Synthesis and application of stereopure guanidine-containing backbone to multiple oligonucleotide modalities in preclinical studies

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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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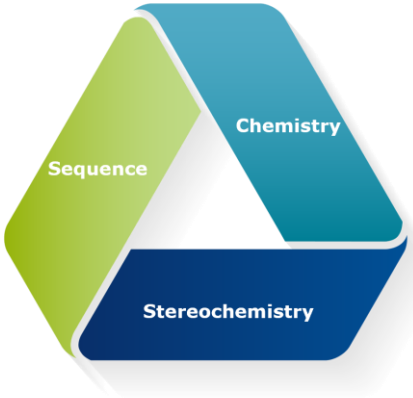
PRISM platform enables rational drug design

Sequence

B: bases

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



Chemistry

R: 2′ modifications

OMe, MOE, F,
other modifications

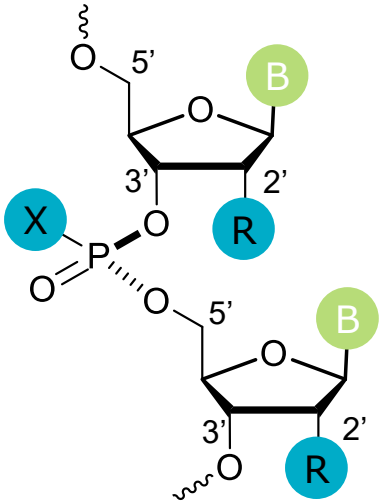
X: backbone chemistry

PO, PS, PN

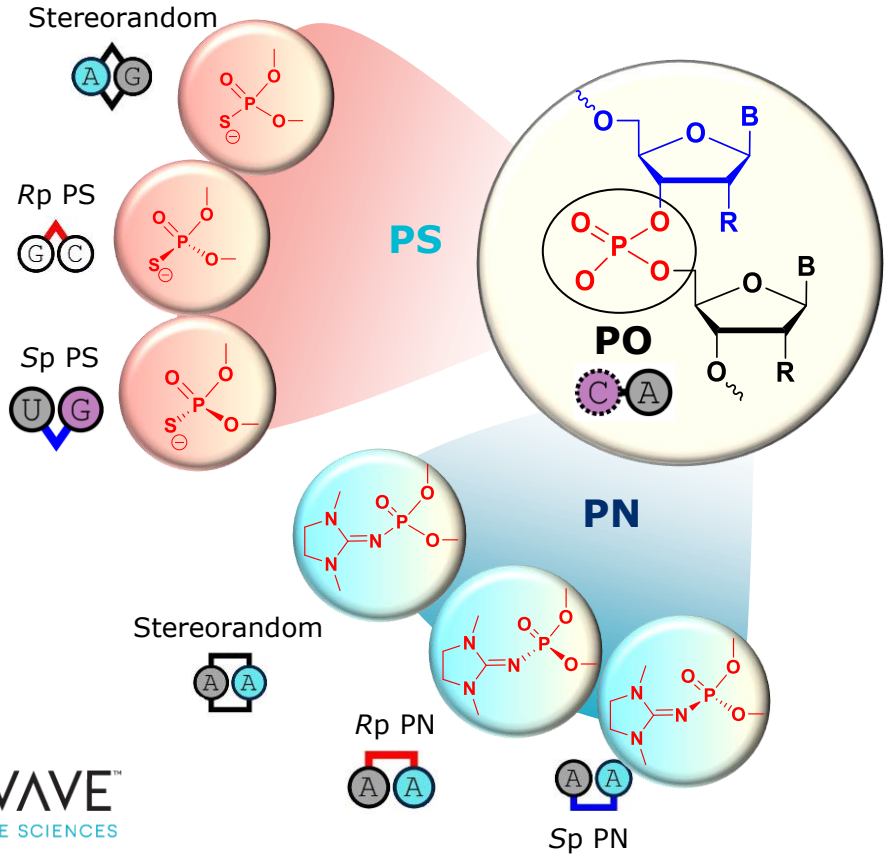
Stereochemistry

Chiral control of
any stereocenter

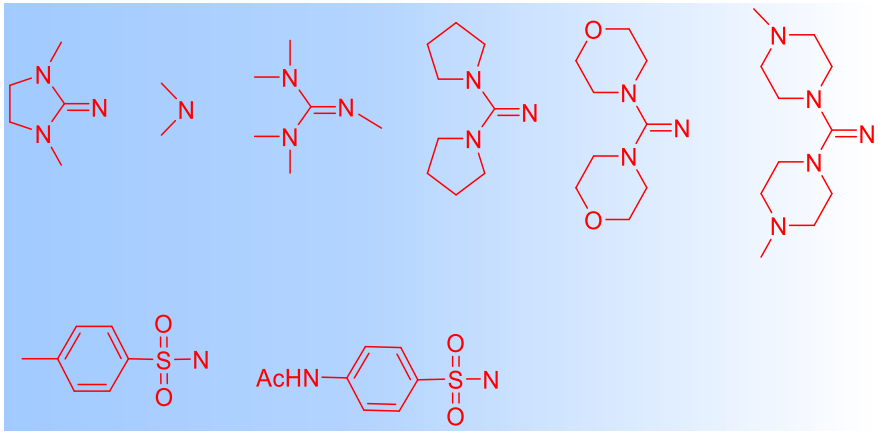
5′ modifications,
backbone modifications



PRISM backbone modifications



PN variations
(eg, targeting ligands)



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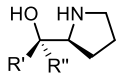
