Building a Therapeutic A-to-I RNA Editing Platform Through Oligonucleotide Chemistry Optimization

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Forward-looking statements

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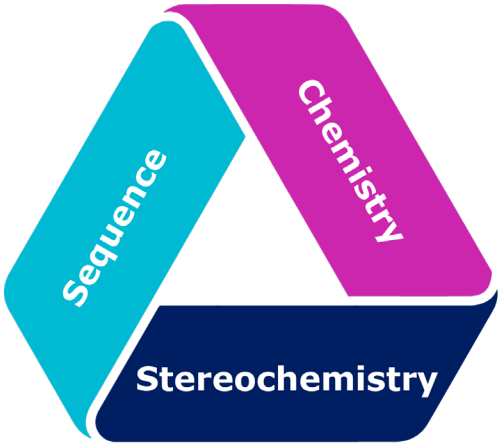
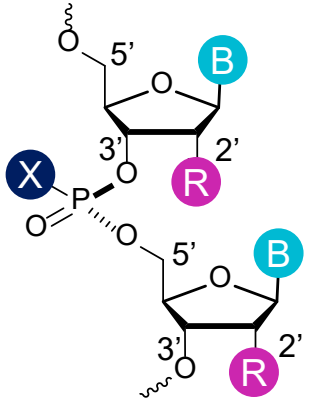
Disclosures

- Ian Harding is an employee of Wave Life Sciences

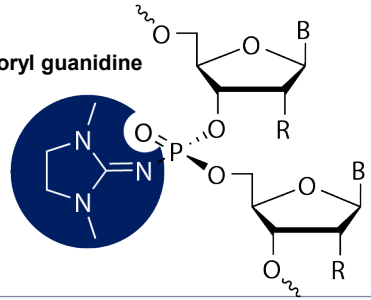
Wave's ability to rationally design oligonucleotides enables access to unique disease targets





- **B** Base
- **R** 2'-Ribose
- **X** Stereochemistry and backbone modification

Phosphoryl guanidine

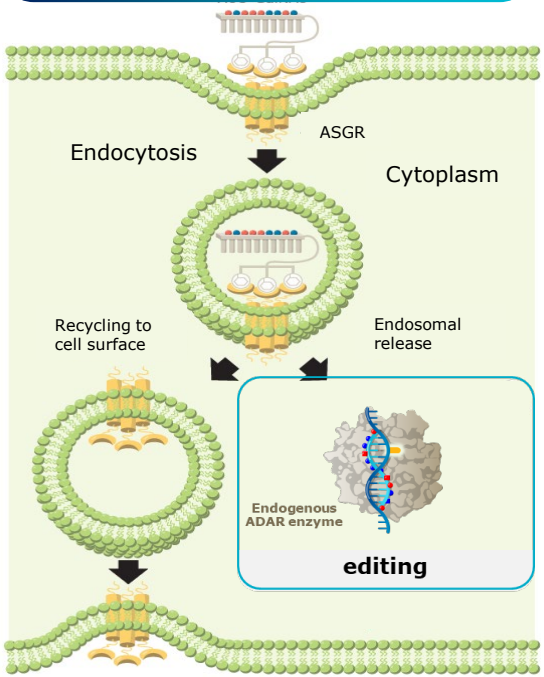


O: Phosphodiester
S: Phosphorothioate
N: Phosphoryl guanidine

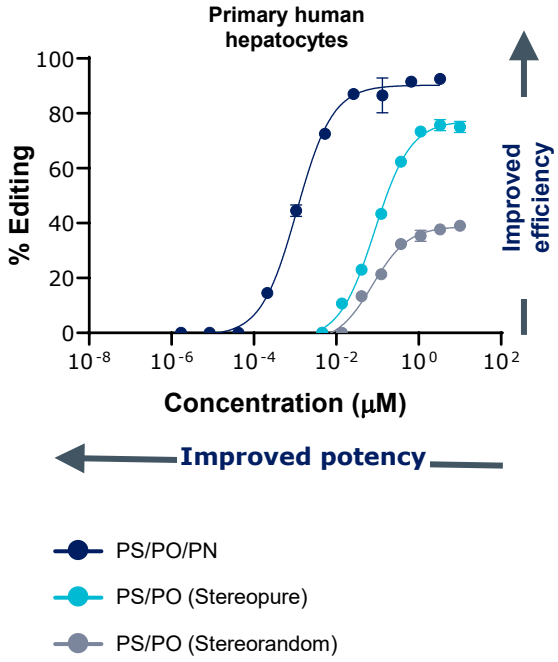
WAVE™ LIFE SCIENCES

Unlocking RNA editing with PRISM™ to develop AIMers: A-to-I editing oligonucleotides

