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Phosphoryl-guanidine backbone chemistry: understanding its impact on stereopure oligonucleotides

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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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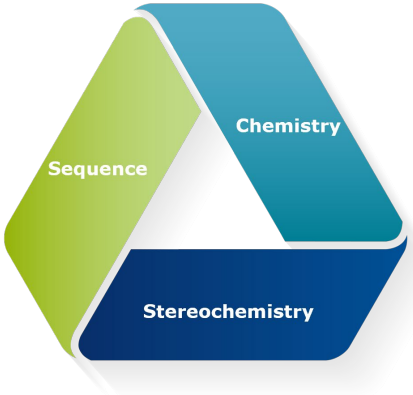
PRISM platform enables rational drug design

Sequence

B: bases

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



Chemistry

R: 2′ modifications

OMe, MOE, F,
other modifications

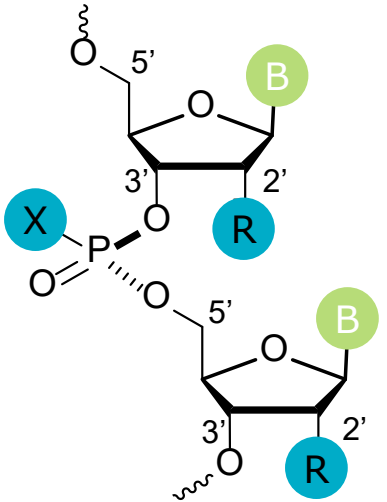
X: backbone chemistry

PO, PS, PN

Stereochemistry

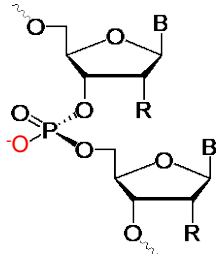
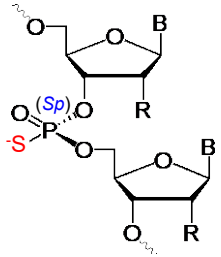
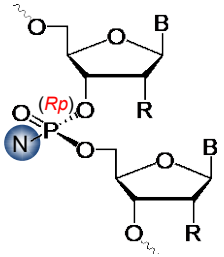
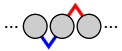
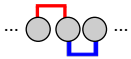
Chiral control of
any stereocenter

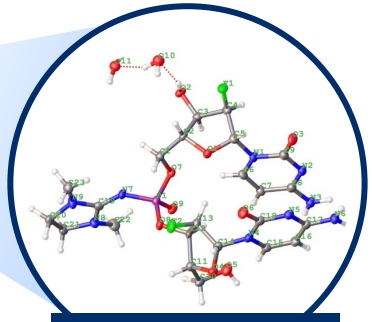
5′ modifications,
backbone modifications



Wave's ability to rationally design oligonucleotides enables access to unique disease targets

PRISM backbone linkages

PO	PS	PN
		
		
Chirality None	**Chirality** PS backbone *Rp* PS backbone *Sp*	**Chirality** PN backbone *Rp* PN backbone *Sp*
Negative charge	**Negative charge**	**Neutral charge**



Phosphoryl guanidine x-ray structure

example

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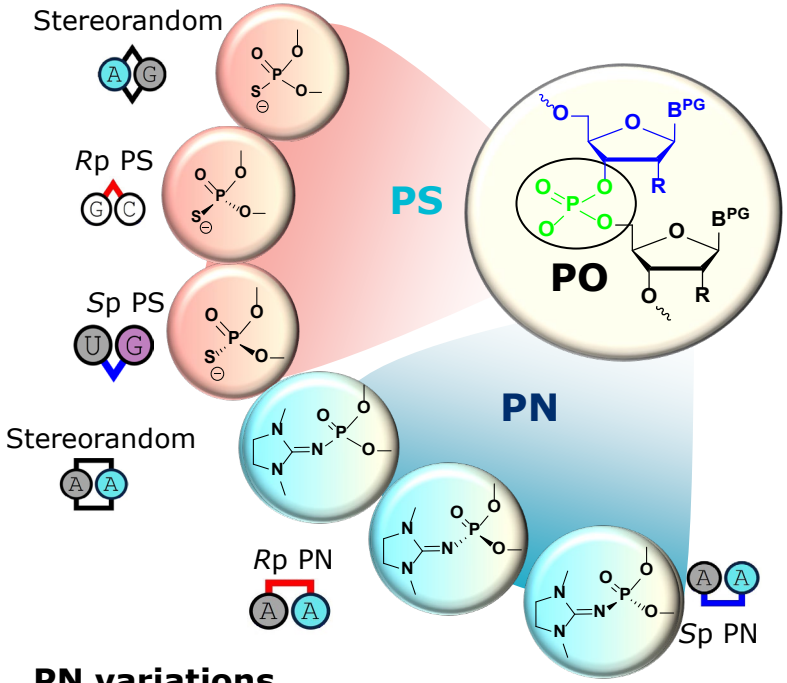
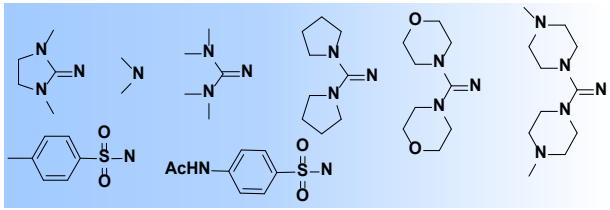
PO: phosphodiester PS: phosphorothioate

PN backbone and monomer library for stereopure oligonucleotide synthesis

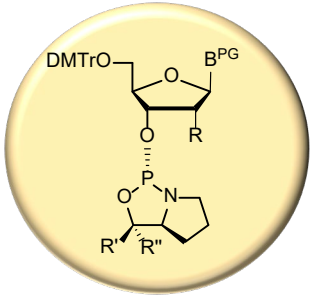


PN variations

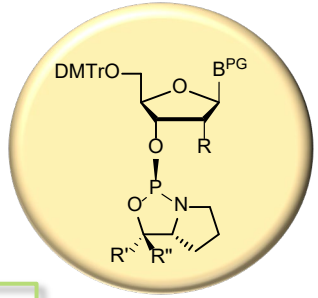


Chiral monomers

- Tunable 'R' groups
- Std base protecting groups
- Various 2'-modifications
- Manufactured in multi kilos



