Experimental and investigational drugs for the treatment of alpha-1 antitrypsin deficiency

Pye, Anita; Turner, Alice

DOI:
10.1080/13543784.2019.1672656

Document Version
Peer reviewed version

Citation for published version (Harvard):
Pye, A & Turner, A 2019, 'Experimental and investigational drugs for the treatment of alpha-1 antitrypsin deficiency', *Expert opinion on investigational drugs*, vol. 28, no. 10, pp. 891-902. https://doi.org/10.1080/13543784.2019.1672656

Experimental and investigational drugs for the treatment of alpha-1 antitrypsin deficiency

Journal:	*Expert Opinion On Investigational Drugs*
Manuscript ID	EOID-2019--0059.R2
Manuscript Type:	Review (Invited)
Keywords:	Alpha 1 antitrypsin deficiency, Chronic obstructive pulmonary disease, Emphysema, Clinical trials, Cirrhosis

Experimental and investigational drugs for the treatment of alpha-1 antitrypsin deficiency

Anita Pye[1] & Alice M Turner[1]

[1]Institute of Applied Health Research, University of Birmingham, B5 2TT

Corresponding author: Alice M Turner, a.m.turner@bham.ac.uk

ABSTRACT

Introduction

Alpha-1 antitrypsin deficiency (AATD) is most often associated with chronic lung disease, early onset emphysema and liver disease. The standard of care in lung disease due to AATD is alpha-1 antitrypsin augmentation but there are several new and emerging treatment options under investigation for both lung and liver manifestations.

Areas covered

We review therapeutic approaches to lung and liver disease in alpha-1 antitrypsin deficiency (AATD) and the agents in clinical development according to their mode of action.

The focus is on products in clinical trials, but data from pre-clinical studies is described where relevant, particularly where progression to trials appears likely.

Expert opinion

Clinical trials directed at lung and liver disease separately are now taking place. Multimodality treatment may be the future, but this could be limited by treatment costs. The next 5-10 years may reveal new guidance on when to use therapeutics for slowing disease progression with personalized treatment regimes coming to the forefront.

Keywords: Alpha-1 antitrypsin deficiency, Chronic obstructive pulmonary disease, Emphysema, Cirrhosis, Clinical trials

Article Highlights

- The main pathogenic mechanisms for therapeutic targeting in AATD are protease imbalance in the lung and polymer burden in the liver
- Intravenous AAT augmentation therapy is a viable option to reduce emphysema progression, but there remain uncertainties over optimal patient and dose selection
- Other approaches to address protease imbalance which are in clinical trials include oral neutrophil elastase inhibitors and nebulized hyaluronan
- Approaches to liver disease in clinical trials include siRNA to reduce transcription of mutant protein, and hence polymer formation, as well as carbamazepine to enhance degradation of mutant protein by autophagy
- Specific molecular approaches targeting polymerization of mutant protein are attractive because of potential to treat lung and liver simultaneously but remain at an early stage of development

1 INTRODUCTION

Alpha-1 antitrypsin deficiency (AATD) was first described when absence of the α1 band on protein electrophoresis of serum taken from a patient at a local respiratory hospital was associated with early onset emphysema (1). AATD is also associated with chronic obstructive pulmonary disease (COPD), bronchiectasis (2), liver fibrosis and cirrhosis (3), vasculitis (4) and panniculitis (5). Although AATD represents a predisposing factor for all of these phenotypes, not all subjects develop disease. Some variation in clinical presentation may be due to environmental factors, such as cigarette smoking or pollution (6), but there may also be genetic modifiers (7). The diagnosis is usually made after investigation of pulmonary or liver disease. Currently neonatal screening, which would allow diagnosis at birth, rather than when symptoms of disease develop is not adopted in routine practice. The classical presentation is with breathlessness and cough, similar to all COPD, though often at a younger age or with less smoke exposure (8).

The observation of AATD in patients with emphysema suggested a role for pathways involving alpha-1 antitrypsin (AAT) in pathogenesis and led to many studies investigating this concept in both animals and man. AAT has a range of functions within the lung, the primary one being to protect the tissues against neutrophil elastase (NE) (9), with a smaller role in defending against damage by other serine proteases, such as proteinase 3. These enzymes induce emphysema and bronchial damage in animal models (10-12), giving rise to the protease-antiprotease hypothesis. This theory suggests that when there is an imbalance of protective anti-proteases and destructive proteases, digestion of elastin, the extra-cellular matrix, and epithelial tissue damage, occurs, which is seen clinically as emphysema and COPD (13). Consequently therapeutic strategies which address protease imbalance, such as NE inhibition, may help to modify the disease trajectory.

Early strategies to treat AATD mostly focused on improving pulmonary AAT levels usually by way of enhancing circulating levels; in non-smokers with the PiSZ phenotype, epidemiological studies have shown little or no increase in the risk of lung disease compared to controls (14), thus their circulating level of AAT (typically 11-14μmol/l) is generally taken to be the minimum needed to protect the lung from NE mediated damage. Historically this has been done by replacing AAT directly through infusions, commonly referred to as augmentation therapy, and this is considered the standard of care in many countries worldwide (15-17). However, even when on augmentation therapy many patients' lung disease continues to progress, such that ways to optimize this treatment remain a research priority. Furthermore this approach may help to stabilise the lung disease, but would have no effect on the liver since the mechanism of liver disease differs.

AATD occurs due to genetically determined abnormalities in the structure of AAT, many of which lead to protein polymerization within the liver, and consequent poor secretion into the blood, such as the PiZ variant (18). More rarely other polymorphisms in the AAT gene (*SERPINA1*) occur which lead to absence of production; genetic aspects of the disease have been reviewed elsewhere (19). The number of individuals with the PiZZ genotype in Northern Europe is thought to total almost 17000, with the highest density of cases being seen in Latvia; spread of the PiZ mutation from this location through the rest of Europe leads to total numbers in excess of 100000 across the region(20). Whilst the deficiency is more common in Caucasians it is seen worldwide (20), but many patients even in areas of relatively high prevalence (eg Europe) are not diagnosed. Patients of the PiZZ or null phenotypes are those with the lowest levels and historically most therapeutic trials have focused on this population. However PiSZ heterozygotes also appear to have significant deterioration in their lungs over time (21), and this genotype is more common (based on allele frequency (20)), hence strategies which can address disease in this group may also be important. The close link between genotype, protein

structure and disease have led to studies of gene therapy and small molecules targeting alterations in protein structure to treat the clinical manifestations of AATD.

Polymerization within the liver, which is seen with the most well known deficiency variants, the PiZ and S alleles, leads to hepatocyte damage and cirrhosis (22, 23). Whilst there is significant heterogeneity of presentation and prognosis of liver disease (3), implying that genetic and environmental modifiers are probably also important, the unmet need for patients with progressive disease, in whom little could be done except transplantation, led to much research into the mechanism of liver damage, which is now leading to trials of investigational products. Reduction of polymer burden, either through reduced protein formation or improved polymer clearance are routes which have led to trials already, and small molecules which block the polymerization process are also an attractive option. Polymers outside the liver may also amplify pulmonary inflammation by acting as neutrophil chemoattractants (24) and increasing apoptosis (25), such that an anti-polymeric agent could also have smaller benefits in the lung. Most evidence suggests that lack of protection against NE is the main driver of lung disease; how much results from polymer effects remains a matter of debate. Mechanisms of disease and consequent routes to therapy in AATD are summarized in Figure 1.

This review will briefly cover the history of augmentation therapy in AATD, and then look at the newer strategies in development, firstly those that may optimize augmentation, and thereafter alternative ways to treat the lung, and the liver. In order to ensure comprehensive coverage of ongoing work in AATD we have conducted reviews of all public clinical trial databases using the search term ‘Alpha-1 antitrypsin deficiency’, as well as contacting other experts in the field, and provide these findings as a table of ongoing or as yet unpublished studies (Table 1). Finally, we conclude with some thoughts about the likely direction of travel for therapy in AATD.

2 THERAPEUTIC APPROACHES IN LUNG DISEASE

2.1 Intravenous augmentation therapy

Intravenous augmentation therapy is derived from human plasma and was first tested at a dose of 60mg/kg in the early 1980's(28), then approved for human use in 1987 by the Food and Drug Administration (FDA) in the USA on the basis that it could raise concentrations of AAT in the epithelial lining fluid of the lung (thus improve local anti-NE protection) and restore normal circulating AAT concentrations (28, 29). Widespread use across the USA, Canada and Europe followed, and it is recommended in American, Canadian and European guidelines (15-17). The first RCT of AAT augmentation(30) used a 4 weekly infusion regimen and a wide range of infusion frequencies were used in the American registry study (31) and Spanish rEXA group study (32). A definitive 4 year trial, which demonstrated benefit of weekly infusions on emphysema progression(33), and biomarkers of NE activity(34), cemented the role of augmentation in management in 2015.