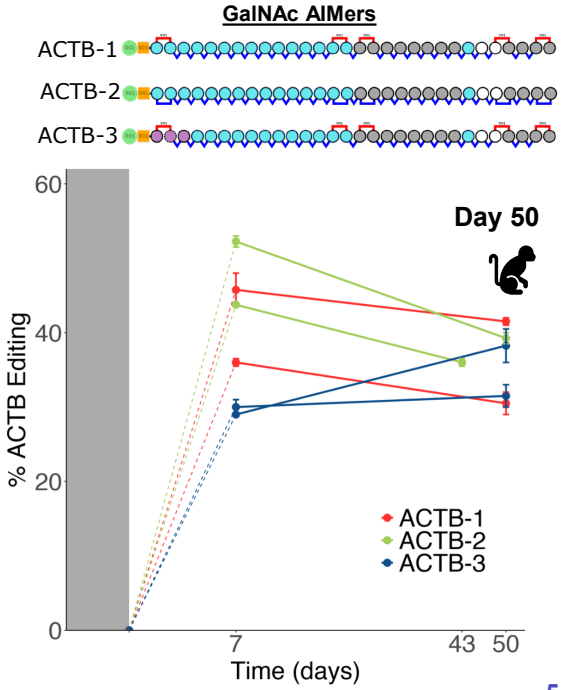
GalNAc-mediated uptake



PRISM™-driven gains



Substantial and durable editing in NHP liver *in vivo*



[1]Woolf *et al.*, 1995 *Proc Natl Assoc Sci* 92:8298-8302;
Monian *et al.*, 2022 *Nature Biotech* published online Mar 7, 2022 doi: 10.1038.s41587-022-01225-1

Overview

1. Optimizing editing activity with PRISM™
2. Achieving RNA editing in multiple tissues
3. Applications

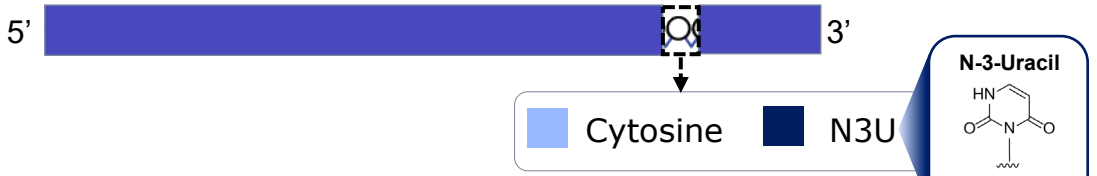
Overview

1. Optimizing editing activity with PRISM™
2.
3.

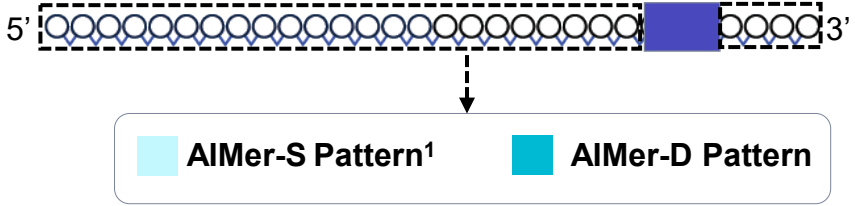
Enhancing editing efficiency across nearest neighbors

Approach: Structure-activity relationship analysis of AIMer backbone, sugar and orphan base