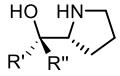
Advances in stereopure monomer synthesis & manufacturing

Chiral auxiliary

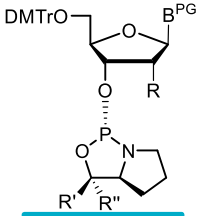


L-Prolinol

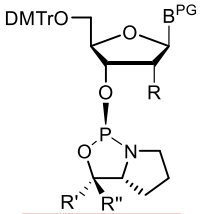


D-Prolinol

R'= Ph, R''= CH_3
R'= $CH_2SiCH_3Ph_2$, R''= H



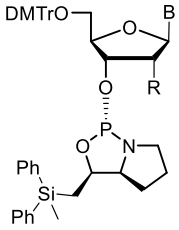
Sp Monomer

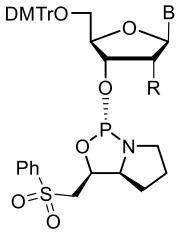


Rp Monomer

R = H, OCH_3, $OCH_2CH_2OCH_3$, F

Amidites





- High yield (up to 95%)
- High selectivity
- Multi-kg scale
- Various 2'-modifications
- Standard protecting groups
  - Bz, iBu, Ac

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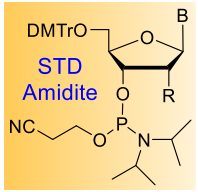
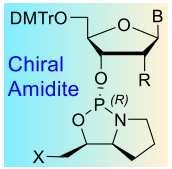
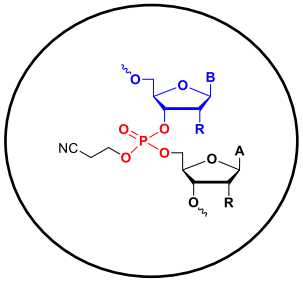
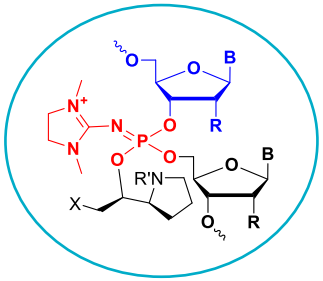
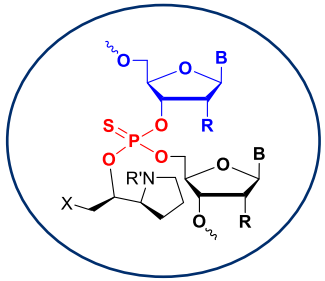
Efficient scalable process for synthesis