Meta-analysis of the effects of augmentation has shown a consistent effect on progression of emphysema as measured by quantitative radiological imaging of the lung (CT densitometry) (35), and CT density has been shown to relate to lung function and other conventional measures of disease severity in COPD and AATD by systematic review and meta-analysis of studies reporting the relationship between density and FEV_1 (36). In addition change in density in the lower zone has been shown to relate to longer term mortality in AATD (37); taken together the literature therefore suggests that CT density is an appropriate surrogate outcome measure for trials targeting lung disease progression in emphysema.

Despite the strength of evidence supporting augmentation to manage progressive lung disease, questions remain about infusion frequency and dose. Head to head studies testing

different infusion frequencies are lacking but pharmacokinetic work suggests that levels remain above 11μM for only 7-10 days (38-40). One study concluded that twice weekly fixed dose regimens (1-2g) rather than per weight (120mg/kg) weekly or fortnightly doses were the best; however data showing levels over time suggested weekly doses would be adequate (38). One modeling study demonstrated that infusions might be given up to 21 days apart (41), but this has not been borne out by patient-derived data. Given that most studies have shown levels becoming sub-therapeutic before 14 days it is likely that weekly regimes will remain the standard with our current iv preparations.

A randomized, placebo-controlled trial (SPARTA) (42) assessing the efficacy and safety of two separate doses (60 and 120 mg/kg) of alpha-1 proteinase inhibitor is currently being conducted with an estimated end date of 2023 (40). In this multi-center, worldwide study augmentation is administered by IV infusion on a weekly basis for 3 years in adults with a diagnosis of AATD and clinical evidence of pulmonary emphysema. The primary outcome measure of efficacy is change from baseline whole-lung 15th percentile lung density (PD15) determined by CT lung densitometry at total lung capacity. Secondary efficacy variables are the evaluation of severe exacerbations, and PD15 of the basal lung region

However, although emphysema progression is clearly reduced by augmentation therapy, the ongoing decline in lung density in treated patients is clearly a concern. In addition the need to derive the product from human plasma means that it is expensive and some healthcare systems have questioned its cost-effectiveness as a result. There are therefore many countries worldwide who do not reimburse augmentation in its current licensed form.

2.2 Recombinant or altered forms of augmentation therapy

One method of making augmentation more economically viable, and without supply limitations, is to generate synthetic AAT. In order for a recombinant AAT product (rAAT) to be viable it would need to be safe, with a good plasma half-life, clinically efficacious and cost-effective relative to other AAT sources. None of these requirements have been met yet, although many groups continue to work in the field. Recent advances include cysteine-pegylation in an *E-coli* production system, which appears to overcome some of the degradation issues of earlier *E-coli* work, though whether it will exhibit immunogenicity is not yet known (43). Several studies of rAAT have been tested in Phase I studies in AATD (NCT00157092, NCT00161707) but in some cases no results have been posted.

A Phase 1 safety investigation of Baxter/Arriva rAAT looked at a total of 32 subjects with AATD who were sequentially assigned to 1 of 4 dose groups (10, 50, 100 or 200mg) randomized in a 6:2 ratio of drug or placebo. Each subject received study drug or placebo (vehicle buffer) on day 1 and was re-challenged with the same dose on day 15. Data demonstrated that Baxter/Arriva rAAT administered by nebulizer at doses up to 200mg was safe and well-tolerated with a total of 79 adverse experiences reported in 26 of the 32 subjects. The rate of AEs was similar in both active and placebo arms of the study and no subjects were discontinued for any reason. The most frequently reported AEs were headache, upper respiratory tract infection, pain in extremity, sinus headache, nasal congestion and rash. Pulmonary function measurements were similar across all treatment days and treatment groups and data indicated that overall airflow was unaffected after administration of aerosolized rAAT. Clinical laboratory parameters showed little change between pre-dose and post-dose measurements, and there were no apparent changes in rAAT or yeast cell protein immunogenicity.

The current status of rAAT production systems and their limitations are shown in Table 2.

Another approach to rAAT is the generation of fusion molecules which fuse the recombinant AAT to another molecule; theoretically this might reduce issues of immune response to non-human protein. A number of studies in mice have been published which have used rAAT fused to the constant region of IgG1 (rAAT-Fc), although interestingly none have addressed concepts specific to AATD. In murine models of myocardial infarction, diabetes and gout rAAT-Fc reduced infarct size, reduced development of hyperglycaemia and ameliorated joint inflammation respectively(70-72). However in several of these studies the authors reported no anti-elastase activity of the fusion molecule, which is clearly a limitation when contemplating treatment of AATD lung disease. The anti-inflammatory properties demonstrated might still be useful for management of panniculitis, a rare skin complication of AATD in which augmentation appears useful, albeit with only case series based evidence to support its use(73).

Further development of the rAAT-Fc concept has led to progress relevant to AATD lung disease. INBRX-101, a recombinant human alpha-1 antitrypsin Fc fusion protein (rhAAT-Fc), is currently being tested in an open-label, 2-part, dose-escalating Phase 1 study in adults with a diagnosis of AATD (NCT03815396). The first part of the study consists of a single ascending dose (SAD) administration of the compound, followed by part 2 where multiple ascending dose (MAD) administrations. Primary outcome measures of this study are the frequency and severity of adverse events, and the half-life, immunogenicity, distribution in bronchoalveolar lavage fluid (BALF) and functional concentration in serum and BALF of INBRX-101 will also be determined. The planned dosing schedule of INBRX-101 is IV administration every 3 to 4 weeks versus weekly infusions with plasma-derived products. This prolonged dosing interval, relative to augmentation, may improve quality of life by reducing treatment frequency.

2.3 Inhaled augmentation therapy

The inhaled approach is inherently attractive since only 2-3% of AAT given intravenously reaches the lung (74). Initial studies demonstrated that this method can augment anti-NE capacity of the respiratory epithelium and that inhaled AAT can pass into the lung interstitium, since trace amounts are detectable in the systemic circulation (75). However, whilst it is possible to deliver AAT to the alveoli using conventional nebulizers, this could take a long time (up to 100 minutes), or be less effective in patients with poor lung function (76, 77). Secondly the amount of AAT needed in the interstitium to prevent disease is not clear; even if some protease anti-protease imbalance remains the effect of small amounts of AAT might reduce inflammation and hence disease progression. Finally, it remains unknown if penetration to the interstitium is the critical element of treatment. Nebulized drug might be used to treat airways disease in those patients with little emphysema but acute or recurrent exacerbations, or even to treat the enhanced inflammation seen at exacerbations. If treating airways disease is the aim then consideration needs to be given to the aerodynamic diameter of particles, as only those with the smallest (<1.5μM) will reach the small airways.

Kamada conducted a European Phase 2 study looking at aerosolized (inhaled) human plasma-derived AAT 160mg dose versus placebo using a rapid nebulizer device which uses novel technology for liquid aerosol generation. Eligible subjects had experienced at least two exacerbations in the previous 18 months from screening and the primary outcome measure was the time to first moderate/severe exacerbation. Secondary endpoints included rate and severity of exacerbations, and effect on lung function. The drug was found to be safe and well tolerated with no adverse events indicating immunogenicity and/or clinical indication of bronchospasm. Despite a previous study in the US comparing 80mg and 160mg doses meeting the primary endpoint, results showed no significant difference between the placebo and AAT group (conference presentation only, no publication or abstract available). There was a trend towards the reduction in the number of Anthonisen Type-1 exacerbations and a statistically significant

reduction in dyspnea score compared to placebo. There was also a trend towards improvement in FEV_1 in the AAT group. Kamada have recently announced plans to start a pivotal phase III study to evaluate the safety and efficacy of the inhaled AAT product in patients with AATD(78). The primary endpoint of the study will be to check the lung function measured by FEV_1 while the secondary endpoints will be to observe the changes in lung density as gauged by CT densitometry.

2.4 Neutrophil elastase inhibition

As described in the introduction protease imbalance plays a key role in lung disease pathogenesis in AATD, driven by the lack of inhibition of NE by AAT. In addition intravenous augmentation therapy, which appears clinically beneficial, reduces markers of NE activity (desmosine)(34), further confirming the importance of this pathway. Alvelestat (MPH-996), an oral NE inhibitor, is currently being trialed in Phase 2 clinical trials ASTRAEUS (NCT03636347) and ATALANTa (NCT03679598) which aim to reduce lung damage and slow the progression of lung disease caused by AATD. The primary endpoint is the change from baseline of desmosine/isodesmosine as biomarkers of NE activity. The rarity of AATD, and consequent need for surrogate outcomes if a study is to be of a reasonable size, has made markers like desmosine attractive as a primary outcome for phase 2 studies treating AATD lung disease.