1 **Optimizing orphan site base**

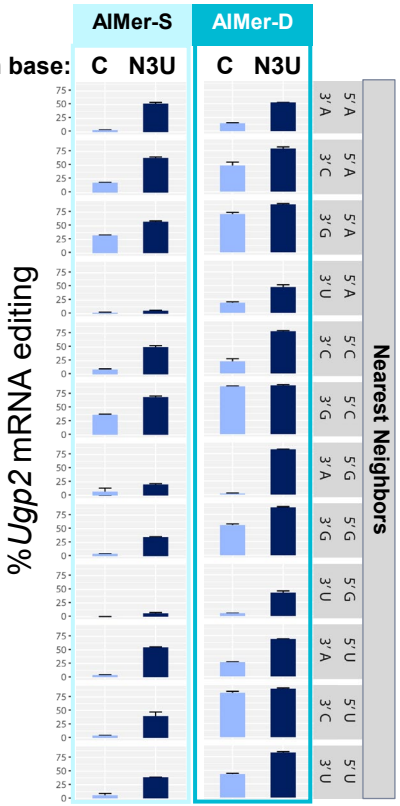


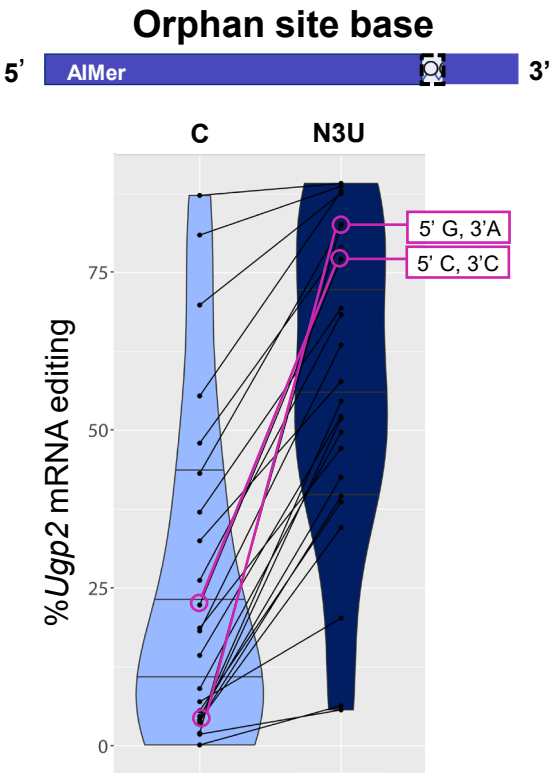
2 **Optimizing sugar and backbone modification pattern**

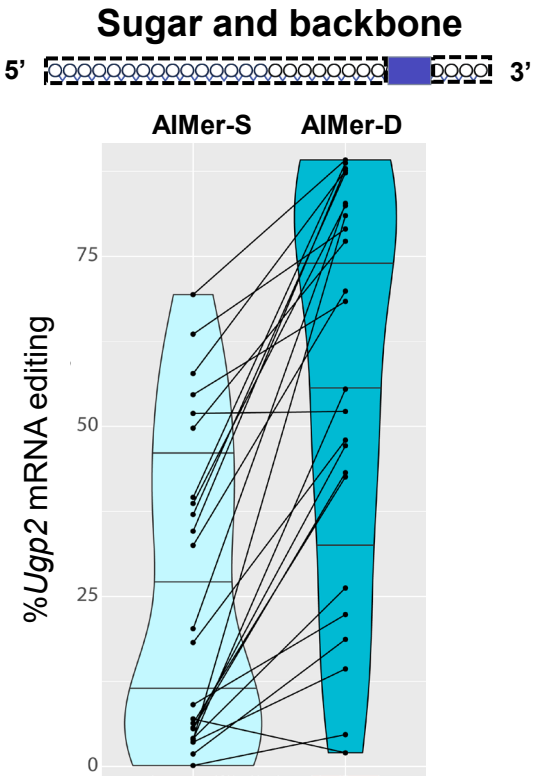


[1]Monian *et al.*, 2022 *Nature Biotech* published online Mar 7, 2022 doi: 10.1038.s41587-022-01225-1

N3U and AIMer-D chemistry mask increase editing across nearest neighbor sequences

