Exploring new oligonucleotide backbone chemistries and their deployment to improve the properties of stereopure oligonucleotides

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Chief Technology Officer

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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.

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PRISM platform enables rational drug design

Sequence

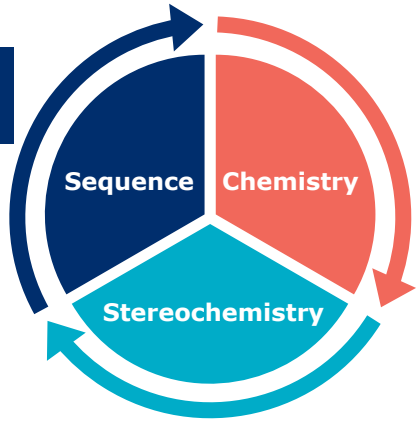
B: bases

A, T, C, mC, G, U,
other modified bases

Stereochemistry

Chiral control of
any stereocenter

Sugar or
backbone modifications



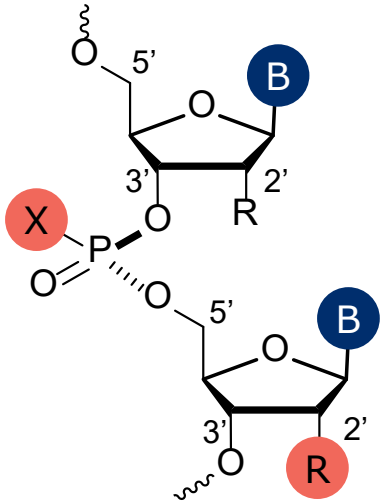
Chemistry

R: 2′ modifications

OMe, MOE, F,
other modifications

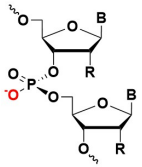
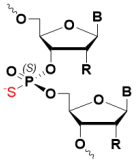
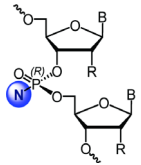
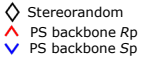
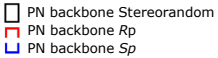
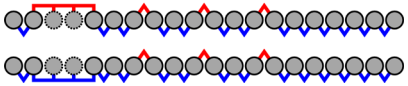
X: backbone chemistry

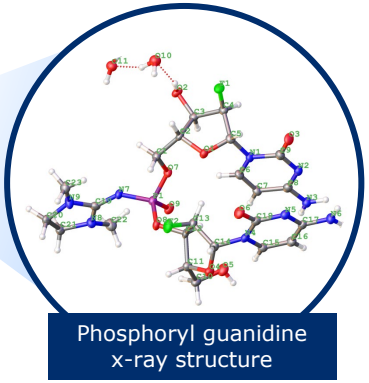
Phosphodiester (PO),
phosphorothioate (PS),
other backbone
modifications



Expanding repertoire of backbone modifications with novel PN backbone chemistry

Backbone linkages

	PO	PS	PN
Backbone modification (X)	Phosphodiester 	Phosphorothioate 	Phosphoramidate diester 
Stereochemistry	Not chiral	Chiral 	Chiral 
Charge	Negative	Negative	Neutral
Depiction			
PRISM backbone modifications	PO/PS		
	PO/PS/PN		

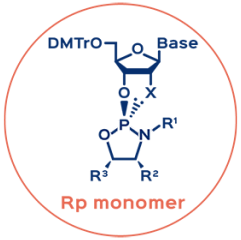


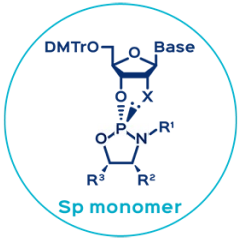
Phosphoryl guanidine x-ray structure

Molecule structure illustrative of backbone modification patterns

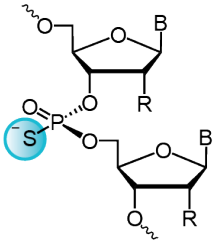
New building blocks and chemistry support synthesis of chimeric stereopure backbones

Versatility in chemistry

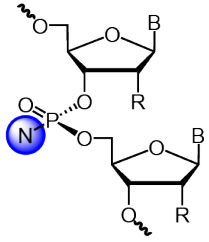




Chiral backbone chemistries

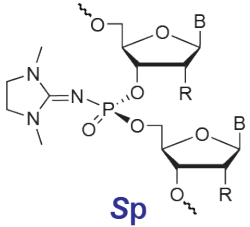


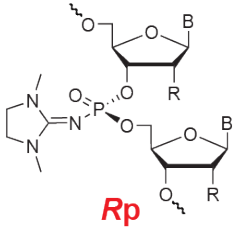
Phosphorothioate diester (PS)



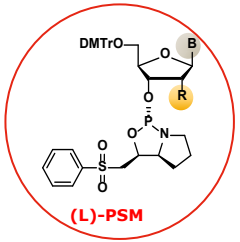
Phosphoroamidate diester (PN)

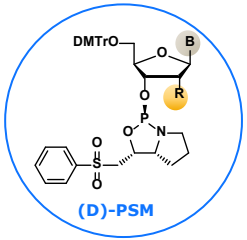
Introducing stereopure PN linkages





Growing library of amidites





B Nucleotide base

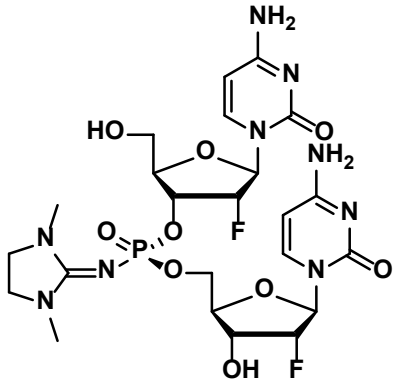
A T G C U ^{5Me}C

R 2′-ribose modification

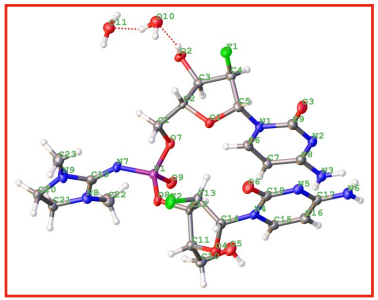
F MOE OMe LNA H

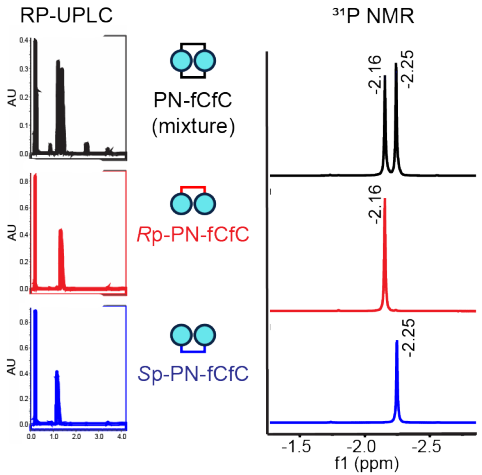
L-PSM (*S*)-2-(phenylsulfonyl)-1-((*S*)-pyrrolidin-2-yl)ethan-1-ol, D-PSM (*R*)-2-(phenylsulfonyl)-1-((*R*)-pyrrolidin-2-yl)ethan-1-ol, L-DPSE (*S*)-2-Diphenylmethylsilylethyl; F 2′-deoxyfluoro; MOE 2′-O-methoxyethyl; Ome 2′-O-Methyl; LNA locked nucleic acid; H 2′-deoxyribose

