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ERJ ADVANCES

A Cure for Alpha-1? Novel Therapeutics in Alpha-1 Antitrypsin Deficiency

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Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder caused by mutations in the *SERPINA1* gene, that encodes alpha-1 antitrypsin (AAT) (1). AAT is an inhibitor of neutrophil serine proteases such as neutrophil elastase (NE) and proteinase-3 (1). AATD is inherited in an autosomal co-dominant pattern, a homozygous mutation causes 95% of severe AAT deficiency involving a single amino acid substitution - Glu342Lys (referred to as the 'Z' allele) (2). Approximately 1 in 25 individuals of Northern and Western European descent carry the Z allele, with lower frequencies reported in Southern Europe (1-2%) and rare occurrence in other populations (3). AAT is primarily synthesized within the liver and released into the bloodstream. The Z mutation leads to the production of misfolded proteins that polymerize and accumulate as Periodic Acid Schiff positive inclusions within hepatocytes (4).The misfolded and polymerized protein triggers the endoplasmic reticulum overload response, hepatocyte injury, inflammation and fibrosis, which may progress to cirrhosis or hepatocellular carcinoma. These polymerized aggregates are a key factor in driving liver disease associated with AATD (3). The liver pathology is distinct from the lung manifestations of AATD as it results from the toxic accumulation of misfolded proteins. The lung disease is predominantly the result of a loss of protease inhibition. Reduced circulating AAT levels compromise lung protection against neutrophil elastase, causing a protease-antiprotease imbalance. This imbalance leads to lung matrix degradation, progressive destruction, and emphysema (5). Additionally, Z-AAT can form polymers within the lung. These polymers have proinflammatory properties, acting as a chemoattractant for neutrophils (6). Beyond lung and liver disease, AATD can contribute to systemic conditions due to circulating Z polymers and protease-antiprotease imbalance (7). Some individuals develop panniculitis, a rare skin disorder that presents as painful subcutaneous nodules (8), others may develop vasculitis (9). This review aims to outline the current landscape of novel

therapeutic strategies for AATD, with a focus on both liver and lung directed approaches that target the AAT protein, RNA, and DNA levels of disease pathogenesis.

Augmentation therapy remains the only currently available pharmacological intervention for AATD. This approach involves weekly intravenous infusions of plasma-derived AAT, aiming to restore protective serum (and therefore tissue) levels and slow the progression of lung disease (10). Recent data also suggest a potential liver benefit, with lower enzyme levels and reduced fibrosis markers observed in treated individuals (11). Clinical trials have shown that augmentation therapy slows emphysema progression using CT densitometry, but benefit on clinical end-points has been harder to define and the therapy has limitations, including high annual costs and burden from frequent infusions (10,12). Augmentation therapy is not available in all countries.

Given the limitations of current augmentation therapy, there is growing interest in innovative treatments that target the underlying pathology of AATD. Whilst developments continue around protease-antiprotease mechanisms, such as neutrophil elastase inhibitors, here we focus on novel mechanisms that target the AAT production and secretion. These emerging approaches fall into three broad categories: protein-targeted therapies, RNA-based drugs, and DNA-editing strategies each aiming to correct or mitigate the disease at different stages of gene expression, translation and protein function (Figure). The following sections explore these novel therapeutics in more detail.

1. Treatments Targeting the Protein

Therapeutic strategies targeting the mutant alpha-1 antitrypsin (Z-AAT) protein focus on promoting correct conformational folding and secretion, promoting its degradation and preventing misfolding with intracellular polymerization. A core mechanism in the liver pathology of AATD is the polymerization of Z-AAT, that results from a sequential C-sheet domain swap, forming toxic polymers that accumulate within hepatocytes and drive liver disease (13–15). Early efforts used chemical chaperones such as 4-phenylbutyric acid to stabilize protein folding and increase secretion of functional AAT. While effective in animal models (16), this failed to significantly increase circulating AAT levels in humans (17). Further development efforts included small-molecule chaperones for both liver and lung

disease led by Vertex Pharmaceuticals, which reached Phase 2 trials. However, these were discontinued due to insufficient clinical efficacy. An alternative therapeutic approach targets the clearance of already misfolded or aggregated Z-AAT through enhanced degradation. carbamazepine, an autophagy-inducing drug has been shown to promote the breakdown of Z-AAT aggregates and reduce liver fibrosis in mouse models of disease (18). However, carbamazepine is linked to Stevens-Johnson syndrome and toxic epidermal necrolysis, rare but potentially life-threatening conditions which warrant close safety monitoring in future use. Carbamazepine was evaluated in individuals with ZZ AATD (ClinicalTrials.gov ID: NCT01379469), but results are not available. Building on mechanistic understanding of Z AAT polymerization, efforts have turned towards identifying small molecules that stabilize Z-AAT and prevent intrahepatic polymerization (19). The most recent drug is BMN349, a novel oral small molecule that is currently in Phase 1 clinical trials (ClinicalTrials.gov ID NCT06738017). In preclinical studies, BMN349 reduced hepatic Z-AAT polymer accumulation and increased plasma levels of total Z-AAT in PiZ mice (20).

