**RNA Single-base Editing Therapy that Treats Genetic Lung and Liver Disease Entered Clinical Trials**

When Peggy's mom was in her forties, she started to have trouble breathing. Although she had never smoked, she had been exposed second-hand earlier in her life. Eventually, her mother ended up at the Mayo Clinic, where she was the 36th patient to be diagnosed with Alpha-1 Antitrypsin Deficiency (AATD). Knowing it was a genetic condition, the doctors tested Peggy and her brother for it. Peggy, 21 years old and an avid runner with complete lung function, had the full deficiency of the disease. Into her thirties, she was able to continue daily life with no breathing problems; it was as her pulmonologist did annual function tests that they started to see a drop in her lung function, and they made the decision to start treatment to preserve as much lung function as possible. The treatment involves a weekly IV infusion that brings Alpha-1 levels to a protective level.

For those with the disease, there have been few treatment options besides weekly AAT protein infusions. However, the therapeutic landscape has started to shift, with several pharmaceutical companies working on reversing the mutation that leaves patients vulnerable to lung and liver disease. In December, healthy volunteers started receiving an RNA editing therapy to treat the genetic disorder. Wave Life Sciences, the company behind the treatment, claims it's the first RNA editing treatment for this disorder to be dosed in humans.

Known as WVE-006, the therapy uses single-base editing to treat the genetic disorder alpha-1 antitrypsin deficiency (AATD). The product is a short chain of nucleotides that directs naturally occurring ADAR enzymes to change a specific letter in each mRNA molecule to correct the mutation that interferes with AAT production (1).

"By using the cell's endogenous machinery to edit that single base, you now make a normal protein. And we've shown that the normal protein can be expressed at high levels," says Paul Bolno, Wave's President and CEO (1).

**Alpha-1 Antitrypsin Deficiency (AATD): a genetic condition that leaves the lungs and liver vulnerable**

In 1997, Mary Beth was at her grandparent's family reunion when her grandpa announced over dinner that he was diagnosed with AADT. Surrounded by his grandkids, he asked them all to get tested, and she chose to get tested out of respect for him. A few weeks later, Mary Beth received a letter in the mail stating she had alpha-1; alongside the letter were pamphlets saying she should not smoke, wear perfume and hairsprays, or use anything that could irritate her lungs. Mary Beth sat there and thought her life was over, being diagnosed with a lung condition while she was young.

Alpha-1 Antitrypsin Deficiency (AATD) is an inherited genetic disorder commonly caused by a G-to-A point mutation in the *SERPINA1* gene. The mutation results in low levels of the circulating AAT, a protein the liver makes to help protect the lungs. As a result, people with the disease are more vulnerable to lung damage from smoking, pollution, or dust from the environment and often develop diseases like chronic obstructive pulmonary disorder or bronchiectasis. Additionally, misfolding can cause the protein to get stuck in the liver and result in liver disease like cirrhosis. Many people with AATD-related liver disease are unaware of their condition until signs or symptoms of cirrhosis appear (2). In a recent study of individuals with AATD who had not previously been diagnosed with liver disease, 35.1% were found to have clinically significant fibrosis (2).

Delayed diagnosis is a significant problem, and both Peggy and Mary Beth are grateful they were diagnosed when they were young and were able to avoid further lung destruction. In contrast to the two women, Eric was diagnosed when he was 57 after a CT scan for abdominal pain showed lower lobe emphysema. Eric was then diagnosed with both alpha-1-related lung and liver disease. It was later discovered that Eric's father suffered from chronic liver disease, and relatives on his mother's side had COPD. However, none had ever been tested for AATD, which may have allowed Eric to receive an earlier diagnosis and begin treatments that could have prevented his lung and liver disease.

While some individuals have no symptoms, those who do typically start experiencing them between the ages of 20 and 50 years old and often receive an asthma diagnosis, which shares the symptom of wheezing.

While any race or ethnicity can have AATD, it is more common in white people of Northern European backgrounds. One per 2,500 people in Europe are estimated to have AATD, and an estimated 80,000 to 100,000 people in the United States have the condition. Currently, the only available treatment for those with AATD lung disease is augmentation therapy, which requires

weekly AAT protein infusions; for those with AATD liver disease, the only option is liver transplantation.

**WVE-006: preclinical results indicate functionally restored protein**

According to Paul Bolno, WVE-006 is a holistic solution different from other developing treatments for AATD in that it provides an opportunity to treat the root genetic cause of the disease, restore functional wild-type M-AAT protein, and reduce the dysfunctional Z-AAT protein levels. The drug, a first-in-class, GalNAc-conjugated RNA editing oligonucleotide (AIMer), does so by correcting the single G-to-A base mutation in the *SERPINA1* allele. WVE-006 is reversible, re-dosable, and has the potential to be dosed subcutaneously.