PHP-303 is a novel NE inhibitor acquired by pH Pharma from Bayer AG in 2017. Bayer conducted clinical studies of the drug in bronchiectasis and demonstrated human safety and efficacy against neutrophil elastase. pH Pharma have been focusing on developing PHP-303 in the nonalcoholic steatohepatitis (NASH) environment in addition to other genetic disease areas indications such as AATD where Phase 1 studies are being planned.

Another way to approach NE inhibition is to use non-specific products which are known to have anti-elastase activity. An example of this is hyaluronan (HA), which binds to pulmonary elastin (79) and limits airspace enlargement in animal models of emphysema (80). In a small (n=11) safety study of nebulised HA in COPD circulating and sputum levels of desmosine (an elastin degradation product) were gradually reduced during the treatment period, suggesting that this treatment has promise (81). Although no effects on lung function were seen this is perhaps not surprising in a short term study. In AATD HA correlates directly with serum AAT levels and lung function, and is reduced in lung tissue, implying that it plays a role in pathogenesis of lung disease in AATD (82), and may be an even more useful treatment in this condition than COPD. Based on this evidence a phase II study of nebulised HA (NCT03114020) is now ongoing in centres in the USA, assessing desmosine in sputum, plasma and urine as the primary outcome, and including lung function amongst the secondary outcomes, measured at 28 days from treatment commencement in a target of 40 patients with AATD (any genotype except PiMZ).

3 THERAPEUTIC APPROACHES IN LIVER DISEASE

3.1 Reduced translation of mutant protein

One route to reduce AAT polymers would be to limit production of AAT by interfering with its mRNA using antisense or RNA interference. This method reduced accumulation of mutant protein globules and inhibited expression of fibrosis-associated genes in the PiZZ transgenic mouse (83), this evidence led eventually to a placebo-controlled phase 1 study in healthy volunteers (n=54) and PiZZ AATD individuals (n=11) administered ARC-AAT, a short interfering RNA (siRNA) therapeutic targeting liver production of AAT, or placebo intravenously. ARC-AAT produced sustained knockdown of AAT, and no safety issues were seen in the study(84). Nevertheless further development of the intravenous form, which used an excipient thought to be responsible for off target effects, was discontinued due to safety

concerns in non-human work. Fortunately the newer product, which is also capable of being administered subcutaneously, hence is more convenient for the repeated dosing likely in chronic liver disease, has been approved to progress to a phase II/III study with the primary outcome based on liver fibrosis appearance within biopsy samples (85). The new product, ARO-AAT, has been tested in 45 healthy volunteers resulting in 90% knockdown of AAT and no safety concerns (86). This, together with evidence about ARC-AAT in PiZZ individuals, aided the application for a more advanced phase of study. Phase I study of a different siRNA (ALN-AAT; NCT02503683) was terminated due to transient liver enzyme rises in some patients. However a newer version of this product (ALN-AAT02) is now being tested in a phase I/II study in a single centre in London, aiming to recruit 96 participants, including both healthy volunteers and PiZZ individuals (NCT 03767829)

One concern facing this strategy is the potential increased risk of lung disease progression if used over long periods of time, since participants with null genotypes who produce no AAT exhibit more severe emphysema (87). To mitigate such concerns AAT augmentation therapy could be used concomitantly with liver-targeted siRNA.

3.2 Improved clearance of mutant protein

Autophagy is increasingly being recognized as an important mechanism in pulmonary disease(88); in AATD it contributes to the degradation of mutant AAT, thus promoting autophagy might reduce the impact of liver disease in particular in these patients. In vitro and murine work using carbamazepine (a known autophagy enhancer) has shown the strategy to be effective in reducing liver damage (89) and a trial of carbamazepine in AATD patients with liver disease has been recruiting for 7 years (NCT01379469; last updated May 2018). However, the dose of carbamazepine used in the mouse study was far in excess of the standard dose in man, which is being used in the trial, therefore whether it will be effective is unclear. A small

study of ursodeoxycholic acid in children with AATD showed promise in those with milder disease, although was uncontrolled(90). Rapamycin also upregulates autophagy and reduced polymer accumulation and hepatocellular injury in a PiZ mouse model (91), though no later phase studies are in the public domain using this product. Many licensed drugs are known to influence autophagy, hence it is an attractive proposition for treatment as we could move rapidly to trials of drugs known to be safe; examples include sirolimus, lithium and valproate. Pre-clinical work using a high throughput system capable of screening a drug library for various effects, including autophagy, has been conducted in models relevant to AATD(92) giving further support to work with agents affecting this process.

4 THERAPEUTIC STRATEGIES WITH POTENTIAL TO TREAT ALL ASPECTS OF DISEASE

4.1 Gene therapy

Gene therapy is a complex area, and its role in AATD has been reviewed comprehensively elsewhere(93), so this section will be limited to the main human trials to date, and future directions. In brief gene therapy aims to correct the underlying genetic abnormality in one or more cell types and through this ameliorate the effects of disease; by introducing normal DNA to cells, ultimately this leads to expression of the normal form of a protein. Typically this is done by use of a vector, which may be viral or liposomal, to carry the desired DNA sequence. AAT has a coding sequence small enough to be easily packaged within a viral vector, hence is an attractive disease in which to use this approach.

4.1.1 Intramuscular adeno-associated viral (AAV) vector studies

The first phase I study of gene therapy for AATD chose AAV2, used a range of doses and was encouraging in that it showed safety of the intervention, however there were rising anti-

AAV titres and insufficient AAT levels (94). Further human trials proceeded using AAV1, a range of vector doses, and a similar gene construct and route of administration to the previous AAV2 study, resulting in a sub-therapeutic but sustained AAT response, alongside an undesirable immune reaction (95). Further phase II study incorporated changes to the vector (a recombinant herpes simplex virus (HSV) complementation system, shown to result in greater vector yields(96)) alongside doses up to $6x10^{12}$vg/kg (97). A dose–response relationship occurred, and was sustained for a year, although levels were well below 11μM. Muscle biopsies demonstrated inflammation, but also a population of regulatory T-cells, present and activated in response to the AAV capsid, which may have aided long-term AAT expression(98). The highest dose was delivered via 100 intramuscular injections to avoid viral aggregation and precipitation at higher vector concentrations; since an even higher dose seemed to be needed if appropriate levels were to be achieved other routes of administration needed to be explored. In other diseases where dosing problems have been encountered with gene therapy isolation of the injection limb, immunosuppression and use of capsids with lesser propensity for immune response have been tested to overcome this problem(93). A non-human primate model has compared peripheral venous limb perfusion (VLP) and an intra-arterial push and dwell (IAPD) approach for the AAV1 vector used in AATD gene therapy to date and shows promising results (99), implying that this form of gene therapy could yet progress to further human studies.

4.1.2 Other routes of administration

A human phase I/II study of an intrapleural route for gene therapy in AATD apparently started in late 2017, but the public record indicates it is not yet recruiting (NCT02168686). It will use a serotype of AAV with lower tendency for immune reaction, based on promising results from animal work(100), however the practicality of dosing in this way in patients has yet to be established. Use of indwelling pleural catheters and avoidance of immune response after repeated doses may be important(93).

Inhaled gene therapy reduced markers of chronic airway inflammation in a phase I trial (101). However, this route of therapy has not progressed perhaps because other routes have shown better AAT induction and therefore therapeutic possibility. In general non-viral gene transfer agents, such as those used by this phase I study, are less efficient than viral vectors and deposition problems may also occur(93).

4.1.3 Stem cells

Several groups have used stem cells as vehicles for gene therapy in animal work pertinent to AATD. Lentivirally transduced stem cells have been used for AAT gene transfer in mice, achieving sustained human AAT expression(102). Lentiviral and rAAV vectors have also delivered promising results when used to transduce bone marrow and adipose derived stem cells before transplantation into mice (103, 104).Differentiated mesenchymal stem cells (MSCs) have also been used to produce hepatocyte-like cells suitable for genetic alteration and then autologous transplantation(105).

Induced pluripotent stem cells (IPSCs) have potential to generate unlimited cells for autologous cell-based treatments for many diseases. Yusa *et al* used this approach to correct the PiZZ mutation in IPSCs from AATD patients, which were then differentiated into functional hepatocyte like cells, and transplanted successfully into a murine model of liver injury (106). This approach has a risk of other mutations arising during prolonged IPSC culture, although it could provide sustained AAT gene expression. Careful screening would therefore be essential for safe use in clinical practice.

