WAVE LIFE SCIENCES

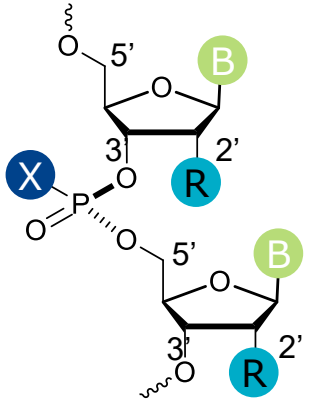
Pioneering New Applications of RNA Editing

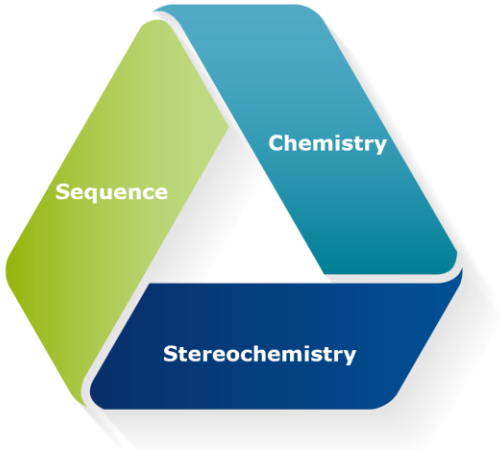
Ian Harding, PhD, Senior Scientist I

July 12, 2023

Enhancing editing activity of AIMers through application of PRISM chemistry

First Generation AIMer Design



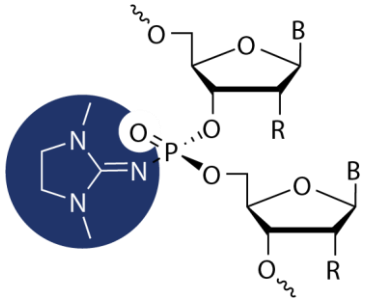


B Base

R 2′-ribose modification

X Stereochemistry & backbone modification

X:
O (Phosphodiester),
S (Phosphorothioate),
N (Phosphoryl guanidine)



Phosphoryl guanidine

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

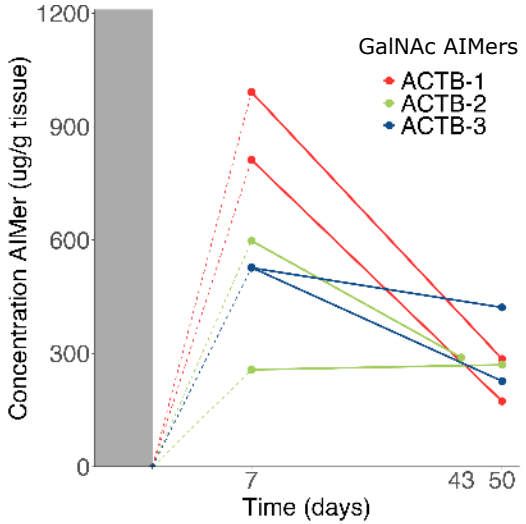
Proof-of-concept preclinical RNA editing data published in *Nature Biotechnology* (March 2022)

nature biotechnology ARTICLES

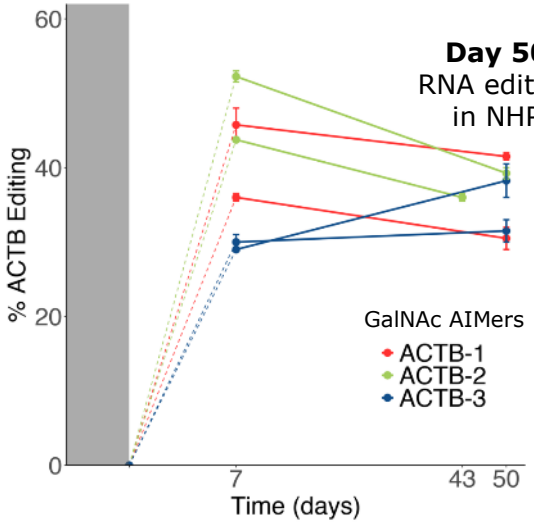
Endogenous ADAR-mediated RNA editing in non-human primates using stereopure chemically modified oligonucleotides

- Specificity *in vitro* & *in vivo* (NHPs)
- *In vitro-in vivo* translation (NHPs)
- GalNAc conjugation
- Foundational AIMer SAR

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

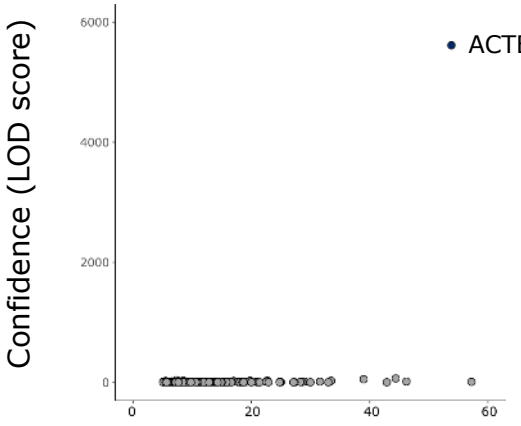


Substantial and durable editing in NHP liver *in vivo* (PD)



ADAR editing with ACTB AIMer is highly specific

RNA editing within full transcriptome (primary human hepatocytes)



RNA editing only detected at editing site in ACTB transcript

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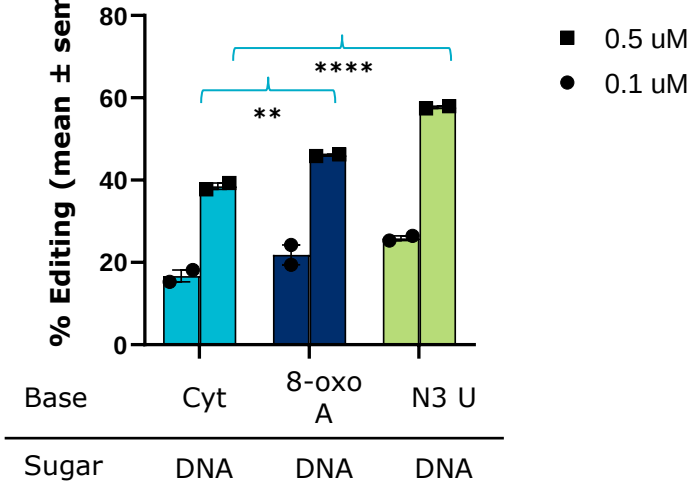
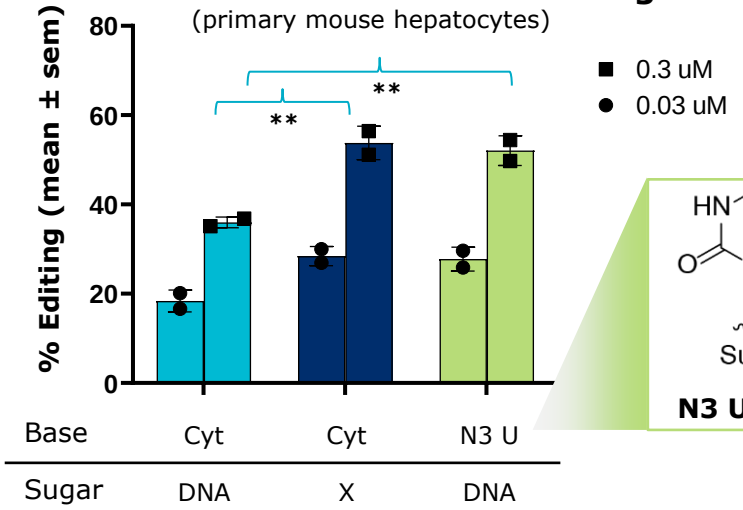
Monian et al., 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1
SAR structure-activity relationship

