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Stereopure AIMers for the treatment of Alpha-1 antitrypsin deficiency (AATD)

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VP Platform Discovery Sciences, Biology

TIDES US: May 9-12, 2022

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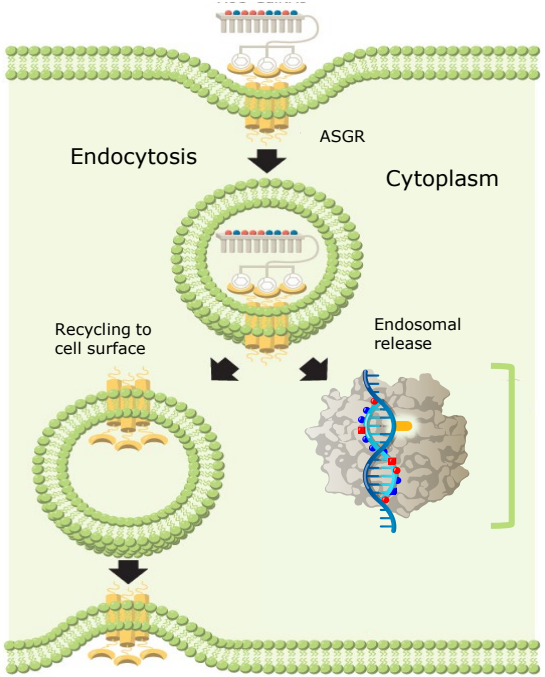
Unlocking RNA editing with PRISM™ to develop GalNAc-AIMers: A-to-I editing oligonucleotides

Optimize AIMer design for endogenous transcripts and GalNAc conjugation

GalNAc-mediated uptake



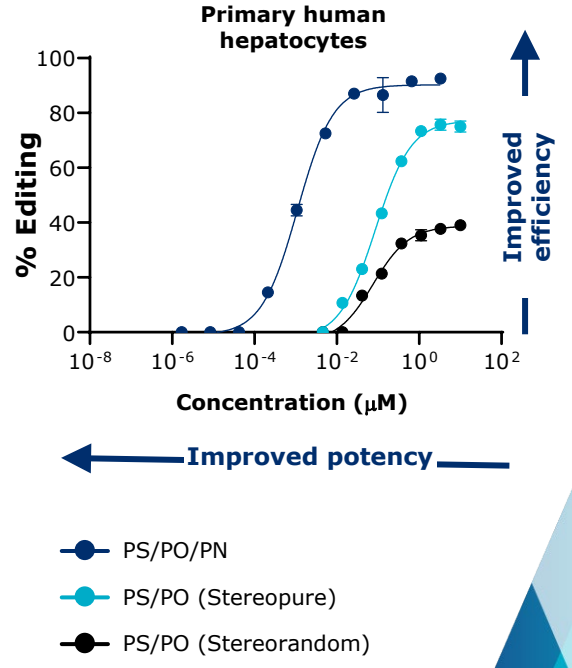
ADAR enzymes

- First publication (1995) using oligonucleotide to edit RNA with endogenous ADAR[1]
- Catalyze conversion of A-to-I (G) in double-stranded RNA substrates
- ADAR1 is ubiquitously expressed across tissues, including liver and CNS – sizeable target space
- Cellular reservoir of ADAR capacity supports directed editing in addition to homeostatic function

Optimize AIMer design

- ✓ Substrate learnings from biology and structures
- ✓ Applied to oligonucleotides
- ✓ Applied PRISM chemistry

PRISM-driven gains



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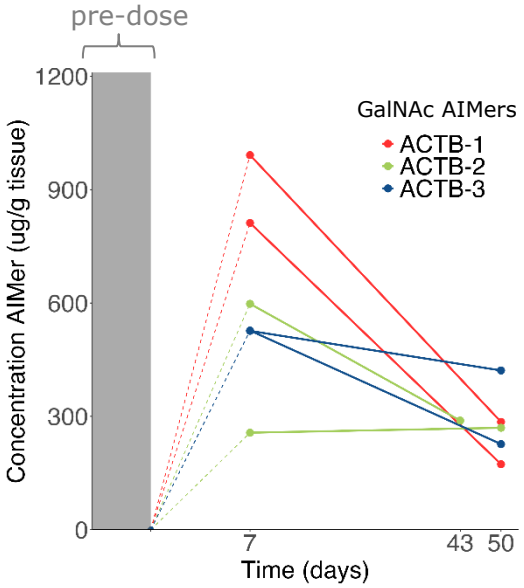
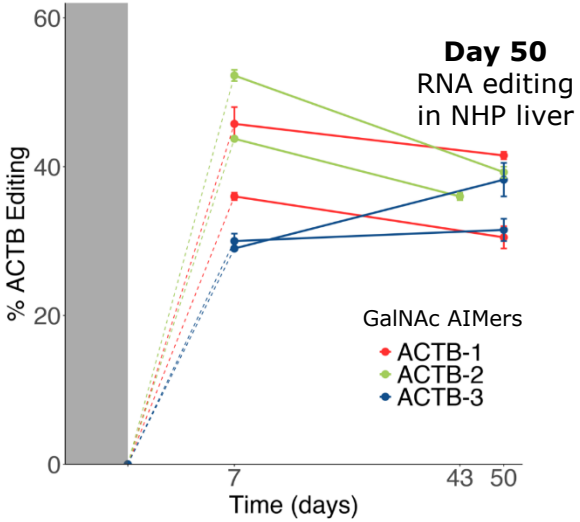
[1]Woolf *et al.*, 1995 *Proc Natl Assoc Sci* 92:8298-8302;
Monian *et al.*, 2022 *Nature Biotech* published online Mar 7, 2022 doi: 10.1038.s41587-022-01225-1