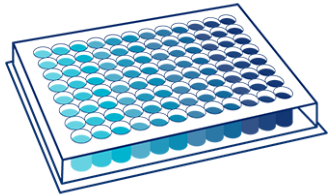
L-Monomer



D-Monomer

Poster #58

Candidate Optimization and Selection



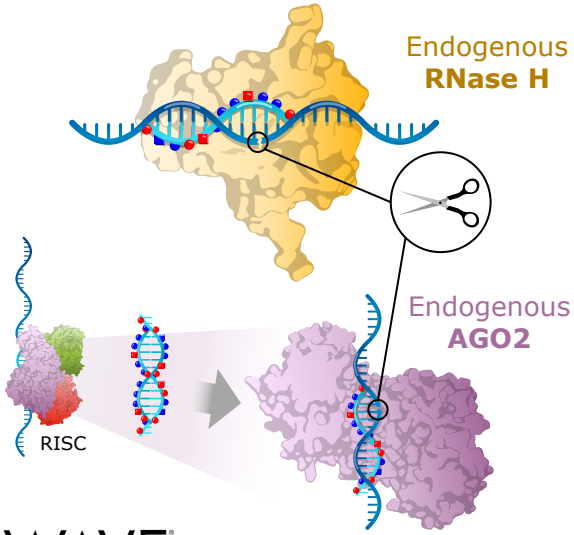
High-throughput scale

GMP Quality

Harnessing the biological machinery in our cells to treat genetic diseases

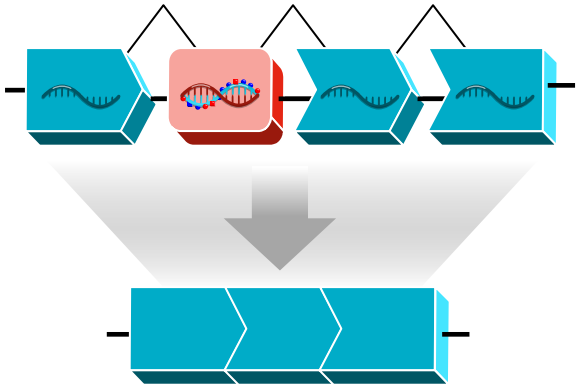
Silencing

- Degradation of RNA transcripts to **turn off** protein production



Splicing

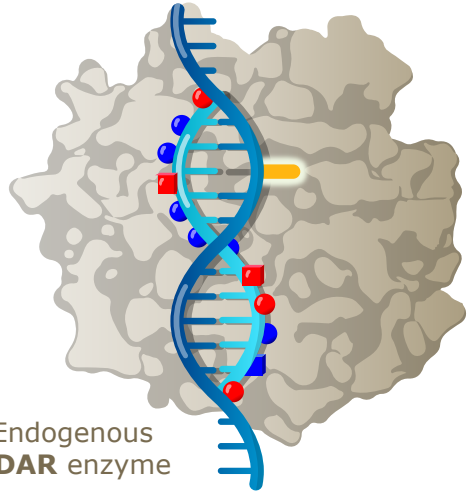
- Restore RNA transcripts and **turn on** protein production



Restored **Reading Frame**

RNA Base Editing

- Efficient editing of RNA bases to **restore** or **modulate** protein production



Endogenous **ADAR** enzyme

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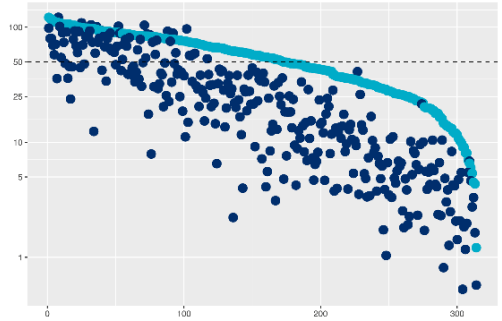
Potency is enhanced with addition of PN modifications across modalities

Silencing

Target knockdown (% remaining)

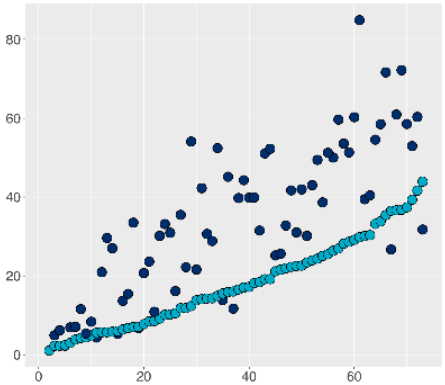


Ranked by potency of reference PS/PO compound

Improved knockdown

Splicing

% Skipping

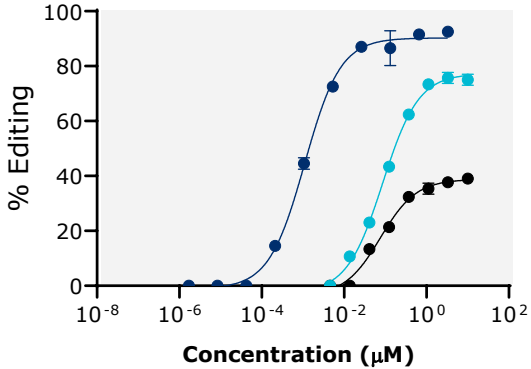


Ranked by potency of reference PS/PO compound

Improved skipping

Editing

% Editing



Improved editing

PS/PO/PN
PS/PO (Stereopure)
PS/PO (Stereorandom)

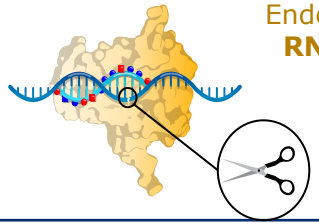
PS/PO reference compound

PS/PN modified compound

Left: Experiment was performed in iPSC-derived neurons *in vitro*; target mRNA levels were monitored using qPCR against a control gene (HPRT1) using a linear model equivalent of the ΔΔCt method; Middle: DMD patient-derived myoblasts treated with PS/PO or PS/PO/PN stereopure oligonucleotide under free-uptake conditions. Exon-skipping efficiency evaluated by qPCR. Right: Data from independent experiments

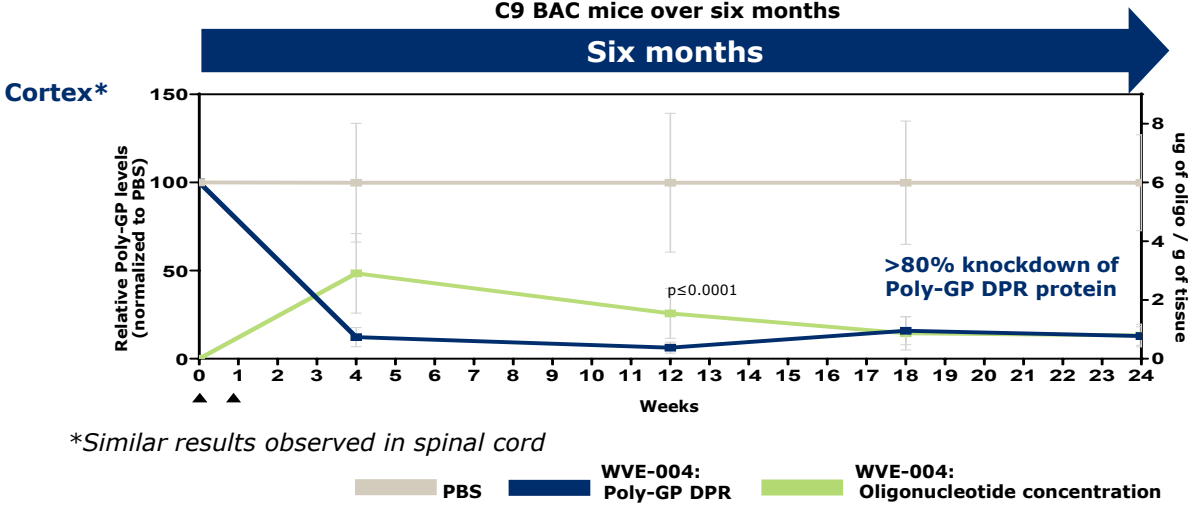
WVE-004 treatment resulted in durable reduction of poly-GP biomarker in mouse spinal cord & cortex[1]

Silencing – RNase H



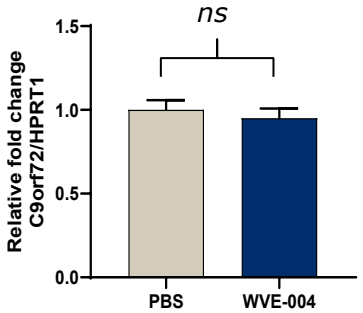
- WVE-004 leads to variant-selective silencing of C9orf72 transcripts
  - Contains PN chemistry
  - Lowers expansion-containing transcripts
  - Preserves healthy C9orf72 protein
- Poly-GP is produced from G_4C_2 expansion-containing transcripts

Change in poly-GP and oligonucleotide concentration in C9 BAC mice over six months