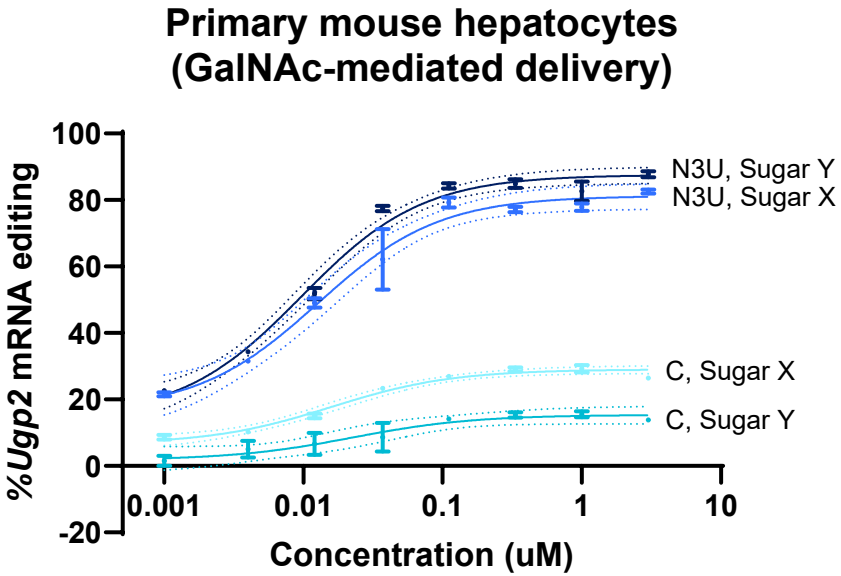


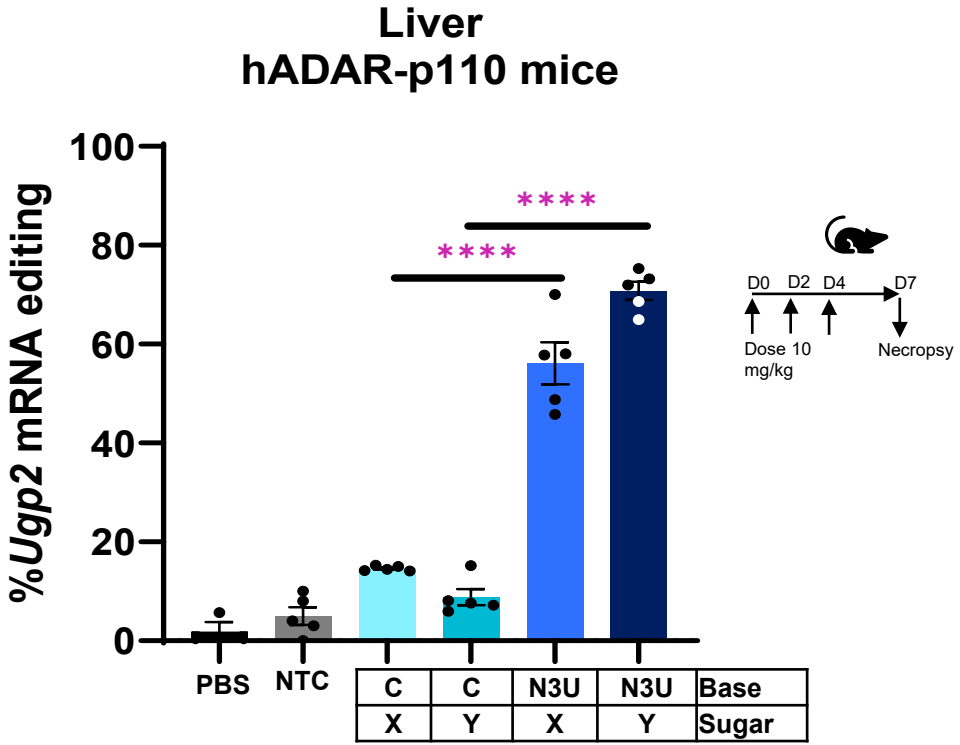




Primary mouse hepatocytes from human ADAR1 transgenic mice were treated with 3.0 uM AIMers (unconjugated), directed toward the *Ugp2* mRNA, that varied by edit region sequence, AIMer chemistry design, and edit site base for 72 hours. UGP2 RNA editing was quantified by Sanger sequencing.

N3U supports enhanced editing efficiency *in vivo*





Left: GalNAc-conjugated AIMers targeting *Ugp2* were dosed, for 72 hours prior to editing assay, in primary hepatocytes isolated from hADAR1-p110 hemizygous knock-in mice. Data shown are the mean ± SEM, n=3 for each condition. Dashed lines represent 95% confidence intervals. Right: 8-week-old transgenic human ADAR-p110 knock-in mice were dosed with PBS (black) or GalNAc-conjugated oligonucleotide (10mg/kg) subcutaneously on day 0, 2, and 4, and evaluated for UGP2 editing on day 7 (n=5/group). NTC: Non-targeting control, targeting *ACTB*. **** p<0.0001

Overview

1

2 Achieving RNA editing in multiple tissues

3

Systemic *in vivo* editing without delivery vehicles

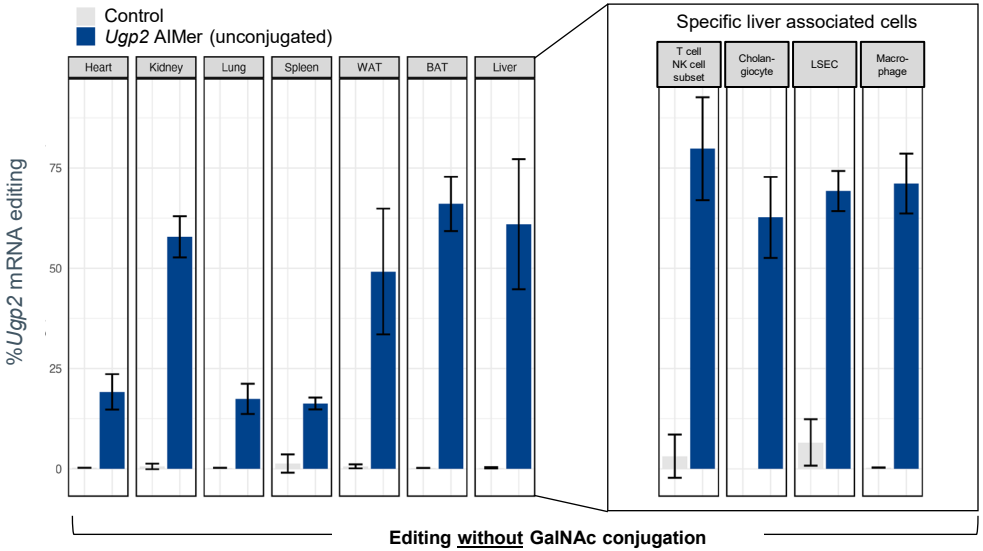
Extrahepatic

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

Delivery: Efficient RNA editing after subcutaneous injection (no delivery vehicle)

