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RNA base editing for the treatment of Alpha-1 antitrypsin deficiency

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Oct. 5, 2022

Presented at 18th Annual Meeting of the Oligonucleotide Therapeutics Society

Forward looking statements

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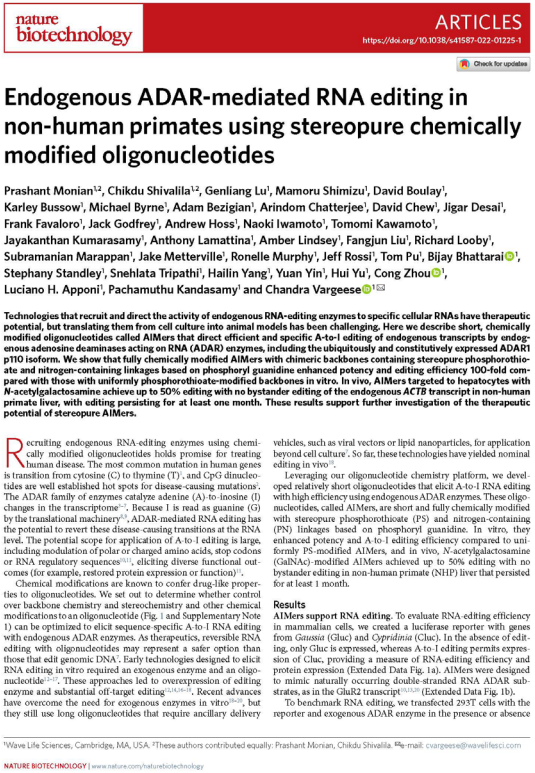
Proof-of-concept preclinical RNA editing data published in *Nature Biotechnology* (March 2022)



nature biotechnology

ARTICLES

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

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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), 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)[12–16].

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[17]. 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[12,14,16–18]. Recent advances have overcome the need for exogenous enzymes in vitro[19–21], 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[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[20,22] (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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- Foundational AIMer SAR
- GalNAc conjugation
- *In vitro-in vivo* translation (NHPs)
- Specificity *in vitro* & *in vivo* (NHPs)

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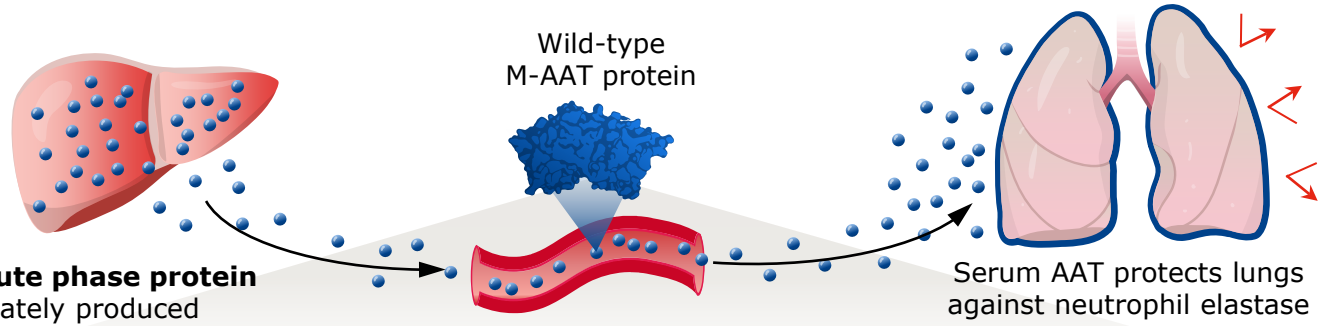
Monian *et al.*, 2022 published online Mar 7, 2022; doi: 10.1038.s41587-022-01225-1
SAR structure-activity relationship

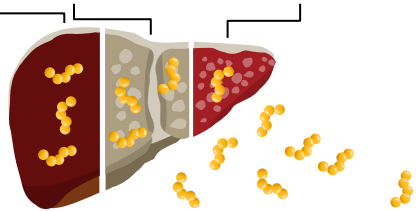
SERPINA1 Z mutation: The most common cause of Alpha-1 antitrypsin deficiency (AATD)



