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Review

Intrapleural Gene Therapy for Alpha-1 Antitrypsin Deficiency-Related Lung Disease

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Abstract

Alpha-1 antitrypsin deficiency (AATD) manifests primarily as early-onset emphysema caused by the destruction of the lung by neutrophil elastase due to low amounts of the serine protease inhibitor alpha-1 antitrypsin (AAT). The current therapy involves weekly intravenous infusions of AAT-derived from pooled human plasma that is efficacious, yet costly. Gene therapy applications designed to provide constant levels of the AAT protein are currently under development. The challenge is for gene therapy to provide sufficient amounts of AAT to normalize the inhibitor level and anti-neutrophil elastase capacity in the lung. One strategy involves administration of an adeno-associated virus (AAV) gene therapy vector to the pleural space providing both local and systemic production of AAT to reach consistent therapeutic levels. This review focuses on the strategy, advantages, challenges, and updates for intrapleural administration of gene therapy vectors for the treatment of AATD.

Abbreviations: alpha-1 antitrypsin deficiency, **AATD**; alpha-1 antitrypsin, **AAT**; adeno-associated virus, **AAV**; serine protease inhibitor, **SERPIN**; epithelial lining fluid, **ELF**; Food and Drug Administration, **FDA**; nonhuman primate, **NHP**; investigational new drug, **IND**

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Introduction

Alpha-1 antitrypsin deficiency (AATD) is an autosomal recessive disorder that affects 1/1500 to 1/5000 people of European ancestry.[1,2] In the United States, approximately 90,000 individuals are affected, with an additional 175,000 worldwide.[1,3,4] The disease manifests primarily in the lung, presenting as early-onset emphysema and a reduced lifespan.[5-11] Smoking accelerates the lung destruction process.[12-14] AATD can also present as other lung diseases including bronchiectasis and asthma, and a subset of individuals develop liver cirrhosis or rarely, hepatocellular carcinoma, panniculitis and vasculitic or autoimmune disorders.[4,15-23] The emphysema associated with AATD is caused by the slow destruction of the lung parenchyma by unregulated neutrophil elastase, which is released by dying or activated neutrophils.[5-7,24]

AAT regulates neutrophil elastase and other proteases, including proteinase 3, α-defensins, and cathepsin G, and has been shown to have anti-inflammatory properties and the ability to modulate immune responses.[25-34] AAT, a serine protease inhibitor (SERPIN), mainly acts in the lower respiratory tract to inhibit the action of these proteases. AAT deficiency results in an imbalance between the proteases and AAT in the lung leading to destruction of the lung matrix and damage to the alveolar structures.[5–7,24,35]

AAT is a 52 kDa protein that is produced and secreted mainly from the liver into the plasma. AAT reaches the lung primarily by diffusion from the circulation,[5–8,10,13,36–38] but a small amount is also produced locally by bronchial epithelial cells, mononuclear phagocytes, and neutrophils.[39-43] The normal range of AAT in the serum is 20 to 53 μM; levels >11 μM are required to protect the lung from destruction.[4,7,13,44,45] The low levels of AAT in AATD are caused by mutations in the SERPINA1 gene that has over 120 naturally occurring allelic variants.[2,4,46-49] The normal M alleles, consisting of M1(Ala213), M1(Val213), M2, M3, and M4, are present in more than 98% of the population.[50] The most prevalent deficient allele is the Z variant, which has a single amino acid substitution of lysine for glutamic acid at position 342 (E342K) that causes the polymerization of the AAT protein during post-translational processing preventing its secretion from hepatocytes.[1,2,8,51–54] Homozygous Z individuals have 10%-15% of the serum AAT levels of individuals with the normal M allele and account for >95% of cases of clinically diagnosed AATD.[2,5-7,13,16,38,55] The S allele, which has an amino acid substitution of valine for glutamic acid at position 264 (E264V), results in an AAT with reduced serum half-life due to instability of the protein.[56-59] Homozygous S individuals have ~50% of normal AAT serum levels and are not at risk, but 15% to 20% of SZ heterozygotes have serum levels <11 μM and are at risk for disease development.[60]

The current therapeutic strategy to protect individuals with AATD from the development and progression of emphysema is to supplement the levels of plasma AAT above the level needed to prevent destruction by proteases. The susceptibility of the lung to destruction in the absence of AAT results from the significant portion of neutrophils that reside in the pulmonary capillaries leaving the lung at high risk from unchecked neutrophil elastase destruction.[61]