2. RNA-Based Therapy

RNA-based therapies for AATD focus on silencing or correcting the expression of the mutant Z allele of the *SERPINA1* gene to prevent the production of misfolded Z-AAT. The leading strategy involves small interfering RNA (siRNA) molecules that target and degrade *SERPINA1* mRNA. A prominent example is Fazirsiran (ARO-AAT), an RNA interference (RNAi) therapeutic to silence Z AAT mRNA expression, thus reducing Z AAT protein synthesis. This drug has demonstrated significant reductions in hepatic Z-AAT accumulation and histologic improvement in liver inflammation and fibrosis in Phase II clinical trials (21,22). While this approach is effective in reducing toxic hepatic burden, it does not increase circulating levels of AAT and is not therefore likely to address the pulmonary component of AATD. As such, siRNA-based treatments may need to be combined with protein augmentation or other therapies to offer comprehensive disease mitigation. Antisense oligonucleotides (ASOs), single-stranded RNA-like molecules designed to bind mRNA and prevent translation or induce degradation, are also being studied, although less extensively than siRNAs. In a preclinical study using the PiZ mouse model, ASO therapy significantly reduced hepatic Z-AAT accumulation, improved liver histology, and decreased fibrosis, supporting its potential as a liver-directed therapeutic option for AATD (23).

In contrast, RNA editing represents an emerging approach that may impact both liver and lung disease by restoring expression of the functional protein. The furthest in development is Wave Life Sciences' WVE-006, a first-in-class RNA editing candidate delivered via N-acetylgalactosamine (GalNAc) conjugation and KRRO-110 capsulated in lipid nanoparticles (LNPs) for intravenous delivery(24,25). These agents are currently in phase 1/2 clinical trial (NCT06405633) (NCT06677307), with preclinical data showing promising editing of the mutant *SERPINA1*, restoration of functional M-AAT protein, and improvement in liver biomarkers (24,25). By restoring circulating M-AAT, these approaches have the potential to address the pulmonary manifestations of AATD. However, as with other RNA-based therapies, efficacy requires ongoing dosing to maintain therapeutic benefit. Overall, RNA therapies now encompass both gene-silencing and gene-correcting approaches and hold significant promise for modifying disease progression.

3. DNA-Editing Therapy

DNA-based therapies for AATD aim to correct or supplement the defective *SERPINA1* gene to restore normal AAT production, decrease mutant protein production and thus address both pulmonary and hepatic manifestations of the disease. One of the earliest strategies investigated was adeno-associated virus (AAV)-mediated gene augmentation, which delivers a functional copy of the human *SERPINA1* gene. Initial clinical trials using recombinant adeno-associated virus rAAV1 vectors via intramuscular injection demonstrated safety and showed sustained gene expression, but serum AAT levels remained below the therapeutic threshold (26). Subsequent preclinical studies with AAV vectors showed 200-fold higher expression levels in skeletal muscle (27). However, the treatment still failed to reach protective serum concentrations in human (27). Challenges such as pre-existing anti-AAV antibodies and the possibility of insertional mutagenesis have limited the clinical impact of rAAV approaches (28). To overcome these limitations, clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/Cas9) technology is now being explored (29). In a transgenic PiZ mouse model of AATD, CRISPR/Cas9-mediated correction of the Glu342Lys mutation via a dual AAV delivery system led to partial restoration of wild-type M-AAT expression, significant reduction in Z-AAT polymer accumulation, and improvement in liver histopathology (30). However, potential concerns such as off-target effects, and immune responses to Cas9 remain barriers to clinical translation. The most recent advance in

DNA-based therapy is base editing, a precision gene editing technology that enables single-base changes in DNA without causing double-stranded breaks (31). In early results from an ongoing Phase 1/2 clinical trial (NCT06389877), a single IV dose of BEAM-302 led to durable, dose-dependent increases in total and functional AAT, production of corrected M-AAT, and reduction of circulating Z-AAT (32). This clinical demonstration of first *in vivo* base editing marks a major milestone in AATD treatment, offering the potential for a single-dose curative intervention that addresses both liver and lung disease at the genetic level. Another emerging approach involves Tessera Therapeutics, which is developing an in vivo gene writing platform that uses RNA to direct precise edits to DNA. In preclinical models, this strategy has shown promising levels of *SERPINA1* correction and restoration of functional AAT protein. It is important to highlight that RNA and DNA editing strategies are mechanism-based interventions that directly modify gene expression or genomic sequences and therefore cannot be meaningfully compared to placebo treatments.

In conclusion, this is an exciting time in AATD research and care. Strategies targeting protease imbalance, protein misfolding, RNA and DNA are offering new interventions to target the major liver and lung manifestations of AATD. These still need to be thoroughly evaluated in clinical trials, and safety needs to be evaluated in long term follow-up, but we may well be on the verge of a cure for AATD.

Declaration of interests. DAL is an inventor on patent PCT/GB2019/051761 that describes the development of small molecules to block the polymerisation of Z a_1-antitrypsin. This includes BMN349 that is described in this manuscript. JRH and AM are investigators on trials of novel therapeutics in AATD.

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Figure: An overview of novel liver-targeted AATD treatment strategies.