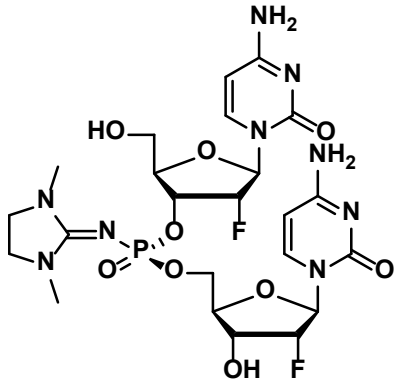
Chemical and structural validation of dimer stereochemistry



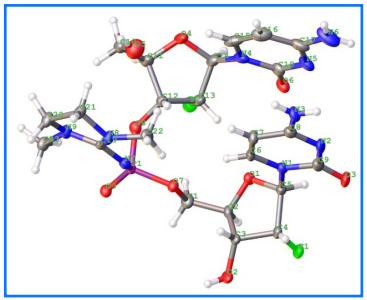
PN-*Rp*-fCfC dimer





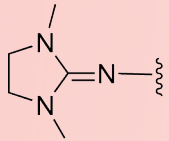


PN-*Sp*-fCfC dimer

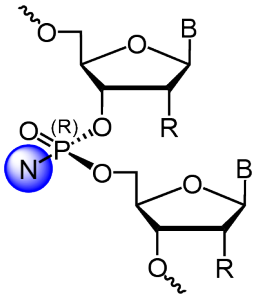


For all dimers evaluated, diastereoselectivity >99%
RP-UPLC, reversed-phase ultra performance liquid chromatography; 31P-NMR phosphorus-31 nuclear magnetic resonance

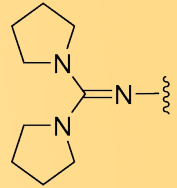
We are exploring multiple types of PN chemistry



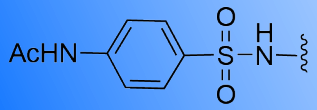
PN-1
(1,3-dimethylimidazolidin-2-ylidene)phosphoramidate



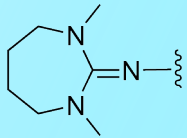
PN-5
(di)pyrrolidine-1-yl)methylene) phosphoramidate



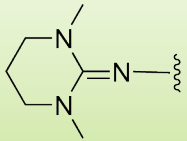
PN-2
((4-acetamidophenyl)sulfonyl) phosphoramidate



PN-4
(1,3-dimethyl-1,3-diazepan-2-ylidene)phosphoramidate



PN-3
(1,3-dimethyltetrahydropyrimidin-2(1H)-ylidene)phosphoramidate



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Kandasamy et al., submitted

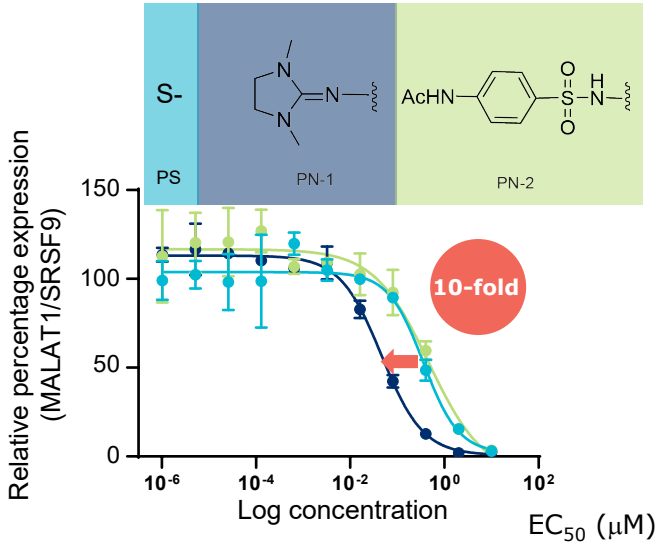
PN chemistry increases potency *in vitro* 10-fold

PRISM

Silencing

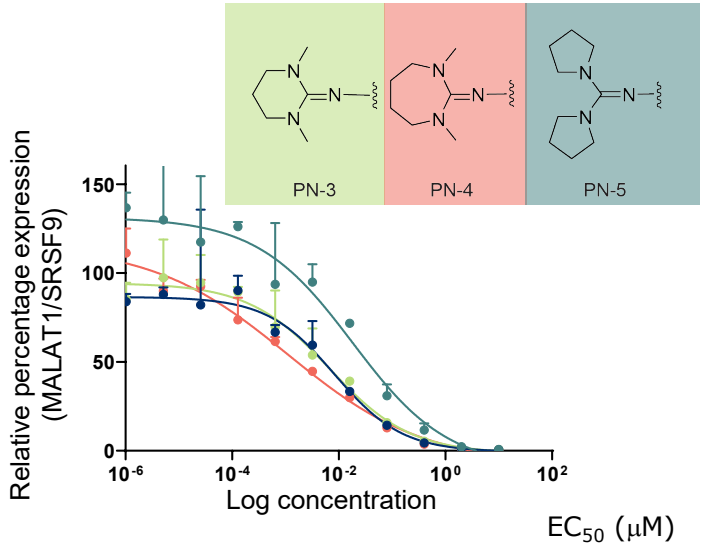
RNase H

Potency



EC_{50} (μM)

		EC_{50} (μM)
MALAT1-200		0.36
MALAT1-384		**0.04**
MALAT1-489		0.46



EC_{50} (μM)

		EC_{50} (μM)
MALAT1-384		0.01
MALAT1-1044		0.01
MALAT1-1045		0.001
MALAT1-1043		0.02

Backbone modifications

- Stereorandom PS linkage
- *Rp* PS linkage
- *Sp* PS linkage
- Phosphodiester linkage
- Stereorandom PN linkage
- *Rp* PN linkage
- *Sp* PN linkage

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iCell neurons were treated with increasing concentrations of oligonucleotide. MALAT1 RNA was normalized to SRSF9. Mean ± sd are shown, n=3 per concentration. Half maximal effective concentrations (EC_{50}) are calculated with GraphPad Prism software.