Proof-of-concept RNA editing in NHP liver is robust, durable, and specific

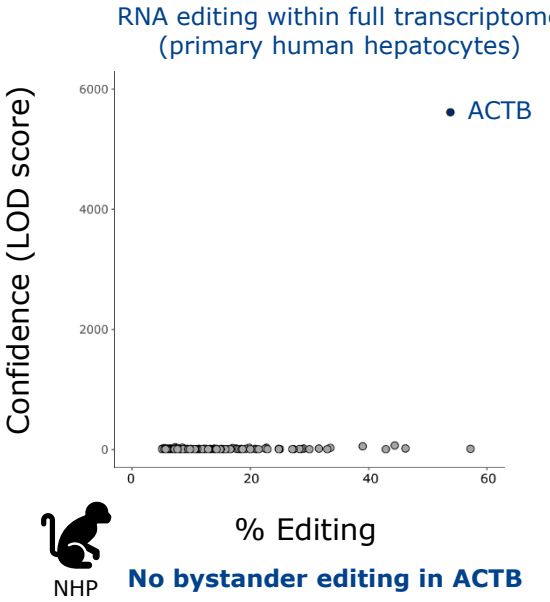
AIMers detected in liver of NHP at day 50



Durable editing in NHP liver *in vivo*

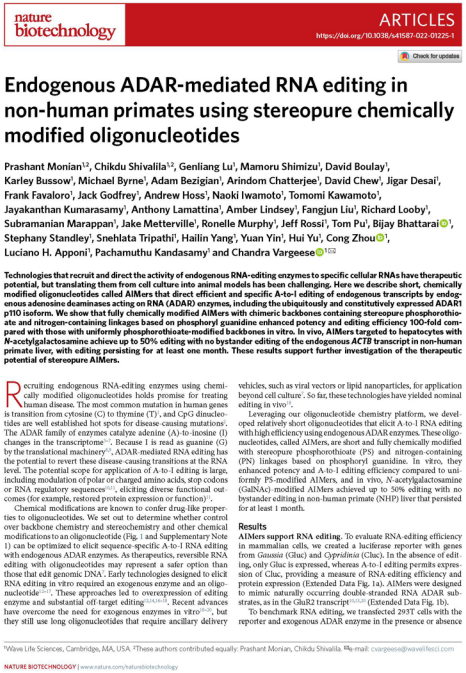


ADAR editing with ACTB AIMer is highly specific



AIMer tissue concentration (left) and editing activity (middle) in liver after 5 mg/kg SC injections on days 1-5; Right: Hepatocytes treated with 1 μM AIMer, 48 hrs later RNA collected, RNAseq conducted using strand-specific libraries to quantify editing; plotted circles represent sites with LOD>3. NHP: non-human primate; ACTB: Beta-actin; Monian et al., 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1

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



nature biotechnology

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https://doi.org/10.1038/s41587-022-01225-1

Check for updates

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

Prashant Monian[1,2], 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], Stephany Standley[1], Snehlata Tripathi[1], Hailin Yang[1], Yuan Yin[1], Hui Yu[1], Cong Zhou[1], Luciano H. Apponi[1], 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 CpG dinucleotides 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[2–5]. Because I is read as guanine (G) by the translational machinery[6,7], 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], eliciting 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[3]. Early technologies designed to elicit RNA editing in vitro required an exogenous enzyme and an oligonucleotide[13–17]. These approaches led to overexpression of editing enzyme and substantial off-target editing[13,15,16]. Recent advances have overcome the need for exogenous enzymes in vitro[18–20], but they still use long oligonucleotides that require ancillary delivery vehicles, such as viral vectors or lipid nanoparticles, for application beyond cell culture[3]. So far, these technologies have yielded nominal editing in vivo[21].

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 *Cypridinia* (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[10,15,16] (Extended Data Fig. 1b).

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

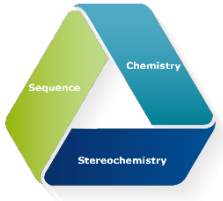
[1]Wave Life Sciences, Cambridge, MA, USA. [2]These authors contributed equally: Prashant Monian, Chikdu Shivalila. ✉e-mail: cvargeese@wavelifesci.com

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

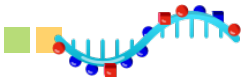
Monian et al., 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1
SAR structure-activity relationship

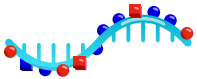
AIMers: Realizing the potential of therapeutic RNA editing by harnessing endogenous ADAR

Solved for key therapeutic attributes for potential best-in-class RNA editing therapeutics