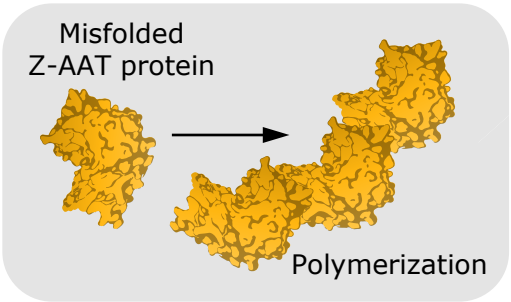
Gain-of-function and loss-of-function disease

Liver Disease

Fibrosis → Cirrhosis → Hepatocellular Carcinoma

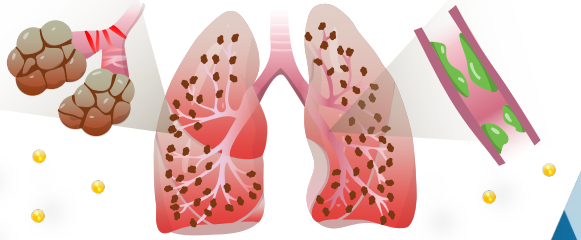


E342K mutation causes AAT proteotoxic stress, leading to progressive liver disease



Lung Disease

Emphysema Bronchiectasis



Low serum AAT leads to lung disease

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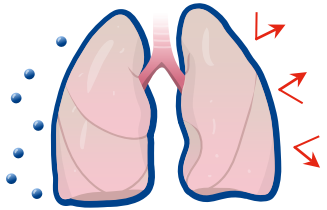
Strnad *et al.*, 2020 *N Engl J Med* 382:1443-55; Stoller et al., 1993 *Alpha-1 Antitrypsin Deficiency* GeneReviews

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

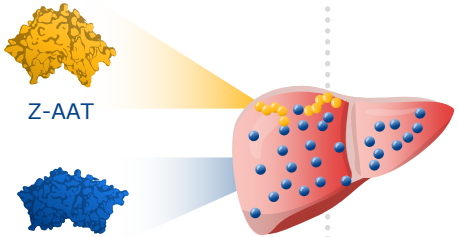
Wave ADAR editing approach addresses all treatment goals:

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



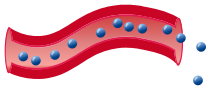
M-AAT reaches lungs to protect from proteases