Wild-type mice

Substantial RNA editing across multiple mouse tissues following single subcutaneous dose of *Ugp2* AIMer

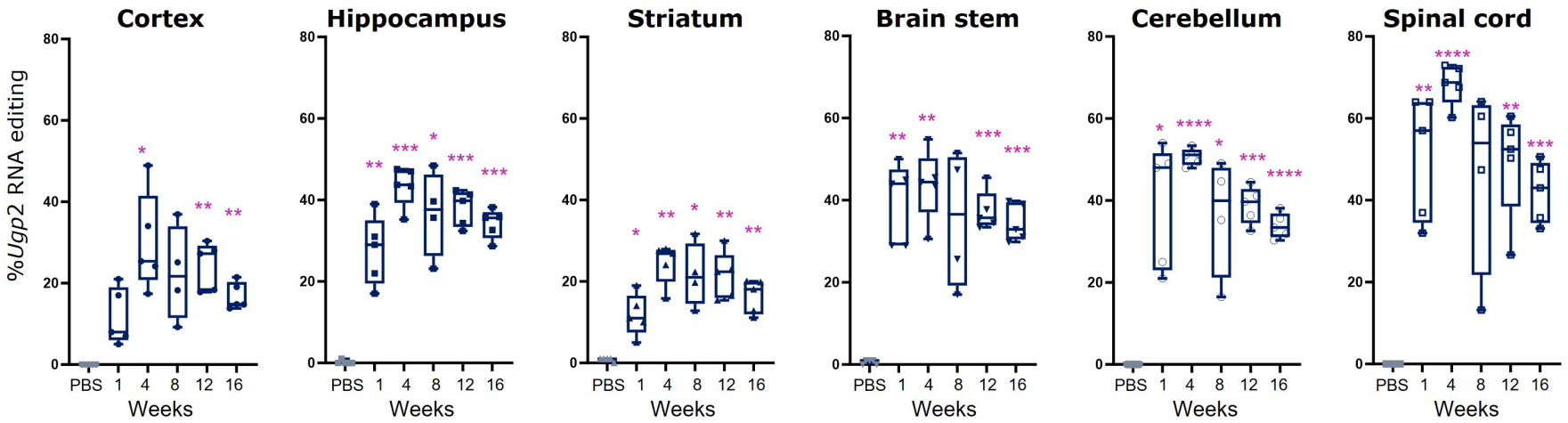


Single dose of 100 mg/kg unconjugated *Ugp2* AIMer, 7 days post dose; Results presented as mean +/- SD. WAT: White adipose tissue; BAT: Brown adipose tissue; LSEC cells: Liver Sinusoidal Endothelial Cells.

Durable editing observed out to 4 months post-single dose

Peak editing observed 4-weeks post-single ICV dose across tissues

hADAR mice

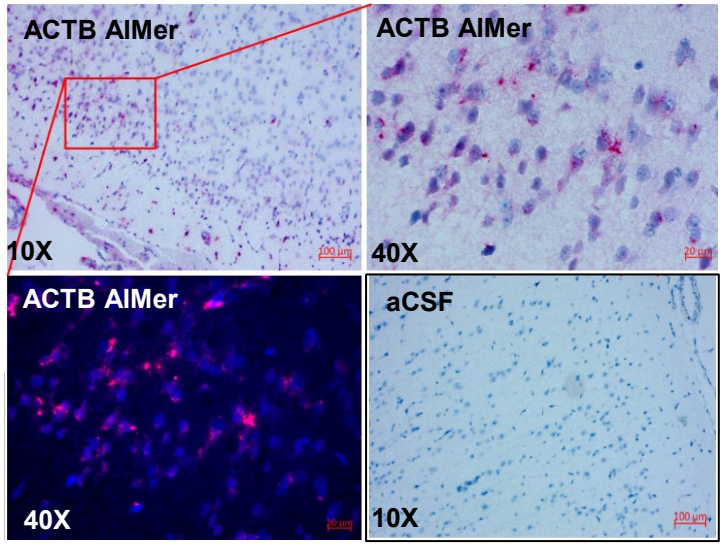


	Cortex	Hippocampus	Striatum	Brain stem	Cerebellum	Spinal cord
Peak editing	30%	>40%	25%	>40%	50%	>65%

