Investigational WVE-006, an RNA Editing Oligonucleotide for the Treatment of Alpha-1 Antitrypsin Deficiency (AATD)

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SUMMARY

- AATD is commonly caused by a G-to-A mutation (PiZ allele) in the *SERPINA1* gene. This single mutation causes misfolding and aggregation of mutant Z-AAT protein in the liver, leading to a decrease in functional AAT protein in circulation and chronic obstructive pulmonary disease (COPD).[1]
- Current standard of care for AATD aims to restore total serum AAT protein levels to an anticipated therapeutic threshold (11 µM) through weekly intravenous protein augmentation therapy with human plasma-purified AAT.[2] No therapy is approved for the treatment of liver disease other than liver transplantation for advanced disease.[1]
- WVE-006 is an investigational *N*-Acetylgalactosamine (GalNAc)-conjugated A-to-I RNA editing oligonucleotide, that was designed to correct the Z mutation in *SERPINA1* mRNA in hepatocytes, increase functional AAT protein in serum, and thereby protect the lungs from damage. Because WVE-006 corrects the Z mutation, it is also designed to address liver disease associated with AATD.
- In a mouse model for AATD, treatment with WVE-006 increased serum levels from ~8 µM at baseline to 20-30 µM by week 13. This was ~7-fold higher than levels in PBS-treated mice (~4 µM), which declined with time. After treatment, ~50% of the *SERPINA1* Z transcript in the liver was edited.
- At baseline, Z-AAT was the only isoform of AAT protein detected in the mice. By 13 weeks, M-AAT, the wild-type AAT protein, accounted for ~50% of total serum AAT in WVE-006-treated mice. Serum from WVE-006-treated mice also displayed a ~3-fold improvement in neutrophil elastase inhibition relative to PBS-treated mice.
- These preclinical data indicate that WVE-006 directed ~50% editing of the *SERPINA1* Z transcript in the mouse liver, which led to the production of functional M-AAT protein, with total AAT in serum reaching levels higher than the 11 µM threshold believed to lower risk for COPD.
- WVE-006 is being evaluated in a two-part clinical program called RestorAATion. The phase 1/2 program is also assessing the safety and tolerability of single- and multiple-ascending doses of WVE-006 in healthy volunteers and patients; the program is also assessing the concentration WVE-006 in plasma, and pharmacodynamic measures, such as serum M-AAT levels and percentage of *SERPINA1* transcript editing in liver.

INTRODUCTION

- We have developed RNA editing oligonucleotides that direct efficient and specific A-to-I RNA editing of mRNA by endogenous ADAR (adenosine deaminase acting on RNA) enzymes (**Figure 1**).[3]
- WVE-006, an investigational GalNAc-conjugated editing oligonucleotide, was designed to correct the Z mutation in *SERPINA1* mRNA in hepatocytes. The Z transcript encodes Z-AAT protein. The corrected transcript encodes the wild-type M-AAT protein (**Figure 1**).
- The Z mutation leads to misfolding and aggregation of mutant Z-AAT protein in the liver, leading to progressive liver injury and culminating in end-stage liver disease.[1] Retention of Z-AAT in the liver decreases circulating serum AAT levels, leaving lung tissue vulnerable to protease-induced damage (e.g., by neutrophil elastase)[1] (**Figure 2A**).
- In AATD, risk for liver and lung disease is highest in patients with two copies of the *SERPINA1* Z mutation (PiZZ) (**Figure 2B**).[1]
- We developed WVE-006 to convert the high-risk ZZ homozygous condition to the low-risk profile of an MZ heterozygote by editing the Z transcript, restoring functional M-AAT protein in serum, and increasing neutrophil elastase inhibition activity in serum, which is expected to protect lung from damage.