Base and ribose modifications at the edit site increase editing

Base modification enhances *SERPINA1* RNA editing
(primary mouse hepatocytes)



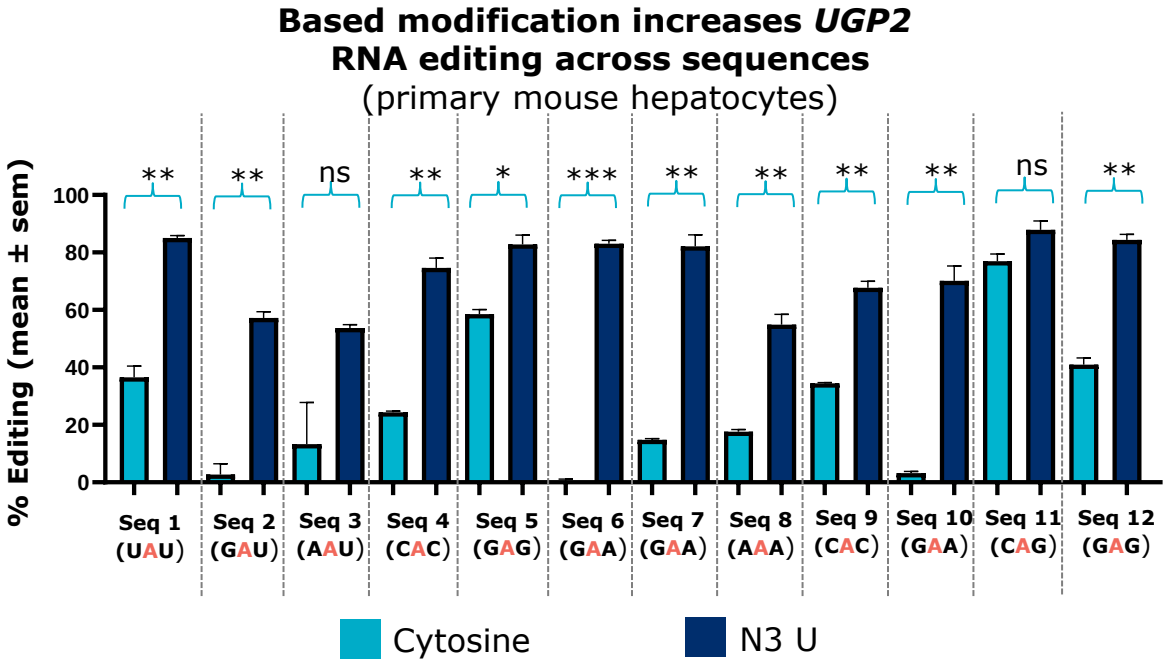
Base and sugar modifications enhance *SERPINA1* RNA editing
(primary mouse hepatocytes)



Primary mouse hepatocytes from human ADAR1/SERPINA1 transgenic mice (Right) Primary mouse hepatocytes treated with AIMers with the indicated edit site modifications. RNA editing was quantified by Sanger sequencing.
Cyt: Cytosine; 8-oxo dA: 8-Oxo-2′-deoxyadenosine; N3 U: N3 Uridine; X: modified sugar; DNA: deoxyribose

Presented at RNA Editing 2023 – Gordon Research Conference (March 2023)

Edit site base modification increases editing across edit region sequences

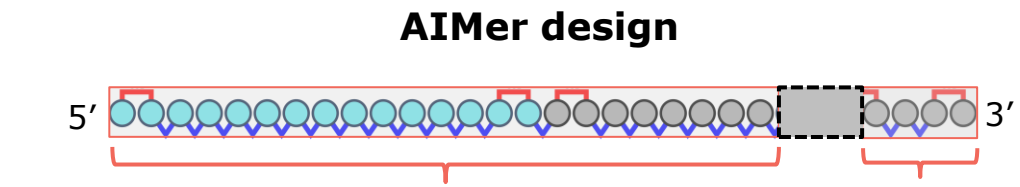


- N3 U consistently increases editing across numerous nearest neighbor pairings
- Pronounced impact for editing GAU and GAA sequences
- Additional sequence screening with N3 U ongoing

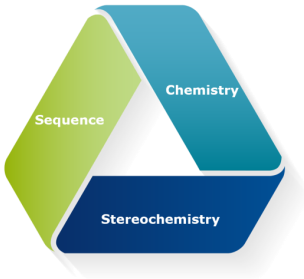
Primary mouse hepatocytes from human ADAR1 transgenic mice were treated with 3 uM AIMers that varied by edit region sequence and edit site base modification for 72 hours. RNA editing was quantified by Sanger sequencing. Stats: Two Tailed T-test: * p<0.05, **p<0.005, ***p<0.001
Seq: Sequence; red font denotes edit site – modified base in AIMer corresponds to edit site in RNA

Presented at RNA Editing 2023 – Gordon Research Conference (March 2023)

Chemical optimization improves RNA editing *in vitro* across sequences



Optimize backbone and 2′-ribose **chemistry** outside the edit region



First-gen
Next-gen

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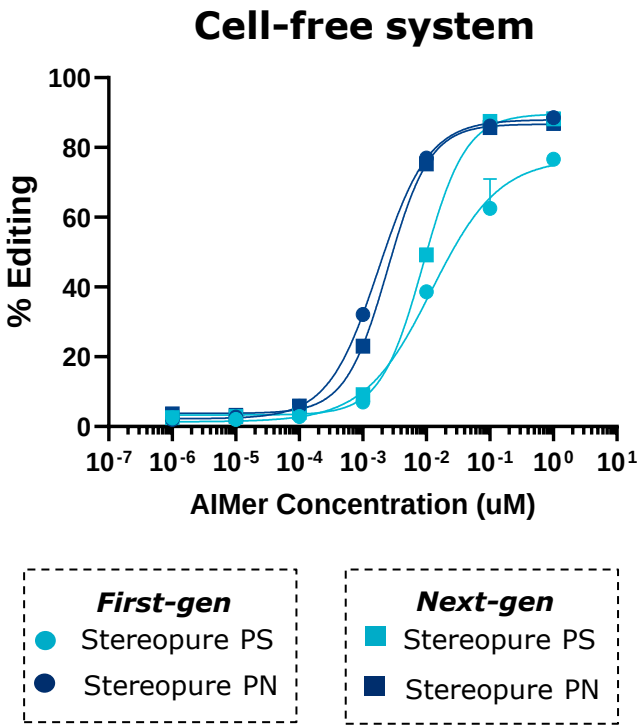
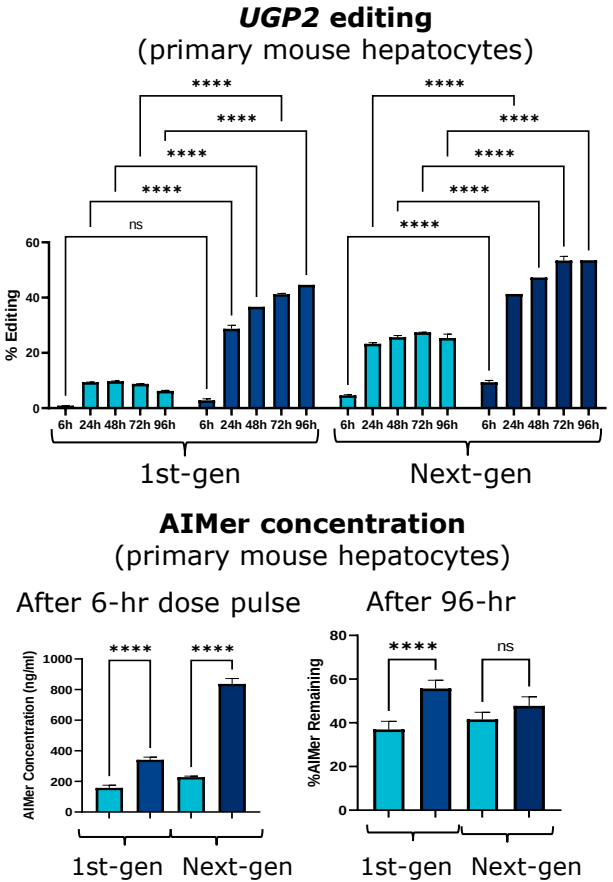
Human SF8628 and primary human astrocytes were treated with old and new AIMers of the same sequence. RNA editing was quantified by Sanger sequencing.
First-gen design based on Monian et al., 2022 *Nature Biotechnol* doi: 10.1038/s41587-022-01225-1
Presented at RNA Editing 2023 – Gordon Research Conference (March 2023)