Across many modalities, PN-1 chemistry enhances potency, exposure, and durability

Modality		Pharmacology	
Silencing	• Efficient engagement of RNase H or AGO2	Potency	• Target knockdown, splicing or editing
Splicing	• Efficient uptake in the cell nucleus	Exposure	• In the right tissues, cells and cellular compartments
Editing	• Efficient engagement of ADAR	Durability	• Enabling infrequent administration

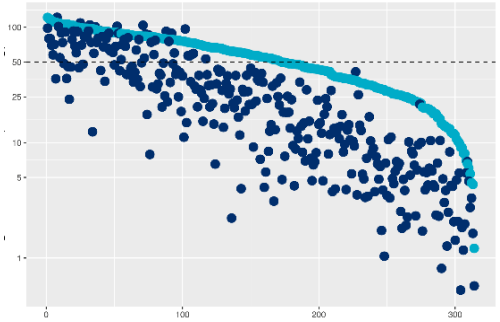
PN-1 chemistry increases potency in silencing, splicing, and editing preclinical studies

PRISM

Potency

Silencing

Target knockdown (% remaining)

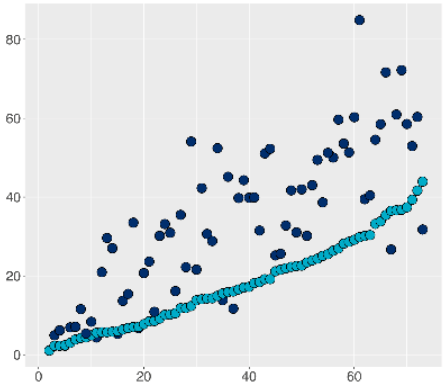


Improved knockdown

Ranked by potency of reference PS/PO compound

Splicing

% Skipping

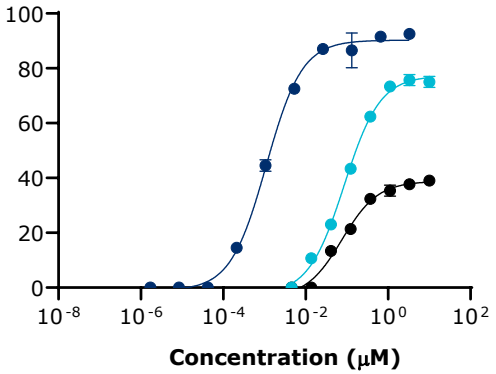


Improved skipping

Ranked by potency of reference PS/PO compound

Editing

% Editing



Improved editing

PS/PO/PN

PS/PO (Stereopure)

PS/PO (Stereorandom)

PS/PO reference compound

PS/PN modified compound

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Presented at Analyst & Investor Research Webcast on August 25, 2020; Right: Data from independent experiments

PN-1 chemistry increases potency and durability in mouse CNS for multiple targets

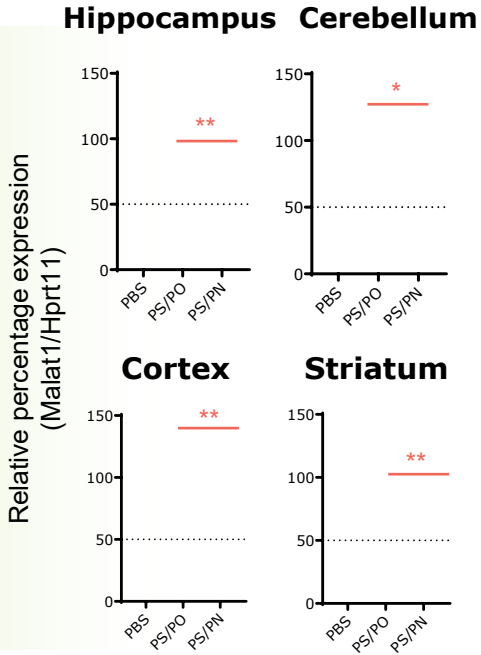
PRISM

Silencing
RNase H
Durability

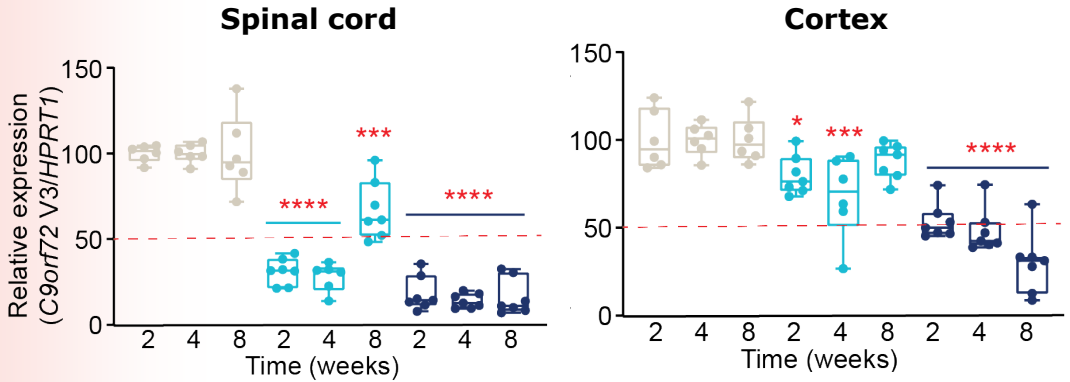
Low dose leads to Malat1 and C9orf72 knockdown in CNS

PBS
PS/PO
PS/PN

MALAT1



C9orf72



- PN-1 increases potency and durability throughout CNS
- Application of PN-1 to WVE-004 (C9orf72) extended duration to at least 6 months

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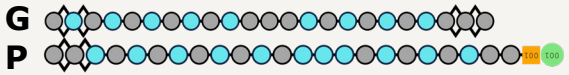
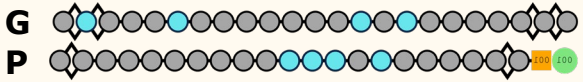
(Left) Mice received a single 100 μg ICV injection (n=3 per group). Relative percentage Malat1 expression (normalized to Hprt1) is shown for the indicated tissues 10-weeks post-dose. Stats: 1-way ANOVA. (Right) C9BAC mice received 2 x 50 μg ICV injection (n=7 per group). C9orf72 V3 is normalized to Hprt1. Stats: 2-way ANOVA *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 PBS, phosphate buffered saline

RNAi-dependent silencing modality

ESC	Advanced ESC
	
• 2’-OMe & 2’-F ribose • Terminal PS modifications • GalNAc-modified passenger strand	• Decreased 2’-F ribose in both strands • Terminal PS modifications • GalNAc-modified passenger strand

Application of PRISM to RNAi

- ✓ Control backbone stereochemistry
- ✓ Introduce PN-1 linkages

ESC & Adv ESC format and TTR sequence (5'- UUAUAGAGCAAGAACACUGUUUU -3') based on Foster et al., 2018 Mol Ther
ESC: Enhanced stability chemistry, 2’-OMe: 2’-O-Methyl, 2’-F: 2’-deoxy-2’fluoro, PS: Phosphorothioate, g: guide strand, P: passenger strand

