group of rare, genetic disorders of the eye resulting in progressive photoreceptor cell death and gradual functional loss
- Currently no cure for RP
- Rhodopsin accounts for >25% of adRP cases
- Approximately half of the RHO-associated adRP cases are caused by the P23H mutation
- Mutant P23H rhodopsin protein is thought to misfold and co-aggregate with wild-type rhodopsin, resulting in a gain-of-function or dominant negative effect in rod photoreceptor cells
- **~1,800 patients in US**



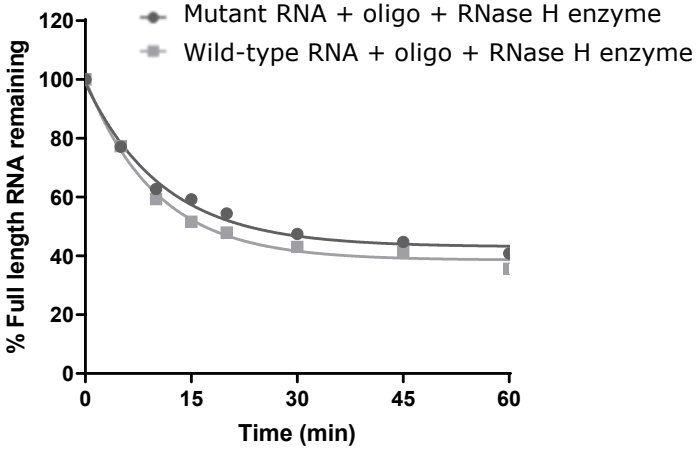
Allele-selective reduction of the mutant P23H allele while maintaining the wild type rhodopsin allele may prevent further cell loss.

WAVE LIFE SCIENCES

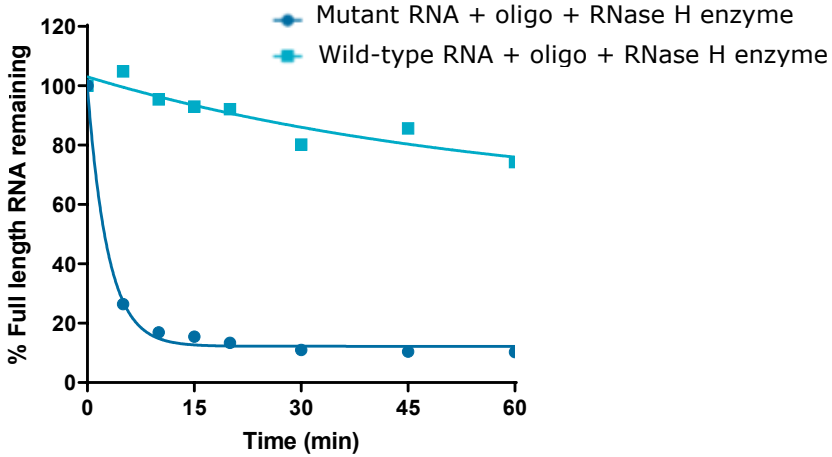
adRP associated with Rhodopsin P23H mutation

Stereopure oligonucleotides achieve allele-selective reduction of SNP-containing RNA in **RNase H biochemical assay**

Stereorandom



Stereopure



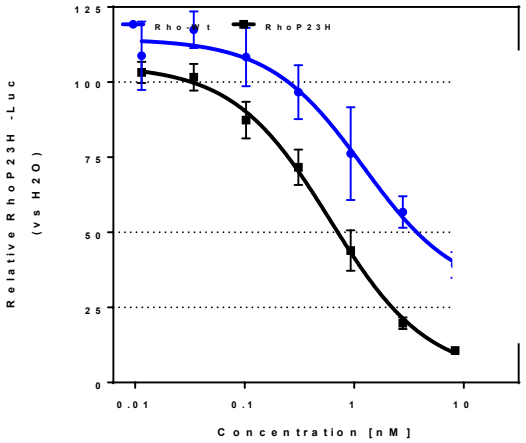
Stereopure oligonucleotide is allele selective; Stereorandom is not

Biochemical assays on a Wave stereopure sequence as well as a sequence described in WO2016138353A1. Oligonucleotide, mutant or wild type RNA and RNase H enzyme were incubated in a ratio of 250 : 1 substrate to enzyme for one hour. % of full-length RNA remaining was quantified by AUC of UV in LC/MS chromatogram.