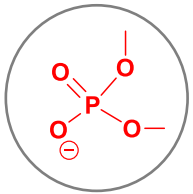




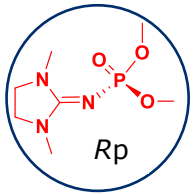




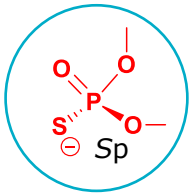
DEA wash



PO



PN



PS

- ❑ Std CNE amidite for PO
- ❑ Chiral amidite for both PS and PN

- ❑ High diastereoselectivity
- ❑ High coupling efficiency

Standard C&D process

- ❖ On column DEA wash
- ❖ Ammonium hydroxide

Scalable process

- ❖ 96-well plate to GMP manufacturing
- ❖ Comparable yield to std oligo

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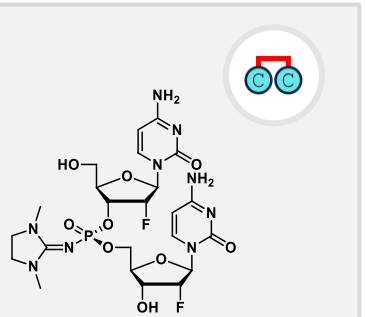
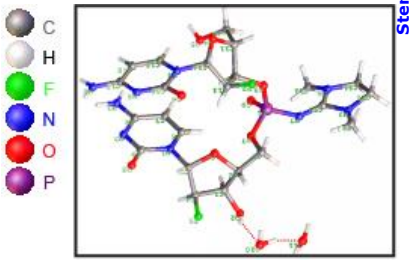
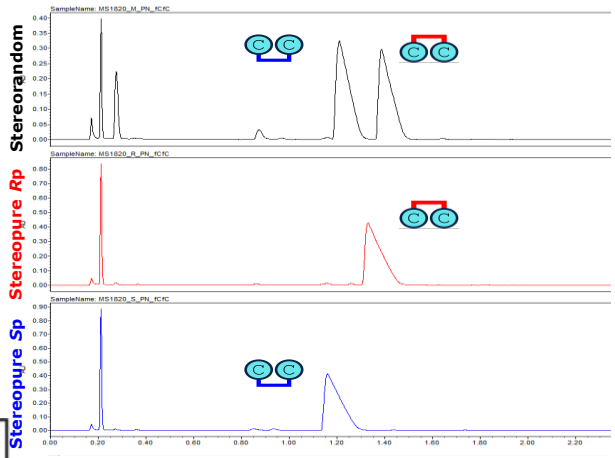
Characterization of stereopure dimers



***R*p-PN-fCfC**

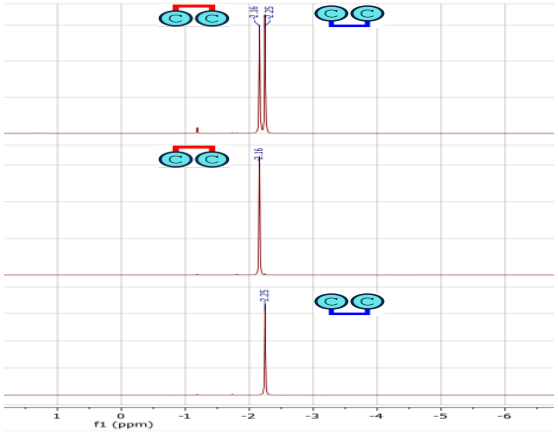


***R*p-PN-fCfC**
X-ray crystal structure
CCDC Refcode: 2113502



RP-UPLC

Yield (*Rp*:*Sp*)
93.26% (47.27: 52.73)
84.97% (99.25:0.75)
83.91% (0.22:99.78)



^{31}P NMR

Dimer	Amidite	*Rp*:*Sp*
2′-MOE-G + 2′OMe-U	L-PSM D-PSM	**99.8**:0.2 0.9:**99.1**
2′-MOE-5MeC + 2′OMe-U	L-PSM D-PSM	**99.9**:0.1 0.4:**99.6**
2′-OMe-G + 2′OMe-U	L-PSM D-PSM	**99.5**:0.5 0.2:**99.8**
2′-OMe-U + 2′-OMe-U	L-PSM D-PSM	**99.5**:0.5 0.2:**99.8**