2) Reduce Z-AAT protein aggregation in liver

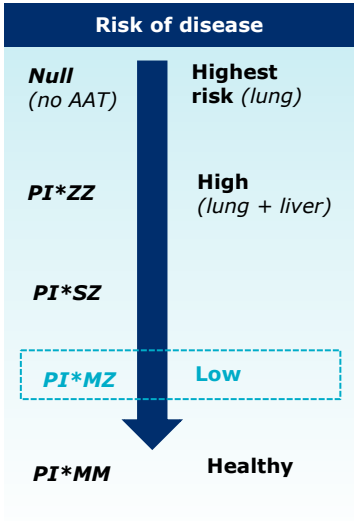


Wild-type M-AAT protein replaces Z-AAT with RNA correction

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 restore wild-type M-AAT*

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

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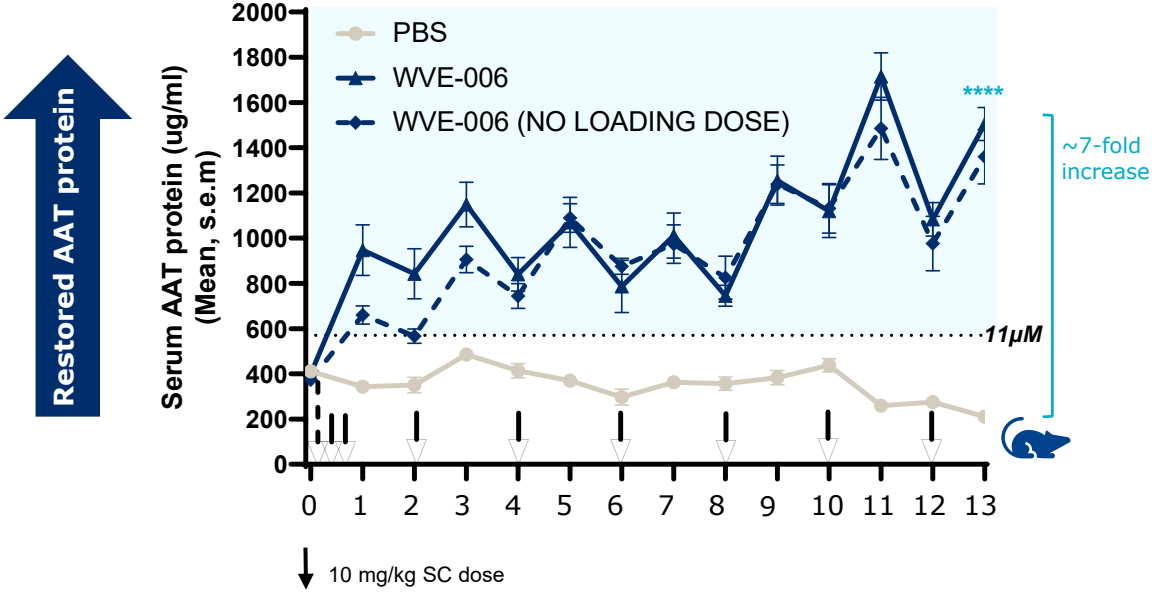
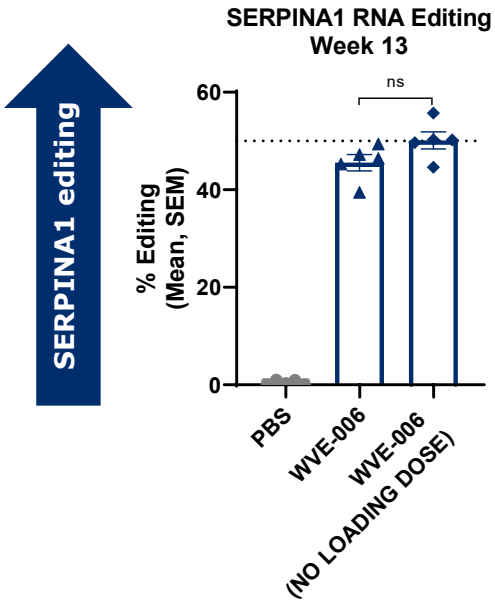
AAT: Alpha-1 antitrypsin Strnad *et al.*, 2020 *N Engl J Med* 382:1443-55; Blanco *et al.*, 2017 *Int J Chron Obstruct Pulmon Dis* 12:561-69; Remih *et al.*, 2021 *Curr Opin Pharmacol* 59:149-56.

WVE-006 results in circulating AAT protein levels well above established 11μM threshold in vivo

WVE-006 treatment results in serum AAT protein levels >11 uM in AATD mouse model (NSG-PiZ mice)



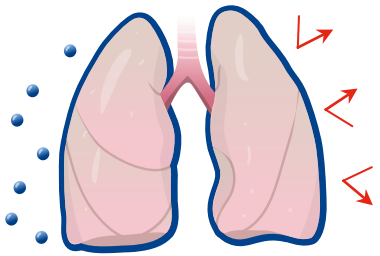
SERPINA1 mRNA editing in liver of AATD mouse model (NSG-PiZ mice) (Week 13)



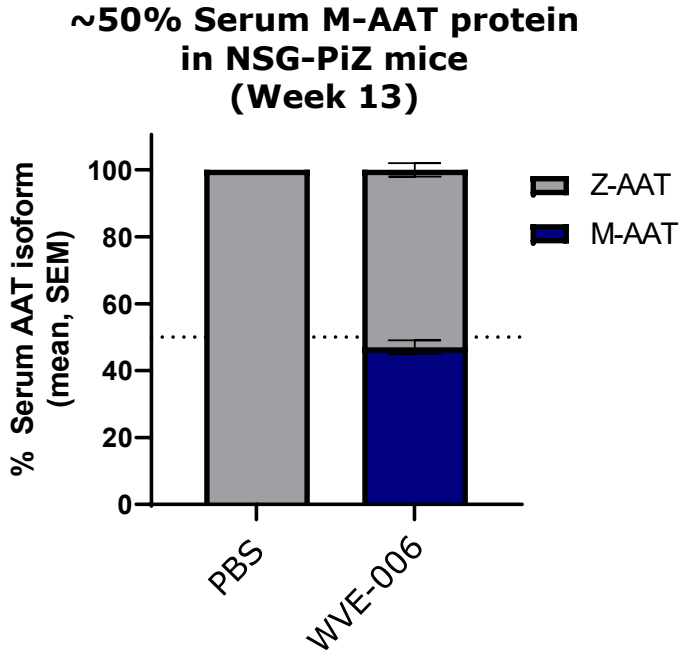
WVE-006 is a GalNAc-conjugated AIMer (A to I(G) RNA base editing oligonucleotide); WVE-006 administered in 7-week old NSG-PiZ mice (n=5 per group); Left: Liver biopsies collected at week 13 (one week after last dose) and SERPINA1 editing was quantified by Sanger sequencing; Stats: One-way ANOVA with adjustment for multiple comparisons (Tukey) **** <0.0001; Right: Total serum AAT protein quantified by ELISA; Stats: Two-Way ANOVA with adjustment for multiple comparisons (Tukey) ns non-significant