2’-chemistry and backbone modifications enhance editing largely through improved uptake



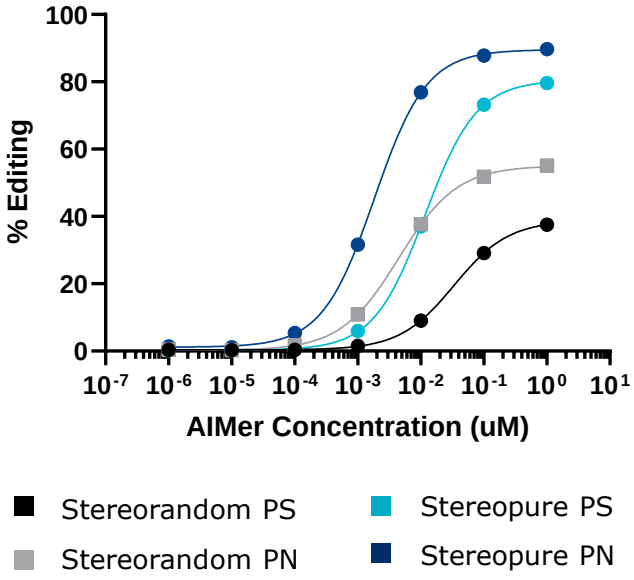


Top right: Hep3B cells were treated gymnotically or by transfection with *UGP2*-targeting AIMers. Bottom right: Primary murine hepatocytes were treated gymnotically (3 μM AIMer) and collected at the indicated time point. RNA editing was quantified by Sanger sequencing. AIMer concentration was quantified by hybridization ELISA immediately after 6-hr pulse or 96-hrs later. Stats: One-way ANOVA or mixed effects models, **** $p<0.0001$, ns significant.

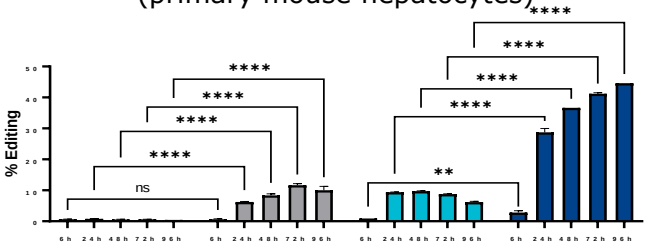
Presented at RNA Editing 2023 – Gordon Research Conference (March 2023)

PN chemistry improves target engagement, AIMer uptake, & editing efficiency in primary mouse hepatocytes

Cell-free system

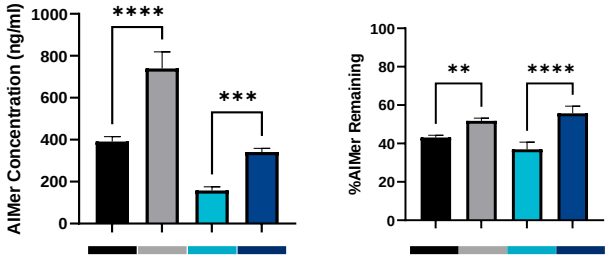


UGP2* **editing following 6 hr AIMer pulse**
(primary mouse hepatocytes)



AIMer abundance
(primary mouse hepatocytes)

After 6-hr dose pulse after 96 hr



Primary murine hepatocytes were treated gymnotically for 6-hr with 3 μM AIMer. Cells were refreshed with maintenance media and collected at the indicated time point. Editing was quantified by Sanger sequencing. AIMer concentration was quantified by hybridization ELISA. Stats: Top left, a mixed effects model Top right, One-way ANOVA * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ns not significant.

Presented at RNA Editing 2023 – Gordon Research Conference (March 2023)

Impact of PN chemistry highlighted in four high-impact publications since 2022

RNA Editing | **Splicing** | **Silencing**

nature biotechnology ARTICLES

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

Prashant Monian[1,4], Chikdu Shivalila[1,2], Genliang Lu[1], Mamoru Shimizu[1], David Boulay[1], Karley Bussow[1], Michael Byrne[1], Adam Bezigian[1], Arindom Chatterjee[1], David Chew[1], Jigar Desai[1], Frank Favaloro[1], Jack Godfrey[1], Andrew Hoss[1], Naoki Iwamoto[1], Tomomi Kawamoto[1], Jayakanthan Kumarasamy[1], Anthony Lamattina[1], Amber Lindsey[1], Fangjun Liu[1], Richard Looby[1], Subramanian Marappan[1], Jake Metterville[1], Ronelle Murphy[1], Jeff Rossi[1], Tom Pu[1], Bijay Bhattarai[1,3], Stephany Standley[1], Snehlata Tripathi[1], Hailin Yang[1], Yuan Yin[1], Hui Yu[1], Cong Zhou[1], Luciano H. Apponi[2], Pachamuthu Kandasamy[1] and Chandra Vargeese[1]

Technologies that recruit and direct the activity of endogenous RNA-editing enzymes to specific cellular RNAs have therapeutic potential, but translating them from cell culture into animal models has been challenging. Here we describe short, chemically modified oligonucleotides called AIMers that direct efficient and specific A-to-I editing of endogenous transcripts by endogenous adenosine deaminases acting on RNA (ADAR) enzymes, including the ubiquitously and constitutively expressed ADAR1 p110 isoform. We show that fully chemically modified AIMers with chimeric backbones containing stereopure phosphorothioate and nitrogen-containing linkages based on phosphoryl guanidine enhanced potency and editing efficiency 100-fold compared with those with uniformly phosphorothioate-modified backbones in vitro. In vivo, AIMers targeted to hepatocytes with *N*-acetylgalactosamine achieve up to 50% editing with no bystander editing of the endogenous *ACTB* transcript in non-human primate liver, with editing persisting for at least one month. These results support further investigation of the therapeutic potential of stereopure AIMers.

Recruiting endogenous RNA-editing enzymes using chemically modified oligonucleotides holds promise for treating human disease. The most common mutation in human genes is transition from cytosine (C) to thymine (T), and C-to-U deaminases are well established hot spots for disease-causing mutations[1]. The ADAR family of enzymes catalyze adenine (A) to inosine (I) changes in the transcriptome[2–4]. Because I is read as guanine (G) by the translational machinery[5–7], ADAR-mediated RNA editing has the potential to revert these disease-causing transitions at the RNA level. The potential scope for application of A-to-I editing is large, including modulation of polar or charged amino acids, stop codons or RNA regulatory sequences[8–11], effecting diverse functional outcomes (for example, restored protein expression or function)[12].