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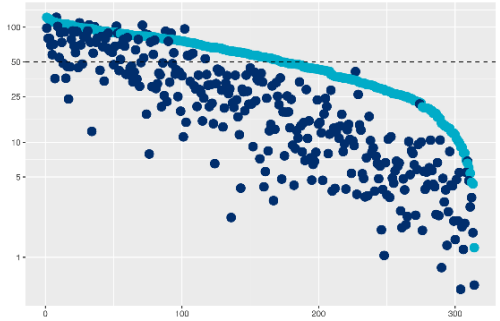
Kandasamy et al., 2022; doi: 10.1093/nar/gkac018

Potency is enhanced with addition of PN modifications across modalities

Silencing

Target knockdown (% remaining)

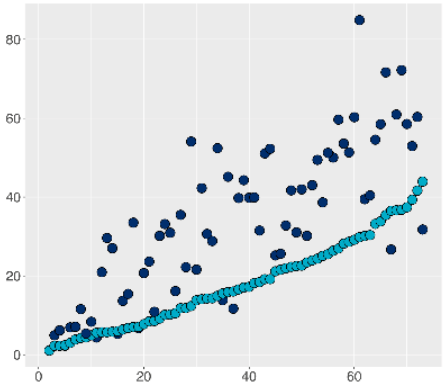


Ranked by potency of reference PS/PO compound

Improved knockdown

Splicing

% Skipping

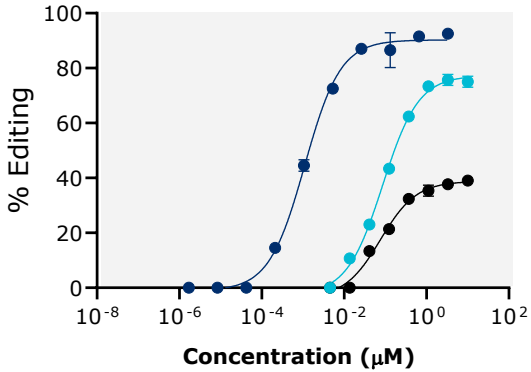


Ranked by potency of reference PS/PO compound

Improved skipping

Editing

% Editing



Improved editing

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

PS/PO reference compound

PS/PN modified compound

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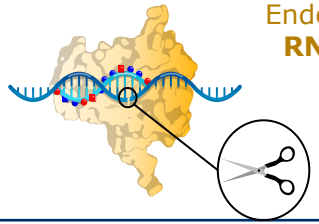
Left: Experiment was performed in iPSC-derived neurons *in vitro*; target mRNA levels were monitored using qPCR against a control gene (HPRT1) using a linear model equivalent of the ΔΔCt method; Middle: DMD patient-derived myoblasts treated with PS/PO or PS/PO/PN stereopure oligonucleotide under free-uptake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

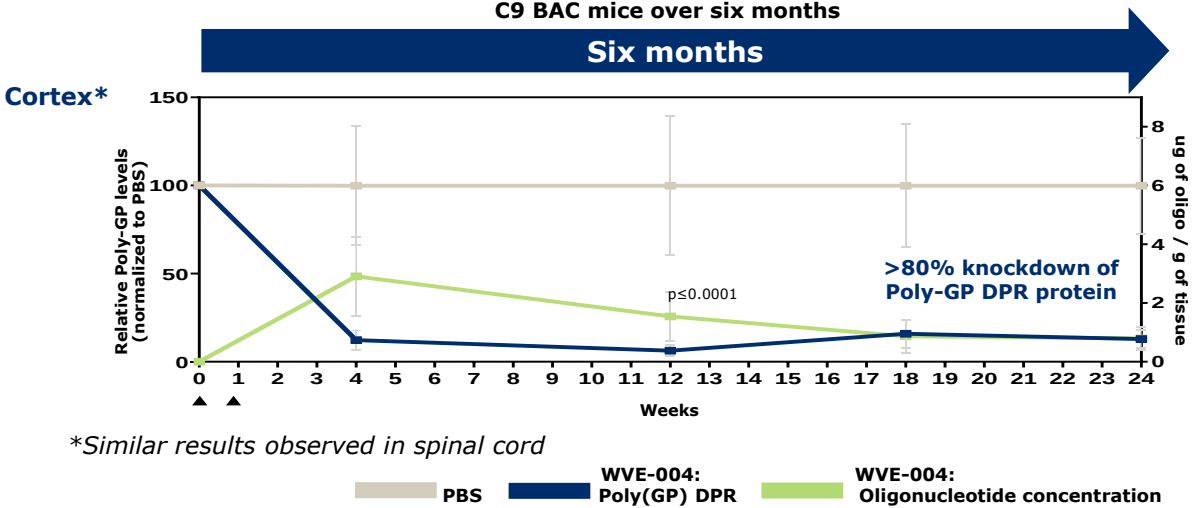
WVE-004 treatment resulted in durable reduction of poly-GP biomarker in mouse spinal cord & cortex[1,2]