4.2 Small molecules

There are a number of molecular approaches which have potential in AATD, largely based on the concept that the problem in the common forms of AATD are polymerization; if this process could be blocked then presumably mutant AAT would not accumulate in the liver – hence lessening the chance of liver disease – and would enter the circulation, thus reducing the impact of deficiency in the lung. This would not be a perfect solution, since Z-AAT has a reduced NE binding capacity, so some functional deficiency would be present, nevertheless it is probably the best current prospect to treat all disease manifestations with one product. Such a product might be non-specific, or specifically designed to suit the molecular alteration known to occur with each mutant form of the protein. The latter approach has been successful in cystic fibrosis(107), and is being actively pursued by several companies. We will now describe some of the progress in these two subtypes of molecular treatment.

4.2.1 Non-specific approaches

Chemical chaperones are a group of molecules that guide folding and may reverse cellular mis-localization of proteins. Several generic chaperones have had an effect in pre-clinical studies relevant to AATD; 4-phenylbutyric acid (PBA) mediated an increase in secretion of functionally active Z-AAT in cell culture and murine models(108) and trimethylamine N-oxide (TMAO) stabilized both native M-AAT and Z-AAT against heat induced polymerization, but did not aid refolding of denatured AAT (109), nor did they augment secretion (108). PBA went on to be used in two small human studies in AATD patients. An open label study in 10 patients over 2 weeks resulted in no change in AAT level, and was poorly tolerated, with multiple side effects being seen(110). Another Phase I study in PiZZ AATD enrolled a total of 11 patients and mean serum AAT levels increased from baseline in the first 5 subjects after receiving 5, 10, 20 g/day of 4-PBA, suggesting a biological effect. However, short-term use of up to 40 g/day did not result in a meaningful increase in AAT levels and again significant adverse events were reported(111).

There is a complex array of chaperones that influence the secretion of Z-AAT in vitro(112) and alterations in intracellular degradation of Z-AAT occur after administration of drugs that interact with them (112), suggesting further potential routes for drug development, despite the failure of PBA.

4.2.2 Specific molecular approaches

Precise inhibition of polymerization of Z-AAT can be achieved by annealing peptides to the reactive loop of β sheet A. Initial studies of molecules 11-13 residues in length found that they were not specific for Z-AAT, hence could not be used for drug design (18). Smaller peptides are both specific and effective at blocking polymerization (113-115). However dissociation from AAT is required in the circulation, otherwise the bound molecule prevents anti-NE activity, hence the end result whilst helpful to the liver, would not afford protection to the lung. Parfrey *et al* demonstrated that some peptides dissociate from Z-AAT under physiological conditions, and the resultant AAT has anti-protease activity (115), but these products have not developed to clinical studies in animals, let alone trials in man. Further development of small peptides targeting a lateral hydrophobic cavity in AAT has been carried out(116), but the strategies have been limited due to lack of maintained secretion of AAT, of failure to adequately block/reverse polymerization. Targeting a different area of the AAT protein with peptides in hepatocyte models resulted in a reduction in intracellular aggregation of Z-AAT, alongside secretion of active AAT (117). Furthermore apoptosis and cellular proliferation were unaffected, which is a prerequisite for clinical trials. This research group, headed by Professor Lomas, have taken their molecular approach to industry and it has been progressing through the development process for 7 years (118, 119).

Ordonez and colleagues generated a monoclonal antibody (4B12) which blocked alpha-1 antitrypsin polymerization *in vitro* and this was found to cause a small increase of the stoichiometry of inhibition for neutrophil elastase (113). A single-chain variable fragment (scFv) intrabody was generated and expression of scFv4B12 within the endoplasmic reticulum reduced intracellular polymerization of Z alpha-1 antitrypsin by 60%. It also increased the secretion of Z alpha-1 antitrypsin that retained inhibitory activity against NE. Ideal therapy should prevent polymer formation while preserving inhibitory activity which the authors report this approach seems to support.

The biopharmaceutical company Vertex Pharmaceuticals is investigating the use of small molecule correctors to potentially treat AATD. They have developed a family of protein correctors that address the underlying cause of AATD by facilitating proper folding of the AAT protein (114). The corrector therapy is designed to restore the body's ability to produce its own AAT, increasing production as needed and has the potential to treat both lung and liver manifestations of AATD. It has received Fast Track designation from FDA but there is limited data in the public domain. Notably the amount of AAT produced in the liver is far in excess of the amount of CFTR (cystic fibrosis transmembrane regulator) produced, thus whether it will be possible for any specific molecular approach to fully correct AAT levels remains unclear, despite the success of the approach in cystic fibrosis.

5 Conclusion

The interventions closest to, or established in, clinical practice for AATD target single organs only, and in the next 5 years it is likely that changes in dose or co-administration of products for lung and liver will be the method of managing symptomatic disease. However the movement toward products capable of targeting lung and liver, and with potential to act as preventative treatments against emphysema and cirrhosis if used early enough is exciting. Of the approaches targeting both organs small molecules are probably those with the greatest

potential, given the fact that gene therapy and stem cells have many hurdles to overcome, and have not become established in other diseases to date, even where research into these routes has been ongoing for more than 30 years (eg cystic fibrosis). Notably molecular approaches have been successful in cystic fibrosis, albeit with questions around cost-effectiveness and patient selection for most health economies.

6 EXPERT OPINION

6.1 Strengths and limitations of current research

The strength of current research programmes in AATD is that they cover all major aspects of disease, and are potentially complementary, in that lung and liver directed routes could be combined. Dialogue is open between investigational product makes in the liver space and those that make augmentation, which is encouraging as it shows that the need for collaboration is recognized. Ongoing work outside augmentation is also a strength, as it is now recognized that replacing AAT in the way we currently do worldwide is not a panacea – indeed there is significant unmet need even for those on intravenous augmentation, many of whom continue to deteriorate from a respiratory perspective. Progress in molecular research, based on our understanding of principles of disease has great potential, and with this and other avenues all reaching clinical phases it is certainly an exciting time in patient facing research in AATD.

The limitations of research in the field stem mainly from the fact that AATD is a rare disease hence recruitment to trials is difficult, particularly when there is so much research in the area, and at least one treatment is available already (augmentation); this means there is competition to get patients into trials, and for many studies stopping augmentation is necessary, which risks deterioration in lung disease if the study is long in duration, and can cause patient anxiety as a result. In addition, gathering data on surrogate outcomes (which are often the only feasible

metric on which to power a study in rare disease) that can be adequately proven to relate to metrics healthcare payers are interested in is challenging. Finally, many healthcare systems ask for cost-effectiveness to be proven, and this may be difficult when data on long term outcomes such as mortality is hard to collect.

6.2 What is the current direction of travel in AATD trials?

AATD is moving toward multimodality treatment initially, and ultimately toward single agents capable of addressing all features of disease via small molecules or gene therapy. For established therapy, namely augmentation, the key question is patient selection and precision medicine. It is recognized that patients continue to decline on therapy, and even within RCTs there has been heterogeneity of response, implying that combination approaches are probably required. With this in mind the fact that research continues into other agents which can target proteolytic imbalance and understanding the inflammatory pathway in AATD is important. Pharmacogenomics is in its infancy and this will be an interesting field to pursue, as well as network biology as a means of identifying patients where one pathogenic mechanism predominates, and thus is a better option to target.

6.3 What is the ultimate goal in AATD and what challenges lie in our way?

The goal in AATD is a cure, by which we mean a treatment which can prevent the onset of the disabling diseases which the condition causes; predominantly this is emphysema, with a smaller proportion of patients having liver fibrosis or cirrhosis. In order to achieve this we not only need drugs which will work to manage both lung and liver disease, but also need to identify patients early. Early identification would allow us to intervene with potentially curative treatments for all organs (eg small molecules) prior to the onset of emphysema or fibrosis, thus

preventing the morbidity which comes with these complications. At present population screening for AATD does not occur; in some countries arguably it does not meet the criteria to screen since they do not have a therapy which could be used early in the disease, as augmentation is not funded, and even in those areas where it is funded its efficacy prior to the onset of emphysema is unknown. Since symptoms of disease often occur late in the disease process case finding is not a viable approach for early detection either. If curative treatments do become available early engagement with policy makers around screening/detection programmes will therefore be important. Finally, appropriate models for assessing cost-effectiveness of treatment, and robust supply chains will be vital – today's treatments are plasma derived and therefore potentially limited in supply. Nevertheless, despite the challenges there is great promise in the programme of research, and our AATD patients can indeed have hope for a cure.

Funding

This paper was not funded.