Figure 1. Introduction to RNA editing

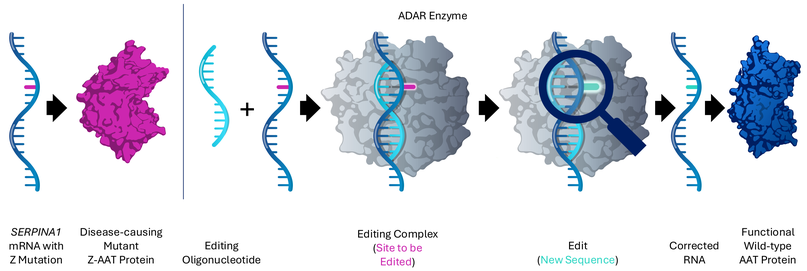


Figure 2. Introduction to AATD

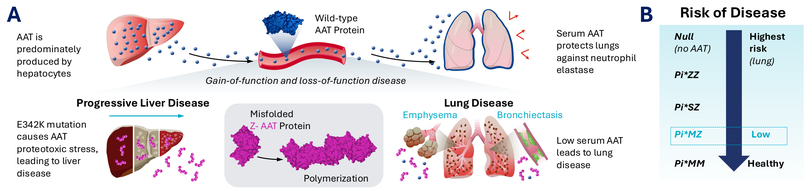
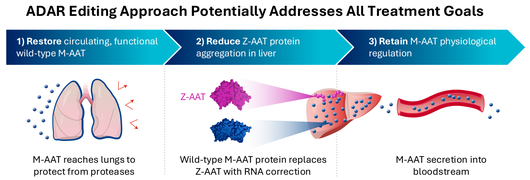


Figure 3. RNA editing is uniquely suited to address the therapeutic goals of AATD



Alternative Approaches Address Only a Subset of Treatment Goals

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

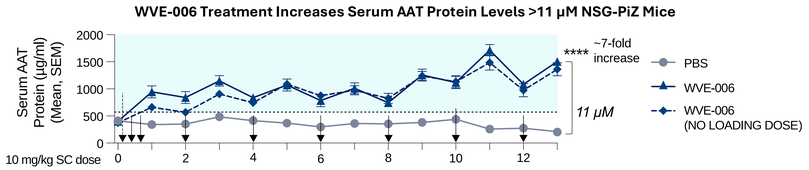
siRNA *approaches address only liver disease*

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

- RNA editing is uniquely suited to address unmet need in AATD, where a single mutation causes both gain-of-function disease in the liver and loss-of-function disease in the lungs. After correction of the Z mutation at the RNA level in hepatocytes, M-AAT protein is expected to replace Z-AAT protein, alleviating protein aggregation in the liver and its associated pathology. Secretion of M-AAT protein in serum will help protect the lungs from protease-induced damage (**Figure 3**).
- Because AAT is an acute-phase response protein, that is dynamically regulated,[1] retaining endogenous control over its expression may be an advantage.
- Alternative approaches are only designed to address liver disease (e.g., silencing with RNAi) or lung disease (e.g., augmentation therapy) but not both.

RESULTS

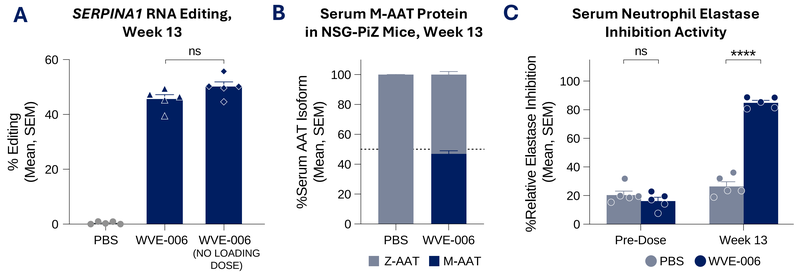
Figure 4. WVE-006 increased total serum AAT levels above the anticipated therapeutic threshold in a repeat-dose mouse experiment



WVE-006 was administered by subcutaneous injection in 7-week-old NSG-PiZ mice (n=5 per group). Total serum AAT protein was quantified by ELISA; Stats: 2-way ANOVA with post-hoc comparison to PBS, ****<0.0001.

- The NSG-PiZ mouse expresses multiple copies of the human *SERPINA1* Z transgene and recapitulates several aspects of AATD liver disease. It has been used to evaluate potential therapies for AATD.[4,5]
- NSG-PiZ mice were treated with WVE-006 or PBS over a 13-week period (**Figure 3**). In the mice treated with PBS, serum AAT levels declined over time, reaching ~4 µM by 13 weeks.
- In NSG-PiZ mice, WVE-006 significantly increased total serum AAT protein levels after one week (up from mean 8 µM at baseline), and serum levels reached 20-30 µM by week 13, ~7-fold higher than levels in PBS-treated mice (**Figure 4**).