Silencing – RNase H



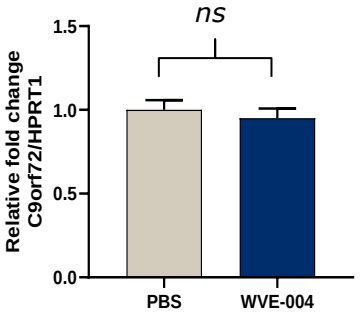
- WVE-004 leads to variant-selective silencing of C9orf72 transcripts
  - Contains PN chemistry
  - Lowers expansion-containing transcripts
  - Preserves healthy C9orf72 protein
- Poly-GP is produced from G_4C_2 expansion-containing transcripts

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



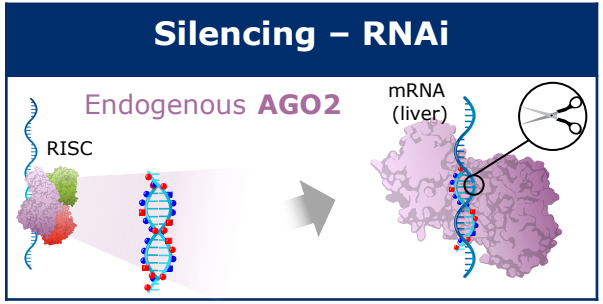
*Similar results observed in spinal cord

C9orf72 protein in C9 BAC mice unchanged at six months



*: p≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001. DPR, dipeptide repeat protein. [1]**Liu et al., 2022** *Mol Ther Nucleic Acids*. [2]**Liu et al**. Poster i182 presented at ENCALS Meeting 2022. Poly-GP assay: **Wilson et al., 2022** *J Neurol Neurosurg Psychiatry.*

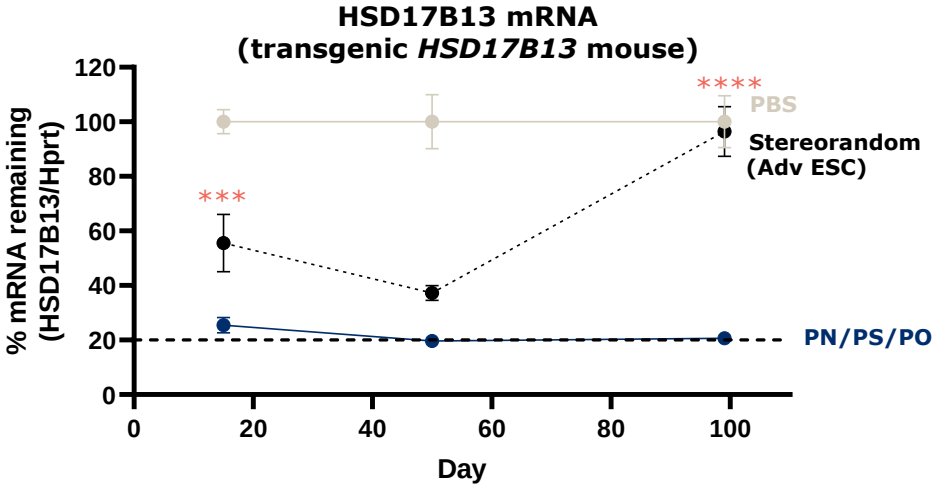
PN modification improves silencing for GalNAc-siRNAs by increasing Ago2 loading in mice