In mice, the drug edited around 50% of the target mRNA in liver cells, which Bolno explains is enough to produce therapeutic effects (1). In the same preclinical study, mice dosed with WVE-006 showed AAT protein levels that were approximately 7-fold greater than PBS-administered controls. The mice also displayed restoration of around 50% wild-type M-AAT protein in serum, which led to a 3-fold increase in neutrophil elastase inhibition activity, indicating the restored protein was working.

Healthy volunteers participating in the RestorAATion clinical trial program began receiving the drug in December 2023. Under the RestorAATion-2 program, individuals with AATD who have the homozygous mutation will be dosed, providing an efficient path to proof-of-mechanism as measured by restoration of M-AAT protein serum levels.

Initiation of the RestorAATion dosing is a significant milestone for the AATD community, which has limited treatment options available and no medicines that address the underlying genetic mutation causing the disease. Wave expects to deliver proof-of-mechanism data in individuals with AATD in 2024. Under its collaboration with GSK, development and commercialization responsibilities will transfer to GSK once Wave completes the RestorAATion-2 study.

**AIMers: Wave's editing tool aimed at a single base**

Wave's A-to-I RNA editing oligonucleotides (AIMers) are designed to correct a single-base mutation in an RNA transcript and recruit naturally available ADAR (adenosine deaminases acting on RNA) enzymes. These enzymes can change an adenine (A) to an inosine (I), which cells read as guanine (G). By correcting only a single base in the RNA transcript, the method avoids permanent genome changes that occur with DNA-targeting approaches and uses an existing system in the body for therapeutic purposes.

Advocates of RNA editing say it's a potentially safer, more flexible alternative to genome editing techniques like CRISPR. Because RNA molecules are transient, the editing doesn't produce permanent changes; while this means the duration of the therapeutic effect could be shorter, it

also makes it potentially safer compared to CRISPR, which has the risk of off-target effects or unintended changes outside the target genome area (1). With the development of the RNA vaccine for COVID-19, a greater understanding and acceptance of the method is present in the field, and according to biologist Andrew Leer, it's now seen as an essential therapeutic molecule (1).

**Wave's Competition: Pharmaceutical companies changing the paradigm of AATD treatment**

Wave Therapeutics isn't the only pharmaceutical company that could change AATD treatment. Krystal Biotech's Investigational New Drug, KB408, has been approved by the FDA to enter a Phase 1 clinical trial, with the first patient expected to receive treatment in the first quarter of 2024. KB408 is a gene therapy using a modified herpes simplex virus 1 (HSV-1) derived vector, which delivers two full-length copies of the *SERPINA1* gene to enable AAT expression. The drug is delivered by nebulization (inhalation).

Arrowhead Pharmaceuticals is also trying to fill the gap for AATD patients and is in its Phase 3 clinical study for RNA-based medicine fazirsiran (ARO-ATT). Fazirsiran is an RNA interference (RNAi) therapy designed to knock down the hepatic production of the mutant alpha-1 antitrypsin protein that causes the disease. Preclinical results demonstrated that mice that received the drug experience a reduction in the disease-causing polymer, restored mitochondrial health, normalized expression of disease-associated genes, reduced inflammation, and prevented tumor formation (2).

"The most striking changes were the normalization of rER throughout the hepatocytes and the appearance of abundant mitochondria resembling those in wild-type mice," the research article states (2). The study adds that the nearly normal appearance of RNAi-treated mouse hepatocytes starkly contrasted the hepatocytes of mice that had not received RNAi treatment (2).

The results also suggest that treating AATD liver disease with RNAi could reduce lung disease progression. The study states that, overall, the results of mice treated with RNAi indicate the drug's potential to effectively treat liver disease in children and adults with AATD (2).

Also in development and in its Phase II trial is INBRX-101, a therapy for AATD being created by Sanofi. The drug is a recombinant human AAT-Fc fusion protein that has the potential to allow patients to achieve normal AAT levels through monthly dosing. The drug's Phase I clinical trial showed favorable safety and pharmacokinetics results.

Vertex Pharmaceuticals has developed three AAT treatments. VX-864, a "corrector molecule" designed to prevent the misfolding of AAT and boost AAT plasma levels, is being discontinued due to non-serious rash events in some patients. Even though all three dose levels of VX-864 performed better than the placebo in increasing AAT levels, Vertex decided the effect was so small that it was unlikely to have a substantial clinical benefit. However, the company is

continuing to enroll and dose healthy patients in its two other AAT corrector treatments, VX-634 and VX-668, which the company says has "significantly improved potency and drug-like properties compared with the first-generation of AATD correctors."