Efficient ADAR recruitment	Stability	Delivery and intracellular trafficking	Beyond liver
• AIMer design principles • SAR developed to design AIMers for different targets	• Decade of investment and learnings to improve stability of single-stranded RNAs	• GalNAc compatible for targeted liver delivery • Endosomal escape and nuclear uptake	• AIMers can be delivered to CNS and reach other tissues in preclinical models[1]
Potent and specific editing *in vivo*	**Potential for infrequent dosing**	**Subcutaneous dosing**	**IT, IVT, systemic dosing**





- **Systematized AIMer design enables rapid advancement of new targets**
- **Strong and broad IP in chemical and backbone modifications, stereochemistry patterns, novel and proprietary nucleosides**

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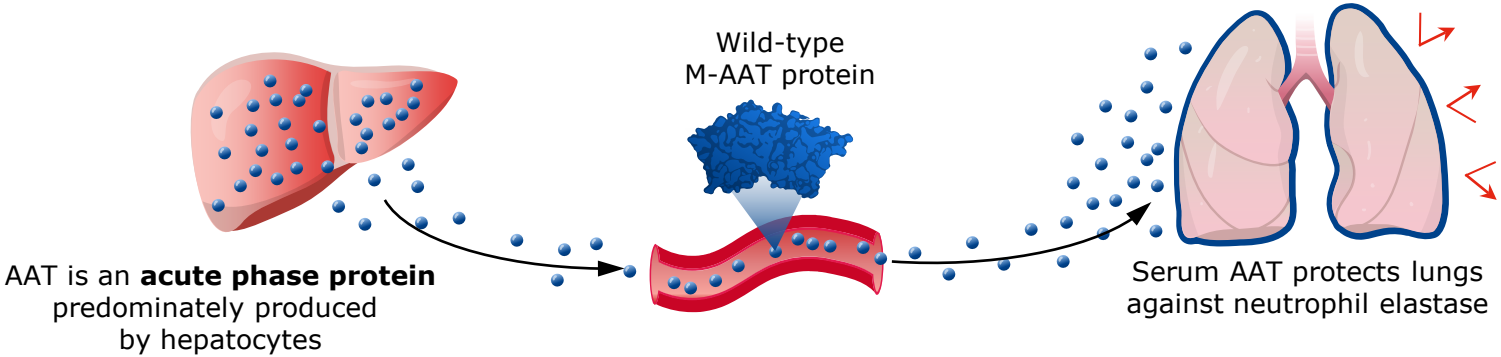
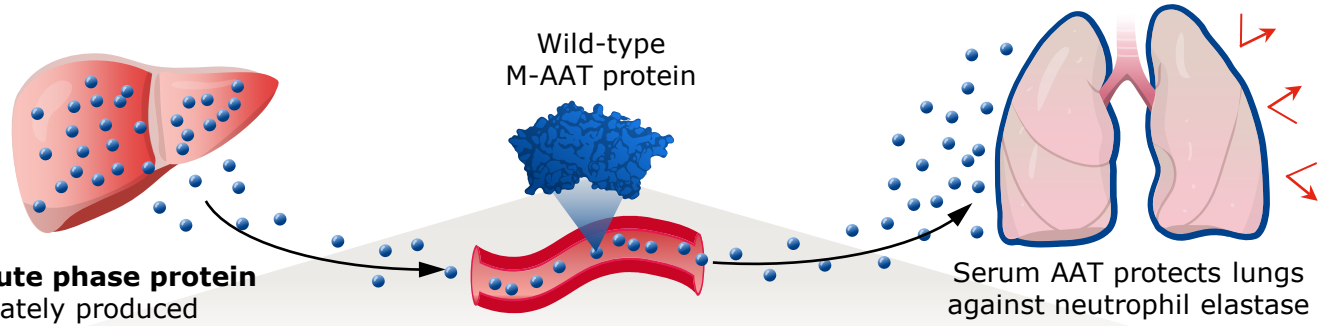
[1]Shivalila *et al.*, presented at 3rd International Conference on Base Editing – Enzymes and Applications Deaminet 2022

SERPINA1 Z mutation: The most common cause of Alpha-1 antitrypsin deficiency (AATD)



Strnad *et al.*, 2020 *N Engl J Med* 382:1443-55; Stoller et al., 1993 *Alpha-1 Antitrypsin Deficiency* GeneReviews

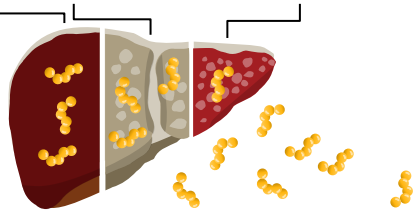
SERPINA1 Z mutation: The most common cause of Alpha-1 antitrypsin deficiency (AATD)



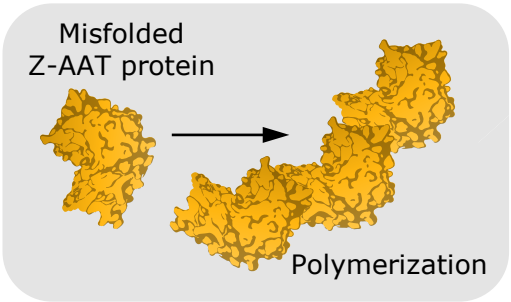
Gain-of-function and loss-of-function disease