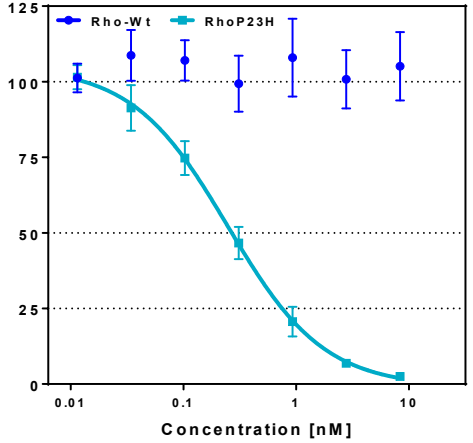
adRP associated with Rhodopsin P23H mutation

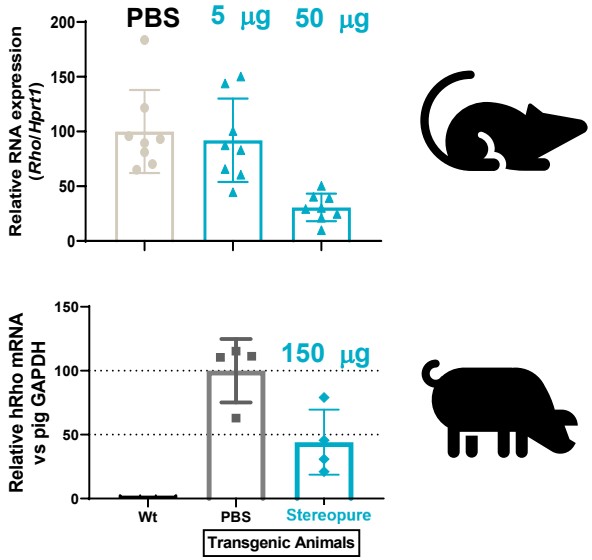
Stereopure oligonucleotide achieves selective knockdown of mutant allele

Stereorandom



Stereopure





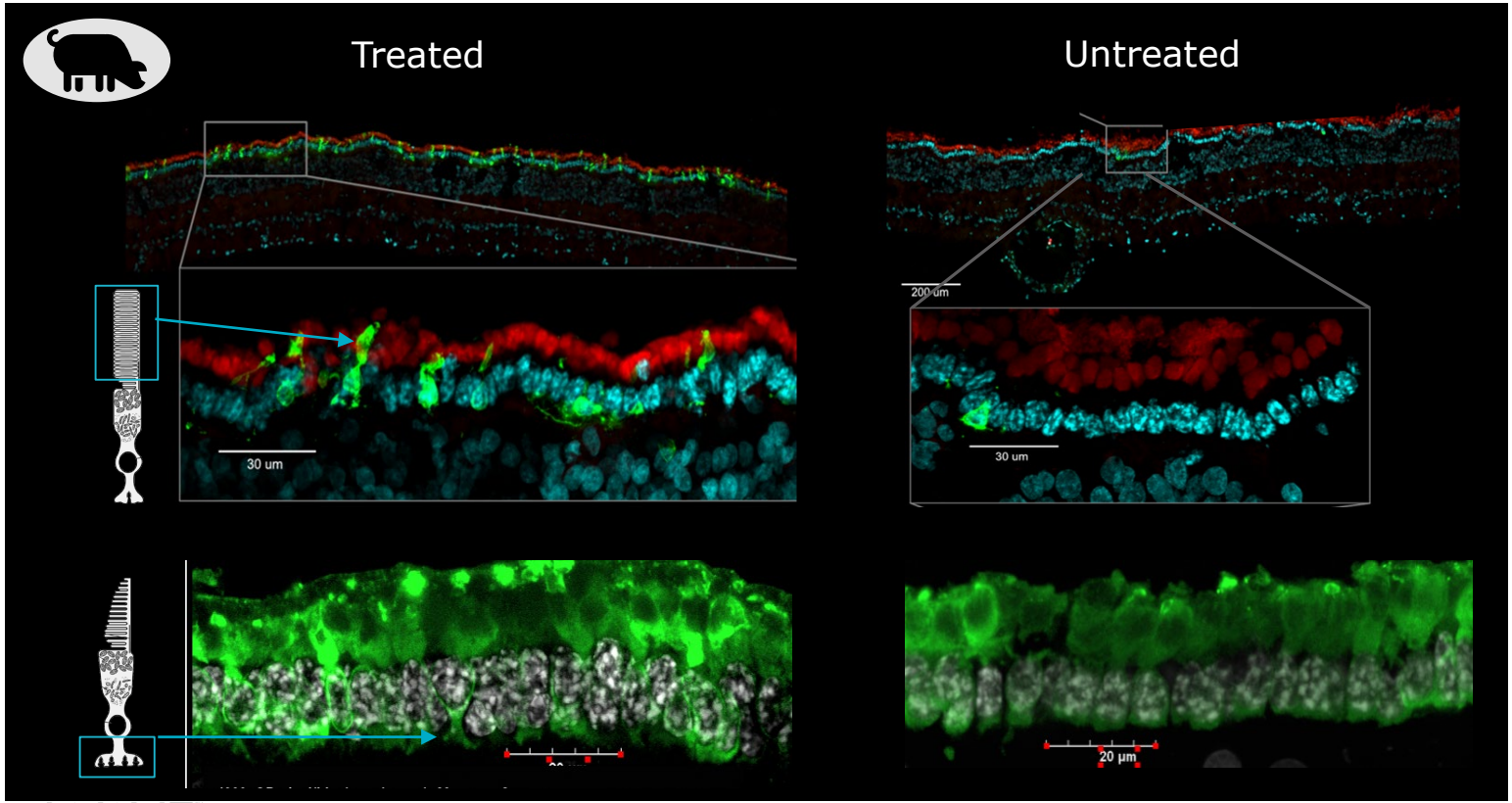
Stereopure oligo exhibits activity *in vivo in* P23H mouse model an human P23H pig model