AAT is produced mainly in liver hepatocytes and circulates systemically, but as the predominant serine protease inhibitor, its main function is to protect the fragile alveolar structures of the lung from neutrophil elastase. The level of AAT required for protection was determined to be 11 μM in the serum based on clinical observation of the development of emphysema in AATD patients.[62] AAT reaches the lung by diffusion from the circulation, and the level in the lung interstitium is ~50% of plasma levels. Further diffusion into the epithelial lining fluid (ELF) is limited by the tight junctions formed by the cells of the alveolar epithelium. The level of AAT in ELF is ~5% to 10% of plasma levels, and the level required for protection is 1.2 μM.[36,62] Thus, the rationale behind the current treatment strategy is to normalize levels of AAT in the ELF and interstitium by infusing AAT into the circulation and allowing it to diffuse to the other compartments, thereby normalizing the AAT levels. As long as the AAT in the plasma is above the protective threshold of 11 μM, the alveolar compartments should receive sufficient AAT to be protected. Based on this hypothesis, the current therapy consists of weekly intravenous infusions of 60 mg/kg of AAT purified from pooled human plasma to boost the level of circulating AAT. With this protein amount, AAT levels in serum are highly increased directly after the infusion but fall to near the protective threshold after one week due to the 4.5 day half-life of AAT.[62] This therapeutic strategy was approved by the Food and Drug Administration (FDA) on the basis of its biochemical efficacy of maintaining the protective level of the AAT in the serum and the corresponding normalization of the AAT level and anti-neutrophil elastase capacity in the lungs.[63] Clinical efficacy of this therapeutic strategy was demonstrated recently using computer tomography lung density scans at total lung capacity to validate the reduced rate of progression of lung destruction.[64,65]

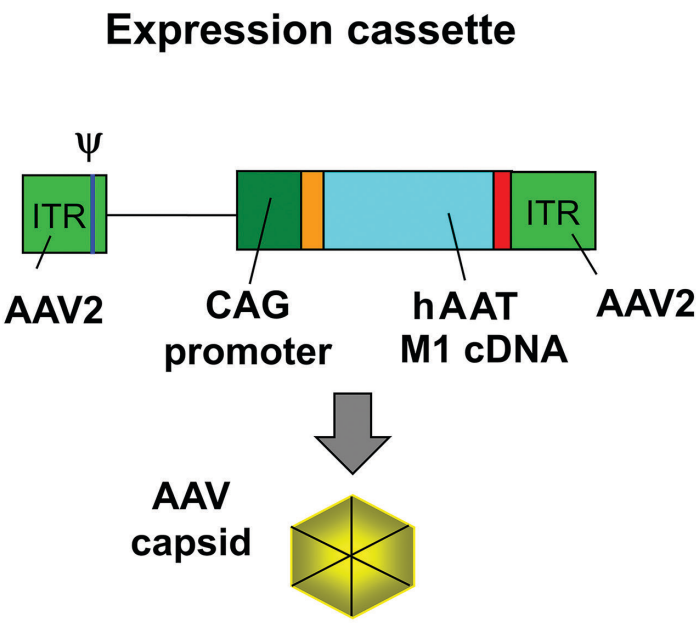
Gene Therapy for Alpha-1 Antitrypsin Deficiency

Although AAT protein augmentation is effective at reestablishing AAT levels in plasma and lung ELF and in slowing the progression of the destructive lung disease caused by AATD,[64,65] it is costly and requires weekly intravenous infusions of purified AAT from pooled human plasma. The necessary repetitive

intravenous therapy derived from a human product presents problems of patient compliance and risks of allergic reactions, viral contamination, or limitations in available supply.[66-68] Gene therapy to treat AATD has the possibility to alleviate all of these issues. The potential for a one-time administration reduces the burden on the patient of weekly time-consuming and invasive procedures and the associated issue of patient compliance. If effective, once gene therapy has been delivered, constant levels of AAT protein would be generated and released into the circulation, eliminating the current pattern of AAT peaks directly after infusion and AAT troughs by the end of the cycle. Thus, gene therapy offers a strategy that will persistently provide protective levels of AAT to the lung and has lower risk with fewer issues for patients and supply.