Application of PN-1 chemistry to siRNA: Improving another class of silencers

PRISM

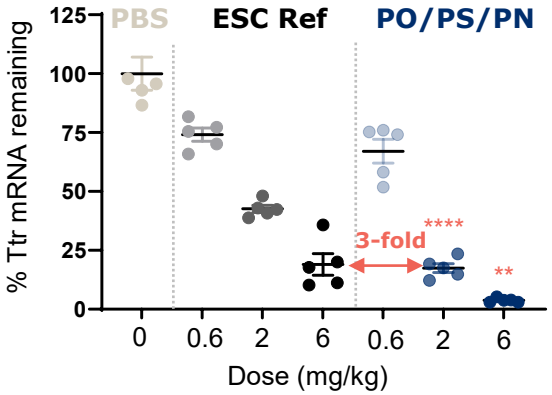
Silencing

RNAi

PN-1 chemistry improves potency and durability of ESC format

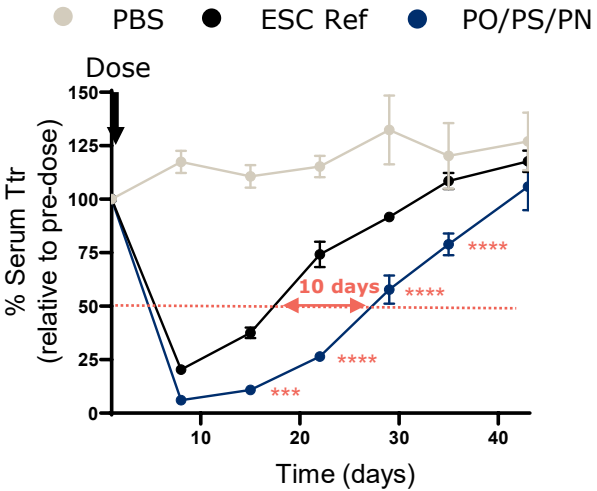
Potency

Ttr mRNA (liver)



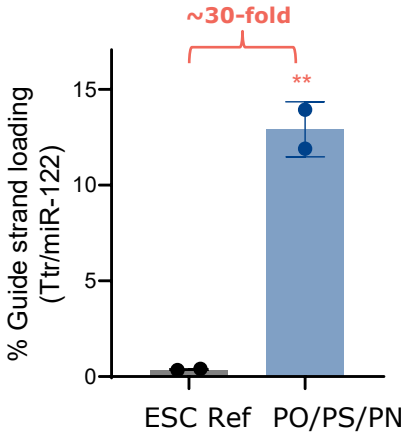
Durability

Ttr protein (serum)



Ago2-loading

Guide strand-Ago2 IP (liver)



(Left) C57Bl/6 mice were administered a single 0.2, 2, or 6 mpk SC dose (day 1). Tissue was harvested (day 8). Stats: 2-way ANOVA with post-hoc comparison to ESC. (Middle) Mice received a single 6 mpk SC dose (day 1). Serum was collected on days 1, 8, 15, 22, 29, 36 & 43. Stats: 2-way Mixed ANOVA with post-hoc comparison to ESC. (Right) As described for left panel (2 mpk); Ago2 loading measured by qPCR after immunoprecipitation (IP) and normalized to miR-122; Stats: Student's 2-tailed T test ** P<0.01, *** P<0.001****, P<0.0001. All post-hoc P values Bonferroni-corrected for multiple hypotheses.

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Application of PN-1 chemistry to siRNA: Improving on the state-of-the-art

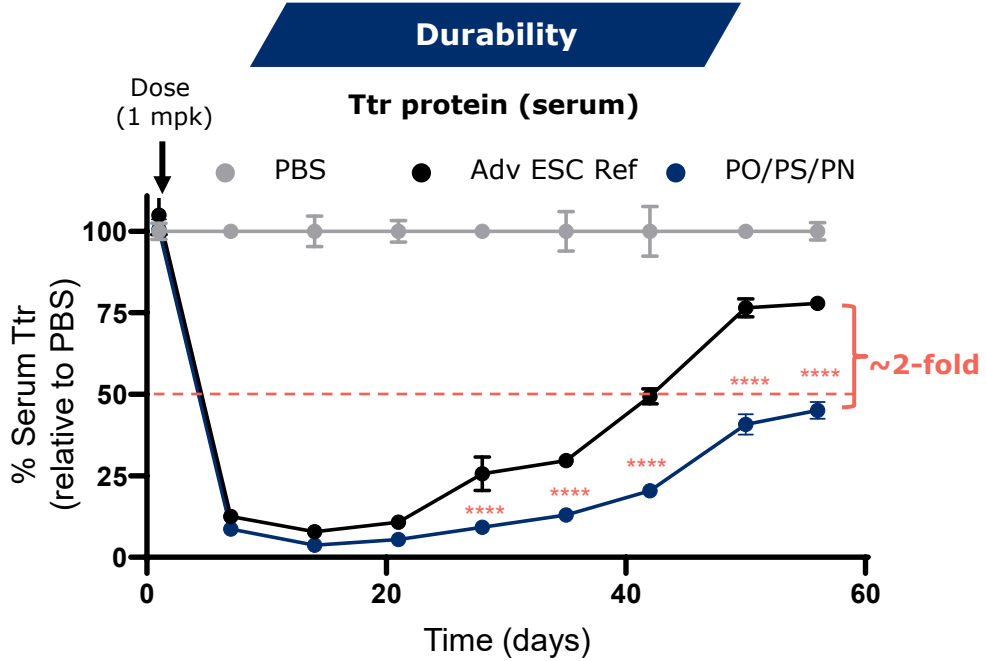
PN-1 chemistry extends duration of Advanced ESC format

PRISM

Silencing

RNAi

Durability



- PN-1 increases potency and durability
- PN-1 extends 50% knockdown period
- Durability experiments at multiple doses are ongoing

Mice received a single 1 mpk subcutaneous dose (day 1). Serum was collected on days 1, 7, 14, 21, 28, 35, 42, 50 & 56. Stats: 2-way Mixed ANOVA with post-hoc comparison to Adv ESC reference, **** P<0.0001. P values Bonferroni-corrected for multiple hypotheses.