Declaration of interest

A Turner has received funding (current or last 3 years) from ATS Foundation, Alpha 1 Foundation, AstraZeneca, Chiesi, CSL Behring and NIHR. Grifols Biotherapeutics and has received personal fees and/or support to attend conferences from Boehringer Ingelheim, CSL Behring, Chiesi, AstraZeneca, Glaxo Smith Kline, Vertex, and pH pharma. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers

1. Eriksson S. Studies in alpha 1 antitrypsin deficiency. Acta MedScand. 1965;177 (Suppl.):432.
2. Parr DG, Guest PG, Reynolds JH, et al. Prevalence and impact of bronchiectasis in alpha1-antitrypsin deficiency. Am J Respir Crit Care Med. 2007;176(12):1215-21. Epub 2007/09/18.
3. Townsend SA, Edgar RG, Ellis PR, et al. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. Aliment Pharmacol Ther. 2018;47(7):877-85. Epub 2018/02/16.
4. Morris H, Morgan MD, Wood AM, et al. ANCA-associated vasculitis is linked to carriage of the Z allele of alpha(1) antitrypsin and its polymers. Ann Rheum Dis. 2011;70(10):1851-6. Epub 2011/08/09.
5. Stone H, Pye A, Stockley RA. Disease associations in alpha-1-antitrypsin deficiency. Respir Med. 2014;108(2):338-43. Epub 2013/11/02.
6. Wood AM, Harrison RM, Semple S, et al. Outdoor air pollution is associated with disease severity in alpha1-antitrypsin deficiency. Eur Respir J. 2009;34(2):346-53. Epub 2009/03/28.
7. Kim WJ, Wood AM, Barker AF, et al. Association of IREB2 and CHRNA3 polymorphisms with airflow obstruction in severe alpha-1 antitrypsin deficiency. Respiratory research. 2012;13:16. Epub 2012/02/24.
8. Stockley RA, Turner AM. alpha-1-Antitrypsin deficiency: clinical variability, assessment, and treatment. Trends in molecular medicine. 2014;20(2):105-15. Epub 2014/01/02.
9. Carrell RW, Jeppsson JO, Laurell CB, et al. Structure and variation of human alpha 1-antitrypsin. Nature. 1982;298(5872):329-34.
10. Sloan B, Abrams WR, Meranze DR, et al. Emphysema induced in vitro and in vivo in dogs by a purified elastase from homologous leukocytes. Am Rev Respir Dis. 1981;124(3):295-301.
11. Senior RM, Tegner H, Kuhn C, et al. The induction of pulmonary emphysema with human leukocyte elastase. Am Rev Respir Dis. 1977;116(3):469-75.
12. Snider GL, Lucey EC, Christensen TG, et al. Emphysema and bronchial secretory cell metaplasia induced in hamsters by human neutrophil products. Am Rev Respir Dis. 1984;129(1):155-60.
13. Stockley RA. Neutrophils and protease/antiprotease imbalance. American journal of respiratory and critical care medicine. 1999;160(5 Pt 2):S49-52. Epub 1999/11/11.
14. Turino GM, Barker AF, Brantly ML, et al. Clinical features of individuals with PI*SZ phenotype of alpha 1-antitrypsin deficiency. alpha 1-Antitrypsin Deficiency Registry Study Group. Am J Respir Crit Care Med. 1996;154(6 Pt 1):1718-25.
15. Miravitlles M, Dirksen A, Ferrarotti I, et al. European Respiratory Society statement: diagnosis and treatment of pulmonary disease in alpha1-antitrypsin deficiency. Eur Respir J. 2017;50(5). Epub 2017/12/02.
16. Marciniuk DD, Hernandez P, Balter M, et al. Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. Canadian respiratory journal : journal of the Canadian Thoracic Society. 2012;19(2):109-16. Epub 2012/04/27.
17. Sandhaus RA, Turino G, Brantly ML, et al. The Diagnosis and Management of Alpha-1 Antitrypsin Deficiency in the Adult. Chronic Obstr Pulm Dis. 2016;3(3):668-82. Epub 2016/06/06.
18. Lomas DA, Evans DL, Finch JT, et al. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature. 1992;357(6379):605-7. Epub 1992/06/18.
19. Salahuddin P. Genetic variants of alpha1-antitrypsin. Current protein & peptide science. 2010;11(2):101-17. Epub 2009/09/16.
20. de Serres FJ, Blanco I. Prevalence of alpha1-antitrypsin deficiency alleles PI*S and PI*Z worldwide and effective screening for each of the five phenotypic classes PI*MS, PI*MZ, PI*SS,

PI*SZ, and PI*ZZ: a comprehensive review. Ther Adv Respir Dis. 2012;6(5):277-95. Epub 2012/08/31.
21. Green CE, Vayalapra S, Hampson JA, et al. PiSZ alpha-1 antitrypsin deficiency (AATD): pulmonary phenotype and prognosis relative to PiZZ AATD and PiMM COPD. Thorax. 2015;70(10):939-45. Epub 2015/07/05.
22. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. The New England journal of medicine. 1986;314(12):736-9. Epub 1986/03/20.
23. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. The New England journal of medicine. 1976;294(24):1316-21. Epub 1976/06/10.
24. Parmar JS, Mahadeva R, Reed BJ, et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. Am J Respir Cell Mol Biol. 2002;26(6):723-30. Epub 2002/05/30.
25. Petrache I, Fijalkowska I, Medler TR, et al. alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. The American journal of pathology. 2006;169(4):1155-66. Epub 2006/09/28.
26. ISRCTN registry [database on the Internet]. BioMed Central. 2019. Available from: https://www.isrctn.com/.
27. Clinicaltrials.gov [database on the Internet]. US National Library of Medicine. 2019. Available from: https://clinicaltrials.gov/.
28. Gadek JE, Klein HG, Holland PV, et al. Replacement therapy of alpha 1-antitrypsin deficiency. Reversal of protease-antiprotease imbalance within the alveolar structures of PiZ subjects. The Journal of clinical investigation. 1981;68(5):1158-65. Epub 1981/11/01.
29. Wewers MD, Casolaro MA, Sellers SE, et al. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. The New England journal of medicine. 1987;316(17):1055-62. Epub 1987/04/23.
30. Dirksen A, Dijkman JH, Madsen F, et al. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. American journal of respiratory and critical care medicine. 1999;160(5 Pt 1):1468-72. Epub 1999/11/11.
31. Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group. American journal of respiratory and critical care medicine. 1998;158(1):49-59. Epub 1998/07/09.
*32. Barros-Tizon JC, Torres ML, Blanco I, et al. Reduction of severe exacerbations and hospitalization-derived costs in alpha-1-antitrypsin-deficient patients treated with alpha-1-antitrypsin augmentation therapy. Therapeutic advances in respiratory disease. 2012;6(2):67-78. Epub 2012/02/23.
This manuscipt described a reduction in the incidence and severity of exacerbations in AAT-deficient patients treated with augmentation therapy
**33. Chapman KR, Burdon JG, Piitulainen E, et al. Intravenous augmentation treatment and lung density in severe alpha1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;386:360-8. Epub 2015/06/01.
This trial demonstrated the positive benefit of weekly infusions of augmentation therapy on the progression of emphysema
34. Ma S, Lin YY, Cantor JO, et al. The Effect of Alpha-1 Proteinase Inhibitor on Biomarkers of Elastin Degradation in Alpha-1 Antitrypsin Deficiency: An Analysis of the RAPID/RAPID Extension Trials. Chronic Obstr Pulm Dis. 2016;4(1):34-44. Epub 2017/08/30.
**35. Edgar RG, Patel M, Bayliss S, et al. Treatment of lung disease in alpha-1 antitrypsin deficiency: a systematic review. Int J Chron Obstruct Pulmon Dis. 2017;12:1295-308. Epub 2017/05/13.
This review described the effects of augmentation on progression of emphysema as measured by quantitative CT scanning of the lung