Restoration of serum M-AAT protein

Correction of loss-of-function phenotypes



Restored M-AAT reaches lungs to protect from proteases

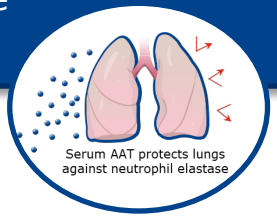


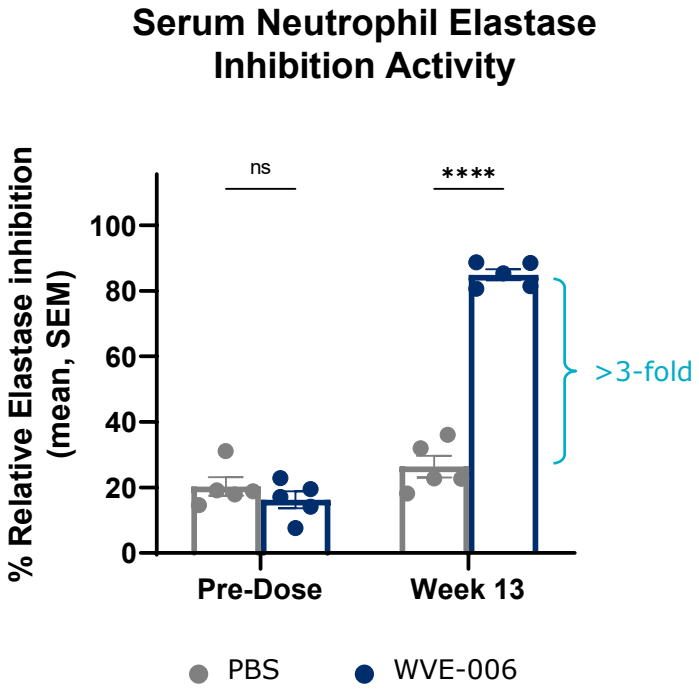
GalNAc-conjugated AIMers administered in 7-week old NSG-PiZ mice (n=5 per group). Proportion of AAT protein in serum measured by mass spec, total AAT protein quantified by ELISA.

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

Increased neutrophil elastase inhibition activity demonstrates functionality of AAT protein

- Increases in neutrophil elastase, a proteolytic enzyme, may cause emphysema and damage the surrounding lung tissue
- Main function of AAT protein is to neutralize/control neutrophil elastase