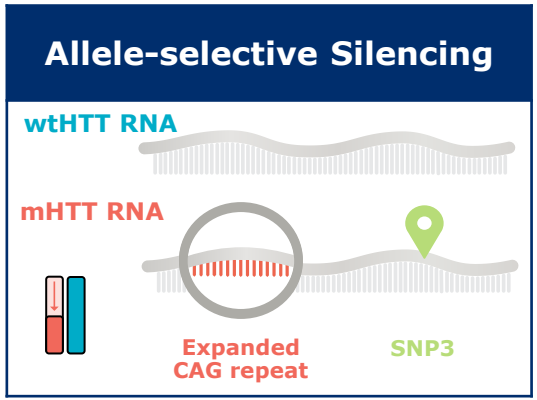
*Similar results observed in spinal cord

C9orf72 protein in C9 BAC mice unchanged at six months

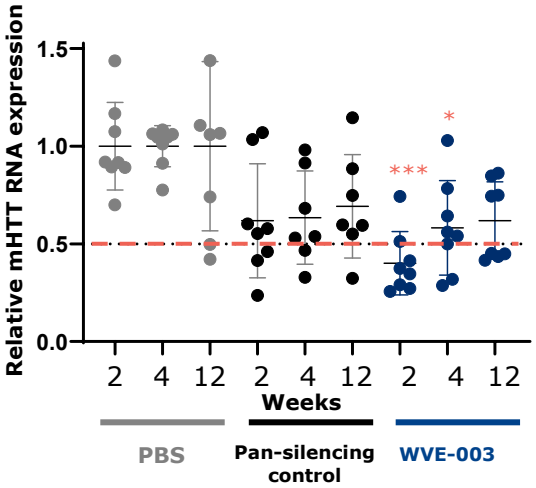


DPR, dipeptide repeat protein. [1]**Liu et al., 2022** *Mol Ther Nucleic Acids*. Poly-GP assay: **Wilson et al., 2022** *J Neurol Neurosurg Psychiatry.*

Potent, durable and allele-selective knockdown demonstrated in preclinical studies

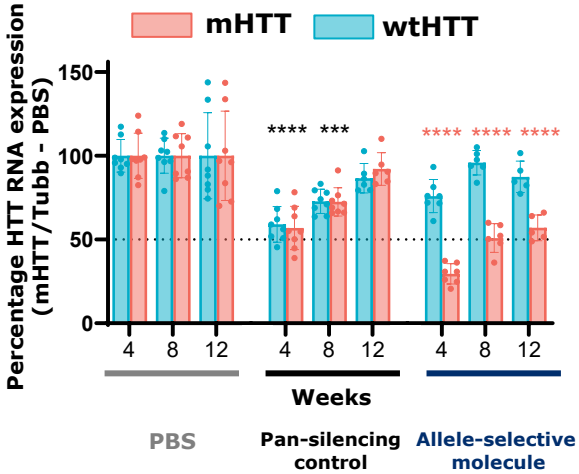


Durable mHTT knockdown for 12 weeks with multiple doses of WVE-003 in cortex*
(BACHD mouse)



*Similar results in striatum

Multiple doses of allele-selective molecule decreases mHTT, spares wtHTT in cortex*
(Hu97/18 mouse)



*Similar results in striatum

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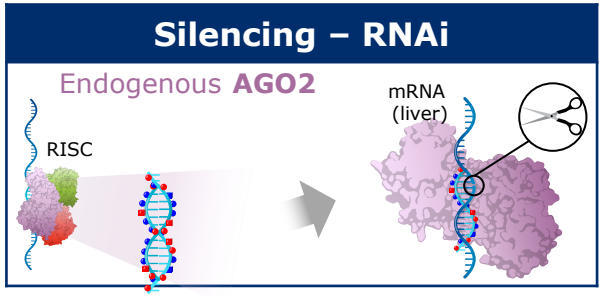
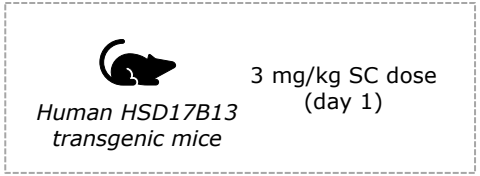
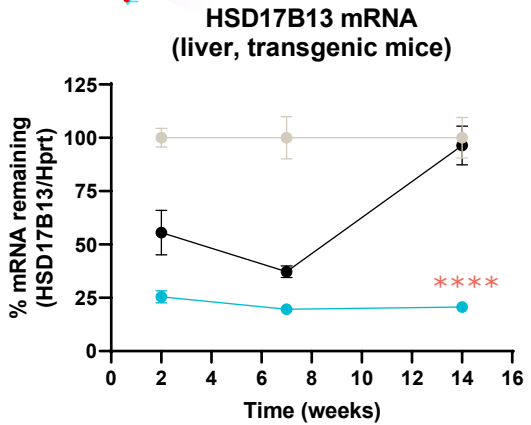
Left: 3x100 μg ICV doses in BACHD mice. Relative mHTT RNA in cortex (2, 4, 12-wks post-dosing). BACHD contains SNP3 only in some mHTT transgenes. Data are mean ± SD, n=8. *P<0.0332, ***P<0.0002, ****P<0.0001 versus PBS unless otherwise noted). P values were calculated via 1-way analysis of variance. Pan-silencing control based on Tabrizi et al, 2019 *NEJM;* Right: 3x100 μg ICV doses in Hu97/18 mice. Relative mHTT RNA in cortex at 4, 8 and 12-weeks post-dosing. Data are mean ± SD, n=8. Stats: *P<0.05, **P<0.01, ***P<0.0001, ****P<0.0001 versus PBS by 1-way ANOVA. mHTT, mutant HTT; wtHTT, wild-type HTT; Tubb, tubulin

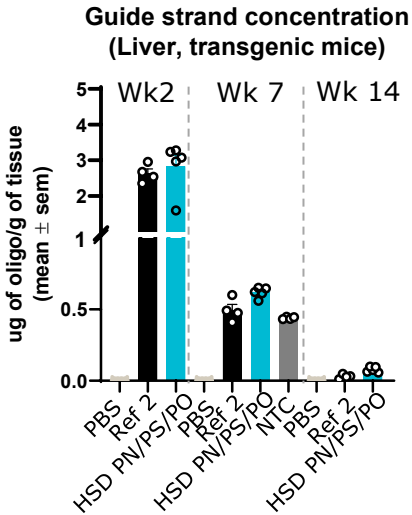
Durable *HSD17B13* silencing is driven in part by Ago2 loading advantage

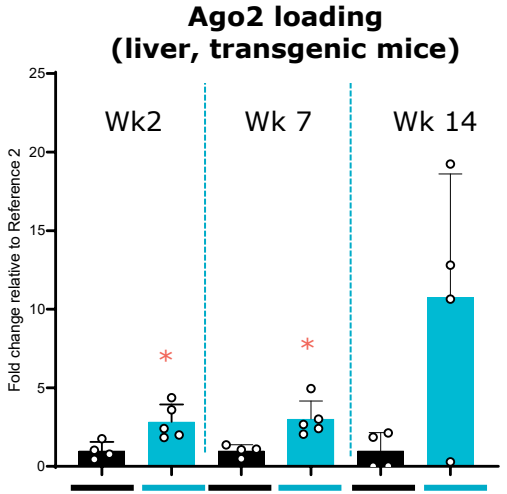




- ~80% silencing *HSD17B13* mRNA 14-weeks post-single dose with PN-containing siRNA
- Significantly more Ago2-loading than comparator siRNA