The current gene therapy strategies for AAT therapy involve delivering the normal human M allele coding sequence under the control of a highly active constitutive promoter using a gene transfer vector (Figure 1). The goal is for transduced cells to secrete sufficient amounts of AAT into the circulation to normalize the levels of protective AAT in the lungs by diffusion after a single administration. Although a number of gene transfer vectors have been tested for delivery of the normal M-type AAT coding sequence in the past 25 years (reviewed in Chiuchiolo and Crystal[61] and Sondhi et al[69]), the adeno-associated virus (AAV) vectors are the delivery vehicles of choice. AAV is a small parvovirus that does not cause disease in humans and causes little toxicity upon administration at doses $<10^{15}$ genome copies.[70,71] AAV vectors are highly effective at transducing a broad range of organs in vivo and provide persistent expression of the protein when delivered to non-proliferating cells.[70] There are 6 classically described human serotypes of AAV and greater than 50 recently identified serotypes from humans and nonhuman primates (NHPs).[72,73] Many AAV serotypes are available for use that have low prevalence in the general population and low or absent levels of pre-existing anti-vector immunity.[72,73] Serotypes AAV1, AAV2, AAV5, AAV6, AAV8, AAV9, and AAVrh.10 have all been used in preclinical studies of AAT therapy,[74-88] and clinical studies have been carried out using AAV1 and AAV2 vectors.[89-91]

Figure 1. Design of an Adeno-Associated Virus Vector



Shown is the expression cassette with the coding sequence for the AAT M1 cDNA driven by the highly active, constitutive CAG promoter, followed by an intron, (orange) the AAT cDNA and the polyA (red) signal flanked by AAV2 inverted terminal repeats (ITR). The genome is packaged in an AAV capsid.

AAV=adeno-associated virus; ITR=inverted terminal repeats; hAAT=human alpha-1 antitrypsin

The Case for Intrapleural Administration for Alpha-1 Antitrypsin Gene Therapy

Depending on the route of administration, the delivery of the AAT gene could allow AAT to be produced by many different cells and organs in addition to hepatocytes where the bulk of natural AAT is produced. This opens an array of possible routes of administration for a gene therapy vector, with the primary goal being to achieve the threshold levels of AAT protein of 11 μM in the serum and 1.2 μM in the alveolar ELF necessary to protect from neutrophil elastase proteolytic activity.[7,13,36,62] Several different routes of administration have been attempted for AAT gene therapy. The earliest attempts targeted the respiratory tract epithelium directly, but the delivered vectors were unable to generate therapeutic levels of AAT.[80,81,92,93] This failure is likely due to the natural defenses of the lung against pathogens and foreign substances, as well as the lack of viral receptors on the apical surface of respiratory epithelial cells.[92-96] Routes of delivery that target the liver directly, including intravenous and intraportal administration, have been assessed in preclinical studies but have not made the transition into humans.[76,85,87] Several studies have evaluated AAV vectors targeted to skeletal muscle in both preclinical animal studies and human clinical trials.[75,79,84,97] A clinical trial utilizing AAV2 yielded very low expression of AAT.[98] A trial with AAV1 met with more success as sustained expression of AAT was achieved, but the levels were much lower than the therapeutic threshold.[89] In a second trial with AAV1, levels of ~2% of the therapeutic level were sustained as long as 5 years.[90,91]