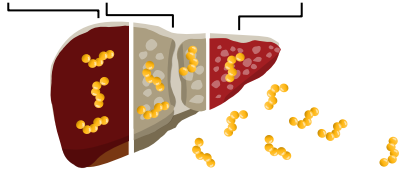


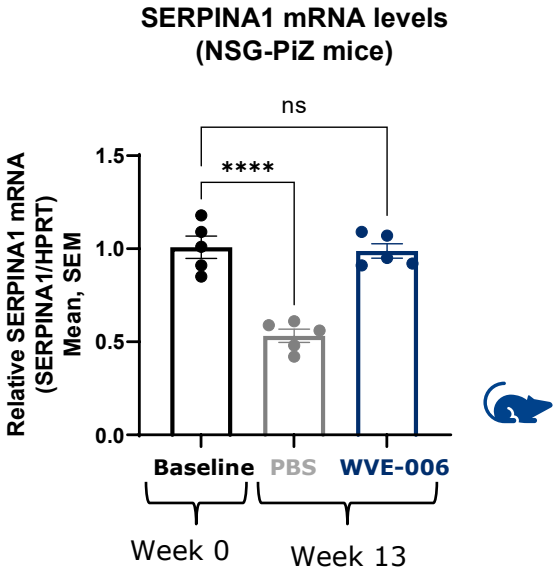
GalNAc-conjugated AIMers administered in 7-week old NSG-PiZ mice (n=5 per group). Serum collected from mice was tested for ability to inhibit fixed concentration of neutrophil elastase in an *in vitro* reaction. Stats: Two-way ANOVA with adjustment for multiple comparisons (Bonferroni)

Treatment with WVE-006 maintains SERPINA1 mRNA levels relative to baseline in NSG-PiZ mice

Z-AAT aggregation causes loss of hepatocytes expressing transgene in this mouse model

Fibrosis → Cirrhosis → Hepatocellular Carcinoma

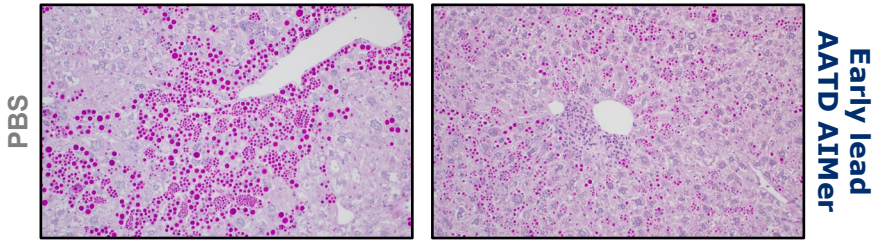




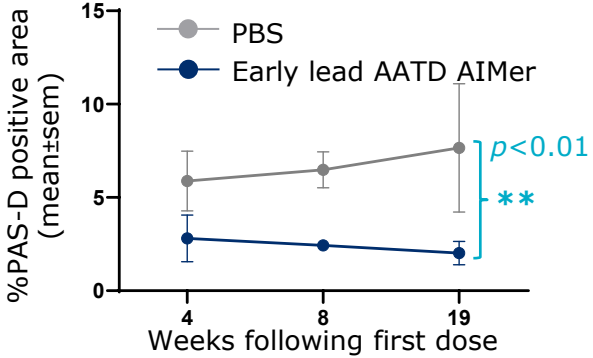
WVE-006 administered in 7-week old NSG-PiZ mice (n=5 per group). Stats: One-ANOVA with adjustments for multiple comparisons (Dunnett)

SERPINA1 AIMer reduces aggregation of Z-AAT and inflammation in mouse liver

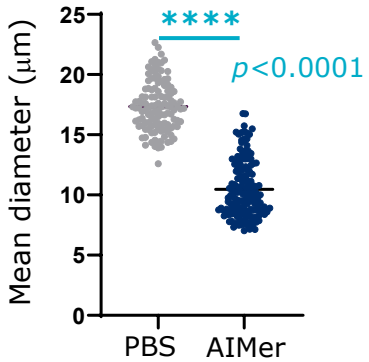
PAS-D staining
(19 weeks)



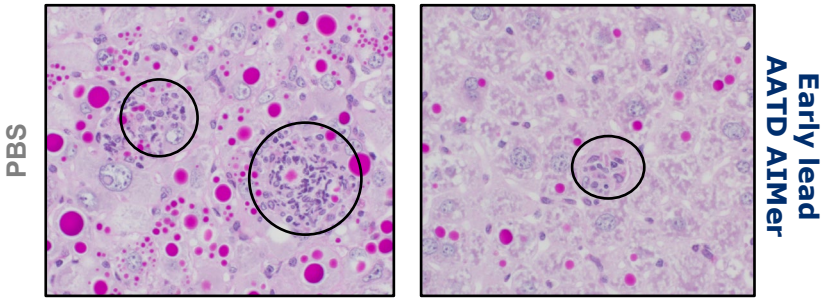
PAS-D-positive area



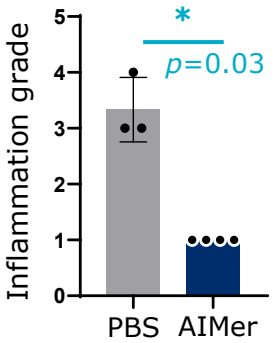
PAS-D globule size
(19 weeks)