Liver Disease

Fibrosis → Cirrhosis → Hepatocellular Carcinoma

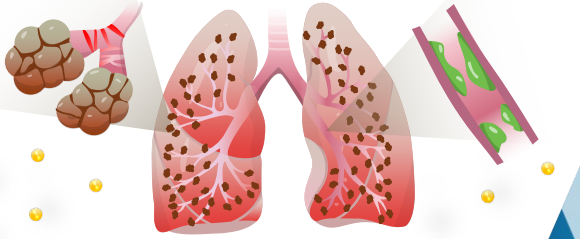


E342K mutation causes AAT proteotoxic stress, leading to progressive liver disease



Lung Disease

Emphysema Bronchiectasis



Low serum AAT leads to lung disease

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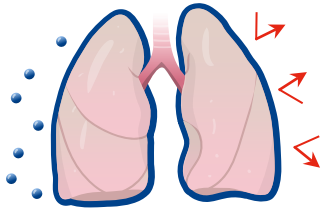
Strnad *et al.*, 2020 *N Engl J Med* 382:1443-55; Stoller et al., 1993 *Alpha-1 Antitrypsin Deficiency* GeneReviews

RNA editing is uniquely suited to address the therapeutic goals of AATD

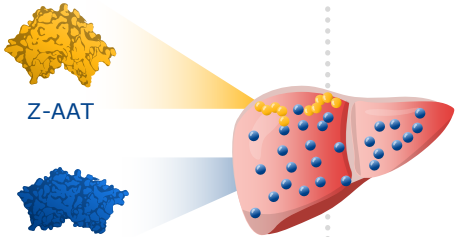
Wave ADAR editing approach addresses all treatment goals:

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



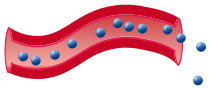
M-AAT reaches lungs to protect from proteases

2) Reduce Z-AAT protein aggregation in liver

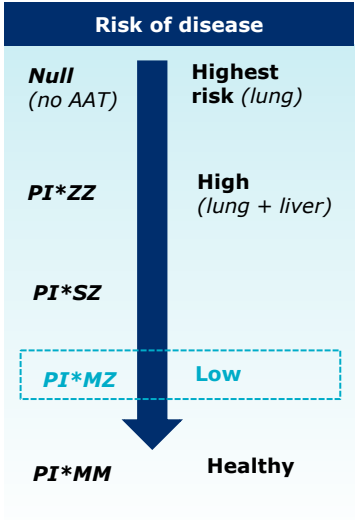


Wild-type M-AAT protein replaces Z-AAT with RNA correction

3) Retain M-AAT physiological regulation



M-AAT secretion into bloodstream



Alternative approaches address only a subset of treatment goals:

Standard of care:* *weekly IV protein augmentation *(11μM) addresses only lung manifestations*

siRNA *approaches address only liver disease*

Small molecule *approaches may address the lung and liver but do not generate wild-type M-AAT*

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

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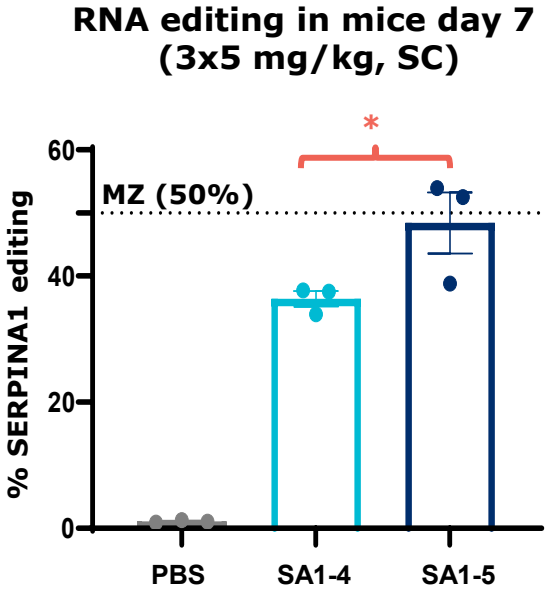
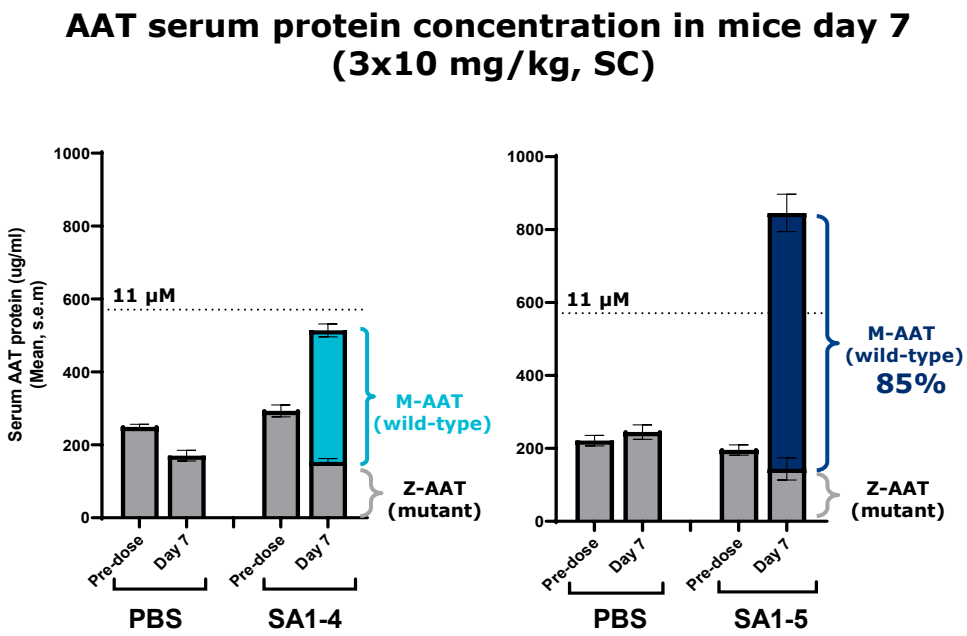
AAT: Alpha-1 antitrypsin Strnad *et al*., 2020 *N Engl J Med* 382:1443-55; Blanco *et al*., 2017 *Int J Chron Obstruct Pulmon Dis* 12:561-69; Remih *et al.,* 2021 *Curr Opin Pharmacol* 59:149-56.