36. Crossley D, Renton M, Khan M, et al. CT densitometry in emphysema: a systematic review of its clinical utility. Int J Chron Obstruct Pulmon Dis. 2018;13:547-63. Epub 2018/02/16.
37. Green CE, Parr DG, Edgar RG, et al. Lung density associates with survival in alpha 1 antitrypsin deficient patients. Respir Med. 2016;112:81-7. Epub 2016/02/15.
38. Piitulainen E, Bernspang E, Bjorkman S, et al. Tailored pharmacokinetic dosing allows self-administration and reduces the cost of IV augmentation therapy with human alpha(1)-antitrypsin. European journal of clinical pharmacology. 2003;59(2):151-6. Epub 2003/05/03.
39. Zamora NP, Pla RV, Del Rio PG, et al. Intravenous human plasma-derived augmentation therapy in alpha 1-antitrypsin deficiency: from pharmacokinetic analysis to individualizing therapy. The Annals of pharmacotherapy. 2008;42(5):640-6. Epub 2008/04/17.
40. Stocks JM, Brantly ML, Wang-Smith L, et al. Pharmacokinetic comparability of Prolastin(R)-C to Prolastin(R) in alpha(1)-antitrypsin deficiency: a randomized study. BMC clinical pharmacology. 2010;10:13. Epub 2010/10/06.
41. Soy D, de la Roza C, Lara B, et al. Alpha-1-antitrypsin deficiency: optimal therapeutic regimen based on population pharmacokinetics. Thorax. 2006;61(12):1059-64. Epub 2006/08/25.
42. Sorrells S, Camprubi S, Griffin R, et al. SPARTA clinical trial design: exploring the efficacy and safety of two dose regimens of alpha1-proteinase inhibitor augmentation therapy in alpha1-antitrypsin deficiency. Respir Med. 2015;109(4):490-9. Epub 2015/03/03.
43. Zhu W, Li L, Deng M, et al. Oxidation-resistant and thermostable forms of alpha-1 antitrypsin from Escherichia coli inclusion bodies. FEBS open bio. 2018;8(10):1711-21. Epub 2018/10/20.
44. Bischoff R, Speck D, Lepage P, et al. Purification and biochemical characterization of recombinant alpha 1-antitrypsin variants expressed in Escherichia coli. Biochemistry. 1991;30(14):3464-72.
45. Bottomley SP, Stone SR. Protein engineering of chimeric Serpins: an investigation into effects of the serpin scaffold and reactive centre loop length. Protein Eng. 1998;11(12):1243-7.
46. Courtney M, Buchwalder A, Tessier LH, et al. High-level production of biologically active human alpha 1-antitrypsin in Escherichia coli. Proc Natl Acad Sci U S A. 1984;81(3):669-73.
47. Karnaukhova E, Ophir Y, Golding B, Shrake A, editors. Recombinant human alpha 1 proteinase inhibitor: glycosylation, stability and biological activity. 10th FDA Science Forum: the critical path from concept to consumer; 2004; Washington DC.
48. Krishnan B, Hedstrom L, Hebert DN, et al. Expression and Purification of Active Recombinant Human Alpha-1 Antitrypsin (AAT) from Escherichia coli. Methods Mol Biol. 2017;1639:195-209. Epub 2017/07/29.
49. Casolaro MA, Fells G, Wewers M, et al. Augmentation of lung antineutrophil elastase capacity with recombinant human alpha-1-antitrypsin. J Appl Physiol. 1987;63(5):2015-23.
50. Kwon KS, Song M, Yu MH. Purification and characterization of alpha 1-antitrypsin secreted by recombinant yeast Saccharomyces diastaticus. J Biotechnol. 1995;42(3):191-5.
51. Tamer IM, Chisti Y. Production and recovery of recombinant protease inhibitor alpha 1 antitrypsin. Enzyme Microb Technol. 2001;29:611-20.
52. Sandoval C, Curtis H, Congote LF. Enhanced proliferative effects of a baculovirus-produced fusion protein of insulin-like growth factor and alpha(1)-proteinase inhibitor and improved anti-elastase activity of the inhibitor with glutamate at position 351. Protein Eng. 2002;15(5):413-8.
53. Terashima M, Ejiri Y, Hashikawa N, et al. Effects of sugar concentration on recombinant human alpha(1)-antitrypsin production by genetically engineered rice cell. Biochem Eng J. 2000;6(3):201-5.
54. Huang J, Sutliff TD, Wu L, et al. Expression and purification of functional human alpha-1-Antitrypsin from cultured plant cells. Biotechnol Prog. 2001;17(1):126-33.
55. Zhang N, Wright T, Caraway P, et al. Enhanced Secretion of Human alpha1-Antitrypsin Expressed with a Novel Glycosylation Module in Tobacco BY-2 Cell Culture. Bioengineered. 2019. Epub 2019/04/09.

56. Silberstein DZ, Karuppanan K, Aung HH, et al. An oxidation-resistant, recombinant alpha-1 antitrypsin produced in Nicotiana benthamiana. Free Radic Biol Med. 2018;120:303-10. Epub 2018/03/20.
57. Huang TK, Falk BW, Dandekar AM, et al. Enhancement of Recombinant Protein Production in Transgenic Nicotiana benthamiana Plant Cell Suspension Cultures with Co-Cultivation of Agrobacterium Containing Silencing Suppressors. International journal of molecular sciences. 2018;19(6). Epub 2018/06/09.
58. Morifuji Y, Xu J, Karasaki N, et al. Expression, Purification, and Characterization of Recombinant Human alpha1-Antitrypsin Produced Using Silkworm-Baculovirus Expression System. Molecular biotechnology. 2018;60(12):924-34. Epub 2018/10/12.
59. Massoud M, Bischoff R, Dalemans W, et al. Expression of active recombinant human alpha 1-antitrypsin in transgenic rabbits. J Biotechnol. 1991;18(3):193-203.
60. Moens A, Betteridge KJ, Brunet A, et al. Low levels of chimerism in rabbit fetuses produced from preimplantation embryos microinjected with fetal gonadal cells. Molecular reproduction and development. 1996;43(1):38-46. Epub 1996/01/01.
61. Archibald AL, McClenaghan M, Hornsey V, et al. High-level expression of biologically active human alpha 1-antitrypsin in the milk of transgenic mice. Proc Natl Acad Sci U S A. 1990;87(13):5178-82.
62. Bischoff R, Degryse E, Perraud F, et al. A 17.6 kbp region located upstream of the rabbit WAP gene directs high level expression of a functional human protein variant in transgenic mouse milk. FEBS letters. 1992;305(3):265-8. Epub 1992/07/06.
63. Zbikowska HM, Soukhareva N, Behnam R, et al. Uromodulin promoter directs high-level expression of biologically active human alpha1-antitrypsin into mouse urine. Biochem J. 2002;365(Pt 1):7-11.
64. Carver AS, Dalrymple MA, Wright G, et al. Transgenic livestock as bioreactors: stable expression of human alpha-1-antitrypsin by a flock of sheep. Biotechnology (N Y). 1993;11(11):1263-70.
65. Wright G, Carver A, Cottom D, et al. High level expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. Biotechnology (N Y). 1991;9(9):830-4.
66. Spencer LT, Humphries JE, Brantly ML. Antibody response to aerosolized transgenic human alpha1-antitrypsin. N Engl J Med. 2005;352(19):2030-1.
67. Ross D, Brown T, Harper R, et al. Production and characterization of a novel human recombinant alpha-1-antitrypsin in PER.C6 cells. Journal of biotechnology. 2012;162(2-3):262-73. Epub 2012/10/06.
68. Wang Z, Hilder TL, Drift KV, et al. Structural Characterization of Recombinant Alpha-1-antitrypsin Expressed in a Human Cell Line. Analytical biochemistry. 2013. Epub 2013/03/07.
69. Jaberie H, Naghibalhossaini F. Recombinant production of native human alpha-1-antitrypsin protein in the liver HepG2 cells. Biotechnology letters. 2016;38(10):1683-90. Epub 2016/06/18.
70. Lee S, Lee Y, Hong K, et al. Effect of recombinant alpha1-antitrypsin Fc-fused (AAT-Fc)protein on the inhibition of inflammatory cytokine production and streptozotocin-induced diabetes. Mol Med. 2013;19:65-71. Epub 2013/04/05.
71. Toldo S, Mauro AG, Marchetti C, et al. Recombinant Human Alpha-1 Antitrypsin-Fc Fusion Protein Reduces Mouse Myocardial Inflammatory Injury After Ischemia-Reperfusion Independent of Elastase Inhibition. Journal of cardiovascular pharmacology. 2016;68(1):27-32. Epub 2016/03/06.
72. Joosten LA, Crisan TO, Azam T, et al. Alpha-1-anti-trypsin-Fc fusion protein ameliorates gouty arthritis by reducing release and extracellular processing of IL-1beta and by the induction of endogenous IL-1Ra. Ann Rheum Dis. 2016;75(6):1219-27. Epub 2015/07/16.
73. Sabbagh D, Barmayehvar B, Nguyen T, et al. Managing panniculitis in AATD: a systematic review of the evidence behind treatment. World Journal of Dermatology. 2018;7:1-8.
74. Hubbard RC, Sellers S, Czerski D, et al. Biochemical efficacy and safety of monthly augmentation therapy for alpha 1-antitrypsin deficiency. JAMA : the journal of the American Medical Association. 1988;260(9):1259-64. Epub 1988/09/02.