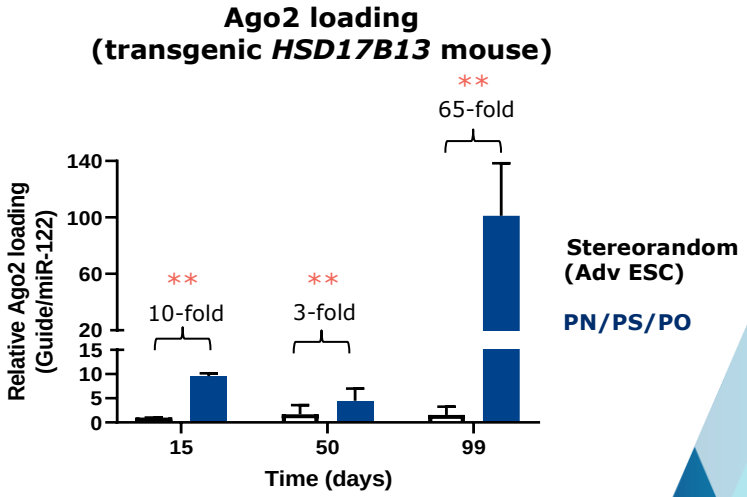


Human HSD17B13 transgenic mice

3 mg/kg SC dose (day 1)

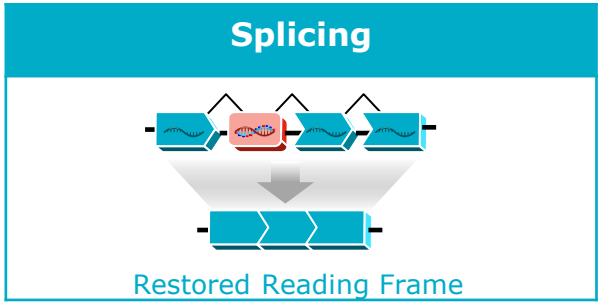
- ~80% silencing *HSD17B13* mRNA 14-weeks post-single dose with PN-containing siRNA
- Significantly greater Ago2-loading than comparator siRNA





Proprietary human *HSD17B13* transgenic mouse model dosed as indicated on day 1 (n≥3) (Left) Percentage HSD17B13 mRNA expression normalized to mouse Hprt. Stats: Linear Mixed Effects Model with Random subject effects *** p<0.001, **** p<0.0001; (Right) Ago2 loading. Stats: ** p<0.01, 2-way ANOVA

PN modification increases exon skipping, restores dystrophin expression and prolongs survival in dKO mice

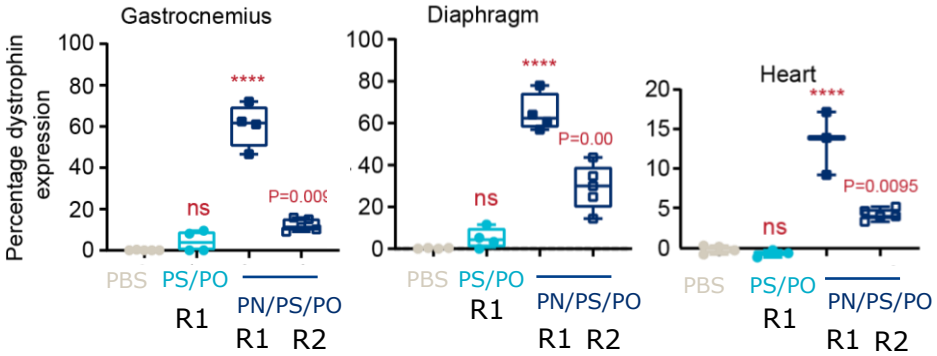


utr/mdx23 dKO mice

PBS
PS/PO
PN/PS/PO

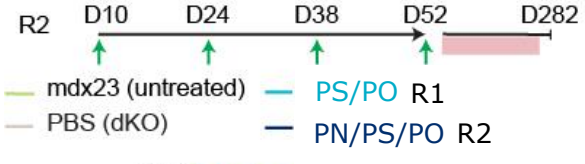
Dosing regimens
R1: 150 mg/kg weekly
R2: 75 mg/kg biweekly