PBS ● HSD Reference 2 ● HSD PN/PS/PO

Reference 2

HSD PN/PS/PO

Poster #101

Mice expressing a human *HSD17B13* transgene were treated with 3 mg/kg of the indicated siRNA or PBS, and liver mRNA, guide strand concentration, and Ago2 loading were quantified at the indicated times post-dose. Stats: Two-way ANOVA with post-hoc test * P<0.05, ****P<0.0001.
HSD Reference 2 is based on Foster, et al., 2018. Mol. Ther. 26, 708-717

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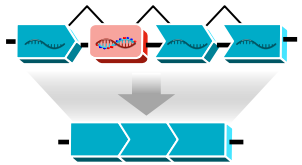
PN-containing molecule restores dystrophin expression and prolongs survival in severe mouse model for DMD

Splicing



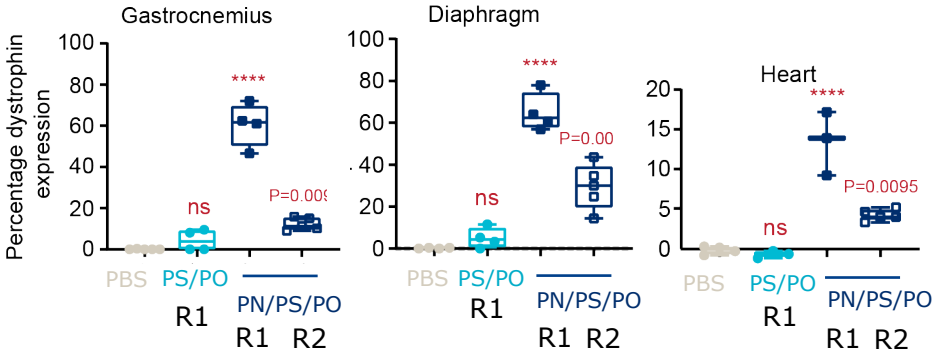
Restored Reading Frame

utr/mdx23 dKO mice

PBS
PS/PO
PN/PS/PO

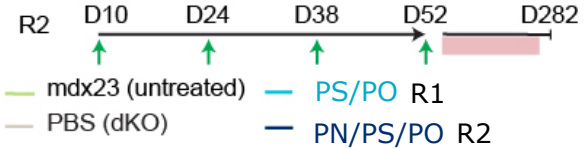
Dosing regimens
R1: 150 mg/kg weekly
R2: 75 mg/kg biweekly

Increased dystrophin protein expression in dKO mice

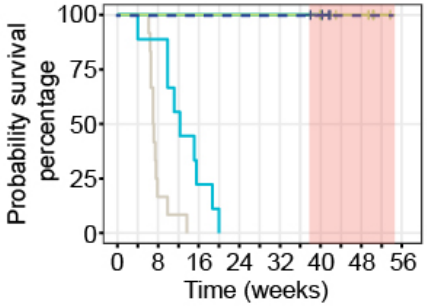


Increased survival in dKO mice

R2: 75 mg/kg, bi-weekly SC



mdx23 (untreated)
PBS (dKO)
PS/PO R1
PN/PS/PO R2



	Median survival	
	PBS	**49 days**
R1	**PS/PO**	86 days
R2	**PN/PS/PO**	**280 days**

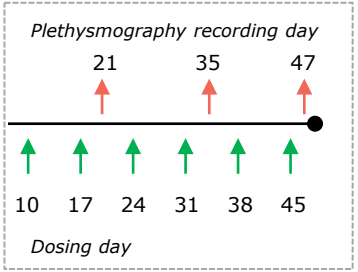
$p=2 \times 10^{-11}$

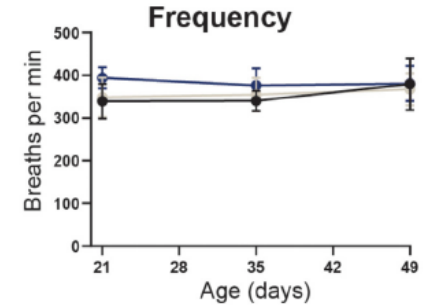
Kandasamy et al., 2022; doi: 10.1093/nar/gkac018

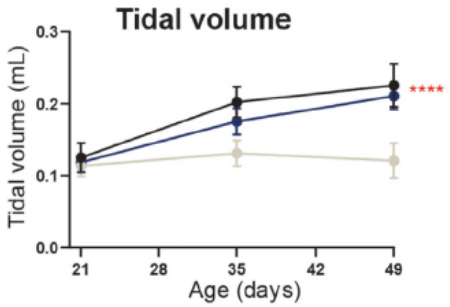
PN-containing molecule restored healthy respiratory function in severe mouse model for DMD

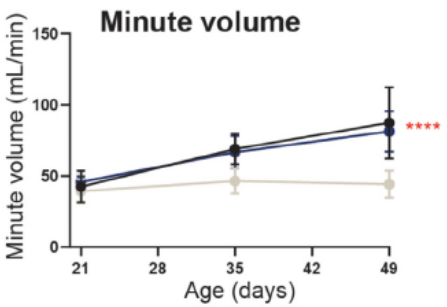
Respiratory profiles in DMD-2788-treated dKO mice matched wild-type mice in 6-week study

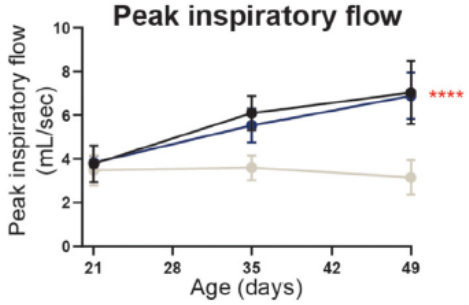
Dosing regimen (150 mg/kg weekly)

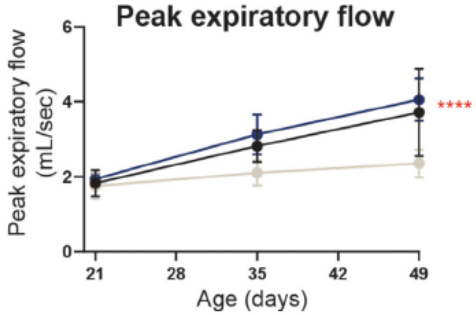












- **PBS** (dKO)
- **Wild-type** (healthy control)
- **DMD-2788** (dKO) PN/PS/PO

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Kandasamy et al., 2022; doi: 10.1093/nar/gkac018

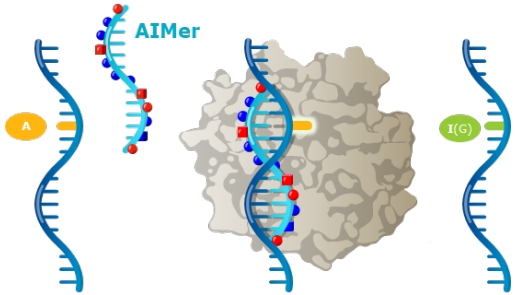
Expanding addressable disease target space using AIMers to activate pathways and upregulate expression

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ✓

WVE-006
(GalNAc AIMer)
AATD

Modulate protein interactions with AIMers



- ☑ **Modulate protein-protein interaction** *Achieved POC*
- ☑ **Upregulate expression** *Achieved POC*
- ☐ Modify function
- ☐ Post-translational modification
- ☐ Alter folding or processing

AIMers have the potential for many different applications to treat diseases beyond correcting single driver genetic mutations