75. Hubbard RC, Crystal RG. Strategies for aerosol therapy of alpha 1-antitrypsin deficiency by the aerosol route. Lung. 1990;168 Suppl:565-78. Epub 1990/01/01.
76. Kropp J, Wencker M, Hotze A, et al. Inhalation of [123I]alpha1-protease inhibitor: toward a new therapeutic concept of alpha1-protease inhibitor deficiency? Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2001;42(5):744-51. Epub 2001/05/05.
77. Brand P, Beckmann H, Maas Enriquez M, et al. Peripheral deposition of alpha1-protease inhibitor using commercial inhalation devices. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 2003;22(2):263-7. Epub 2003/09/04.
78. Kamada announces FDA acceptance of Inhaled AAT program path forward. 2019 [8th August 2019]; Available from: https://www.kamada.com/news/kamada-announces-fda-acceptance-of-inhaled-aat-program-path-forward/.
79. Cantor JO, Shteyngart B, Cerreta JM, et al. The effect of hyaluronan on elastic fiber injury in vitro and elastase-induced airspace enlargement in vivo. Proc Soc Exp Biol Med. 2000;225(1):65-71. Epub 2000/09/21.
80. Cantor JO, Cerreta JM, Ochoa M, et al. Aerosolized hyaluronan limits airspace enlargement in a mouse model of cigarette smoke-induced pulmonary emphysema. Exp Lung Res. 2005;31(4):417-30. Epub 2005/07/20.
81. Cantor J, Ma S, Turino G. A pilot clinical trial to determine the safety and efficacy of aerosolized hyaluronan as a treatment for COPD. Int J Chron Obstruct Pulmon Dis. 2017;12:2747-52. Epub 2017/10/28.
82. Cantor J, Armand G, Turino G. Lung hyaluronan levels are decreased in alpha-1 antiprotease deficiency COPD. Respir Med. 2015;109(5):656-9. Epub 2015/04/12.
83. Guo S, Booten SL, Aghajan M, et al. Antisense oligonucleotide treatment ameliorates alpha-1 antitrypsin-related liver disease in mice. The Journal of clinical investigation. 2014;124(1):251-61. Epub 2013/12/21.
*84. Turner AM, Stolk J, Bals R, et al. Hepatic-targeted RNA interference provides robust and persistent knockdown of alpha-1 antitrypsin levels in ZZ patients. J Hepatol. 2018;69(2):378-84. Epub 2018/03/25.
This study reported the safety of a new short interferring RNA molecule with the potential to reduce AATd polymer fromation in the liver
85. Arrowhead Pharmaceuticals Receives FDA Clearance to Begin Phase 2/3 Study of ARO-AAT for Treatment of Alpha-1 Liver Disease. Arrowhead Pharmaceuticals; 2019; Available from: http://ir.arrowheadpharma.com/news-releases/news-release-details/arrowhead-pharmaceuticals-receives-fda-clearance-begin-phase-23.
86. Schwabe C, Hamilton J, Christianson D, et al. A phase I single and multiple dose escalating study to evaluate the safety, tolerability, pharmacokinetics and effect of ARO-AAT on serum AAT levels in normal adult volunteers. Hepatology. 2018;68(S1):LB-9.
87. Fregonese L, Stolk J, Frants RR, et al. Alpha-1 antitrypsin null mutations and severity of emphysema. Respir Med. 2008;102(6):876-84. Epub 2008/03/21.
88. Mizumura K, Cloonan SM, Haspel JA, et al. The emerging importance of autophagy in pulmonary diseases. Chest. 2012;142(5):1289-99. Epub 2012/11/08.
89. Hidvegi T, Ewing M, Hale P, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science. 2010;329(5988):229-32. Epub 2010/06/05.
90. Lykavieris P, Ducot B, Lachaux A, et al. Liver disease associated with ZZ alpha1-antitrypsin deficiency and ursodeoxycholic acid therapy in children. J Pediatr Gastroenterol Nutr. 2008;47(5):623-9. Epub 2008/10/29.
91. Kaushal S, Annamali M, Blomenkamp K, et al. Rapamycin reduces intrahepatic alpha-1-antitrypsin mutant Z protein polymers and liver injury in a mouse model. Exp Biol Med (Maywood). 2010;235(6):700-9. Epub 2010/06/01.

92. Gosai SJ, Kwak JH, Luke CJ, et al. Automated high-content live animal drug screening using C. elegans expressing the aggregation prone serpin alpha1-antitrypsin Z. PLoS One. 2010;5(11):e15460. Epub 2010/11/26.
*93. Turner AM. Gene Therapy. In: Kalsheker N, Stockley RA, editors. Alpha 1 Antitrypsin deficiency: biology, diagnosis, clinical significance and emerging therapies: Elsevier; 2017. p. 262. **This was a comprehensive review of gene therapy to treat all aspects of AATD.**
94. Brantly ML, Spencer LT, Humphries M, et al. Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 alphal-antitrypsin (AAT) vector in AAT-deficient adults. Human gene therapy. 2006;17(12):1177-86. Epub 2006/11/23.
95. Brantly ML, Chulay JD, Wang L, et al. Sustained transgene expression despite T lymphocyte responses in a clinical trial of rAAV1-AAT gene therapy. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(38):16363-8. Epub 2009/08/27.
96. Kang W, Wang L, Harrell H, et al. An efficient rHSV-based complementation system for the production of multiple rAAV vector serotypes. Gene therapy. 2009;16(2):229-39. Epub 2008/10/17.
97. Flotte TR, Trapnell BC, Humphries M, et al. Phase 2 clinical trial of a recombinant adeno-associated viral vector expressing alpha1-antitrypsin: interim results. Human gene therapy. 2011;22(10):1239-47. Epub 2011/05/26.
98. Mueller C, Chulay JD, Trapnell BC, et al. Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. J Clin Invest. 2013;123(12):5310-8. Epub 2013/11/16.
99. Gruntman AM, Gernoux G, Tang Q, et al. Bridging from Intramuscular to Limb Perfusion Delivery of rAAV: Optimization in a Non-human Primate Study. Molecular therapy Methods & clinical development. 2019;13:233-42. Epub 2019/03/05.
100. De BP, Heguy A, Hackett NR, et al. High levels of persistent expression of alpha1-antitrypsin mediated by the nonhuman primate serotype rh.10 adeno-associated virus despite preexisting immunity to common human adeno-associated viruses. Molecular therapy : the journal of the American Society of Gene Therapy. 2006;13(1):67-76. Epub 2005/11/02.
101. Brigham KL, Lane KB, Meyrick B, et al. Transfection of nasal mucosa with a normal alpha1-antitrypsin gene in alpha1-antitrypsin-deficient subjects: comparison with protein therapy. Hum Gene Ther. 2000;11(7):1023-32. Epub 2000/05/16.
102. Wilson AA, Kwok LW, Hovav AH, et al. Sustained expression of alpha1-antitrypsin after transplantation of manipulated hematopoietic stem cells. Am J Respir Cell Mol Biol. 2008;39(2):133-41. Epub 2008/03/08.
103. Li H, Zhang B, Lu Y, et al. Adipose tissue-derived mesenchymal stem cell-based liver gene delivery. Journal of hepatology. 2011;54(5):930-8. Epub 2010/12/21.
104. Li H, Lu Y, Witek RP, et al. Ex vivo transduction and transplantation of bone marrow cells for liver gene delivery of alpha1-antitrypsin. Molecular therapy : the journal of the American Society of Gene Therapy. 2010;18(8):1553-8. Epub 2010/06/17.
105. Ghaedi M, Lotfi AS, Soleimani M. Establishment of lentiviral-vector-mediated model of human alpha-1 antitrypsin delivery into hepatocyte-like cells differentiated from mesenchymal stem cells. Tissue & cell. 2010;42(3):181-9. Epub 2010/05/04.
*106. Yusa K, Rashid ST, Strick-Marchand H, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011;478(7369):391-4. Epub 2011/10/14.
This manuscriptdescribed the correction of PiZZ mutation in induced pluripotent stem calles in AATD patients
107. Gentzsch M, Mall MA. Ion Channel Modulators in Cystic Fibrosis. Chest. 2018;154(2):383-93. Epub 2018/05/12.
108. Burrows JA, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proc Natl Acad Sci U S A. 2000;97(4):1796-801.