Robust SERPINA1 RNA editing in mouse model for AATD restores M-AAT protein in serum

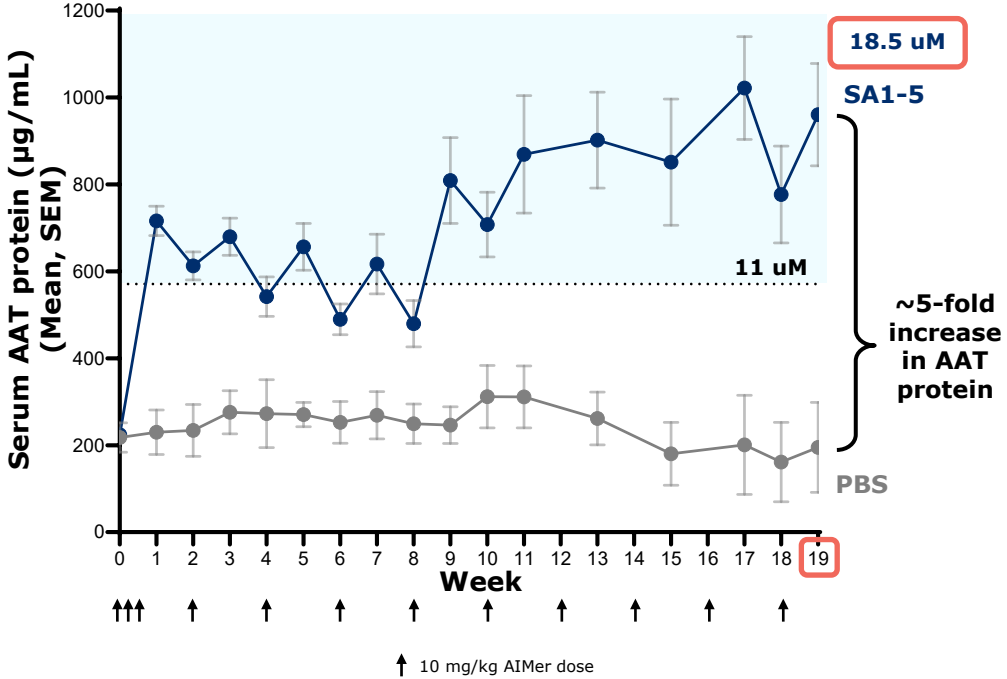




Left: AIMers administered hu*ADAR/SERPINA1* mice (3x5 mg/kg) on days 0, 2, and 4. Livers collected on day 7, and SERPINA1 editing was quantified by Sanger sequencing (shown as mean ± sem) Stats: One-way ANOVA was used to test for differences in editing between SA1-4 and other oligos * P<0.05. Right: hu*ADAR/SERPINA1* mice administered PBS or 3x10 mg/kg AIMer (days 0, 2, and 4) SC. Proportion of AAT protein in serum measured by mass spec, total AAT protein quantified by ELISA.

Bi-weekly AIMer treatment results in serum AAT protein levels in mice above anticipated therapeutic threshold[1]

GalNAc-AIMer results in serum AAT protein levels >11 uM at week 19



SA1-5 administered in hu*ADAR/SERPINA1* mice (8–10 weeks old) Left: Total serum AAT protein quantified by ELISA. Right: Liver biopsies collected at week 19 (one week after last dose); SERPINA1 editing was quantified by Sanger sequencing; Proportion of AAT protein in serum measured by mass spec, total AAT protein quantified by ELISA