**The future of treating AATD**

Peggy, along with others like her, dreams of a cure for AATD within her lifetime, and with therapies like WVE-006 in clinical testing, this brings forward hope that is more than just a dream. As data from the RestorAATion program emerges later in 2024, Wave Therapeutics and patients with AATD hope the results provide a paradigm shift in treatment options. Regardless, with an array of companies working on therapies, alternative treatments to weekly infusions look like a potential option for AATD patients.

**Sources:**


1. Lenharo M. Move over, CRISPR: RNA-editing therapies pick up steam. Nature. 2024 Feb;626(8001):933-934. doi: 10.1038/d41586-024-00275-6. PMID: 38366220.
2. Wooddell CI, Blomenkamp K, Peterson RM, Subbotin VM, Schwabe C, Hamilton J, Chu Q, Christianson DR, Hegge JO, Kolbe J, Hamilton HL, Branca-Afrazi MF, Given BD, Lewis DL, Gane E, Kanner SB, Teckman JH. Development of an RNAi therapeutic for alpha-1-antitrypsin liver disease. JCI Insight. 2020 Jun 18;5(12):e135348. doi: 10.1172/jci.insight.135348. PMID: 32379724; PMCID: PMC7406265.
3. Arrowhead Pharmaceuticals, Inc. (2023, April 4). *First patient treated in alpha-1 antitrypsin deficiency liver disease phase 3 study triggering $40 million milestone payment from Takeda to Arrowhead*. Business Wire. https://www.businesswire.com/news/home/20230404005248/en/First-Patient-Treated-in-Alpha-1-Antitrypsin-Deficiency-Liver-Disease-Phase-3-Study-Triggering-40-Million-Milestone-Payment-from-Takeda-to-Arrowhead
4. Eckford, C. (2024, January 24). *Sanofi bets $1.7b on potential best-in-class rare disease therapy*. European Pharmaceutical Review. https://www.europeanpharmaceuticalreview.com/news/204118/sanofi-bets-1-7b-on-potential-best-in-class-rare-disease-therapy/
5. The France Foundation. (2019, September 17). *A closer look at alpha-1 antitrypsin deficiency: Peggy's story*. YouTube. https://www.youtube.com/watch?v=JfRP1AoWyao
6. Philpott, J. (2023, September 22). *FDA clears Krystal Biotech's IND for Type 1 AATD treatment KB408*. Pharmaceutical Technology. https://www.pharmaceutical-technology.com/news/fda-clears-krystal-biotechs-ind-for-type-1-aatd-treatment-kb408/
7. U.S. Department of Health and Human Services. (2023, October 25). *Alpha-1 antitrypsin deficiency*. National Heart Lung and Blood Institute. https://www.nhlbi.nih.gov/health/alpha-1-antitrypsin-deficiency
8. Vertex Pharmaceuticals. (2023, February 14). *Living with alpha-1 antitrypsin deficiency (AATD): Mary Beth's story | vertex pharmaceuticals*. YouTube. https://www.youtube.com/watch?v=iKtEItlEqKM
9. Waldron, J. (2023, November 7). *Vertex drops phase 2 lung therapy, rethinks DMD drug's journey toward clinic*. Fierce Biotech. https://www.fiercebiotech.com/biotech/vertex-drops-lung-therapy-rethinks-dmd-drugs-journey-towards-clinic
10. *Wave Life Sciences announces initiation of dosing in restoraation clinical program evaluating first-ever RNA editing candidate, WVE-006, for alpha-1 antitrypsin deficiency*. Wave Life Sciences. (2023, December 6). https://ir.wavelifesciences.com/news-releases/news-release-details/wave-life-sciences-announces-initiation-dosing-restoraation
11. Wave Life Sciences USA, Inc. (2022, September 28). *Wave Life Sciences highlights therapeutic potential for WVE-006 for alpha-1 antitrypsin deficiency and progress bringing RNA editing to the clinic during analyst and Investor Event*. GlobeNewswire News Room. https://www.globenewswire.com/en/news-release/2022/09/28/2524648/0/en/Wave-Life-Sciences-Highlights-Therapeutic-Potential-for-WVE-006-for-Alpha-1-Antitrypsin-Deficiency-and-Progress-Bringing-RNA-Editing-to-the-Clinic-During-Analyst-and-Investor-Event.html