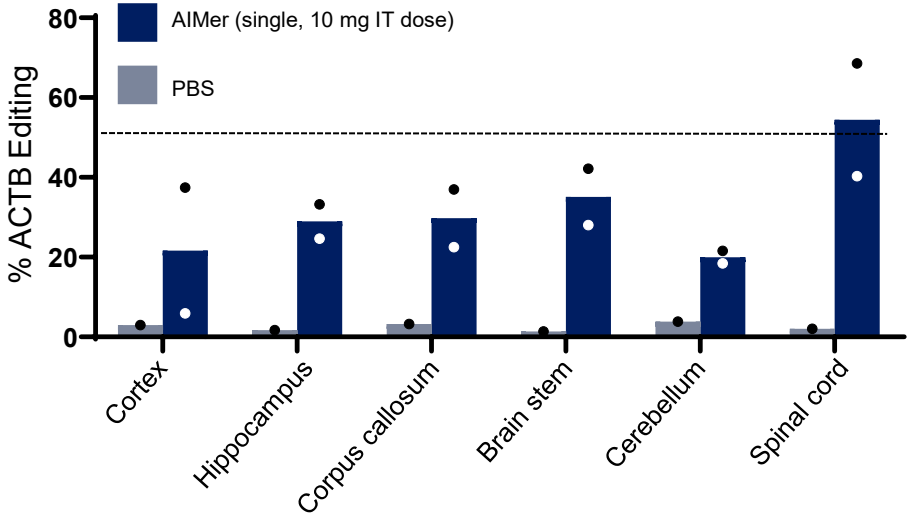
Transgenic human ADAR mice were administered 100 mg AIMer or PBS on day 0 and evaluated for *Ugp2* RNA editing across CNS tissues at 1, 4, 8, 12 and 16-weeks post dose. Percentage *Ugp2* RNA editing determined by Sanger sequencing. Stats: 2-way ANOVA with post-hoc comparison to PBS (n=5 per time point per treatment) *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ICV: intracerebroventricular

AIMer directs widespread RNA editing in CNS of NHP

Distribution to Frontal Cortex



In vivo CNS editing in NHP (*ACTB*, 1 week)



Cynomolgus monkeys (NHPs) were administered 10 mg AIMer or artificial CSF (aCSF) by IT administration on day 0 with distribution and editing evaluated across CNS 1-week post dose. Left: Sections from treated NHPs. ViewRNA (red, Fast red) was used to detect oligonucleotides; sections are counterstained with hematoxylin (blue nuclei). Right: Percentage of UGP2 editing determined by Sanger sequencing. IT: Intrathecal; SN: Substantia Nigra