Chemical modifications are known to confer drug-like properties to oligonucleotides. We set out to determine whether control over backbone chemistry and stereochemistry and other chemical modifications to an oligonucleotide (Fig. 1 and Supplementary Note 1) can be optimized to elicit sequence-specific A-to-I RNA editing with endogenous ADAR enzymes. As therapeutics, reversible RNA editing with oligonucleotides may represent a safer option than those that edit genomic DNA[13]. Early technologies designed to elicit RNA editing in vitro required an exogenous enzyme and an oligonucleotide[14–16]. These approaches led to overexpression of editing enzyme and substantial off-target editing[17–19,20–22]. Recent advances have overcome the need for exogenous enzymes in vitro[23–26], but they still use long oligonucleotides that require ancillary delivery vehicles, such as viral vectors or lipid nanoparticles, for application beyond cell culture[27]. So far, these technologies have yielded minimal editing in vivo[27].

Leveraging our oligonucleotide chemistry platform, we developed relatively short oligonucleotides that elicit A-to-I RNA editing with high efficiency using endogenous ADAR enzymes. These oligonucleotides, called AIMers, are short and fully chemically modified with stereopure phosphorothioate (PS) and nitrogen-containing (PN) linkages based on phosphoryl guanidine. In vitro, they enhanced potency and A-to-I editing efficiency compared to uniformly PS-modified AIMers, and in vivo, *N*-acetylgalactosamine (GalNAc)-modified AIMers achieved up to 50% editing with no bystander editing in non-human primate (NHP) liver that persisted for at least 1 month.

Results

AIMers support RNA editing. To evaluate RNA-editing efficiency in mammalian cells, we created a luciferase reporter with genes from Gaussia (Gluc) and *Cypridina* (Cluc). In the absence of editing, only Gluc is expressed, whereas A-to-I editing permits expression of Cluc, providing a measure of RNA-editing efficiency and protein expression (Extended Data Fig. 1a). AIMers were designed to mimic naturally occurring double-stranded RNA ADAR substrates, as in the GluR2 transcript[28–30] (Extended Data Fig. 1b).

To benchmark RNA editing, we transfected *293T* cells with the reporter and exogenous ADAR enzyme in the presence or absence

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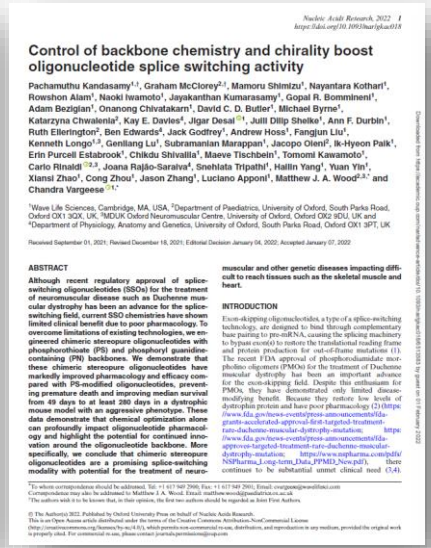

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https://doi.org/10.1093/nar/gkac018

Control of backbone chemistry and chirality boost oligonucleotide splice switching activity

Pachamuthu Kandasamy[1,†], Graham McClorey[2,†], Mamoru Shimizu[1], Nayantara Kothari[1], Rowshon Alam[1], Naoki Iwamoto[1], Jayakanthan Kumarasamy[1], Gopal R. Bommineni[1], Adam Bezigian[1], Onanong Chivatakarn[1], David C. D. Butler[1], Michael Byrne[1], Katarzyna Chwalenia[2], Kay E. Davies[4], Jigar Desai[1,*], Julil Dilip Shelke[1], Ann F. Durbin[1], Ruth Ellerington[2], Ben Edwards[4], Jack Godfrey[1], Andrew Hoss[1], Fangjun Liu[1], Kenneth Longo[1,3], Genliang Lu[1], Subramanian Marappan[1], Jacopo Oieni[2], Ik-Hyeon Paik[1], Erin Purcell Estabrook[1], Chikdu Shivalila[1], Maeve Tischbein[1], Tomomi Kawamoto[1], Carlo Rinaldi[2,3], Joana Rajão-Saraiva[4], Snehlata Tripathi[1], Hailin Yang[1], Yuan Yin[1], Xianxi Zhao[1], Cong Zhou[1], Jason Zhang[1], Luciano Apponi[1], Matthew J. A. Wood[2,‡,*] and Chandra Vargeese[1,*]

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ABSTRACT

Although recent regulatory approval of splice-switching oligonucleotides (SSOs) for the treatment of neuromuscular disease such as Duchenne muscular dystrophy has been an advance for the splice-switching field, current SSO chemistries have shown limited clinical benefit due to poor pharmacology. To overcome limitations of existing technologies, we engineered chimeric stereopure oligonucleotides with phosphorothioate (PS) and phosphoryl guanidine-containing (PN) backbones. We demonstrate that these chimeric stereopure oligonucleotides have markedly improved pharmacology and efficacy compared with PS-modified oligonucleotides, preventing premature death and improving median survival from 49 days to at least 280 days in a dystrophic mouse model with an aggressive phenotype. These data demonstrate that chemical optimization alone can profoundly impact oligonucleotide pharmacology and highlight the potential for continued innovation around the oligonucleotide backbone. More specifically, we conclude that chimeric stereopure oligonucleotides are a promising splice-switching modality with potential for the treatment of neuromuscular and other genetic diseases impacting difficult to reach tissues such as the skeletal muscle and heart.

INTRODUCTION

Exon-skipping oligonucleotides, a type of a splice-switching technology, are designed to bind through complementary base pairing to pre-mRNA, causing the splicing machinery to bypass exon(s) to restore the translational reading frame and protein production for out-of-frame mutations (1). The recent FDA approval of phosphorodiamidate morpholino oligomers (PMOs) for the treatment of Duchenne muscular dystrophy has been an important advance for the exon-skipping field. Despite this enthusiasm for PMOs, they have demonstrated only limited disease-modifying benefit. Because they restore low levels of dystrophin protein and have poor pharmacology (2) (https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation; https://www.fda.gov/news-events/press-announcements/fda-approves-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation; https://www.nspharma.com/pdfs/NSPharma_Long-term_Data_PPMD_New.pdf), there continues to be substantial unmet clinical need (3,4).

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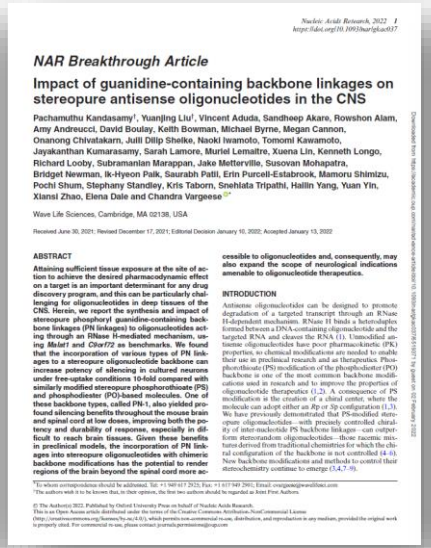
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.