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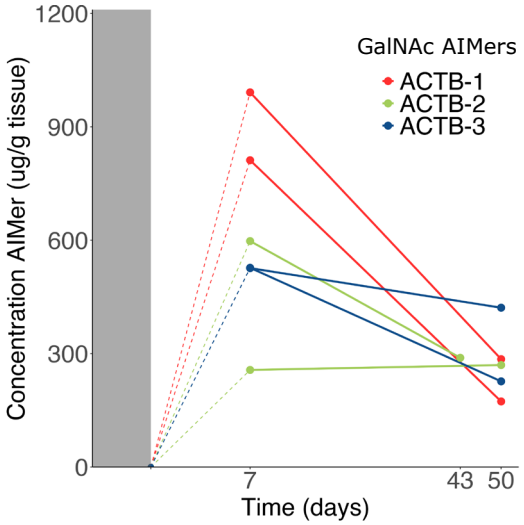
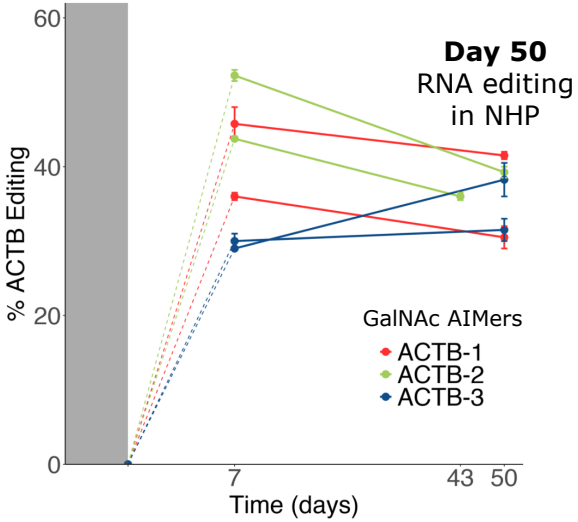
POC: proof of concept

Stability of AIMers enables durable and specific editing out to Day 50 in liver of NHPs

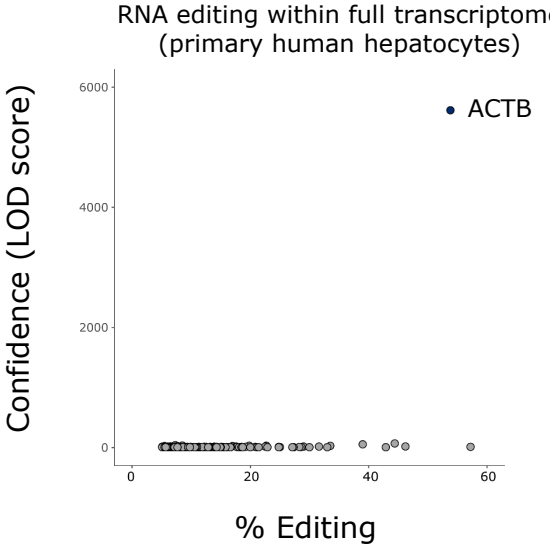
AIMers detected in liver of NHP at Day 50 (PK)



Substantial and durable editing in NHP liver *in vivo* (PD)



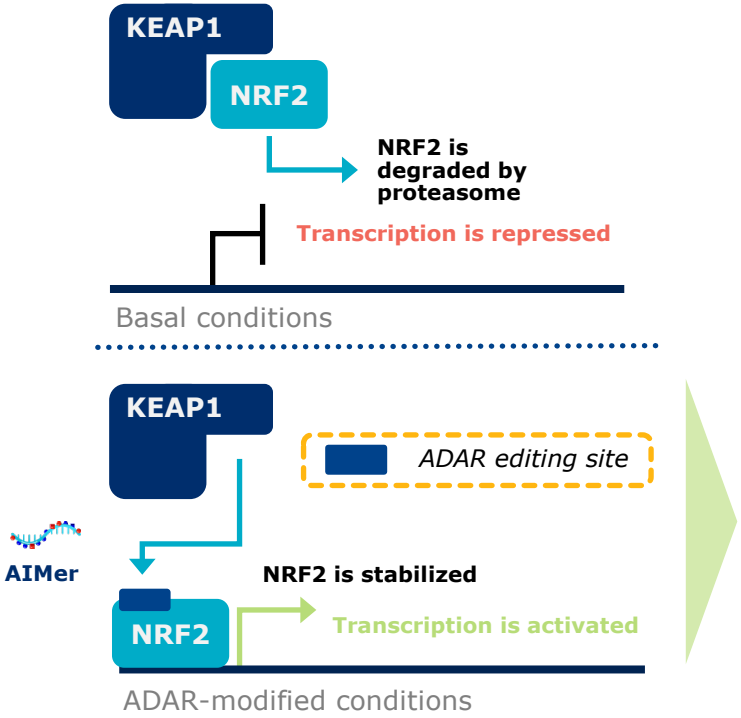
ADAR editing with ACTB AIMer is highly specific



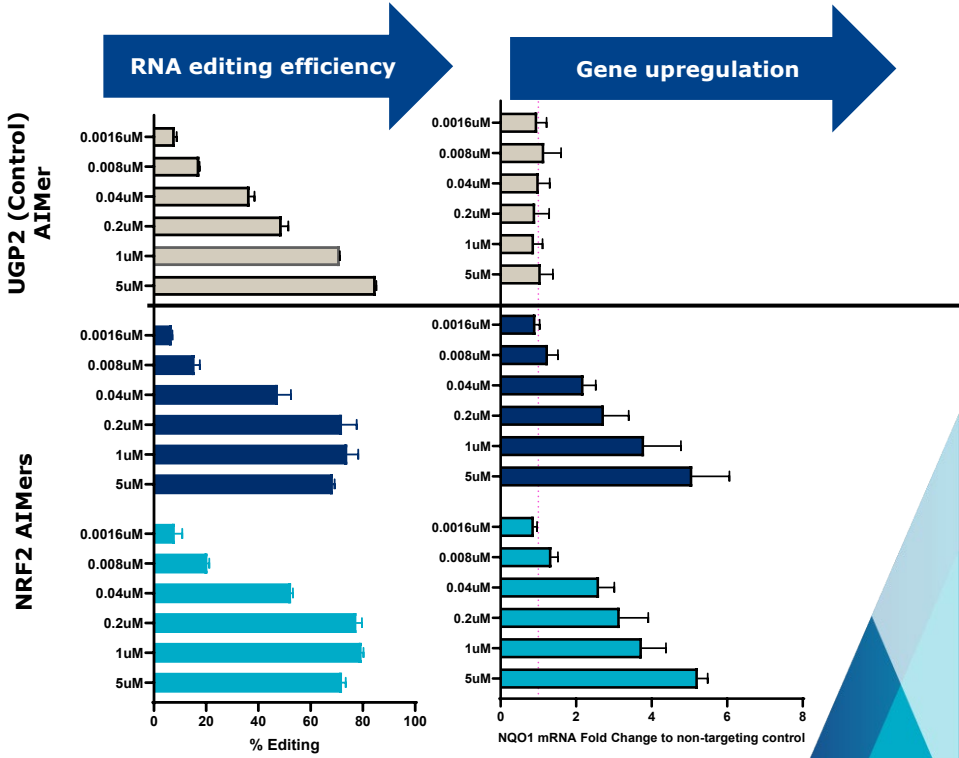
RNA editing only detected at editing site in ACTB transcript