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PN chemistry improves exon-skipping potency and increases cellular uptake in myoblasts

PRISM

Splicing

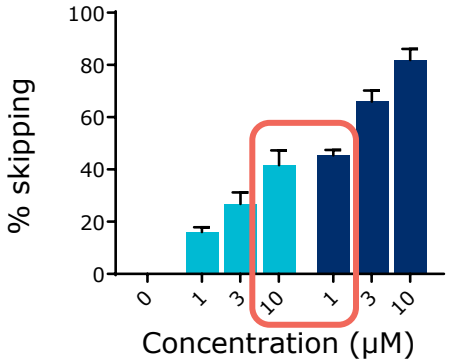
Potency

Exposure

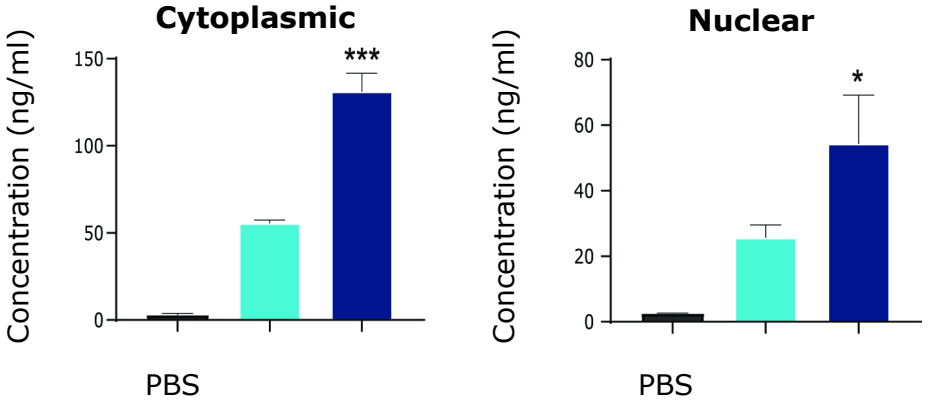
PS/PO backbone

PS/PO/PN backbone

DMD mRNA skipping (Exon 23, H2K mouse myoblasts)



Cellular uptake (H2K mouse myoblasts)



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Left: Cultured H2K myoblasts treated with increasing concentrations of PS/PO or PS/PO/PN stereopure oligonucleotide under free-uptake conditions. Skipping efficiency evaluated by TaqMan assay. Right: Cultured H2K cells treated with 0.5 uM oligonucleotide. Uptake quantified in cytoplasmic and nuclear extracts by hybridization ELISA. **: P ≤ 0.01, ***: P ≤ 0.001

PS *Rp* PS *Sp*

PO PN *Rp*

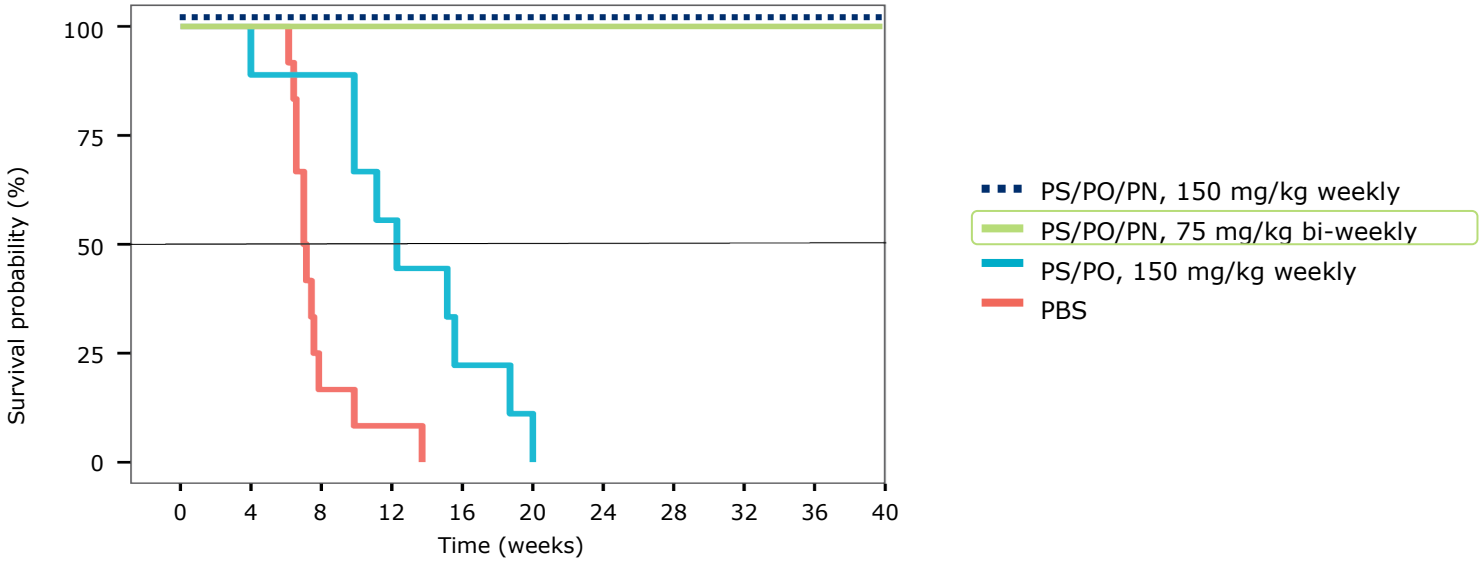
PN-based exon skipping molecule led to overall survival benefit in *Utr/Dmd* mouse model

PRISM

Splicing

Durability

PN-containing molecules led to 100% dKO survival at time of study termination



Note: Untreated, age-matched mdx mice had 100% survival at study termination [not shown]

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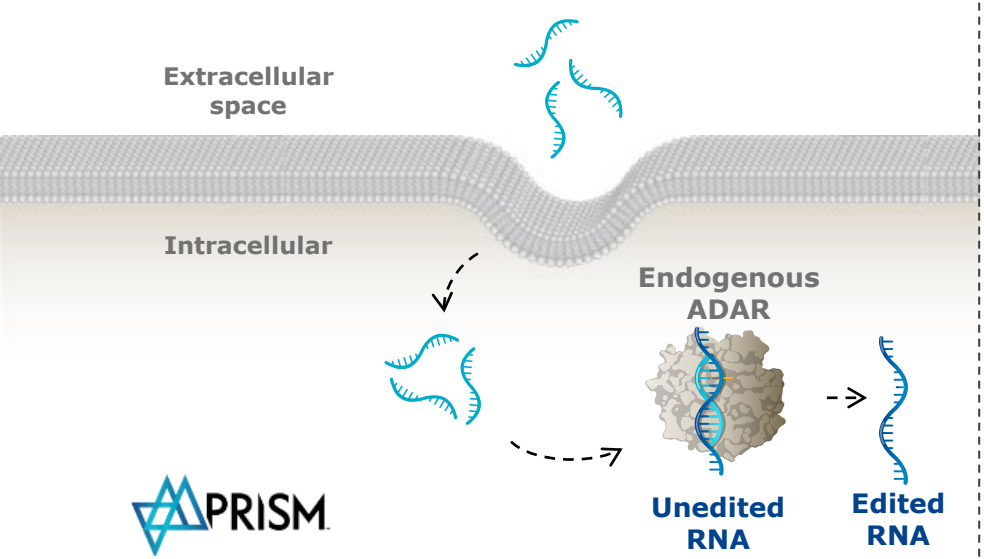
dKO; double knockout mice lack dystrophin and utrophin protein. mdx mice lack dystrophin. Left: Mice with severe disease were euthanized. dKO: PS/PO/PN 150 mg/kg n= 8 (p=0.0018); PS/PO/PN 75 mg/kg n=9 (p=0.00005); PS/PO n=9 (p=0.0024), PBS n=12 Stats: Chi square analysis with pairwise comparisons to PBS using log-rank test