Lobular inflammation
(19 weeks)



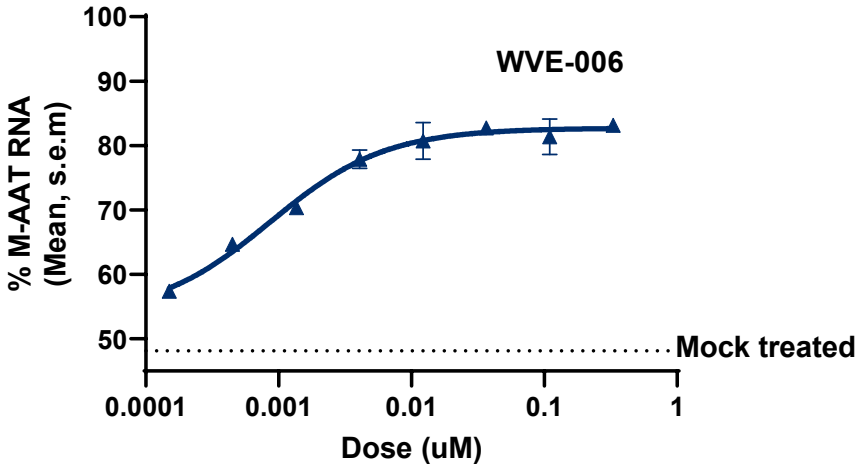
Lobular inflammation
(19 weeks)



Early lead pre-optimization AATD AIMer (SA1-5) administered in hu*ADAR/SERPINA1* mice (8–10 wKs old); lower left: 20x liver images PAS-D stained, 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/ animal) with HALO. Stats Wilcox rank-sum tests

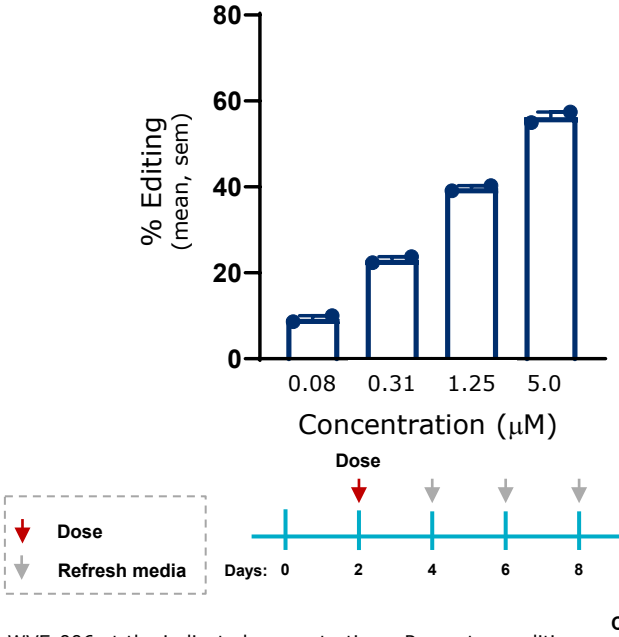
WVE-006 supports dose-dependent RNA editing in human preclinical model systems

Efficient SERPINA1 editing in donor-derived primary human hepatocytes with WVE-006 (MZ genotype)



Note: Due to MZ genotype, Y-axis ranges from ~50-100%

Editing in iPSC-derived human hepatocytes with WVE-006 (ZZ genotype)



Primary human hepatocytes with MZ (left) or ZZ (right) genotype treated with WVE-006 at the indicated concentrations. Percentage editing was determined by Sanger sequencing.

CTA submissions for WVE-006 expected in 2023

- 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 to an "*MZ*" heterozygous state
- Investigational lead, WVE-006, drives serum M-AAT protein levels in mouse models above 11 μM – the anticipated therapeutic threshold[1]
- Restored serum M-AAT inhibits neutrophil elastase, indicating the protein is functional and may protect lungs from damage
- SERPINA1 AIMer reduces aggregation of Z-AAT and inflammation in mouse liver
- WVE-006 supports dose-dependent RNA editing in human cellular models for AATD

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