[1]Brantly et al., 2018 Chronic Obstr Pulm Dis

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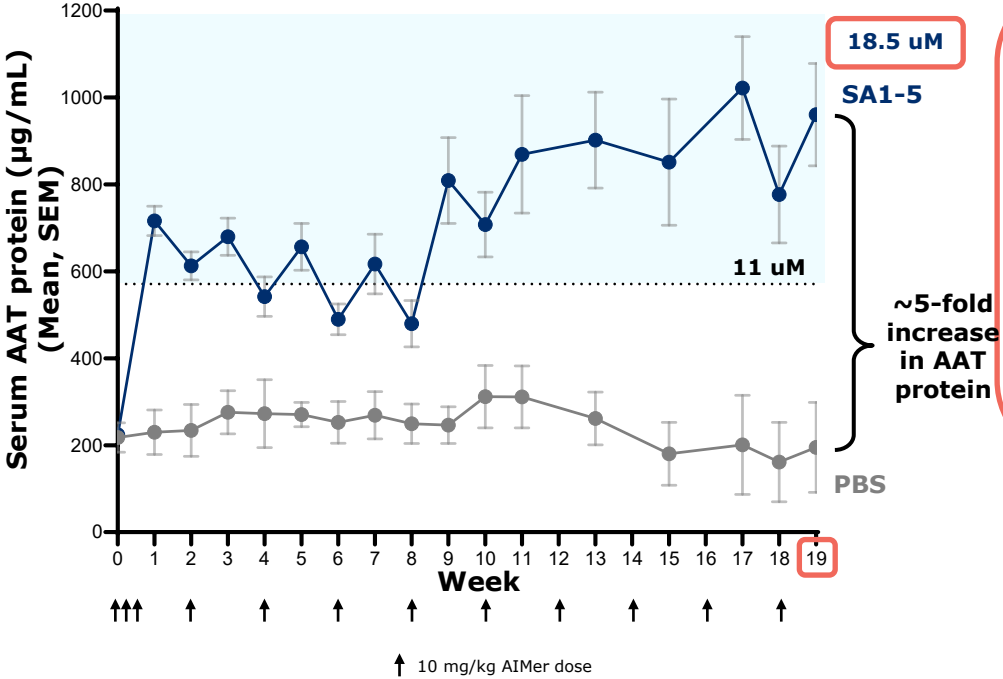
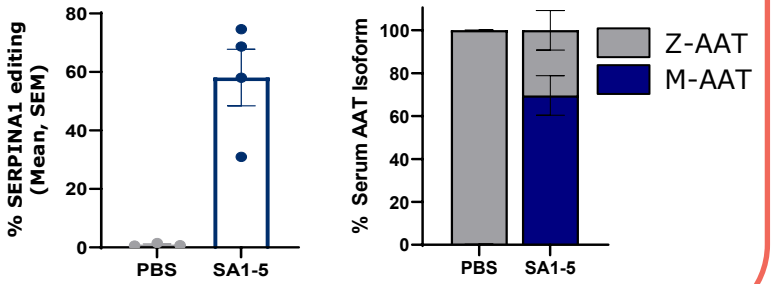
Bi-weekly AIMer treatment supports robust RNA editing and M-AAT protein expression in mice

GalNAc-AIMer results in serum AAT protein levels >11 uM at week 19



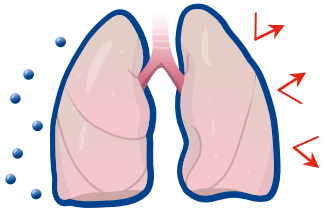
~60% RNA editing & ~70% serum M-AAT protein in mice (week 19 data)



SA1-5 administered in hu*ADAR/SERPINA1* mice (8–10 weeks old) Left: Total serum AAT protein quantified by ELISA. Right: Liver biopsies collected at week 19 (one week after last dose); SERPINA1 editing was quantified by Sanger sequencing; Proportion of AAT protein in serum measured by mass spec, total AAT protein quantified by ELISA

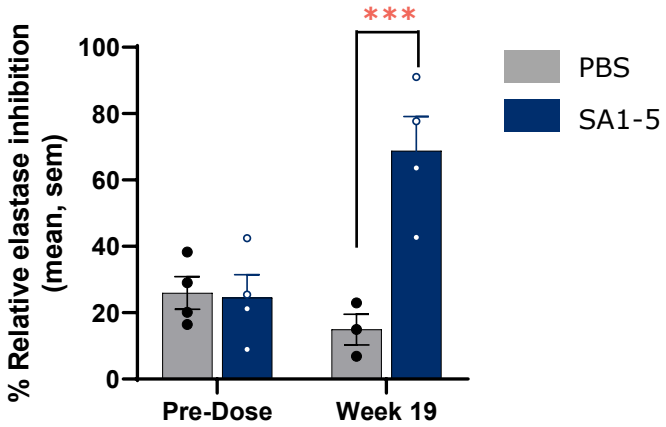
Restoration of functional serum M-AAT protein that neutralizes protease activity in mice

Correction of loss-of-function phenotypes



Restored M-AAT reaches lungs to protect from proteases

~3-fold in increase in neutrophil elastase inhibition at week 19

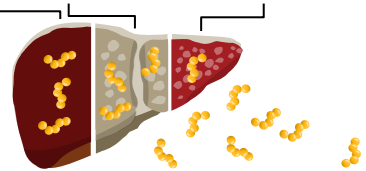


SA1-5 administered in hu*ADAR/SERPINA1* mice (8–10 weeks old); Neutrophil elastase inhibition assay performed with pre-dose and week 19 serum samples, Stats: Mixed effects analysis P<0.001