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NAR Breakthrough Article

Impact of guanidine-containing backbone linkages on stereopure antisense oligonucleotides in the CNS

Pachamuthu Kandasamy[†], Yuanjing Liu[†], Vincent Aduda, Sandheep Akare, Rowshon Alam, Amy Andreucci, David Boulay, Keith Bowman, Michael Byrne, Megan Cannon, Onanong Chivatakarn, Julil Dilip Shelke, Naoki Iwamoto, Tomomi Kawamoto, Jayakanthan Kumarasamy, Sarah Lamore, Muriel Lemaitre, Xuena Lin, Kenneth Longo, Richard Looby, Subramanian Marappan, Jake Metterville, Susovan Mohapatra, Bridget Newman, Ik-Hyeon Paik, Saurabh Patil, Erin Purcell-Estabrook, Mamoru Shimizu, Pochi Shum, Stephany Standley, Kris Taborn, Snehlata Tripathi, Hailin Yang, Yuan Yin, Xianxi Zhao, Elena Dale and Chandra Vargeese[*]

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ABSTRACT

Attaining sufficient tissue exposure at the site of action to achieve the desired pharmacodynamic effect on a target is an important determinant for any drug discovery program, and this can be particularly challenging for oligonucleotides in deep tissues of the CNS. Herein, we report the synthesis and impact of stereopure phosphoryl guanidine-containing backbone linkages (PN linkages) to oligonucleotides acting through an RNase H-mediated mechanism, using *Malat1* and *Cikrf72* as benchmarks. We found that the incorporation of various types of PN linkages to a stereopure oligonucleotide backbone can increase potency of silencing in cultured neurons under free-uptake conditions 10-fold compared with similarly modified stereopure phosphorothioate (PS) and phosphodiester (PO)-based molecules. One of these backbone types, called PN-1, also yielded profound silencing benefits throughout the mouse brain and spinal cord at low doses, improving both the potency and durability of response, especially in difficult to reach brain tissues. Given these benefits in preclinical models, the incorporation of PN linkages into stereopure oligonucleotides with chimeric backbone modifications has the potential to render regions of the brain beyond the spinal cord more accessible to oligonucleotides and, consequently, may also expand the scope of neurological indications amenable to oligonucleotide therapeutics.

INTRODUCTION

Antisense oligonucleotides can be designed to promote degradation of a targeted transcript through an RNase H-dependent mechanism. RNase H binds a heteroduplex formed between a DNA-containing oligonucleotide and the targeted RNA and cleaves the RNA (1). Unmodified antisense oligonucleotides have poor pharmacokinetic (PK) properties, so chemical modifications are needed to enable their use in preclinical research and as therapeutics. Phosphorothioate (PS) modification of the phosphodiester (PO) backbone is one of the most common backbone modifications used in research and to improve the properties of oligonucleotide therapeutics (1,2). A consequence of PS modification is the creation of a chiral center, where the molecule can adopt either an *Rp* or *Sp* configuration (1,3). We have previously demonstrated that PS-modified stereopure oligonucleotides—with precisely controlled chirality of internucleotide linkages—can outperform stereorandom oligonucleotides—those racemic mixtures derived from traditional chemistries for which the chiral configuration of the backbone is not controlled (4–6). New backbone modifications and methods to control their stereochemistry continue to emerge (3,4,7–9).

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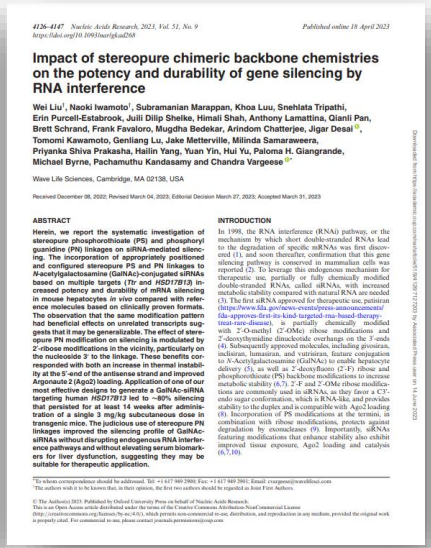
†The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors.



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https://doi.org/10.1093/nar/gkad268
Published online 18 April 2023

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

Wei Liu[†], Naoki Iwamoto[†], Subramanian Marappan, Khoa Luu, Snehlata Tripathi, Erin Purcell-Estabrook, Julil Dilip Shelke, Himali Shah, Anthony Lamattina, Qianli Pan, Brett Schrand, Frank Favaloro, Mugdha Bedekar, Arindom Chatterjee, Jigar Desai[*], Tomomi Kawamoto, Genliang Lu, Jake Metterville, Milinda Samarasekera, Priyanka Shiva Prakasha, Hailin Yang, Yuan Yin, Hui Yu, Paloma H. Giangrande, Michael Byrne, Pachamuthu Kandasamy and Chandra Vargeese[*]

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ABSTRACT

Herein, we report the systematic investigation of stereopure phosphorothioate (PS) and phosphoryl guanidine (PN) linkages on siRNA-mediated silencing. The incorporation of appropriately positioned and configured stereopure PS and PN linkages to *N*-acetylgalactosamine (GalNAc)-conjugated siRNAs based on multiple targets (*Ttr* and *HSD17B13*) increased potency and durability of mRNA silencing in mouse hepatocytes *in vivo* compared with reference molecules based on clinically proven formats. The observation that the same modification pattern had beneficial effects on unrelated transcripts suggests that it may be generalizable. The effect of stereopure PN modification on silencing is modulated by 2′-ribose modifications in the vicinity, particularly on the nucleoside 3′ to the linkage. These benefits corresponded with both an increase in thermal instability at the 5′-end of the antisense strand and improved Argonaute 2 (Ago2) loading. Application of one of our most effective designs to generate a GalNAc-siRNA targeting human *HSD17B13* led to ∼80% silencing that persisted for at least 14 weeks after administration of a single 3 mg/kg subcutaneous dose in transgenic mice. The judicious use of stereopure PN linkages improved the durability profile of GalNAc-siRNAs without disrupting endogenous RNA interference pathways and without elevating serum biomarkers for liver dysfunction, suggesting they may be suitable for therapeutic application.

INTRODUCTION

In 1998, the RNA interference (RNAi) pathway, or the mechanism by which short double-stranded RNAs lead to the degradation of specific mRNAs was first discovered (1), and soon thereafter, confirmation that this gene silencing pathway is conserved in mammalian cells was reported (2). To leverage this endogenous mechanism for therapeutic use, partially or fully chemically modified double-stranded RNAs, called siRNAs, with increased metabolic stability compared with natural RNA are needed (3). The first siRNA approved for therapeutic use, patisiran (https://www.fda.gov/news-events/press-announcements/fda-approves-first-its-kind-targeted-rna-based-therapy-treat-rare-disease), is partially chemically modified with 2′-O-methyl (2′-OMe) ribose modifications and 2′-deoxythymidine dinucleotide overhangs on the 3′-ends (4). Subsequently approved molecules, including givosiran, inclisiran, lumasiran, and vutrisiran, feature conjugation to *N*-Acetylgalactosamine (GalNAc) to enable hepatocyte delivery (5), as well as 2′-deoxyfluoro (2′-F) ribose and phosphorothioate (PS) backbone modifications to increase metabolic stability (6,7). 2′-F and 2′-OMe ribose modifications are commonly used in siRNAs, as they favor a C3′-endo sugar conformation, which is RNA-like, and provides stability to the duplex and is compatible with Ago2 loading (8). Incorporation of PS modifications at the termini, in combination with ribose modifications, protects against degradation by exonucleases (9). Importantly, siRNAs featuring modifications that enhance stability also exhibit improved tissue exposure, Ago2 loading and catalysis (6,7,10).