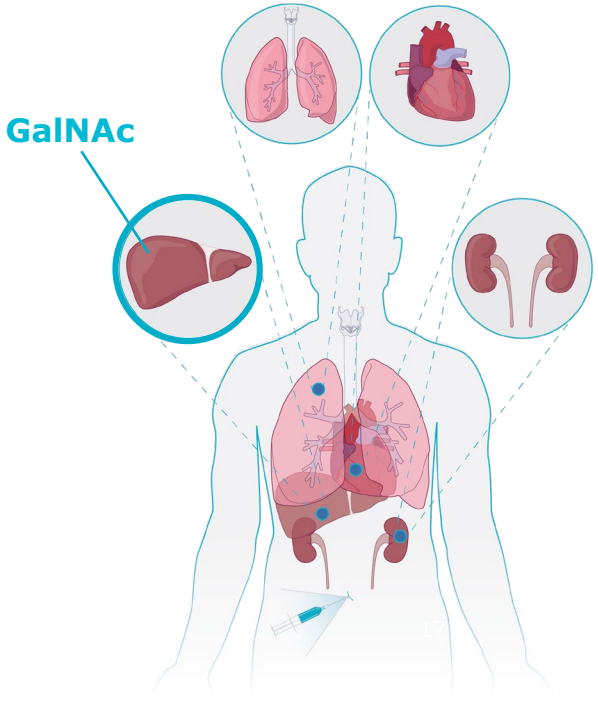
PRISM enables practical approach to RNA editing without need for viruses or exogenous protein

Wave ADAR-editing oligonucleotides



PRISM

- ✓No delivery vehicle required
- ✓No exogenous proteins necessary
- ✓Potential for reduced off-target effects



Subcutaneous

WAVE LIFE SCIENCES

GalNAc-conjugated oligonucleotides support efficient and durable ADAR editing in NHPs

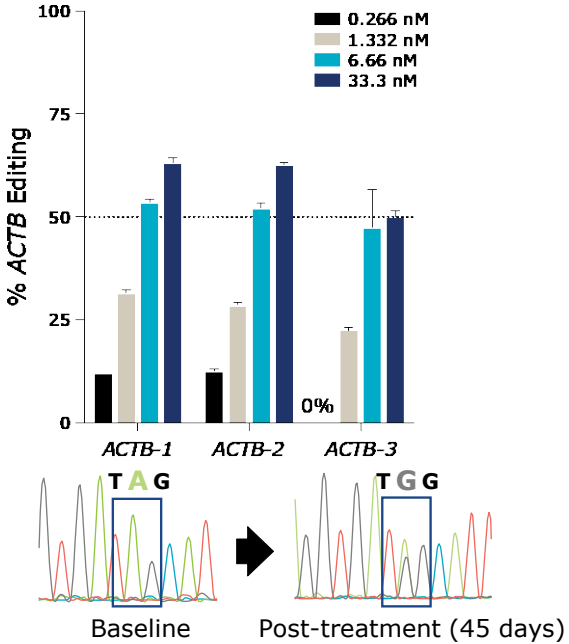
PRISM

ADAR

Systemic

Liver

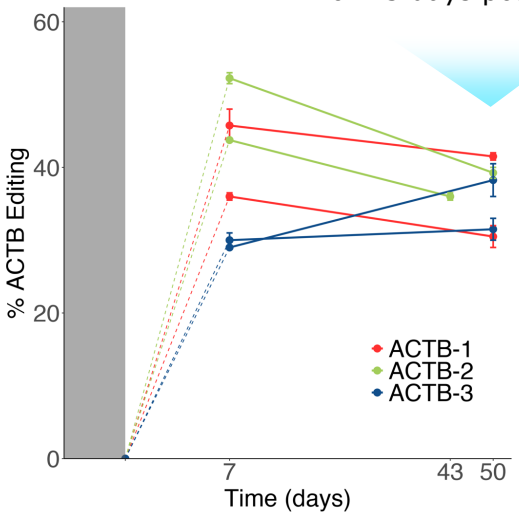
In vitro dose-response in NHP hepatocytes



Substantial and durable editing *in vivo* in NHP liver

(multiple 5 mpk, SC doses)

RNA editing out to **at least day 50**, or 45 days post-last dose



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Left: Primary hepatocytes (NHP) were treated with GalNAc-conjugated oligo. Middle: GalNAc-conjugated oligo was administered once daily on days 1-5. Liver biopsies were collected on days 7 and 50. Right: Unconjugated oligo was administered once on day 1. Tissue biopsies were collected on day 8.
NHP nonhuman primate; ACTB β-actin; mpk mg/kg; SC subcutaneous; oligo A-to-I editing oligonucleotide
Stats: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; all comparisons to PBS-treated group by t test

Leading RNA editing program provides optimal approach for treatment of AATD

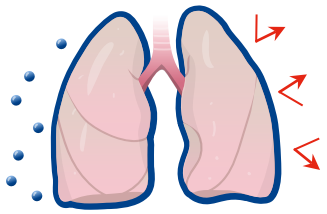
PRISM

Editing

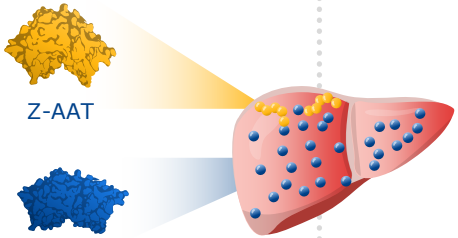
1) Restore circulating, functional wild-type M-AAT

2) Reduce Z-AAT protein aggregation in liver

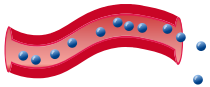
3) Retain M-AAT physiological regulation



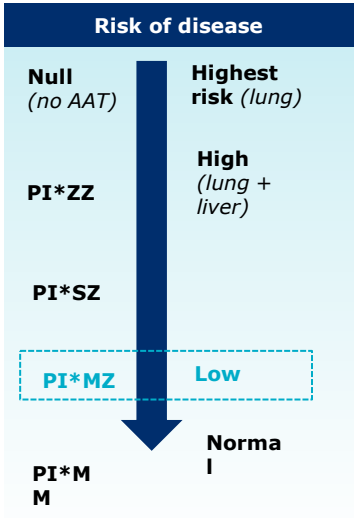
M-AAT reaches lungs to protect from proteases



Wild-type M-AAT protein replaces Z-AAT with RNA correction



M-AAT secretion into bloodstream



Wave ADAR editing approach

GalNAc-conjugated for subcutaneous delivery

~200K people in US and EU with mutation in *SERPINA1* Z allele (PI*ZZ)