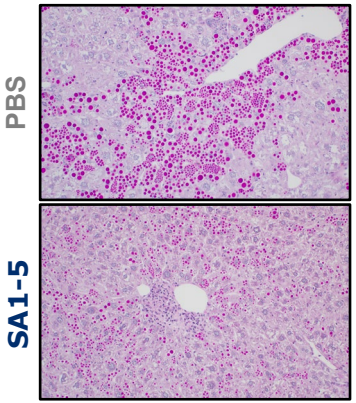
SA1-5 alleviates aggregation of Z-AAT and inflammation in mouse liver

Correction of gain-of-function phenotypes
Z-AAT aggregation causes progressive liver disease

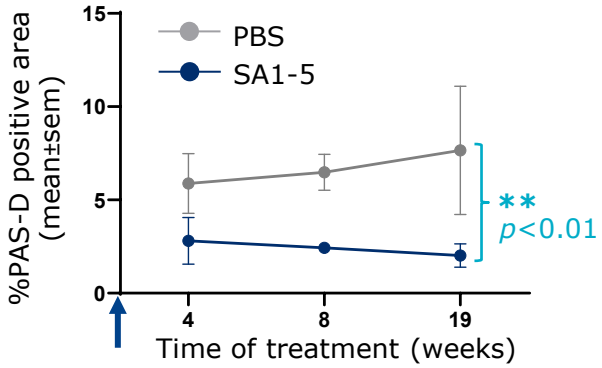
Fibrosis → Cirrhosis → Hepatocellular Carcinoma



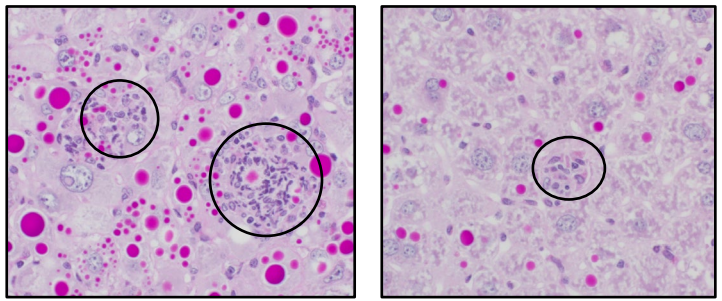
PAS-D staining
(19 weeks)



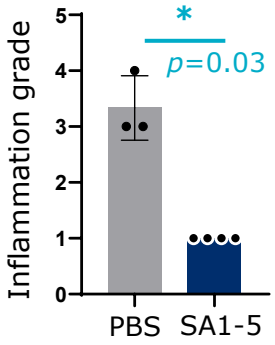
PAS-D-positive area



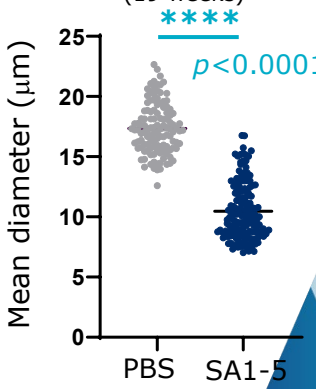
Lobular inflammation
(19 weeks)



Lobular inflammation
(19 weeks)



PAS-D globule size
(19 weeks)



SA1-5 administered in hu*ADAR/SERPINA1* mice (8–10 weeks old); lower left: 20x images from liver stained with PAS-D at 19 weeks; Quantification of PAS-D positive staining, Stats 2-way ANOVA; Right: Quantification lobular inflammation grade (Grade based on # of inflammatory foci in lobules: Grade 0: 0; G1 1-5; G2 6-10; G3 11-15; G4 ≥16) and mean globular diameter (40 largest globules per animal) with HALO. Stats Wilcox rank-sum tests

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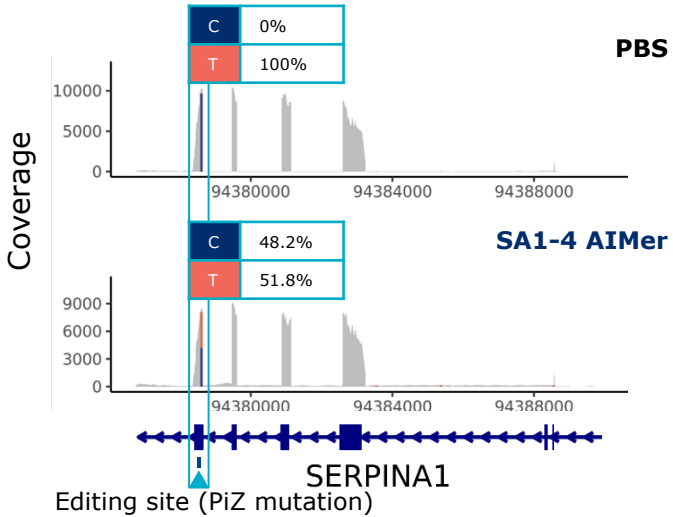
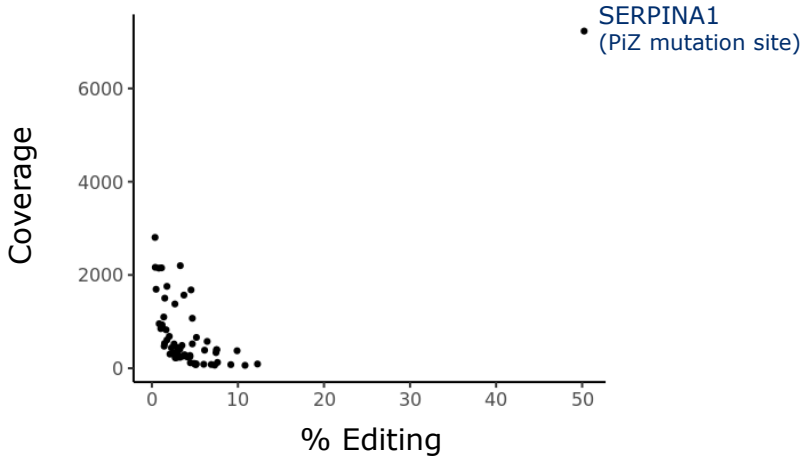
AIMer-directed editing is highly specific in mice