Monian et al., 2022 doi:10.1038/s41587-022-01225-1

Dose-dependent modulation of protein-protein interactions



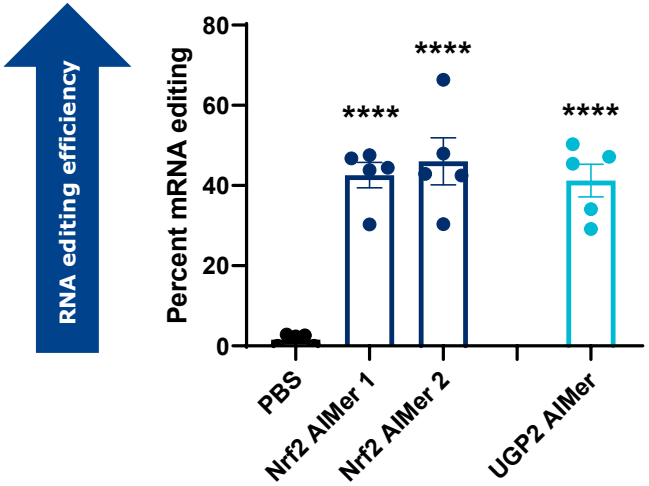
Dose-dependent gene upregulation (NQO1) *in vitro* following Nrf2 editing to disrupt protein/protein interaction



n=2; Primary hepatocytes 48h of treatment with the indicated dose concentration of AIMers

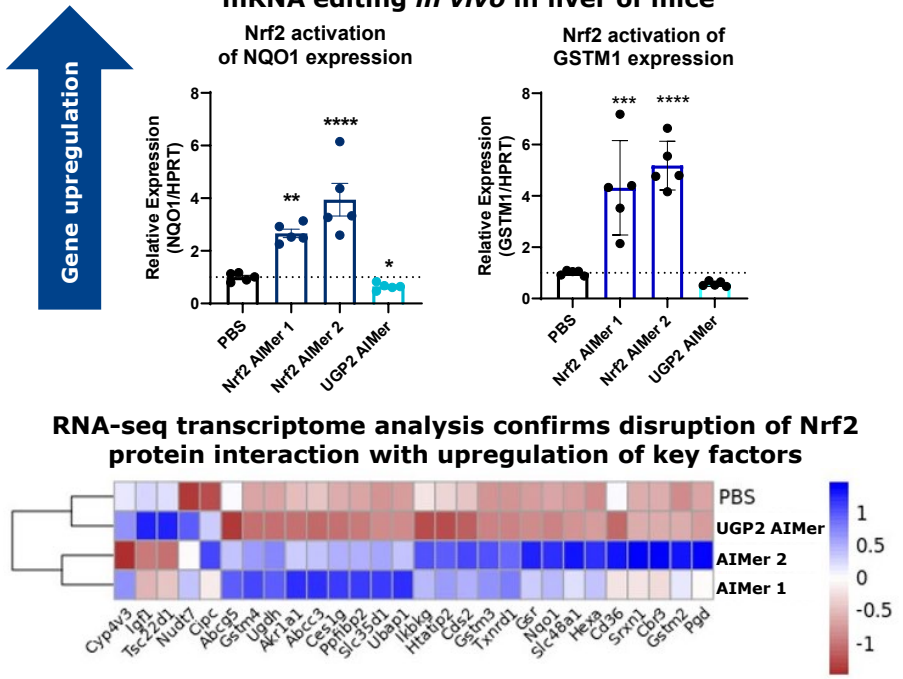
AIMers enable activation of gene pathway *in vivo* with single edit

Nrf2 mRNA editing *in vivo* in liver of mice with GalNAc AIMers



Note: Editing percentage for UGP2 control AIMer indicates editing of UGP2 mRNA

Nrf2 downstream gene upregulation following GalNAc-AIMer mRNA editing *in vivo* in liver of mice

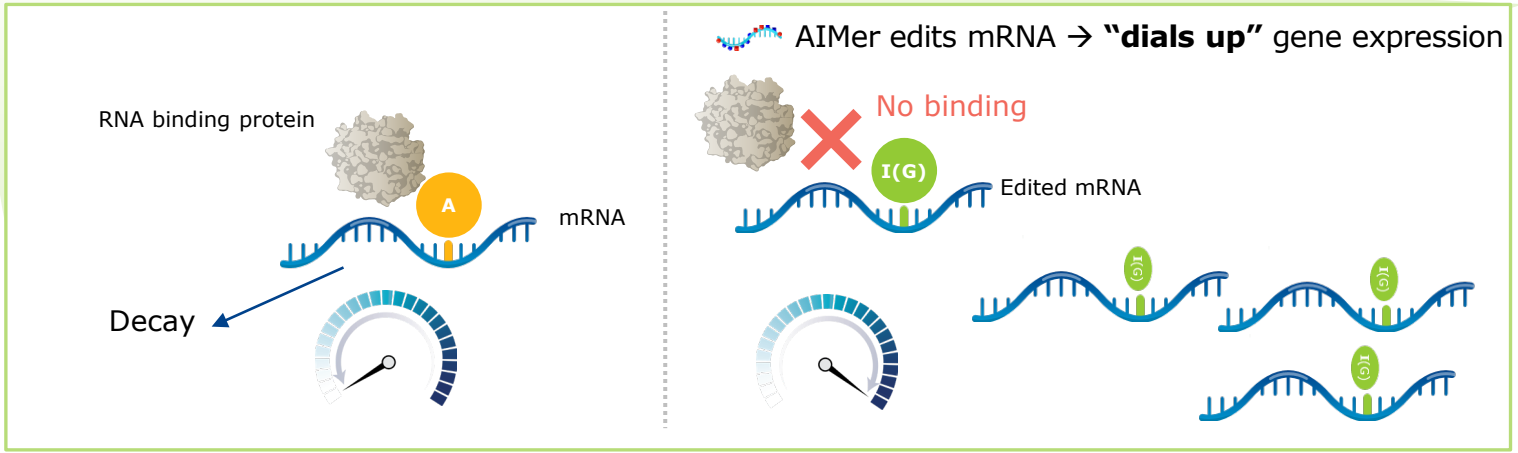


Methods: hADAR C57BL/6 mice dosed subQ (days 0, 2, 4) at 10mg/kg GalNAc-conjugated AIMers. Livers harvested (day 7), analyzed for editing and NQO1 expression via Sanger sequencing or qPCR, respectively. Data analyzed via One-way ANOVA with Tukey's multiple comparison test. Asterisks indicate statistical significance to PBS-treated animals as follows: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001

Upregulation: AIMers can edit RNA motifs to restore or upregulate gene expression

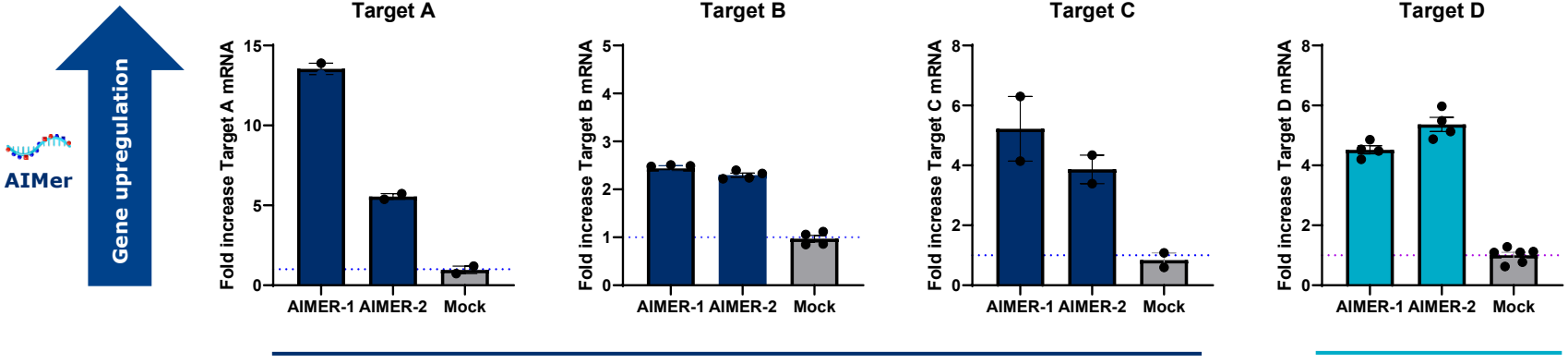
RNA binding proteins recognize sequence motifs to regulate various mRNA properties

Stability	Transport	Processing	Protein production
• Enhance or inhibit mRNA decay	• Intracellular localization	• Splicing • Capping • PolyA usage	• Translational efficiency



AIMers can edit RNA motifs to upregulate gene expression in hepatocytes and T cells *in vitro*

Editing RNA motifs to regulate RNA half-life to upregulate RNA expression is possible for clinically-relevant targets, including both metabolic and immune targets



Primary human hepatocytes (*in vitro*)

Primary human T cells (*in vitro*)

Achieving >2-fold mRNA upregulation *in vitro* across multiple different targets with AIMer editing

Primary human cells (hepatocytes, left; T cells, right) were treated with AIMers. RNA expression quantified by RT-PCR, n≥2.