(Left) Reporter assays on a sequence described in WO2016138353A1. Oligonucleotide and luciferase reporter plasmids (wild-type and mutant *RHO*) are transfected into Cos7 cells. Cells are harvested after 48 hrs, and relative luminescence is measured. (Right) Single IVT injection (1 µL) in mouse Rho P23H mouse model or (150 µL) in human P23H pig model. Eyes collected 1-week post injection for mouse or 2-weeks post injection for pig; RNA isolated; *Rho, Hprt1, and Gapdh* levels determined by qPCR.

WAVE LIFE SCIENCES

adRP associated with Rhodopsin P23H mutation

Stereopure oligonucleotide treatment retain rod outer segments and cone pedicles 16 weeks post single treatment



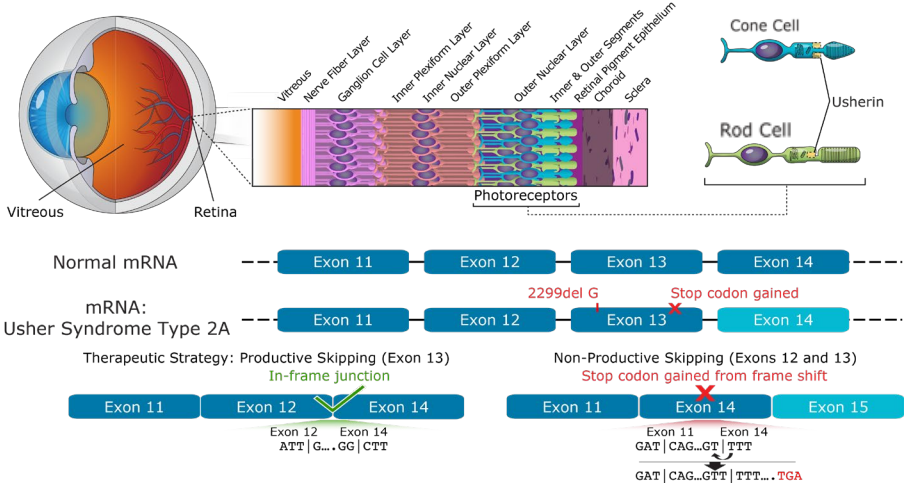
UNIVERSITY OF LOUISVILLE
McCall Lab

WAVE LIFE SCIENCES

Single IVT injection of 25 µL of human P23H targeting oligo in human P23H pig model. Eyes collected 16-weeks post injection. Eyes were enucleated and retina processed for immunohistochemistry. TOP: Red= PNA (cone cell marker); Green = rhodopsin (rod cell marker); Blue= Dapi (nuclear marker). Bottom: Green = Gnat (cone morphology).

Usher Syndrome Type 2A: a progressive vision loss disorder

- Autosomal recessive disease characterized by hearing loss at birth and progressive vision loss beginning in adolescence or adulthood
- Caused by mutations in USH2A gene (72 exons) that disrupt production of usherin protein in retina, leading to degeneration of the photoreceptors
- No approved disease-modifying therapies
- **~5,000 addressable patients in US**