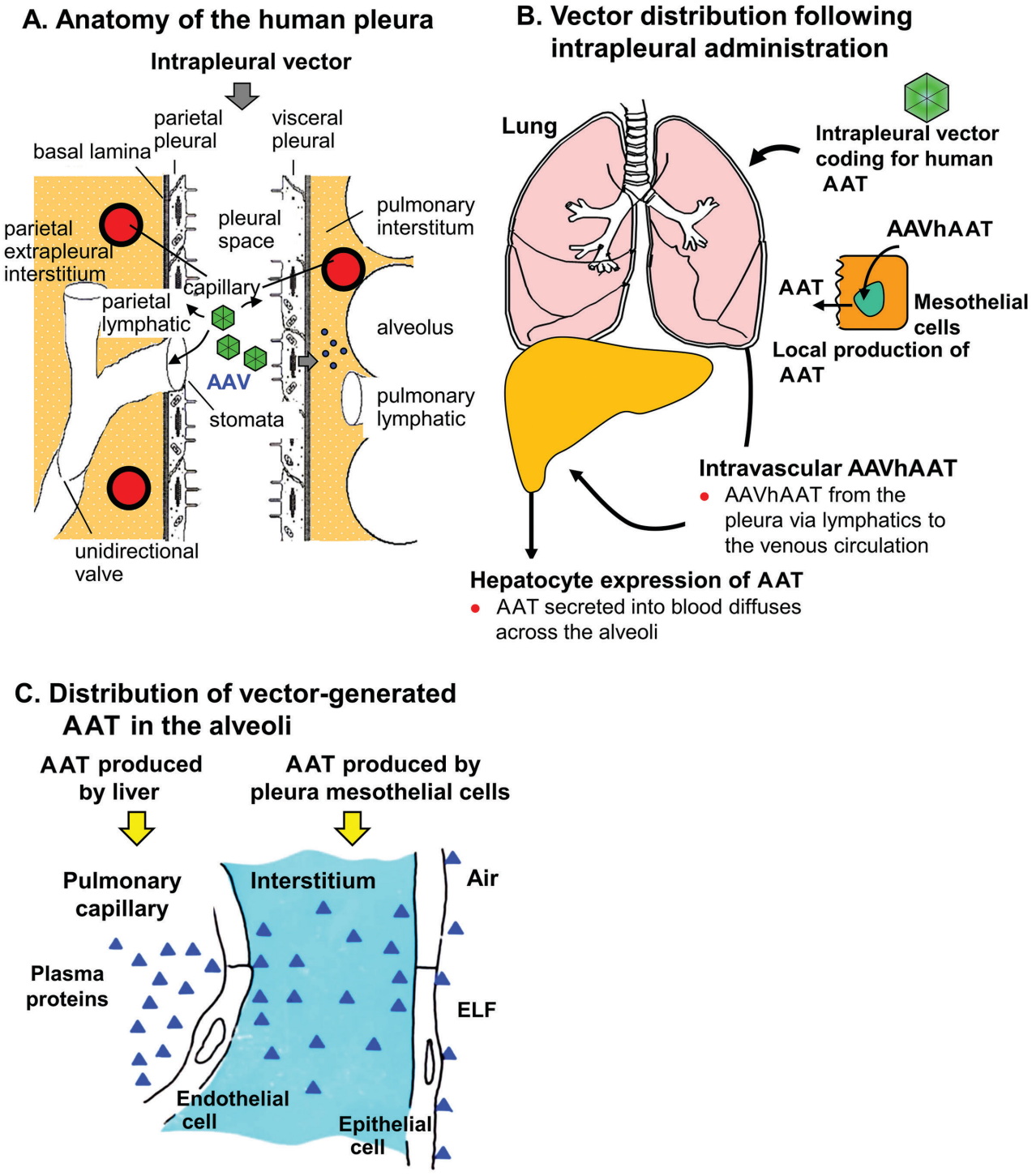
While each of these strategies target generally a single organ for vector transduction and AAT expression, intrapleural administration allows for both targeting of the lung directly and systemic delivery of the AAV gene transfer vector. The pleura is a thin serous membrane that encloses the chest cavity attaching the chest wall (parietal pleura) to the lung parenchyma (visceral pleura)[99,100] (Figure 2A). Both the parietal and visceral pleura contain a single layer of mesothelial cells surrounded by a thin layer of connective tissue rich in lymphatic and blood vessels that are connected to the systemic circulation. The pleura layers are separated by a pleural fluid (0.5 to 1 ml in humans).[100-105] Intrapleural gene transfer vector delivery has the benefits of both local lung delivery by transduction of the mesothelial cells lining the pleura and systemic delivery from vector passing through open stomata in the visceral pleural lymphatics to the systemic circulation and then primarily to liver hepatocytes (Figure 2B). AAT produced by the mesothelial cells is secreted and diffuses into the lung parenchyma. Because the lymphatic system of the parietal pleura connects directly from the pleural space through stomata, this allows for parallel systemic distribution of the gene therapy vector to primarily the liver via the circulation.[99,100,106] AAT produced in the liver can travel back to the lung through the circulatory system. The major advantage of intrapleural administration is that both the lung and liver are targeted by the gene therapy vector, increasing the possibility of producing a sufficient amount of AAT to provide a therapeutic effect (Figure 2C).

Preclinical Efficacy of Intrapleural Administration of AAV Vectors

In a mouse preclinical model, De et al[77] demonstrated that an AAV serotype 5-based vector expressing the human AAT gene produced higher levels of serum AAT via the intrapleural route of delivery compared to intramuscular administration at the same dose. Moreover, the AAV5-based vector produced about 8-fold higher levels of AAT compared to the levels achieved by an AAV2-based vector via both the intramuscular and intrapleural route. Intrapleural delivery of AAV5 coding for human AAT (administered dose of 10^{11} gc) mediated AAT serum levels of 900±50 μg/ml that were sustained up to 40 weeks post-administration. This level is significantly greater (1.6-fold) than the therapeutic threshold level of 570 μg/ml (11 μM). The AAT levels in the bronchoalveolar lavage fluid were similar to that in serum, indicating local production of AAT in the lungs.[77] These observations lend support to the concept that AAV5-mediated intrapleural delivery of the AAT transgene can provide sufficient amounts of AAT to be able to protect the lung from proteolytic damage.