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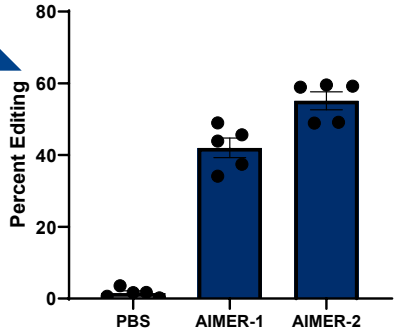
AIMers upregulate mRNA and downstream serum protein *in vivo* in mice

Target A (undisclosed liver target)

- High unmet need with potential for multiple large indications
- Preserves endogenous protein function
- Serum protein with biomarkers of pathway activation
- Potential benefit 3-fold+ upregulation in mouse

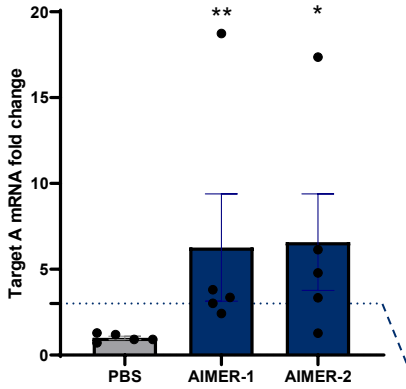
RNA editing

mRNA editing
7-days post-initial dose in mice
GalNAc-AIMer

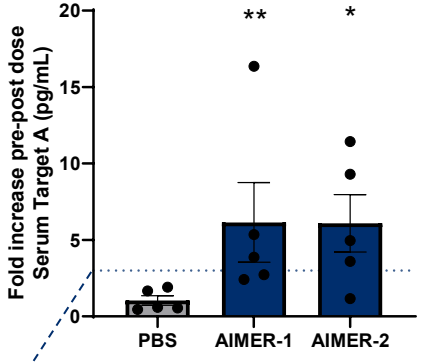


Upregulation

mRNA upregulation
7-days post-initial dose in mice
GalNAc-AIMer



Protein upregulation
7-days post-initial dose in mice
GalNAc-AIMer



Potential threshold for benefit

- ✓ *In vitro* to *in vivo* translation of mouse Target A mRNA upregulation
- ✓ *In vivo* mRNA upregulation corresponds to an upregulation of Target A protein in serum at day 7

hADAR mouse dosed subcutaneously 3 x 10 mg/kg GalNAc-conjugated AIMer or PBS days (0, 2, 4), taken down at day 7

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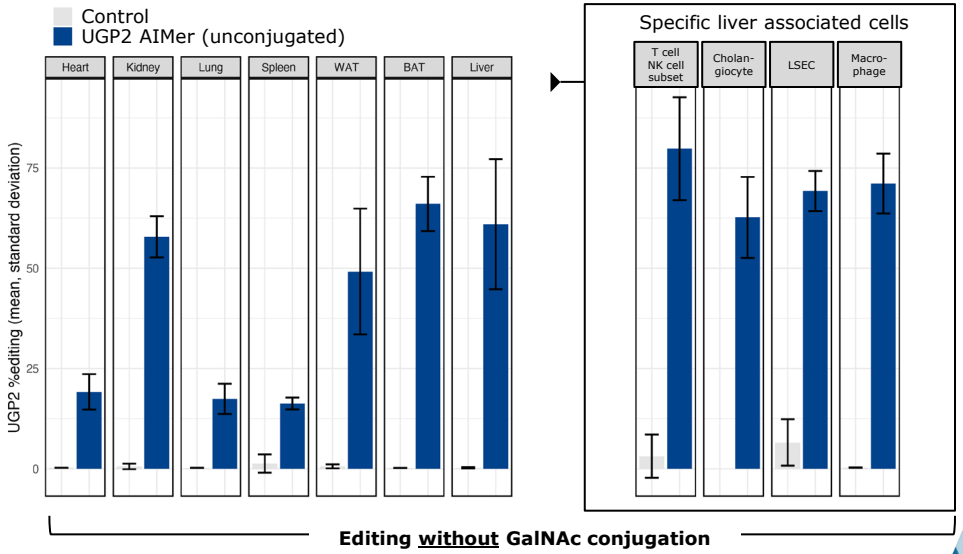
Systemic *in vivo* editing without delivery vehicles

Editing: Potent, durable, specific A → I (G) RNA editing

Delivery: Efficient RNA editing in preclinical *in vivo* models:

- ✓ Targeted delivery (GalNAc)
- ✓ Systemic delivery
- ✓ Local delivery (IT, IVT, others)

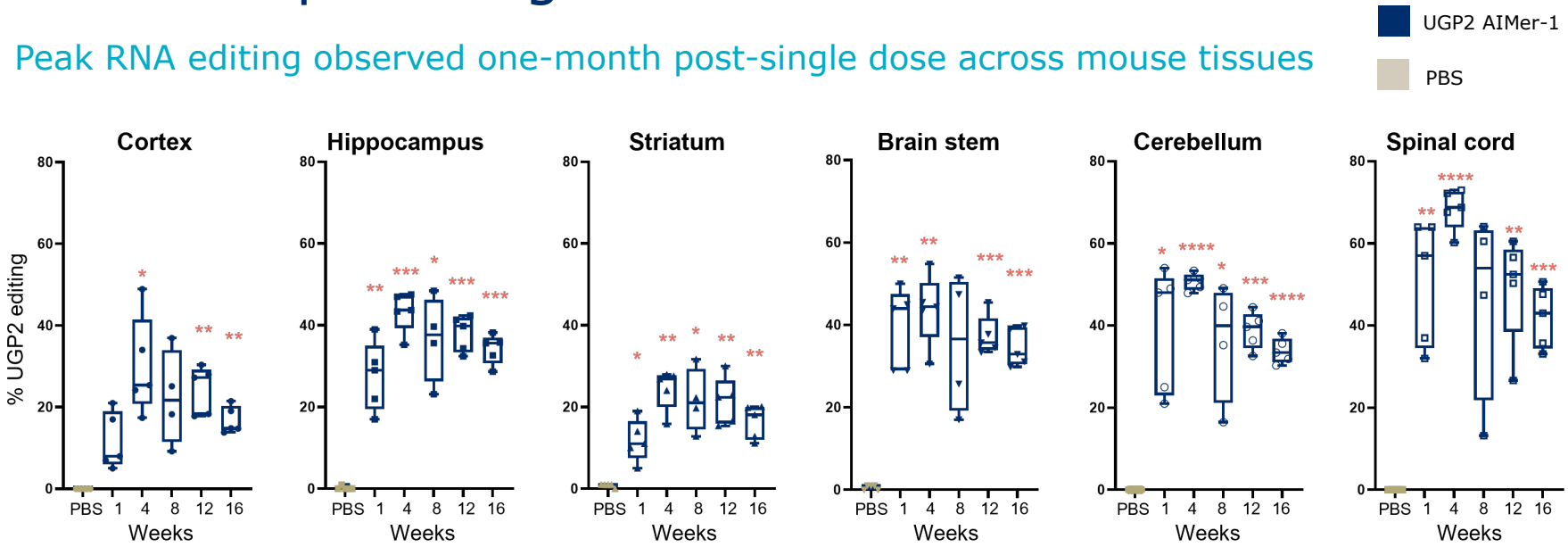
Substantial RNA editing across multiple tissues in mice following single subcutaneous dose of UGP2 AIMer