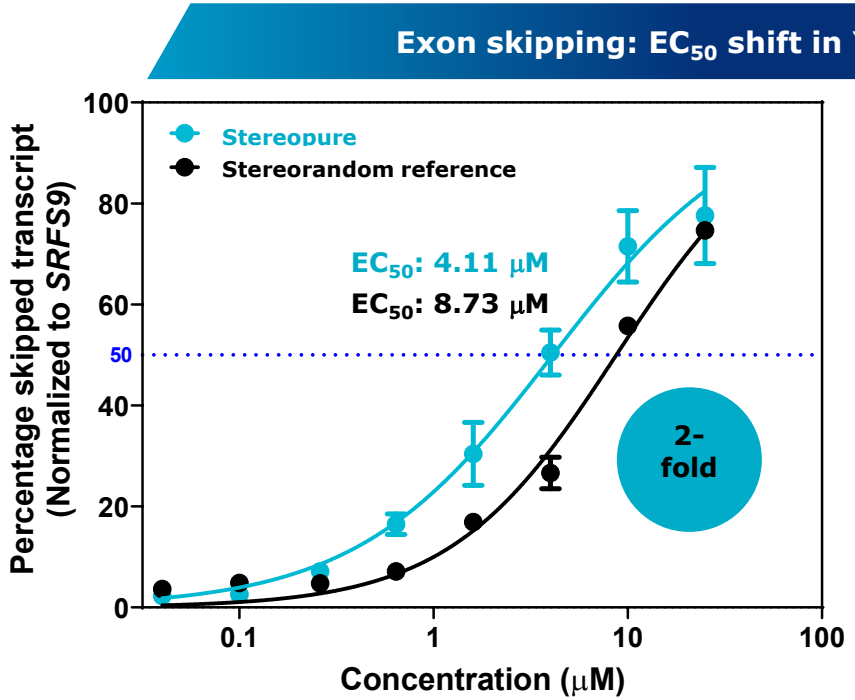


Oligonucleotides that promote *USH2A* exon-13 skipping may restore production of functional usherin protein

WAVE LIFE SCIENCES

Sources: Boughman et al., 1983. *J Chron Dis.* 36:595-603; Seyedahmadi et al., 2004. *Exp Eye Res.* 79:167-173; Liu et al., 2007. *Proc Natl Acad Sci USA* 104:4413-4418.

Stereopure oligonucleotide is more potent *in vitro*

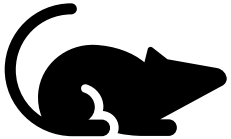


- **Stereorandom reference** from van Diepen *et al.*, 2018
- Dose-response curves in Y79 cells show potency benefit for **stereopure oligonucleotide**
- Oligonucleotides have different sequences and different chemistries, but both elicit *USH2A* exon-13 skipping

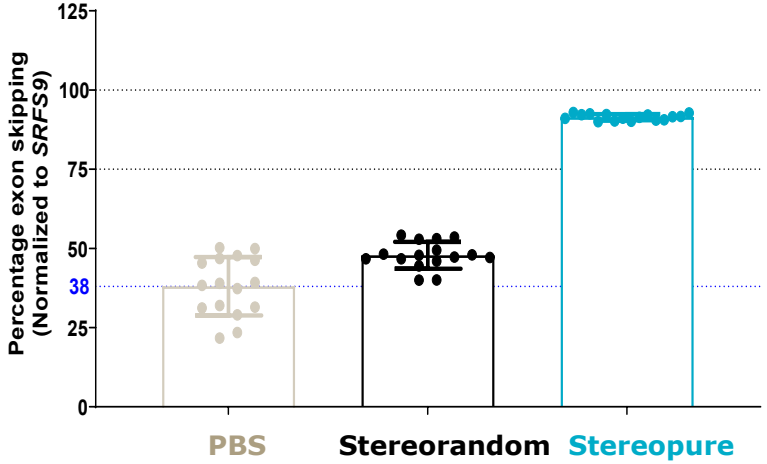
Oligonucleotides were added to Y79 cells under free-uptake conditions. Exon skipping was evaluated by Taqman assays. *USH2A* transcripts were normalized to *SRSF9*. Data are mean±s.d., n=2. Stereorandom: Compound identified in van Diepen *et al.* 2018. Antisense oligonucleotides for the treatment of eye disease. W02018055134A1. Stereopure: is a stereopure antisense oligonucleotide.