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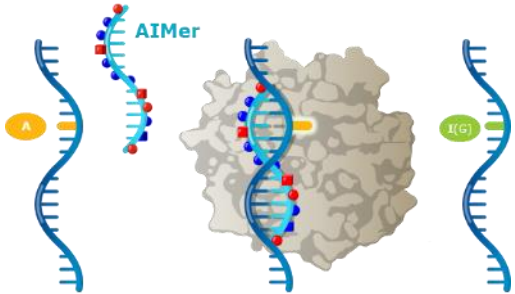
Expanding addressable disease target space using AIMers to activate pathways and upregulate expression

Correct G-to-A driver mutations with AIMers

Restore or correct protein function ✓

WVE-006
(GalNAc AIMer)
AATD



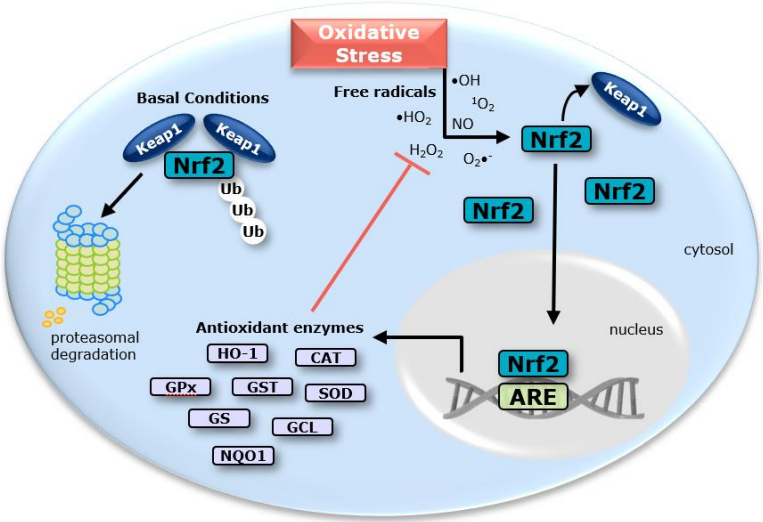
Novel applications beyond mutation correction

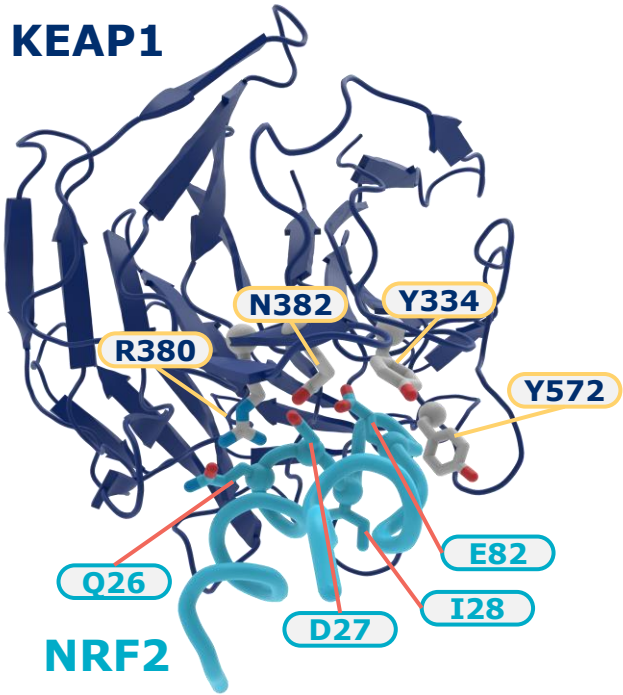
- ☑ **Modulate protein-protein interaction** *Achieved POC*
- ☑ **Upregulate expression** *Achieved POC*
- ☐ Modify function
- ☐ Post-translational modification
- ☐ Alter folding or processing

AIMers provide dexterity, with applications beyond precise correction of genetic mutations, including upregulation of expression, modification of protein function, or altering protein stability

POC: proof of concept

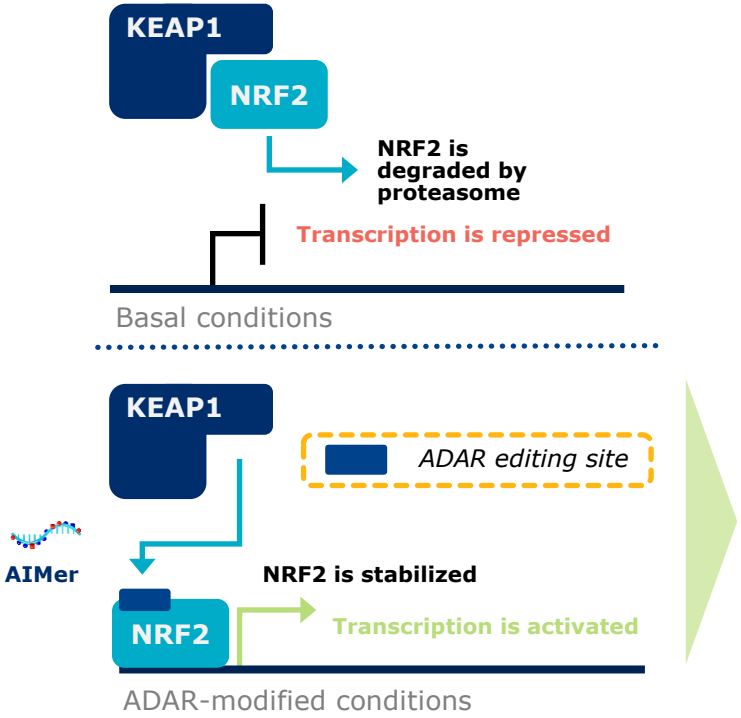
Nrf2 is an antioxidant transcription factor negatively regulated by Keap1 through Nrf2-Keap1 binding



