Chemically-optimized Stereopure Oligonucleotides Direct ADAR-mediated RNA Editing of SERPINA1 Transcripts, Yielding Functional α-1 Antitrypsin Protein in a Mouse Model for α-1 Antitrypsin Deficiency

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Summary

- Wave's PRISM™ platform allows for the development of stereopure oligonucleotides, which enhances our ability to optimize the pharmacologic properties and activities of these molecules.
- Wave is developing oligonucleotides to recruit endogenous adenosine deaminase acting on RNA (ADAR) enzyme to correct the Z mutation in SERPINA1 transcripts. These A-to-I editing oligonucleotides are known as AIMers. The *SERPINA1* Z allele causes α-1 antitrypsin deficiency(AATD)[1].
- We observe up to 50% editing of SERPINA1 Z mRNA in mouse liver, approximating correction to heterozygous (MZ) condition, where individuals have a much lower risk for disease.
- Z mRNA editing leads to an increase in circulating functional wild-type M-AAT protein in transgenic mouse models for AATD. The observed levels of M-AAT restoration are expected to be therapeutically meaningful.
- M-AAT protein restoration in mice is durable, lasting for at least 35 days.
- The production of M-AAT and subsequent increase in serum Z-AAT levels suggests clearance of pathogenic Z-AAT from liver.
- Our ongoing optimization efforts continue to improve editing efficiency and increase M-AAT serum protein in mice.

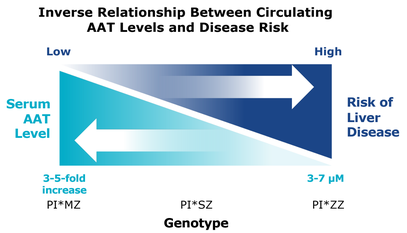
Introduction

- AATD is an autosomal co-dominant genetic disorder caused in part by a G-to-A point mutation in the *SERPINA1* gene. The resulting E342K or Z mutation leads to misfolding and aggregation of Z-AAT protein in hepatocytes, which can cause liver damage and cirrhosis. It also depletes circulating M-AAT protein, leaving the lungs vulnerable to disease.[1]
- We aim to restore physiological levels of functional M-AAT comparable to low-risk heterozygous (MZ) condition by using AIMers to recruit endogenous ADAR enzymes to edit SERPINA1 Z mRNA to M (wild type), which has the potential to address both liver and lung manifestations of AATD (**Figure 1**).

Figure 1. Background and objectives

Objectives

- Recruit endogenous ADAR enzyme to edit SERPINA1 Z mRNA
- Restore circulating M-AAT protein
- Confirm functionality of M-AAT protein
- Confirm specificity of SERPINA1 editing activity
- Optimize design and dosing strategy to achieve levels of M-AAT protein predicted to be therapeutic



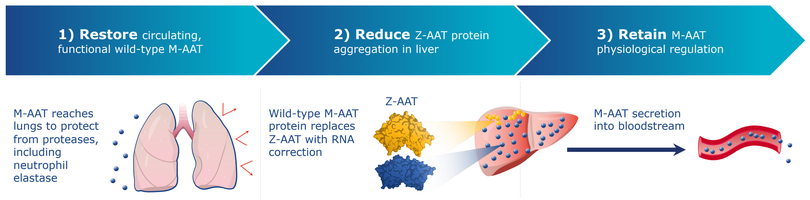
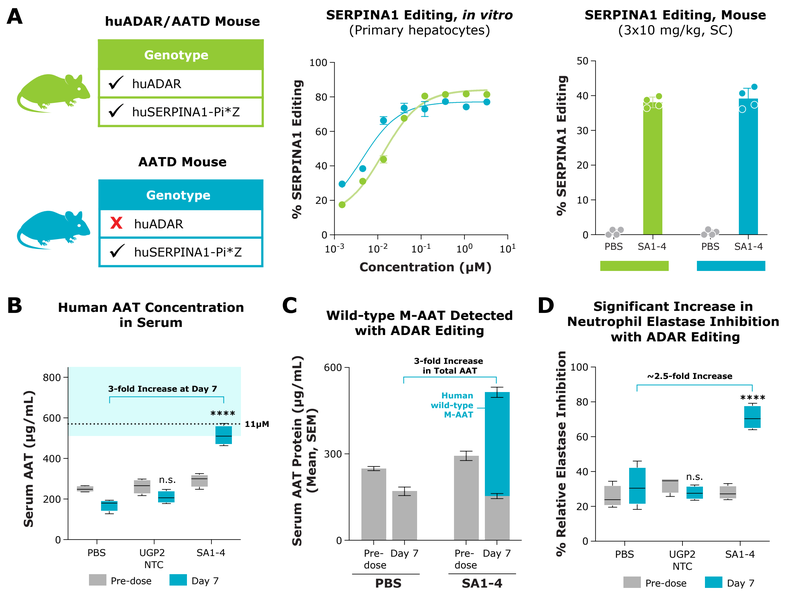


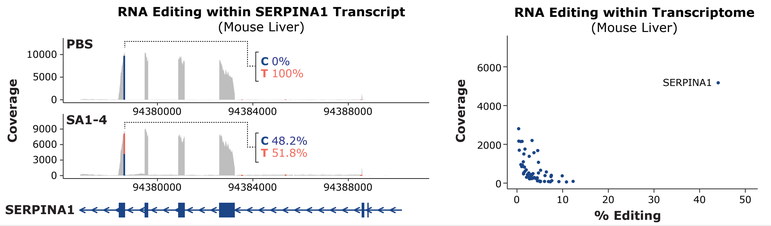
Figure 2. SA1-4 demonstrates proof of concept that SERPINA1 editing can approximate correction to MZ heterozygote



(A, left) dose-response curve in primary hepatocytes treated with increasing concentrations of SA1-4. (A, right) HuADAR/AATD or AATD mice were administered PBS or 10 mg/kg SA1-4 on days 0, 2, and 4. Liver samples were collected on day 7. Stats: One-way ANOVA **** P<0.0001. (B) Serum AAT levels were quantified by ELISA. (C) Mass spectrometry distinguished Z-AAT and M-AAT. (D) Neutrophil elastase inhibition assay. Stats B&D: Matched 2-way ANOVA with correction for multiple comparisons (Bonferroni), ns nonsignificant, *** P<0.001, **** P<0.0001.