Overview

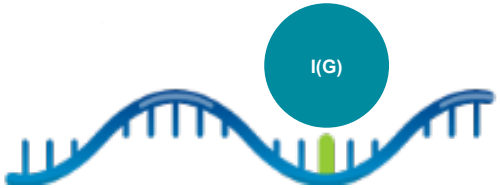
1

2

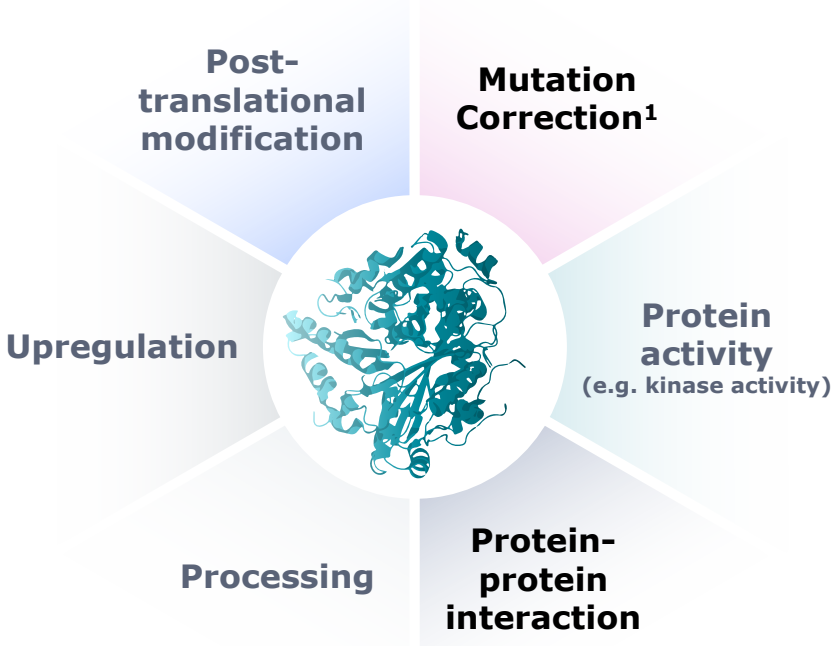
3 Applications

ADAR editing for therapeutic applications

ADAR editing of mRNA

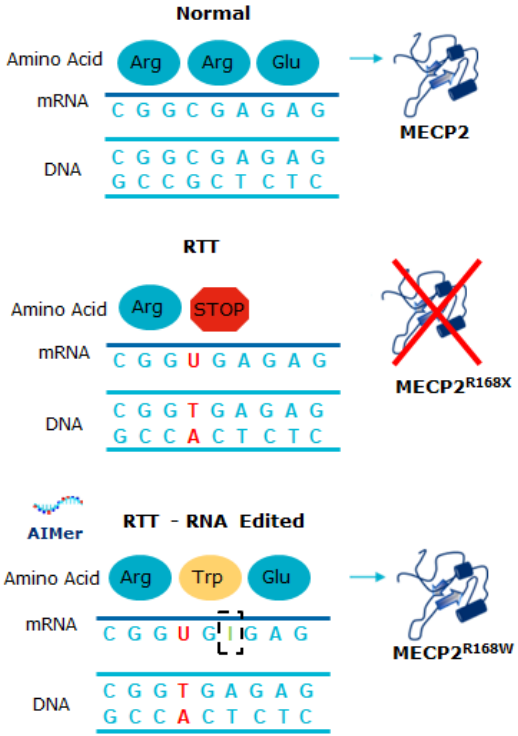


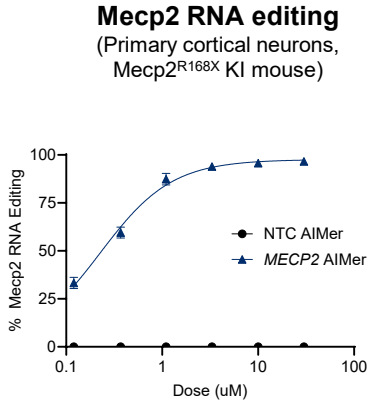
Downstream Applications

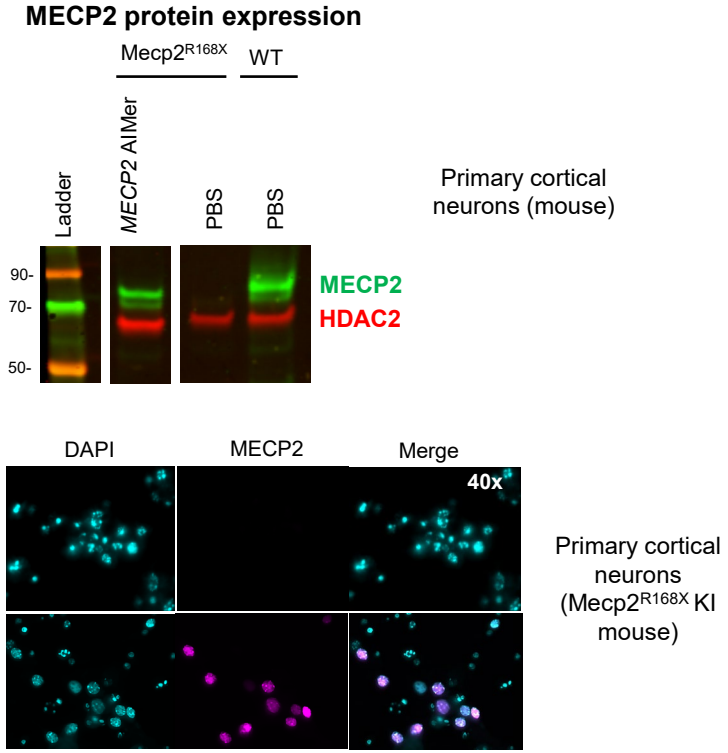


[1]Monian *et al*., 2022. *Nat Biotechnol* DOI: 10.1038/s41587-022-01225-1

AIMers can restore protein expression

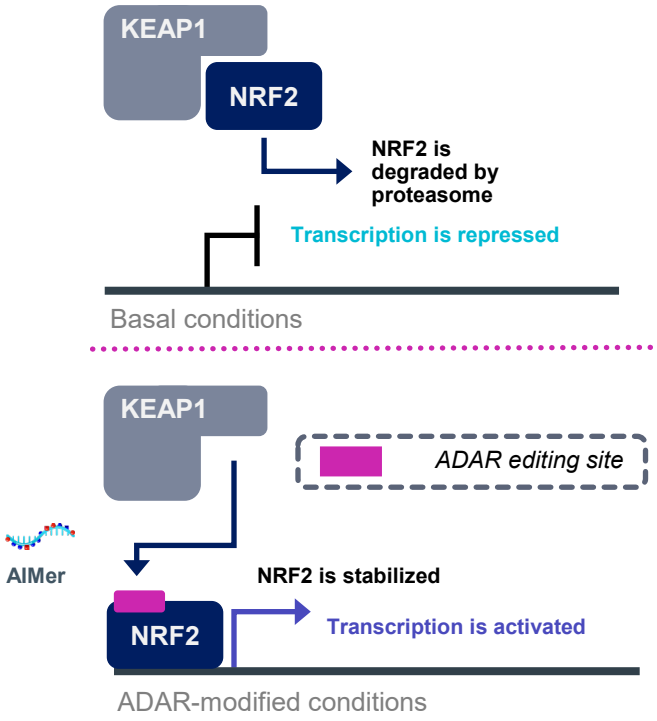


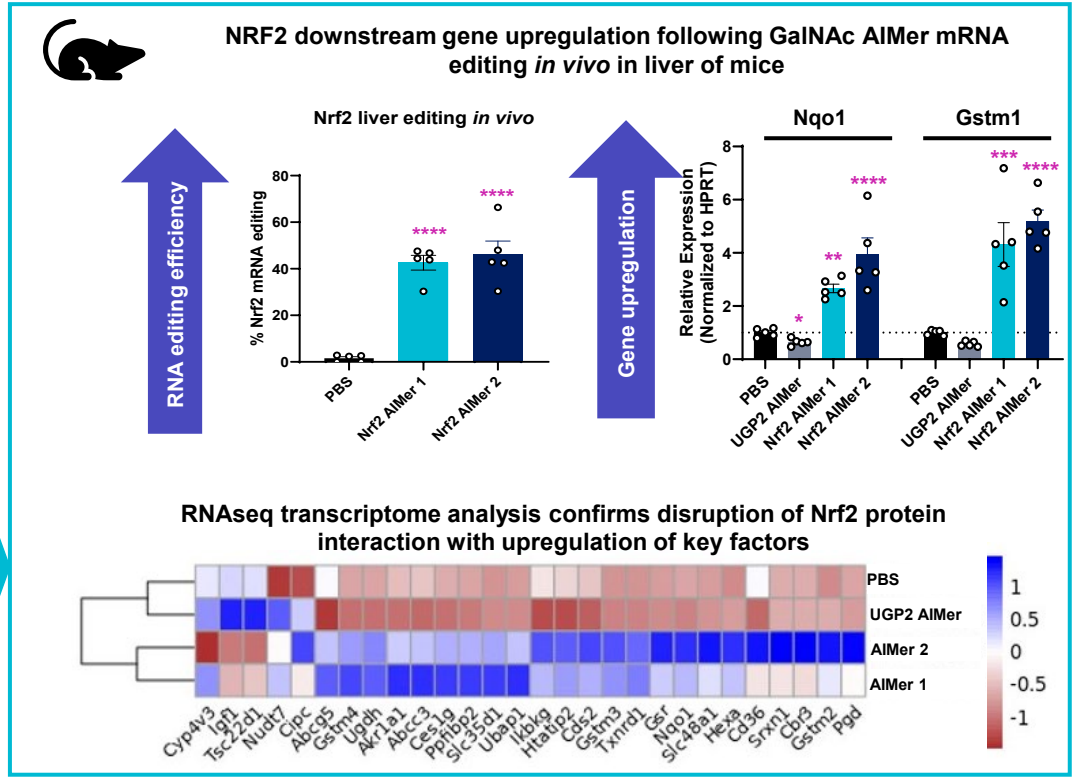




Left: Primary cortical neurons from Mecp2^{R168X} knock-in (KI) mice (E18), treated gymnotically with AIMer for 5 days (mean ± SEM; n=3 per dose/condition). Right, top: Western blot of nuclear extracts from mouse primary cortical neurons (E18) treated gymnotically with 10 µM AIMer. Right, bottom: Primary cortical neurons from Mecp2^{R168X} KI mice (E18) treated with PBS or gymnotic 1 µM AIMer for 5 days. Immunofluorescence staining for nuclei (DAPI, blue) and MECP2 (magenta) Magnification 40X. RTT: Rett Syndrome. NTC: non-targeting control.

AIMers disrupt protein-protein interaction *in vivo*





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

Summary