Stereopure oligonucleotide yields greater knockdown *in vivo*

2-fold efficacy improvement in mouse eye



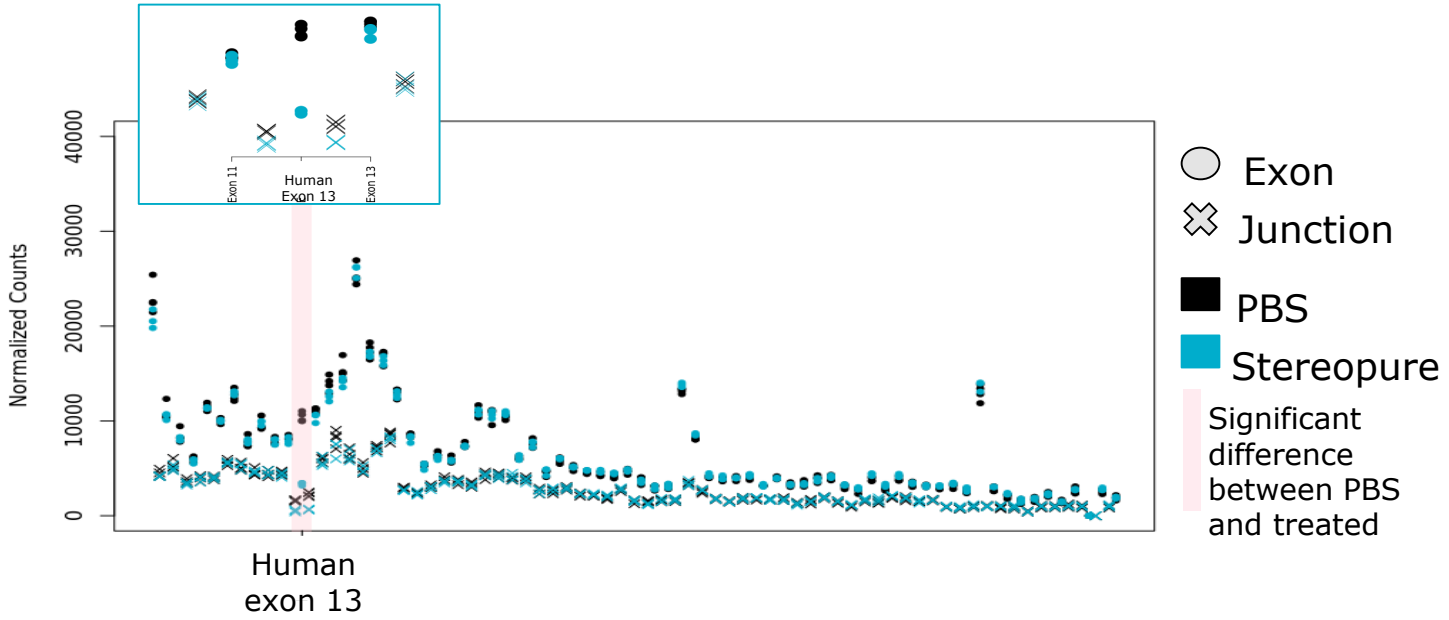
- Oligonucleotides tested in new mouse model with human *USH2A* exon 13
- Evaluated 1-week post-single 50 μg IVT injection
- Efficacy improvement with **stereopure** oligonucleotide



PBS or oligonucleotide (1 x 50 μg IVT) was injected to C57BL6 mice carrying human *USH2A* exon 13. One-week post injection, exon skipping was evaluated by Taqman assays. *USH2A* skipped transcript levels were normalized to *SRSF9*. Data presented are mean± s.e.m. Stereorandom compound is from van Diepen et al. 2018. Antisense oligonucleotides for the treatment of eye disease. W02018055134A1. Stereopure is an *USH2A* exon-13 skipping stereopure antisense oligonucleotide. PBS, phosphate buffered saline; IVT, intravitreal

WAVE LIFE SCIENCES

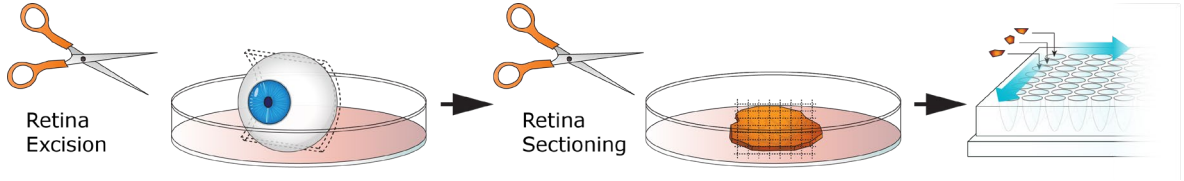
RNA-seq confirms integrity of RNA transcript *in vivo*