Increased dystrophin protein expression in dKO mice

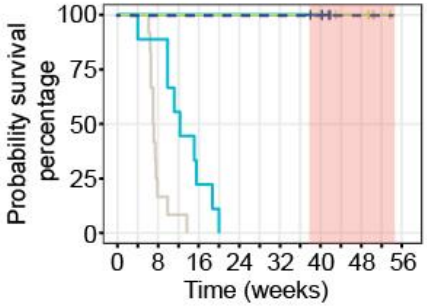


Increased survival in dKO mice

R2: 75 mg/kg, bi-weekly SC



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



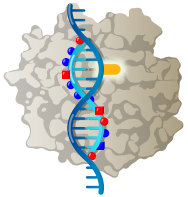
	Median survival	
	PBS	**49 days**
R1	**PS/PO**	86 days
R2	**PN/PS/PO**	**280 days**

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

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

WVE-006 directs RNA base editing to increase serum AAT protein above potential therapeutic threshold in mice

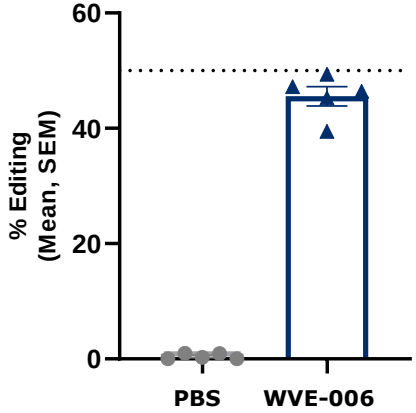
Editing



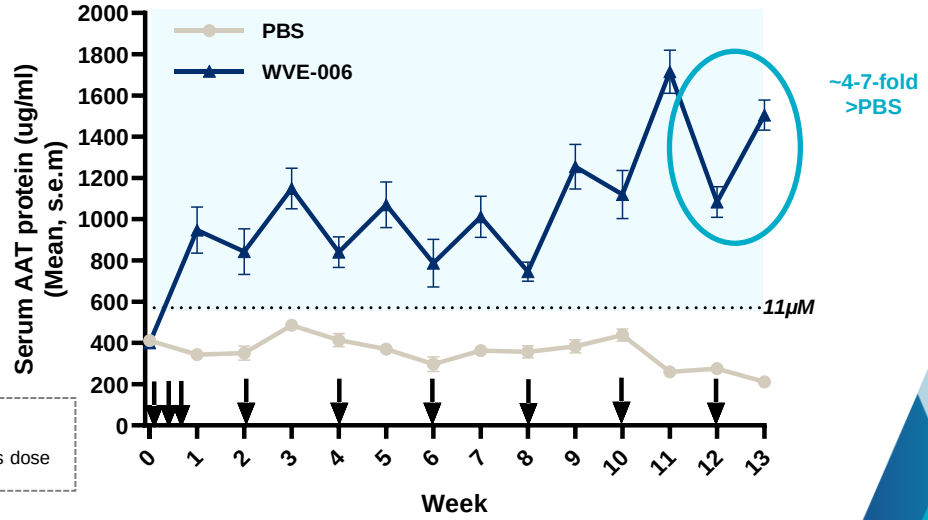
AIMers engage endogenous **ADAR** enzymes

- WVE-006: PN-modified AIMer for *SERPINA1* RNA editing
- *~50% SERPINA1* mRNA editing in liver of AATD mouse model
- WVE-006 increases serum AAT protein levels >11 uM in AATD mouse model

SERPINA1 mRNA editing (liver, NSG-PiZ mice, week 13)



Serum AAT protein levels (NSG-PiZ mice)



WVE-006: GalNAc-conjugated AIMer; NSG-PiZ mice dosed with WVE-006 (n=5).
(Left) SERPINA1 editing quantified from 13-week liver biopsies by Sanger sequencing; (Right) Total serum AAT protein quantified by ELISA

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*

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