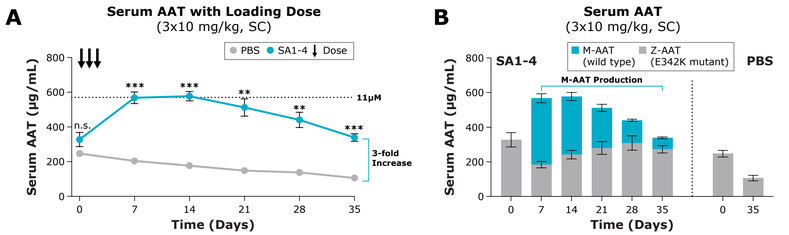
- SA1-4 is a GalNAc-AIMer designed to recruit endogenous ADAR enzymes and direct editing of the SERPINA1 Z transcript to restore the wild-type sequence.
- We developed two mouse models for AATD:
  - huADAR/AATD expresses human *ADAR1* and mutated *SERPINA1* (green);
  - AATD lacks human ADAR1 (expressing only mouse *Adar1*) and expresses human mutated SERPINA1 (blue).
- In primary heptatocytes derived from both mouse models, SA1-4 leads to ~80% editing of the Z transcript back to M (**Figure 2A**, left).
- In mice treated with SA1-4, up to 40% editing of the Z transcript back to M is detected in the liver (**Figure 2A**, right). Comparable levels of editing are detected in the two models.
- In the same experiment in huADAR/AATD mice, human AAT levels in serum increase ~3-fold compared with PBS-treated animals (**Figure 2B**), which matches the lowest level of protein restoration expected to provide a therapeutic benefit (**Figure 1**). 11 µM is the current standard of care concentration for human AAT serum levels for augmentation therapies.[2]
- After treatment, a substantial proportion of serum AAT in huADAR/AATD mice was M-AAT (**Figure 2C**).
- Serum in treated huADAR/AATD mice is functional in a neutrophil elastase inhibition assay (**Figure 2D**), suggesting increase circulating M-AAT has potential to protect lungs from pathology associated with AAT-loss of function.

Figure 3. SA1-4 supports specific editing *in vivo*



Mice were at dosed with 3 x 10 mg/kg SA1-4 on days 0, 2, and 4. Liver biopsies were collected on day 7. RNA-seq was conducted using strand-specific libraries. To quantify on-target SERPINA1 editing, reads were mapped to human SERPINA1, and to quantify off-target editing reads were mapped to entire mouse genome; plotted circles represent sites with LOD>3 (N=4).

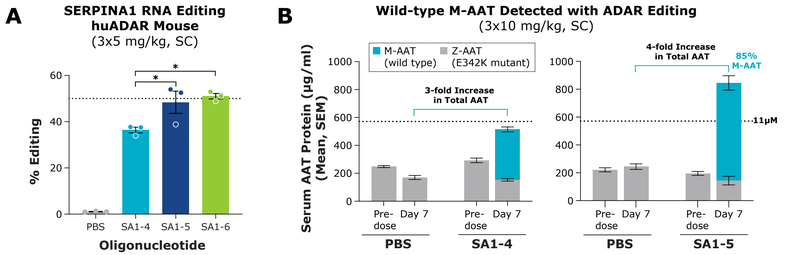
Figure 4. Editing by SA1-4 leads to durable increases in serum AAT in mouse model for AATD



(A) huADAR/AATD mice were administered 3x10 mg/kg doses SA1-4 on days 0, 2 and 4. Serum was collected on days 7, 14, 21, 28, and 35. AAT levels quantified by ELISA. Data are presented as mean ± sem. Stats: Matched 2-way ANOVA ns nonsignificant, ** P<0.01, *** P<0.001. (B) Proportion of AAT in serum that is E type (mutant) or M type (wild type) as measured by mass spectrometry.

- In huADAR/AATD mice treated with SA1-4, serum levels of human AAT increase 3-fold compared with PBS-treated animals for at least 35 days (**Figure 4A**).
- During this time, the proportion of M-AAT in serum increases dramatically by 1-week. The proportion of Z-AAT in serum increases over time, which is consistent with Z-AAT protein being cleared from the liver (**Figure 4B**).

Figure 5. AIMer optimization efforts improve editing activity



(A) AIMers were administered to mice (3x5 mg/kg) on days 0, 2 and 4. Livers were 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 (B) Experiments performed as described in Figure 2.

References: 1. Strnad, et al. 2020. Alpha(1)-Antitrypsin Deficiency. *N. Engl. J. Med.* 382: 1443-1455; 2. Stoller, et al. 2006. Alpha-1 Antitrypsin Deficiency. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. *GeneReviews®*. University of Washington, Seattle, 1993-2020. **Acknowledgments:** The authors are grateful to Eric Smith and Amy Donner (Wave Life Sciences) for graphical and editorial support, respectively.