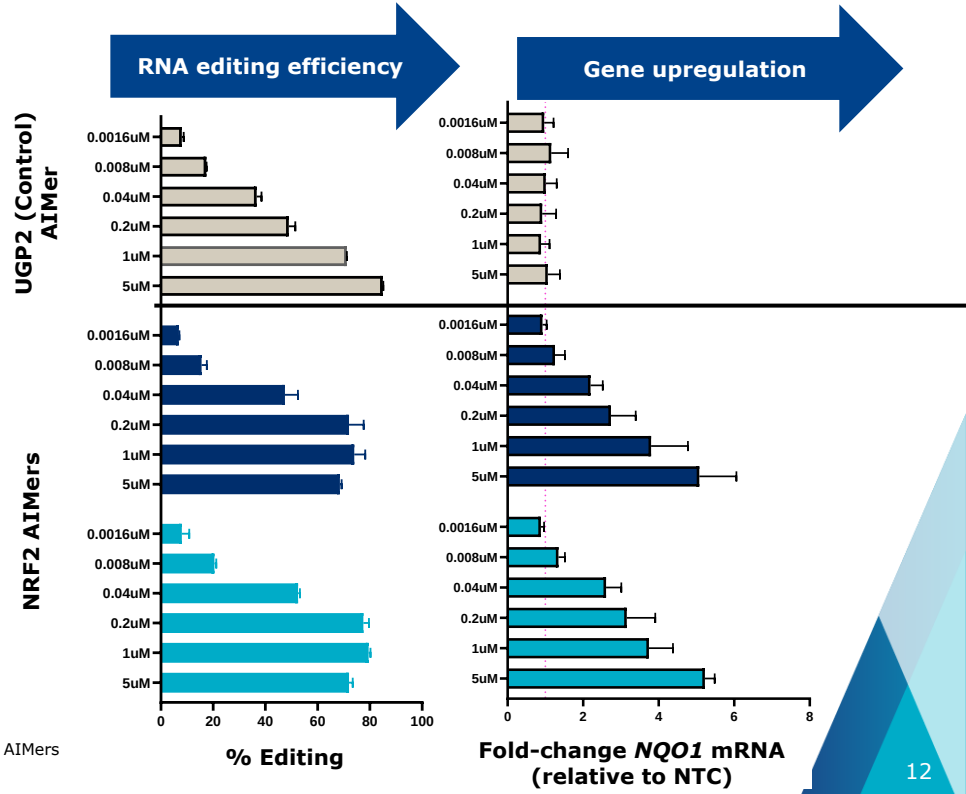


Left: Adapted from Int. J. Mol. Sci. 2019, 20(13), 3208; https://doi.org/10.3390/ijms20133208
Right: Adapted from Free Radical Biology and Medicine, 2015, 88, 101-107; https://doi.org/10.1016/j.freeradbiomed.2015.05.034
Note: Q26, D27, and I28 appear on the Nrf2 DLG motif; E82 appears on the Nrf2 ETGE motif.

Dose-dependent modulation of protein/protein interactions *in vitro*



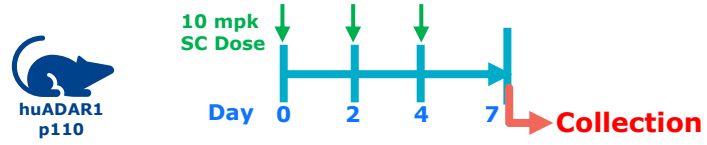
Dose-dependent gene upregulation (NQO1) *in vitro* following Nrf2 editing to disrupt protein/protein interaction (primary hepatocytes)

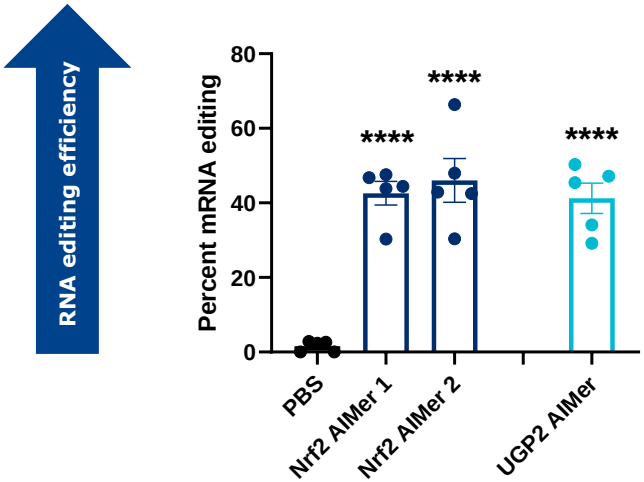


n=2; Primary hepatocytes 48h of treatment with the indicated dose concentration of AIMers
NTC: non-targeting control

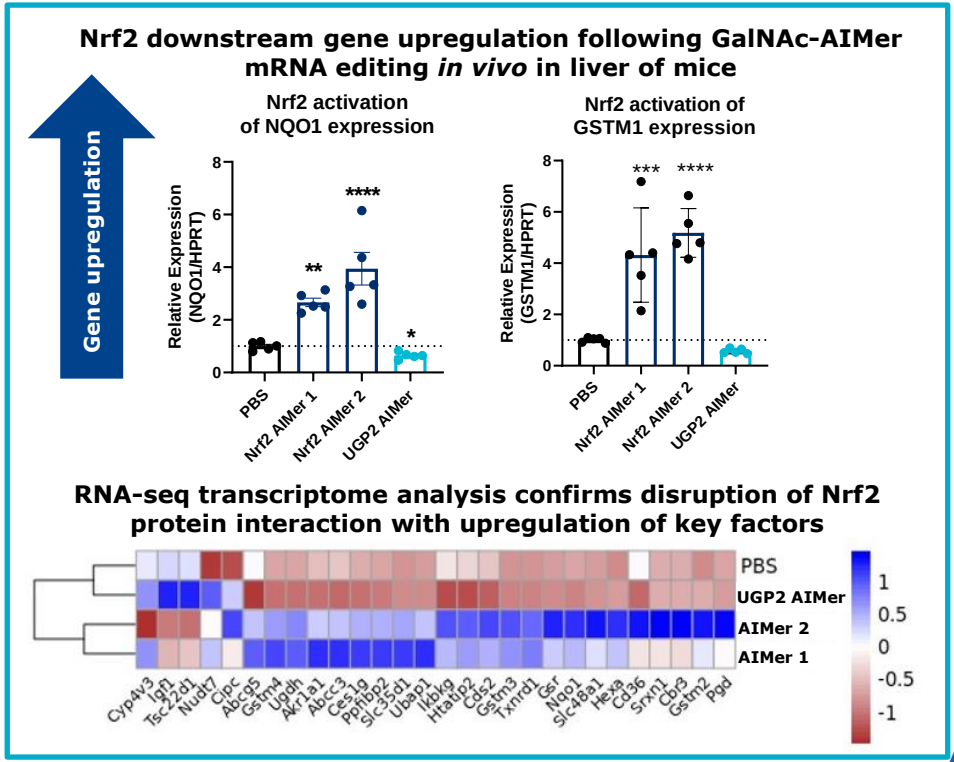
Presented at ASGCT 26th Annual Meeting (May 2023)

Modulation of protein-protein interactions: AIMers enable activation of gene pathway *in vivo* with single edit





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



Methods: hADAR1 p110 C57BL/6 mice dosed subcutaneously (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

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

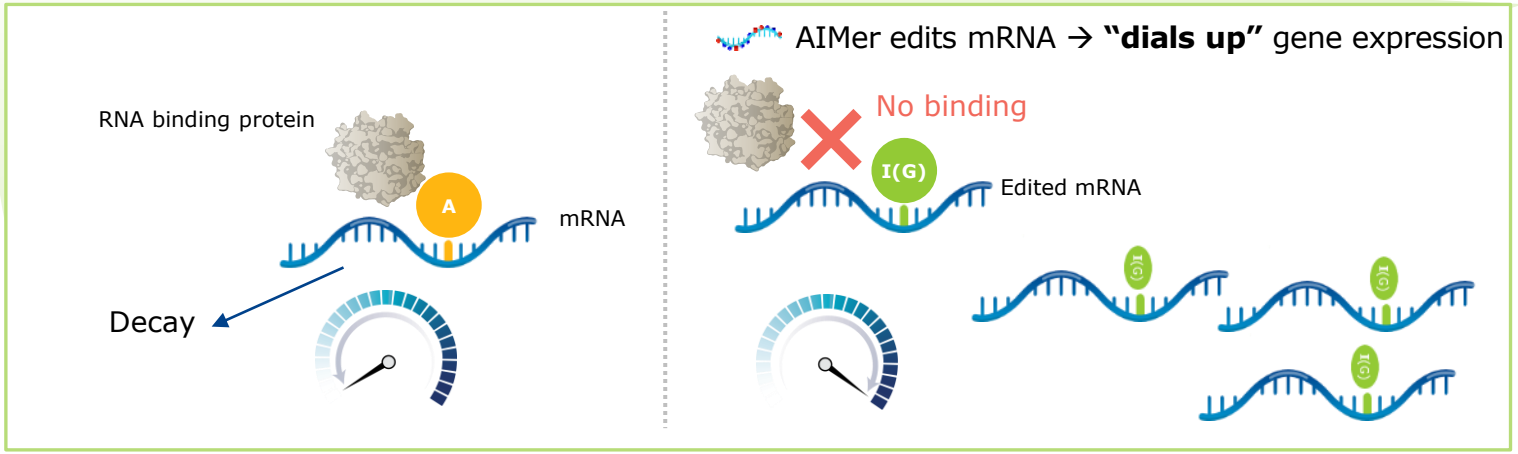
RNA binding proteins recognize sequence motifs to regulate various mRNA properties