- Optimized AIMer design enhances editing *in vitro* and *in vivo*
  - Design improvements include N3U orphan base modification and optimization of sugar modification and backbone modification patterns
  - Editing efficiency improved across nearest neighbor combinations
- AIMers support RNA editing across multiple extrahepatic tissues including kidney, lung, and the CNS
- AIMer-based editing in the CNS is observed in mice and NHPs and is durable up to 16 weeks in mice
- AIMers can be used to disrupt protein-protein interactions and restore protein expression.

Acknowledgements

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- Nicole Neuman, Wave Life Sciences, assisted in preparing this presentation.

2022-2023 Publications

Silencing


Nucleic Acids Research, 2023

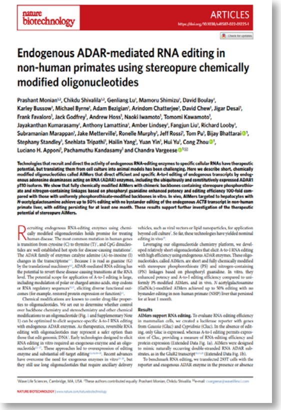
Impact of stereopure chimeric backbone chemistries on the potency and durability of gene silencing by RNA interference

Splicing


Nucleic Acids Research, 2022

Control of backbone chemistry and chirality boost oligonucleotide splice switching activity

RNA Editing


nature biotechnology ARTICLES

Endogenous ADAR-mediated RNA editing in non-human primates using stereopure chemically modified oligonucleotides