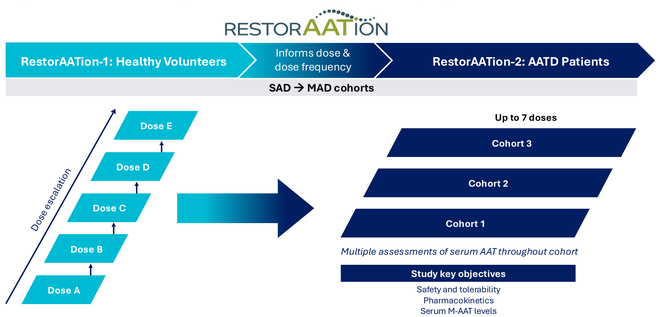
Figure 5. WVE-006 supports ~50% RNA editing in liver and restores functional AAT protein in serum



WVE-006 was administered as in Figure 4. Liver biopsies were collected at week 13 (one-week post-last dose). **A**) *SERPINA1* RNA editing was quantified by Sanger sequencing; Stats: 1-way ANOVA with post-hoc comparisons between all groups (only differences between dose groups shown) ns=non-significant. **B**) Proportion of AAT protein in serum measured by mass spectrometry. **C**) Serum tested for ability to inhibit fixed concentration of neutrophil elastase *in vitro*. Stats: 2-way ANOVA with Bonferroni post-hoc for comparisons between PBS and WVE-006, ns nonsignificant; ****<0.0001.

- After treatment, ~50% of the *SERPINA1* Z transcript in the liver was edited (**Figure 5A**).
- At baseline, Z-AAT was the only protein isoform of AAT detected. By 13 weeks, M-AAT accounted for ~50% of total serum AAT in WVE-006-treated mice (**Figure 5B**).
- At this same time, serum from WVE-006-treated mice displayed a ~3-fold improvement in neutrophil elastase inhibition relative to PBS-treated mice (**Figure 5C**).
- These data indicate that WVE-006 supports the production of functional AAT protein at levels consistent with the heterozygous MZ state in a mouse model for AATD.

Figure 6. Introducing the RestorAATion clinical program



Key Eligibility Criteria

- Homozygous for PiZ allele
- Male or female, 18-70 years old
- Healthy or with stable moderate AATD-induced lung disease (FEV1 ≥50%), and/or stable mild AATD-induced liver disease (≤F2 (≤10 kPa) on FibroScan)

- In December 2023, Wave initiated the RestorAATion clinical program, which includes a healthy volunteer study (RestorAATion-1, NCT06186492) and a phase 1b/2a study in individuals with a ZZ genotype (RestorAATion-2, NCT06405633) (**Figure 6**).[6]
- Both clinical trials are underway.
- RestorAATion-2 is enrolling participants who are homozygous for the *SERPINA1* Z mutation (also known as the PiZZ genotype) and are either healthy (normal lung and/or liver function) or have mild-to-moderate damage in their lungs and/or mild damage in their liver.
- Proof of mechanism data from RestorAATion-2 are expected in the fourth quarter of 2024.

References: 1. Strnad et al., 2020 *New Engl J Med* doi: 10.1056/NEJMra1910234; 2. Brantly et al., 2018 *Chronic Obstr Pulm Dis* doi: 10.15326/jcopdf.6.1.2017.0185; 3. Monian et al., 2022. *Nat Biotechnol* doi: 10.1038/s41587-022-01225-14; 4. Woodell et al., 2020. *JCI Insight* doi: 10.1172/jci.insight.135348; 5. Sifers, 1987. *Nucleic Acids Res* doi: 10.1093/nar/15.4.1459; 6. https://ir.wavelifesciences.com/news-releases/news-release-details/wave-life-sciences-announces-initiation-dosing-restoration. **Acknowledgments:** The authors are grateful to Amy Donner (Wave Life Sciences) for editorial support and Eric Smith for graphical support. This work was funded by Wave Life Sciences.