Stability
- Enhance or inhibit mRNA decay

Transport
- Intracellular localization

Processing
- Splicing
- Capping
- PolyA usage

Protein production
- Translational efficiency



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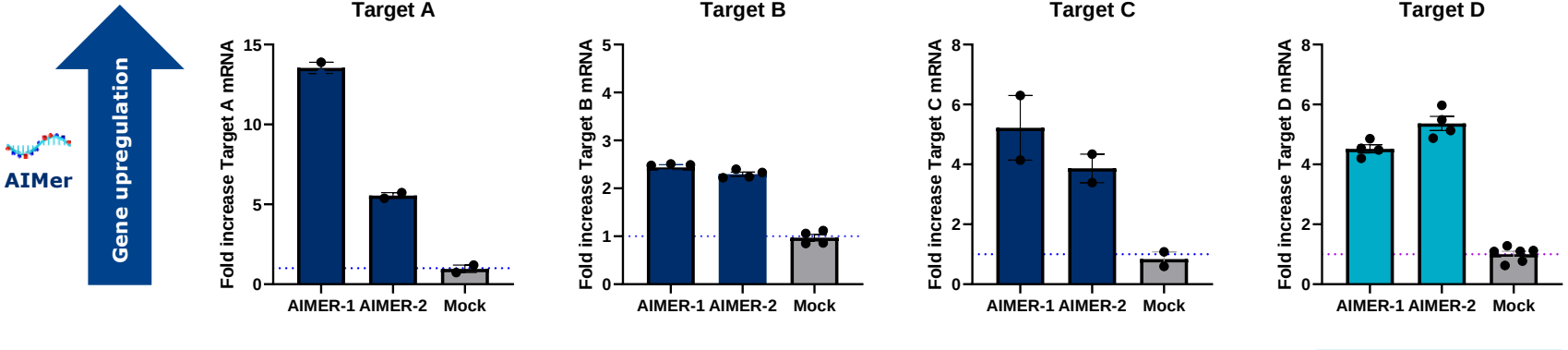
AIMers can edit RNA motifs to upregulate gene expression in hepatocytes and T-cells *in vitro*

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

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Presented at ASGCT 26th Annual Meeting (May 2023)

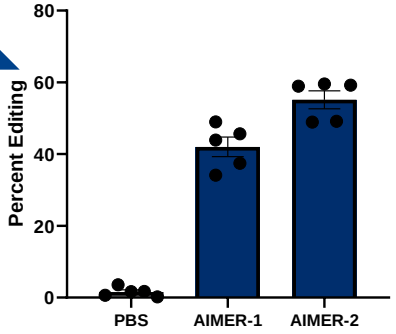
AIMers upregulate mRNA and downstream serum protein *in vivo* above anticipated threshold

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

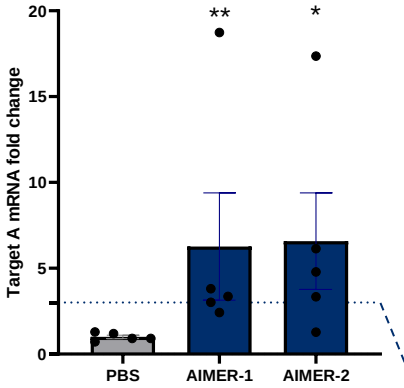
RNA editing

mRNA editing
7 days post-initial dose
GalNAc AIMer

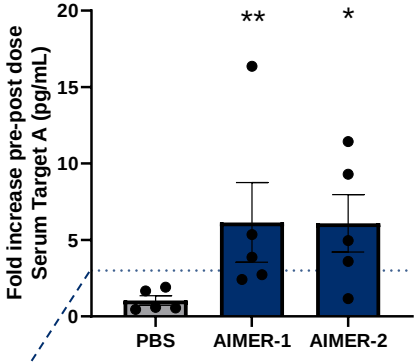


Upregulation

mRNA upregulation
7 days post-initial dose
GalNAc AIMer



Protein upregulation
7 days post-initial dose
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 demonstrating proof-of-concept

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

Presented at ASGCT 26th Annual Meeting (May 2023)

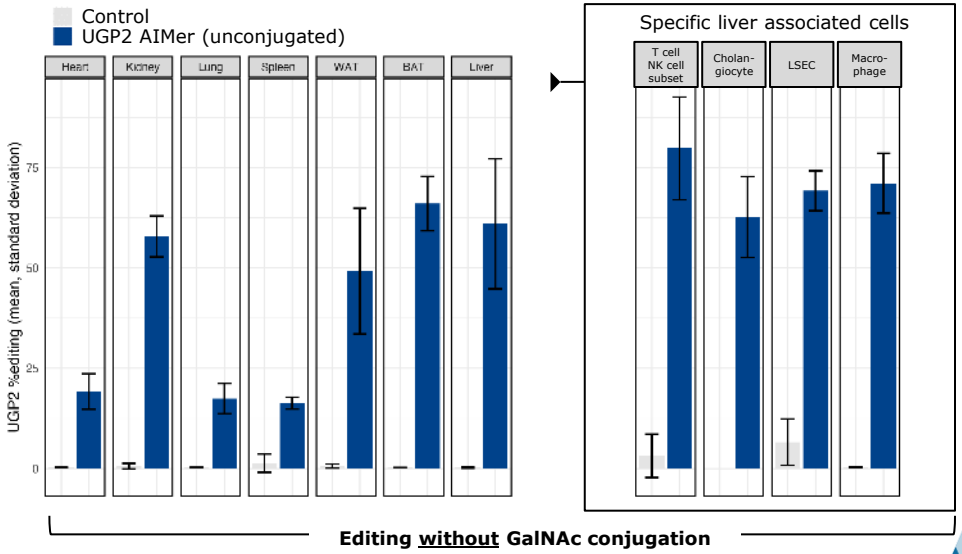
Systemic *in vivo* editing without delivery vehicles

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 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

Vast opportunity for AIMers across disease areas

Potential to address diseases with large patient populations, independent of genetic mutation status

GSK collaboration provides Wave with proprietary genetic insights to expand our pipeline with both partnered and wholly owned Wave programs

Anticipate investor event in 3Q 2023 during which Wave will demonstrate how it is continuing to extend its leadership in RNA editing and share preclinical data

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Conclusions

- AIMers incorporate Wave's best-in-class, proprietary oligonucleotide chemistry, including PN backbone linkages which increase potency, durability and distribution and carry a neutral charge to stabilize AIMer constructs
- AIMer design principles have enabled Wave to rapidly expand RNA editing capabilities and advance first RNA editing clinical candidate for correction of SERPINA1 transcript point mutation
- Enormous opportunity exists for downstream applications of AIMers, which can significantly increase target universe and unlock access to novel disease biology
- Wave is pioneering proprietary therapeutic approaches by using AIMers to upregulate mRNA and increase levels of endogenous proteins
- Disrupting protein-protein interactions or upregulation approaches have potential to address diseases with large patient populations and may allow mutation-independent strategies
- Wave is actively exploring downstream applications of AIMers
- Investor event anticipated in 3Q 2023

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