109. Devlin GL, Parfrey H, Tew DJ, et al. Prevention of polymerization of M and Z alpha1-Antitrypsin (alpha1-AT) with trimethylamine N-oxide. Implications for the treatment of alpha1-at deficiency. Am J Respir Cell Mol Biol. 2001;24(6):727-32.
110. Teckman JH. Lack of effect of oral 4-phenylbutyrate on serum alpha-1-antitrypsin in patients with alpha-1-antitrypsin deficiency: a preliminary study. J Pediatr Gastroenterol Nutr. 2004;39(1):34-7. Epub 2004/06/10.
111. Gonzalez-Peralta RP, Leonard S, Harvey R, et al. 4-Phenyl butyrate mediated secretion rescue in patients with alpha 1-antitrypsin (AAT) deficiency: A pilot study. Hepatology. 2006;44(4):211A.
112. Schmidt BZ, Perlmutter DH. Grp78, Grp94, and Grp170 interact with alpha1-antitrypsin mutants that are retained in the endoplasmic reticulum. Am J Physiol Gastrointest Liver Physiol. 2005;289(3):G444-55.
113. Chang YP, Mahadeva R, Chang WS, et al. Identification of a 4-mer peptide inhibitor that effectively blocks the polymerization of pathogenic Z alpha1-antitrypsin. Am J Respir Cell Mol Biol. 2006;35(5):540-8. Epub 2006/06/17.
*114. Mahadeva R, Dafforn TR, Carrell RW, et al. 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization. Implications for the prevention of Z alpha(1)-antitrypsin-related cirrhosis. J Biol Chem. 2002;277(9):6771-4.
This manuscript described protein correctors that address the underlying cause of AATD by facilitating proper folding of the AAT protein
115. Parfrey H, Dafforn TR, Belorgey D, et al. Inhibiting polymerization: new therapeutic strategies for Z alpha1-antitrypsin-related emphysema. Am J Respir Cell Mol Biol. 2004;31(2):133-9.
116. Mallya M, Phillips RL, Saldanha SA, et al. Small molecules block the polymerization of Z alpha1-antitrypsin and increase the clearance of intracellular aggregates. Journal of medicinal chemistry. 2007;50(22):5357-63. Epub 2007/10/09.
117. Alam S, Wang J, Janciauskiene S, et al. Preventing and reversing the cellular consequences of Z alpha-1 antitrypsin accumulation by targeting s4A. Journal of hepatology. 2012;57(1):116-24. Epub 2012/03/20.
118. New collaboration to develop treatments for liver disease. University of Cambridge; 2012; Available from: https://www.cam.ac.uk/research/news/new-collaboration-to-develop-treatments-for-liver-disease.
119. University College London Collaboration Aims to Treat Alpha-1-Antitrypsin Deficiency. Glao Smith Kline; 2017; Available from: http://www.dpac.gsk.com/case-studies-universitycollegelondon.html.

Table 1: Active and unpublished clinical trials in alpha-1 antitrypsin deficiency

All listed studies are registered on clinicaltrials.gov or ISRCTN(26, 27).

Trial identifier	**Active/complete**	**Phase**	**Primary outcome**
NCT00377416	Active, not recruiting	1	Presence of rAAV2-CB-hAAT vector in blood and semen using recombinant adeno-associated virus vectors
NCT01379469	Recruiting	2	To determine the effect of Carbamazepine on hepatic AAT polymers
NCT01983241 SPARTA	Recruiting	3	Change from Baseline in Whole lung PD15 using 2 dose regimens of 60mg/kg and 120mg/kg alpha-1 proteinase inhibitor versus placebo
NCT02168686 ADVM-043	Active, not recruiting	1/2	Number and proportion of subjects experiencing adverse events using IV adeno-associated virus gene transfer vectors expressing human AAT
NCT02503683 ALN-AAT	Terminated	1	The safety of ALN-AAT evaluated by the proportion of subjects experiencing adverse events (AEs), serious adverse events (SAEs), and AEs leading to study drug discontinuation
NCT02525861	Recruiting	3	Number of adverse events (AEs) considered potentially related to the presence of particle load in the alpha-1 proteinase inhibitor solution
NCT02722304	Terminated	3	Rate of change in lung density for all ARALAST NP recipients versus placebo recipients; and all alpha-1 proteinase inhibitor recipients versus placebo recipients
NCT02870309	Completed	1/2	Safety of 60 mg/kg Alpha-1 MP assessed by Adverse events, ADRs, serious AEs (SAEs), discontinuations due to AEs or SAEs, and COPD exacerbations
NCT02870348	Enrolling by invitation	1/2	Safety of 60 mg/kg Alpha-1 MP as assessed by Adverse events, ADRs, serious AEs (SAEs), discontinuations

			due to AEs or SAEs, and COPD exacerbations using alpha-1 proteinase inhibitor
NCT03008915	Active, not recruiting	2	Pulmonary microvascular blood flow using aspirin versus placebo
NCT03114020	Recruiting	2	Measurement of sputum, plasma and urine concentrations of desmosine and isodesmosine using hyaluronic acid inhalation versus placebo
NCT03362242 ARO-AAT	Active, not recruiting	1	Number of Participants With Adverse Events (AEs) Possibly or Probably Related to Treatment
NCT03385395	Withdrawn	2	Non-inferiority of OctaAlpha1 compared to alpha-1 proteinase inhibitor in terms of the serum trough levels at steady state
NCT03636347 ASTRAEUS	Recruiting	2	Change from baseline on blood biomarkers of neutrophil elastase activity using oral Alvelestat versus placebo
NCT03679598 ATALANTa	Recruiting	2	To evaluate the effect of alvelestat (MPH996) administered twice daily for 12 weeks on blood markers of neutrophil elastase activity
NCT03767829 ALN-AAT02	Recruiting	1/2	Percentage of Participants with Treatment Emergent Adverse Events using small interfering RNA to reduce hepatic AAT
NCT03815396 INBRX-101	Not yet recruiting	1	Frequency and severity of adverse events using open-label dose-escalation of Fc fusion protein (rhAAT-F)

Table 2: Status of selected rAAT production systems

[#] Tobacco and tobacco-like plants included in one category in table

* Further trials abandoned

Host	Protein location	Yield	Stage of testing	Limitation	References
E.coli	Intracellular (IC)	≤38mg/l	Animals	AAT not glycosylated → aggregation + short ½ life	(44-48)
Yeasts	IC or secreted	≤1230mg/l	Animals	Hypermannosylation → immune responses	(49-51)
Insect cells	Secreted	Not reported	In vitro	Non-human glycans → immune responses	(52)
Transgenic rice	Secreted	4.6-5.7 mg/l	Animals	Immune response to rAAT	(53, 54)
Transgenic tobacco[#]	Secreted	≤34.7mg/l	In vitro	Proteolytic processing of rAAT	(55-57)
Silkworm baculovirus construct	Serum	15mg/10ml	In vitro	Immunogenicity unknown	(58)
Transgenic rabbits	Milk	4 g/l	In vitro Animal	Low levels of chimerism in implanted embryos	(59, 60)
Transgenic mice	Milk Urine	≤10mg/ml ≤ 65 mg/l	In vitro Animals	Immune response to rAAT	(61-63)
Transgenic sheep	Milk	≤35g/l	Phase I*	Immune response to rAAT	(64-66)
Human cells	Secreted	2.5g/l	In vitro	Industrial scalability	(67-69)

Figure 1: Mechanisms of disease and consequent routes to therapy in AATD

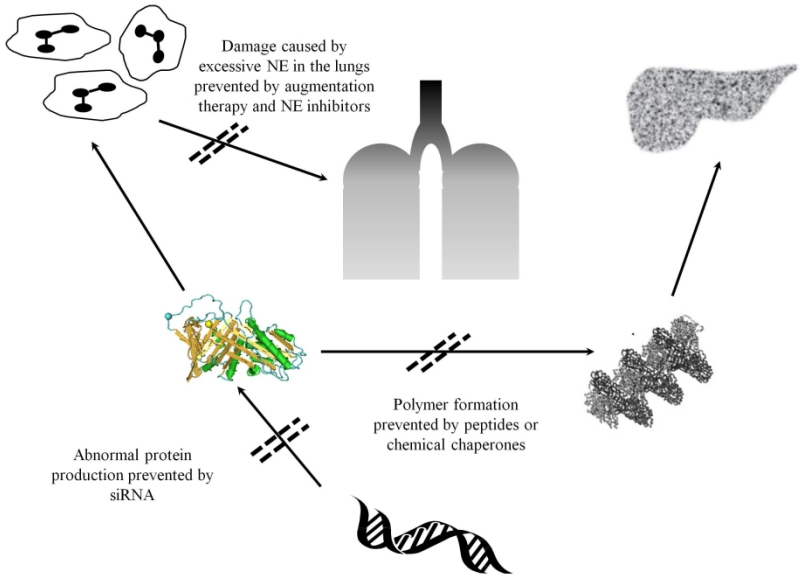

Figure 1: Mechanisms of disease and consequent routes to therapy in AATD

254x190mm (300 x 300 DPI)