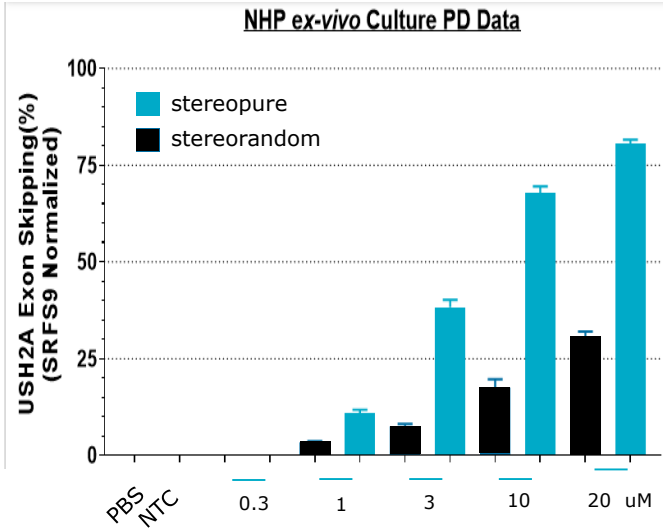
- **RNA-seq** performed
- Confirms **specific human exon-13 skipping** *in vivo* (in mouse model)
- Validates generation of **correct full *USH2A*-skipped transcript** post treatment

WAVE LIFE SCIENCES

Stereopure oligonucleotide yields greater knockdown *ex vivo*



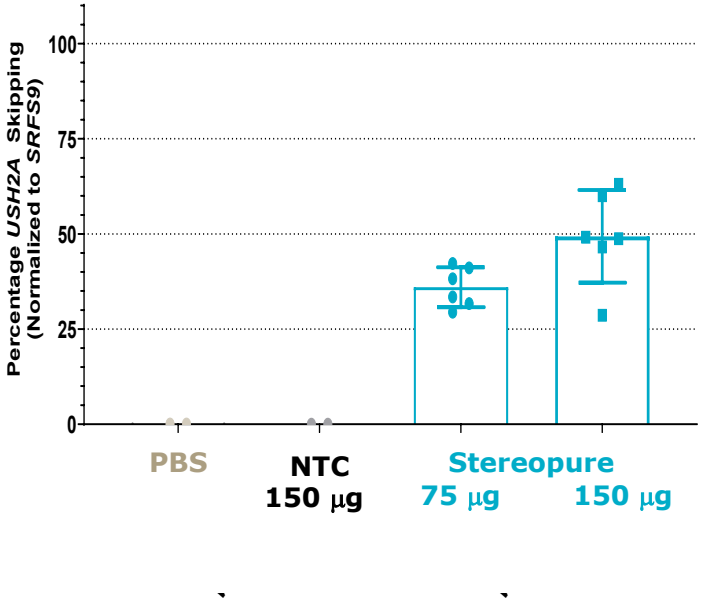
~ 3-fold efficacy improvement *ex vivo* in NHP retinal cultures



Whole NHP were enucleated (n=4) and compounds (1–20 μM) were added to extracted retinas under free-uptake conditions. Exon skipping was evaluated by Taqman assays on RNA. *USH2A* transcript levels were normalized to *SRSF9*. Data presented are mean± s.e.m. stereorandom compound is from van Diepen et al. 2018. Antisense oligonucleotides for the treatment of eye disease. W02018055134A1. Compound-1 is a stereopure antisense oligonucleotide.

Stereopure oligonucleotide elicits dose-dependent exon skipping in NHP *in vivo*

Dose-dependent and specific exon skipping in NHP eye



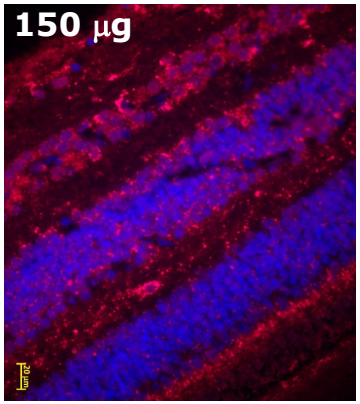
- Oligonucleotide is complementary to NHP *USH2A* exon 12*
- Evaluated 1-week post-single IVT injection
- Dose-dependent activity of **stereopure** oligonucleotides
- Substantial exposure in retina
- Exon-skipping integrity confirmed by RNA-seq at both doses

*NHP exon 12 = human exon 13

Stereopure *USH2A* skipping oligonucleotide, PBS or NTC antisense oligonucleotide was delivered to NHP by single IVT injection. One-week post-injection, retina was isolated and exon skipping was evaluated by Taqman assays. *USH2A* skipped transcript levels were normalized to *SRSF9*. Data are mean± s.e.m. Stereopure is an *USH2A* exon-13 skipping stereopure antisense oligonucleotide. PBS, phosphate buffered saline; NTC, non-targeting control; IVT, intravitreal

Visualization of oligonucleotide, skipped *USH2A* transcript and safety *in vivo* in NHP retina