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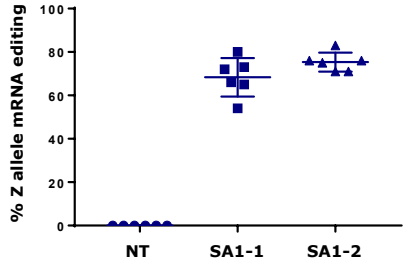
AAT: Alpha-1 antitrypsin; Sources: Strnad 2020; Blanco 2017

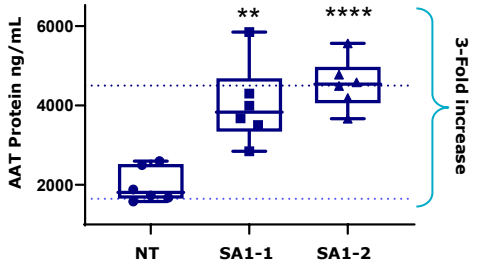
Focused on restoring wild-type M-AAT *in vivo*

In vitro proof of concept

SERPINA1 Z allele mRNA editing



AAT protein concentration in media



In vivo proof of concept

AATD mouse

Genotype
✓ huSERPINA1-Pi*Z
Human Z-AAT protein expressed in liver

huADAR mouse

Genotype
✓ huADAR
Human ADAR expressed in all tissues

huADAR/AATD mouse

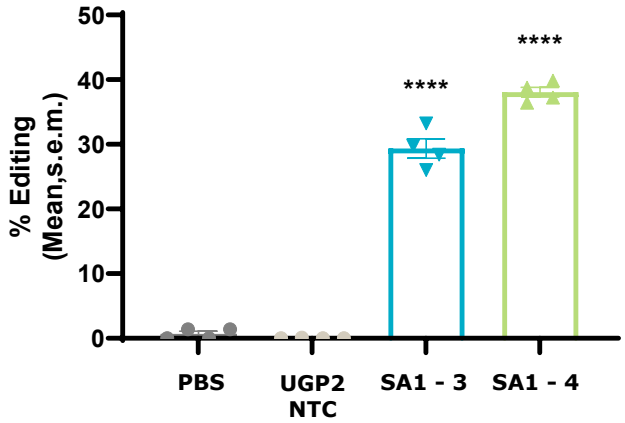
Genotype	Pathology
✓ huADAR	Liver pathology, Z-AAT protein in serum and liver
✓ huSERPINA1-Pi*Z	

AATD: Alpha-1 antitrypsin deficiency, Z-AAT: mutated protein, M-AAT: wild-type human AAT protein

Achieving 40% editing of Z allele mRNA at initial timepoint

SERPINA1 Z allele mRNA editing levels nearing correction to heterozygote (MZ)

In vivo Z allele mRNA editing



- GalNAc-conjugated compounds
- Up to 40% editing of Z allele mRNA in liver of transgenic human ADAR mice at day 7
- Highly specific editing (no bystander edits)

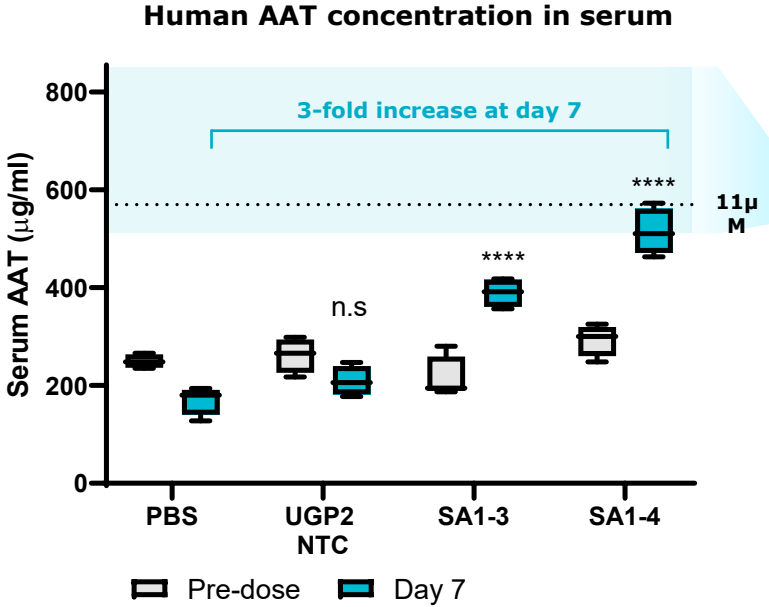
huADAR/AATD mouse

✓ **Z allele mRNA editing *in vivo*** | **AAT protein increase** | **Wild-type M-AAT functional**

Statistics: One-way ANOVA with correction for multiple comparisons (Dunnett's) was used to test for differences SERPINA1-Z allele editing in treated vs. PBS groups; 10 mg/kg dose administered day 0, 2, 4, sample collected on day 7; NTC: non-targeting control