Right: Single dose of 100mg/kg unconjugated UGP2 AIMer, seven days post dose; WAT: White adipose tissue; BAT: Brown adipose tissue; CD3+: T-cells and subset of NK cells; EpCAM+(Epithelial cell adhesion molecule): mainly cholangiocytes within liver; LSEC cells (Liver Sinusoidal Endothelial Cells); M0 cells: macrophages

Substantial *in vivo* RNA editing out to at least 4 months post-single dose in CNS tissues

Peak RNA editing observed one-month post-single dose across mouse tissues

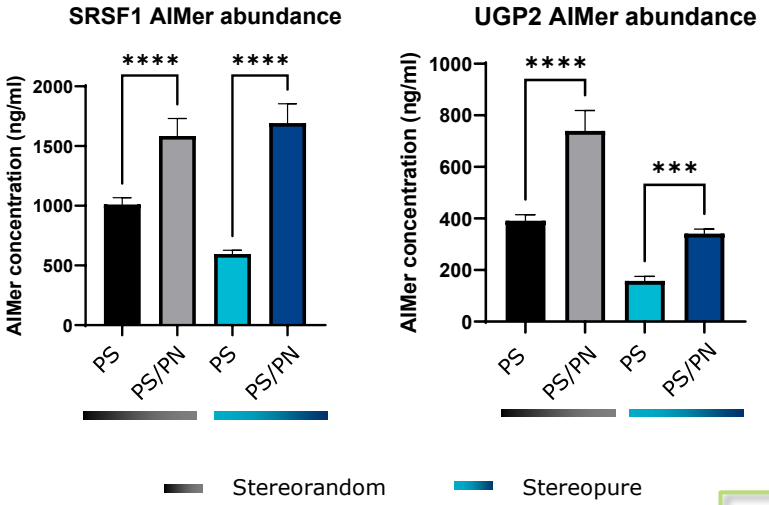


Peak editing	**30%**	**>40%**	**25%**	**>40%**	**50%**	**>65%**

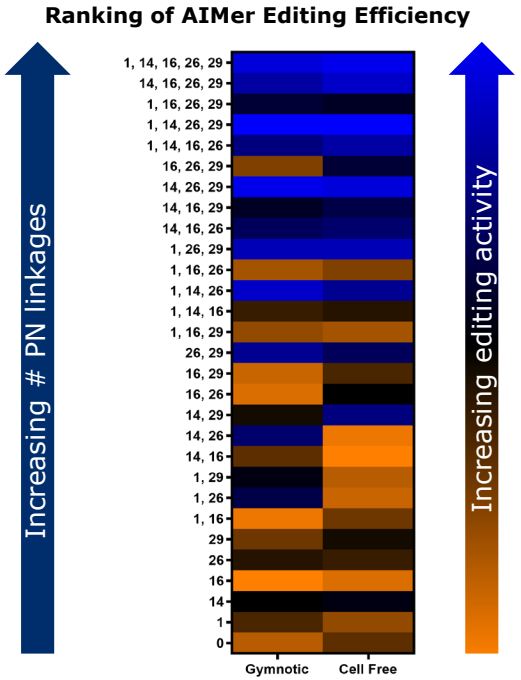
Transgenic huADAR mice administered 100 μg AIMer or PBS on day 0 and evaluated for UGP2 editing across CNS tissues at 1, 4, 8, 12, and 16-weeks post dose. Percentage UGP2 editing determined by Sanger sequencing. Stats: 2-way ANOVA compared to PBS (n=5 per time point per treatment) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ICV intracerebroventricular; PBS phosphate buffered saline

PN modification improves cellular uptake and target engagement for AIMers

Increases AIMer abundance following 6-hr treatment pulse, suggesting improved cellular uptake
(primary mouse hepatocytes)



Increases % editing independent of delivery, suggesting improved target engagement



Poster #52

(Left) Primary murine hepatocytes were treated gymnotically for 6-hr with 3 μM AIMer. AIMer concentration was quantified using a hybrid ELISA. Stats: One-way ANOVA *** p<0.001, **** p<0.0001; (Right) AIMer editing was measured in primary murine hepatocytes or in cell-free system. Sanger sequencing was used to quantify RNA editing. The ranked ratio of % editing for each AIMer:AIMer containing all five PN linkages is depicted.

Summary

- We've developed methods that support stereopure oligonucleotide synthesis
  - Scalable (high-throughput to commercial manufacture)
  - Stereopure
  - Enables chimeric backbone structure
  - Excellent yields
- Backbone chemistry and stereochemistry profoundly impact oligonucleotide pharmacology
- PN backbone chemistry and stereochemistry improve **potency** and **durability** across modalities to deliver meaningful biological outcomes in preclinical studies
  - Enhance activity in mouse CNS, with early data supporting clinical translation (silencing)
  - Enhance Ago2 loading of GalNAc-siRNAs in mouse liver (silencing)
  - Substantial survival benefit in severely dystrophic dKO mouse (splicing)
  - Translation of ADAR RNA-editing modality in NHPs *in vivo* (editing)
- Advancing RNA base editing capabilities
  - Correction of protein-coding mutation in mice
  - Modulation of protein-protein interactions in mice and RNA-protein interactions in primary human cells to upregulate gene expression
  - Editing in a diversity of tissues with a variety of delivery methods in mice

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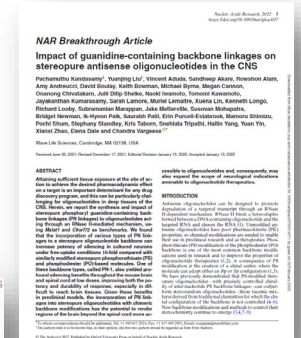
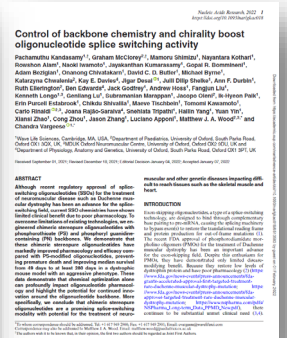
Impact of PN chemistry highlighted in three high-impact publications this year

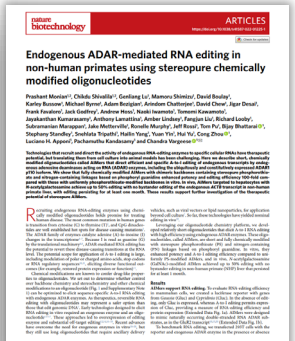
Silencing

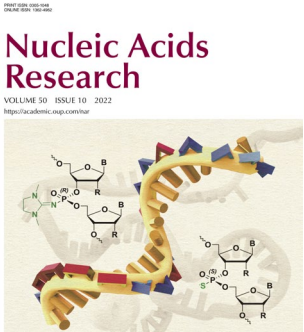


Splicing



RNA Editing





Thanks to all colleagues and contributors from Wave Life Sciences and our collaborators

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