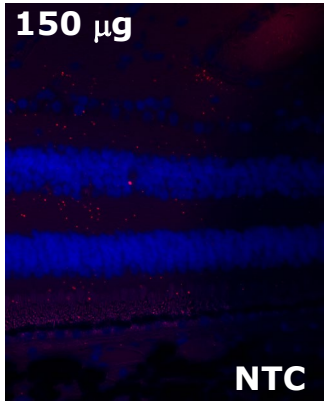
Stereopure oligonucleotide

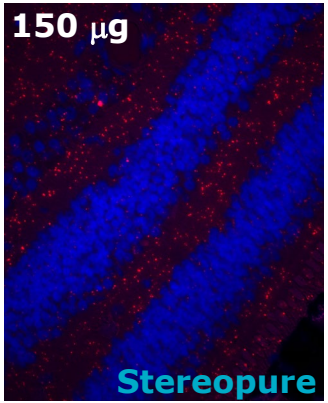


nuclei

oligonucleotide

USH2A-skipped transcript

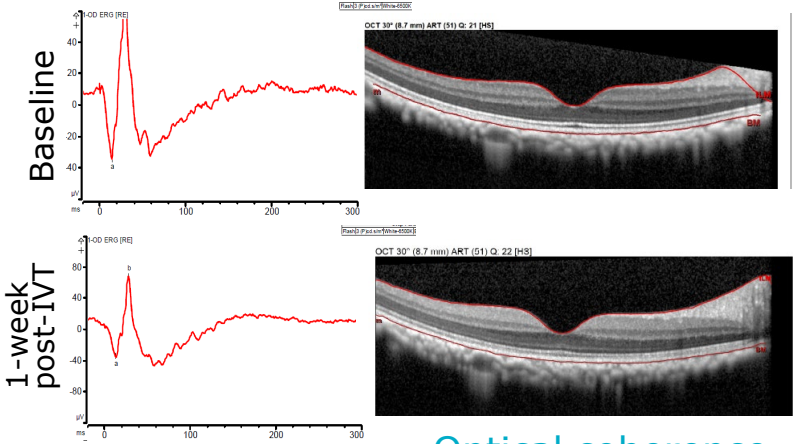




nuclei

USH2A transcript

Safety



Electroretinography

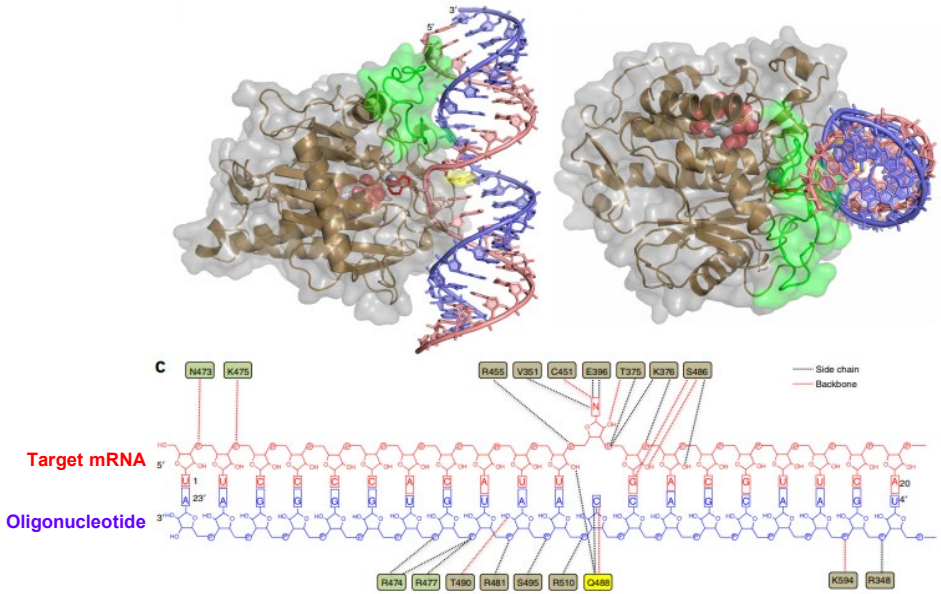
Optical coherence tomography

Stereopure *USH2A* skipping oligonucleotide oligonucleotide was delivered to NHP by single IVT injection. One-week post-injection, eyes were processed for histology. After sectioning, slides were stained with hematoxylin (blue) to identify nuclei and ViewRNA (red) for stereopure oligonucleotide (left) or skipped transcript (right). Staining is Fast Red substrate. Images are 40X under oil. 20 μm scale bars are shown.