Achieving biologically meaningful increases in circulating human AAT protein

3-fold increase in circulating human AAT as compared to PBS at initial timepoint



AAT serum levels by genotype

PI*MZ	~3 to 5-fold increase
PI*SZ	~2-fold increase
PI*ZZ	~3 – 7 uM

huADAR/AATD mouse

✓ **Z allele mRNA editing *in vivo***

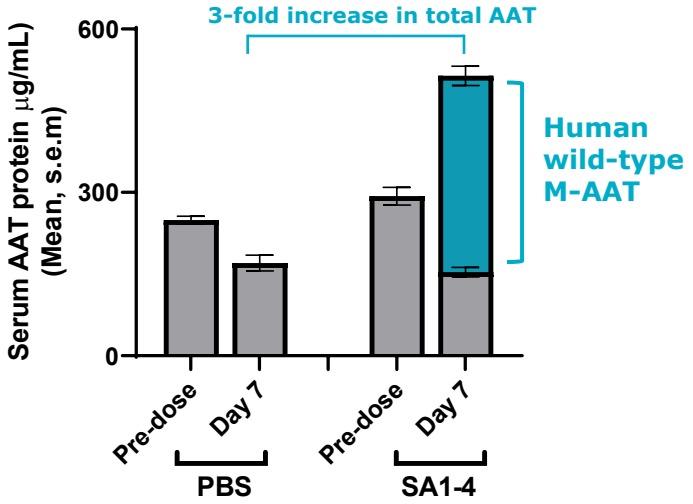
✓ **AAT protein increase**

Wild-type M-AAT functional

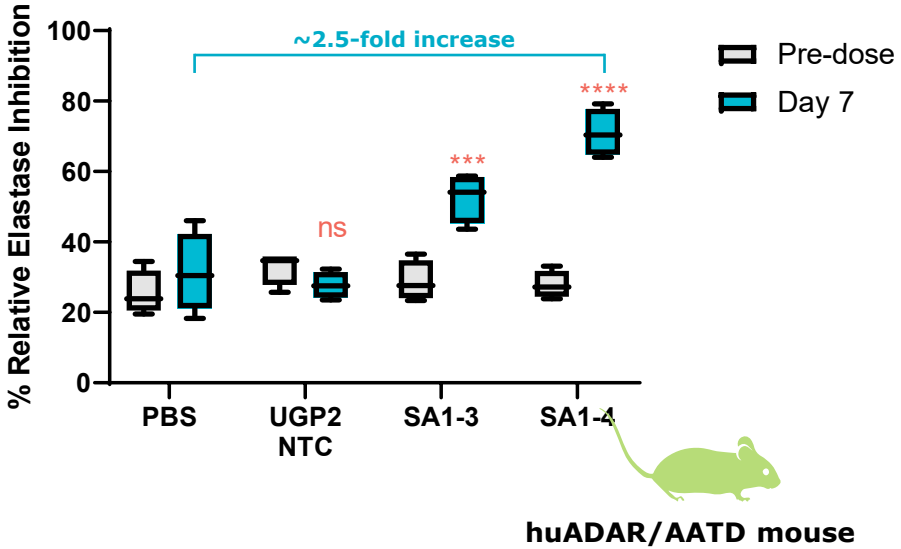
Statistics (ELISA): Matched 2-way ANOVA with correction for multiple comparisons (Bonferroni) was used to test for differences in AAT abundance in treated samples compared to PBS Statistics; de Serres et al., J Intern Med. 2014; NTC: non-targeting control

ADAR editing restores circulating, functional M-AAT

Wild-type M-AAT detected with ADAR editing



Significant increase in neutrophil elastase inhibition with ADAR editing



- ✓ Z allele mRNA editing *in vivo*
- ✓ AAT protein increase
- ✓ Wild-type M-AAT functional

Left: Mass spectrometry and ELISA Right: (Elastase inhibition): Matched 2-way ANOVA with correction for multiple comparisons (Bonferroni) was used to test for differences in elastase inhibition activity in serum collected at day 7 vs pre-dose for each treatment group; NTC: non-targeting control

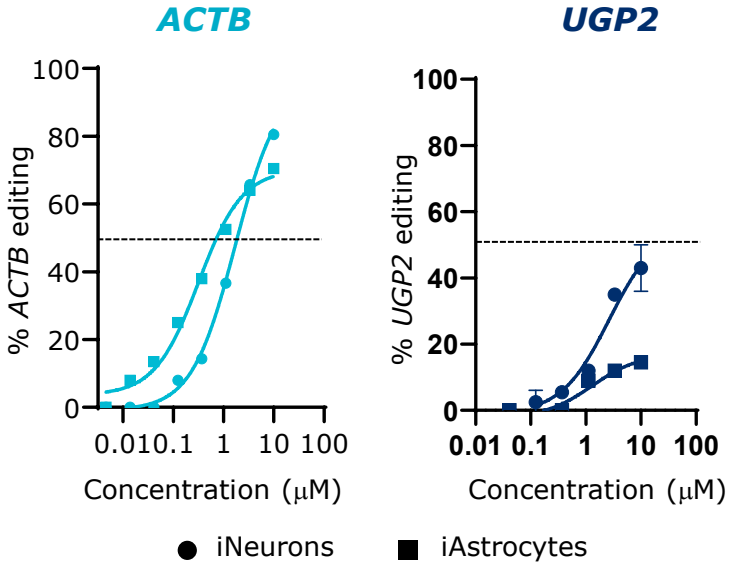
RNA editing throughout CNS of huADAR mouse

PRISM

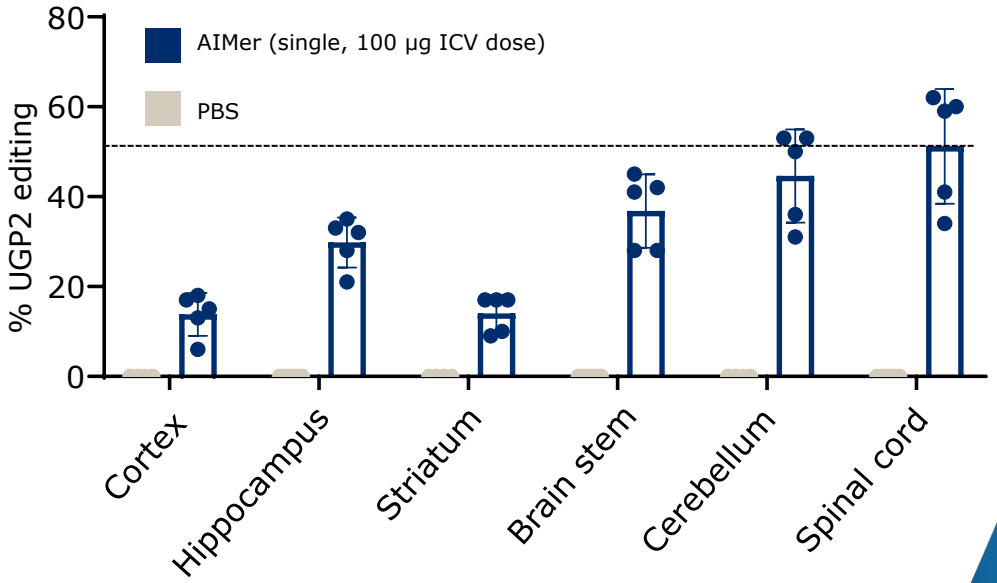
Editing

PN-containing molecules direct editing of *UGP2 in vivo*

In vitro dose-response curves



In vivo CNS editing in proprietary hADAR transgenic mouse (1 week)



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hADAR: human ADAR; UGP2: Glucose Pyrophosphorylase 2; CNS: central nervous system; Editing observed across all tested tissues of human-ADAR-transgenic mice by intracerebroventricular (ICV) injection. 5 mice in each group were injected with PBS or a single 100 μg dose on day 0. Animals were necropsied on day 7. RNA was harvested and editing measured by Sanger sequencing. UGP2 UDP-glucose pyrophosphorylase 2; ACTB β-actin, AIMer A-to-I editing oligonucleotide

Summary

Sustained exploration has yielded a novel modality and pharmacologic advances in preclinical studies

- Backbone chemistry and stereochemistry profoundly impact pharmacology
  - Introducing PN backbone chemistry with stereopure synthesis
    - Improves potency and durability across modalities
    - Increases intracellular exposure
- Application of PN chemistry provides meaningful biological outcomes
    - Substantial survival benefit in severely dystrophic dKO mouse (splicing)
    - Enhances potency & durability throughout CNS (silencing)
    - Enhances potency, durability and Ago2 loading of siRNAs (silencing)
    - Enabled translation of ADAR RNA-editing modality *in vivo* (editing)
- Advancing ADAR capabilities
    - Efficient and durable editing *in vivo* in NHP liver with GalNAc
    - Enables potential therapeutic approach to address lung & liver manifestations of AATD
    - Editing throughout CNS of hADAR mouse

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