No bystander editing observed on SERPINA1 transcript

RNA editing only detected at PiZ mutation site in SERPINA1 transcript
(mouse liver)



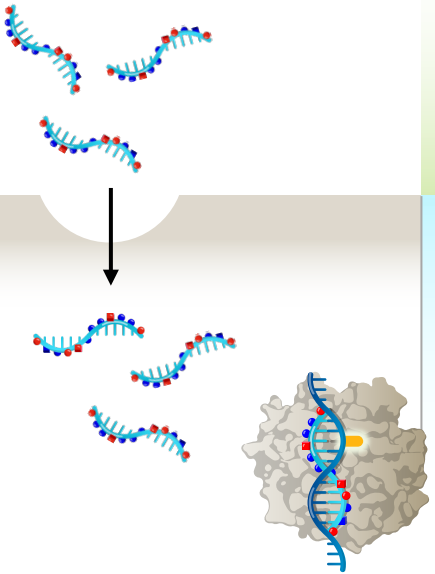
RNA editing across transcriptome
(mouse liver)



Dose 3x10 mg/kg (days 0, 2, 4) SC. Liver biopsies day 7. RNA-seq to quantify on-target SERPINA1 editing, to quantify off-target editing reads mapped to entire mouse genome; plotted circles represent sites with LOD>3 (N=4), SERPINA1 edit site is indicated

Opportunity for novel and innovative AIMer therapeutics

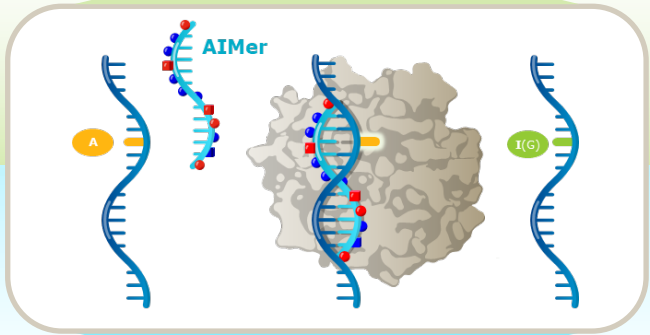
Free-uptake of chemically modified oligonucleotides



Endogenous ADAR

Correct driver mutations with AIMers

Restore or correct protein function



Examples

AATD

Rett syndrome

Recessive or dominant genetically defined diseases

Upregulate expression

Modify function

Modulate protein-protein interaction

Post-translational modification

Alter folding or processing

Examples

Haploinsufficient diseases

Loss of function

Neuromuscular

Dementias

Familial epilepsies

Neuropathic pain

Modulate protein interactions with AIMers

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Summary

- Our RNA base editing platform capability allows for correction of the most common causative mutation for AATD in preclinical models
- We have developed RNA editing oligonucleotides – **AIMers** – intended to correct homozygous "*ZZ*" mutations back to "*MZ*" heterozygous state
- Investigational AIMer leads to durable M-AAT protein levels at or above expected therapeutic threshold
- Restored serum M-AAT inhibits neutrophil elastase, suggesting potential to protect lungs from damage
- Restoration of M-AAT prevents accumulation of Z-AAT aggregates in mouse liver, alleviating phenotypes associated with liver pathology, including inflammation

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

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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)[1], and CpG dinucleotides are well established hot spots for disease-causing mutations[2]. The ADAR family of enzymes catalyze adenine (A)-to-inosine (I) changes in the transcriptome[3–7]. Because I is read as guanine (G) by the translational machinery[8,9], 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[10,11], eliciting diverse functional outcomes (for example, restored protein expression or function)[11].

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[7]. Early technologies designed to elicit RNA editing in vitro required an exogenous enzyme and an oligonucleotide[12–17]. These approaches led to overexpression of editing enzyme and substantial off-target editing[13,14,16–18]. Recent advances have overcome the need for exogenous enzymes in vitro[18–20], but they still use long oligonucleotides that require ancillary delivery vehicles, such as viral vectors or lipid nanoparticles, for application beyond cell culture[7]. So far, these technologies have yielded nominal editing in vivo[18].

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 *Cypridinia* (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[10,13,20] (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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