PBS, phosphate buffered saline; NTC, non-targeting control; IVT, intravitreal

Using PRISM to unlock ADAR-mediated RNA editing

Structure of ADAR deaminase domain bound to dsRNA substrate

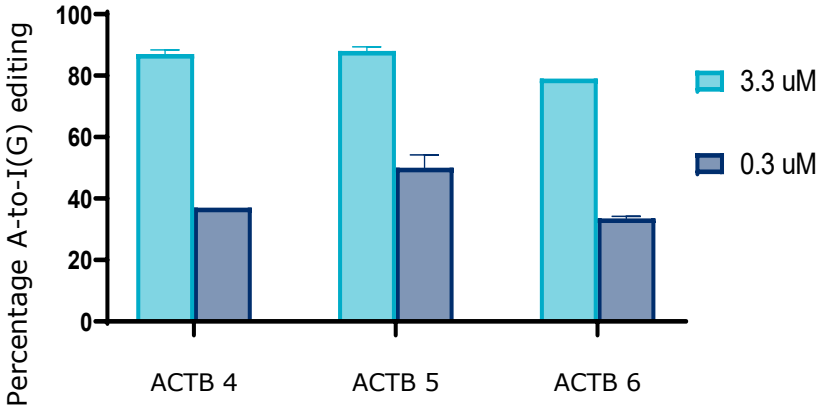


- ADAR is an endogenously expressed class of enzymes that catalyzes conversion of adenosine (A) to inosine (I) on dsRNA substrates
- Inosine (I) is recognized as guanosine (G) by cellular translation and splicing machinery
- ADAR makes multiple contacts with oligonucleotide backbone, sugar and bases
- Using PRISM platform, we rationally designed and screened oligonucleotides to optimize:
  - 2' sugar chemistry
  - Backbone chemistry and stereochemistry
  - Size and structure
  - Modified nucleobases

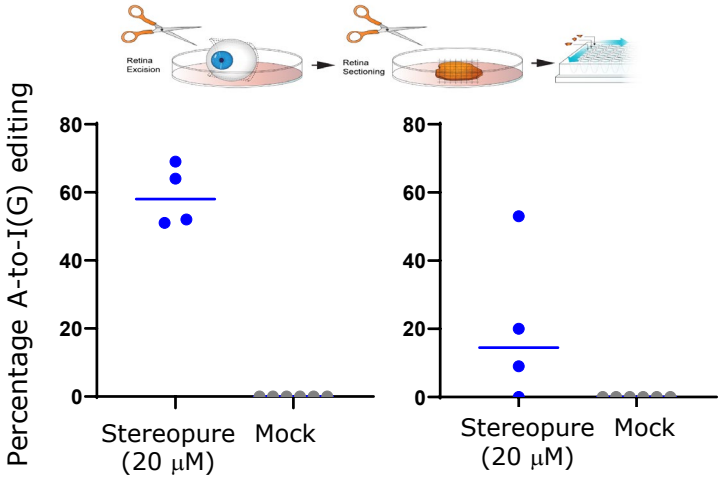
Structure adapted from Matthews et al., Nat Struct Mol Biol. (2016); SAR, structure-activity relationship; ADAR, Adenosine Deaminase Acting on RNA; dsRNA, double-stranded RNA

Stereopure oligonucleotides direct sequence-specific RNA editing *in vitro* and *ex vivo*

Dose-dependent *ACTB* editing
iCell neurons



***ACTB* editing in NHP retina**
Ex vivo



Oligonucleotides were added to iCell neurons for 7 days. RNA was collected, reverse transcribed to cDNA, relevant target region on *ACTB* transcript was PCR amplified and analyzed by Sanger sequencing. Editing was determined using EditR program (Kluesner et al., 2018, The CRISPR Journal).
Whole NHP were enucleated (n=4-5) and compounds (20 uM) were added to retinas under free-uptake conditions. RNA was collected 48 hrs later. Editing determined as described above.

Summary

- Using *MALAT1* for Proof of concept, we show that stereopure RNase H-active oligonucleotides:
  - Are more potent *in vitro* and *in vivo* in mice and NHP
  - Are more durable *in vivo* in mice and NHP
  - Have superior tissue exposure profile in the eye compared with stereorandom
  - Have improved durability through PRISM-driven chemistry advances
- Based on *MALAT1* studies
  - Generated allele-selective oligonucleotide targeting *RHO* P23H, with potential application in ad retinitis pigmentosa
- Application of PRISM to exon skipping oligonucleotides
  - Generated oligonucleotide targeting *USH2A* exon 13, with potential application for Usher syndrome type II
  - Demonstrated exon skipping in multiple *in vitro* and *in vivo* models
  - Showed skipped transcript contains all expected exons
- Application of PRISM to ADAR editing oligonucleotides
  - Demonstrated *in vitro*-active oligos exhibit editing in NHP retina *ex vivo*