To identify a more potent AAV serotype than AAV5, De et al[78] compared 25 different AAV serotypes (16 NHPs and 9 human AAV serotypes; all using the AAV2 inverted terminal repeats flanking the same human AAT cDNA driven by the CAG promoter) in mice (Figure 1). The authors demonstrated that intrapleural

Figure 2. Intrapleural Administration of an Adeno-Associated Virus Vector Coding for Alpha-1 Antitrypsin



A. Anatomy of the lung pleura. **B.** Vector distribution following intrapleural administration, combining local lung delivery via vector transduction of mesothelial cells lining the pleura, and systemic delivery via vector leaking to the systemic venous system and then primarily to liver hepatocytes. **C.** Delivery to the alveoli of AAT produced by AAV gene therapy to the pleura. The endothelial junctions are relatively loose, such that the levels of AAT (MW 52 kDa) in the interstitium are 60% of that in plasma. The epithelial junctions are tight, resulting in ELF AAT levels 5% to 10% of plasma. The locally (mesothelial cell) expressed AAT is delivered directly to the alveolar interstitium, while the liver (hepatocyte) expressed AAT diffuses from plasma to the interstitium, and then to alveolar ELF.

AAV=adeno-associated virus; ELF-epithelial lining fluid; AAT=alpha-1 antitrypsin

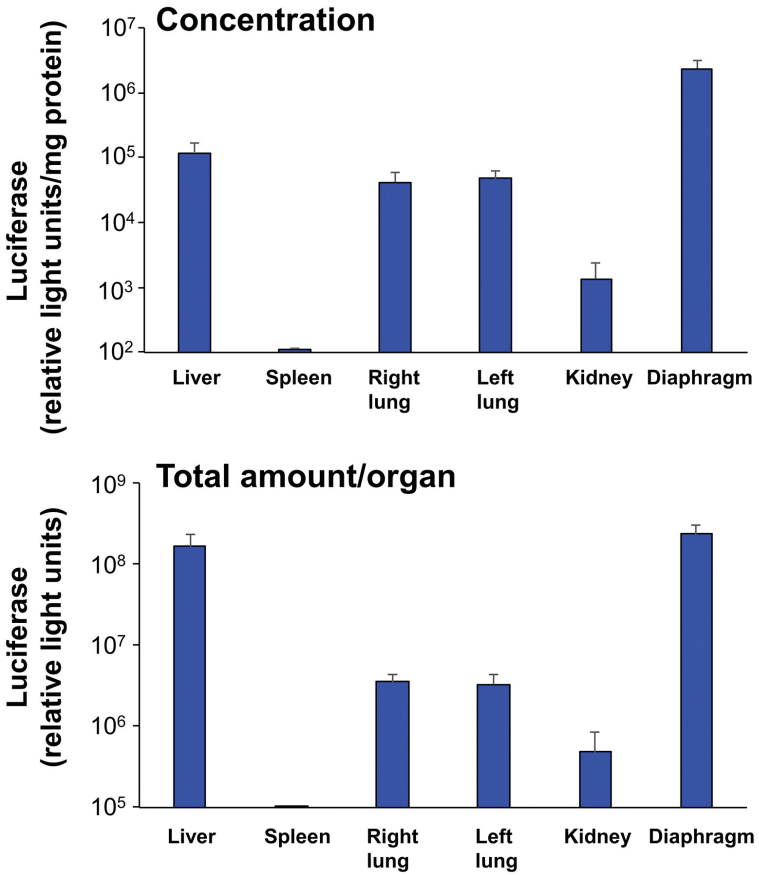
administration of the AAV nonhuman primate derived serotypes AAVrh.10 and AAV8 (both clade E) were the most effective at providing high serum AAT levels. Of the other serotypes tested, 11 were derived from rhesus macaques (AAV7, AAVrh.2, AAVrh.8, AAVrh.13, AAVrh.16, AAVrh.20, AAVrh.21, AAVrh.22, AAVrh.24, AAVrh.34 and AAVrh.43), and 1 was derived from cynomolgus macaque (AAVcy.5), 1 from baboon (AAVbb.2), 1 from chimpanzee (AAVch.5); and 9 from humans (AAV2, AAV5, AAV9, AAVhu.1, AAVhu.11, AAVhu.13, AAVhu.37, AAVhu.41, and AAVhu.47). The AAVrh.10 vector was chosen for further study. Administration of the AAVrh.10 vector (10^{11} gc) via the intrapleural route in the left lung resulted in high levels of transgene expression in the lungs, diaphragm, and liver (Figure 3). Additionally, this route of administration with AAVrh.10 produced sustained therapeutic levels of serum AAT (>2.5-fold above the minimum of 570μg/ml, compared to the 1.6-fold levels produced by AAV5) up to 24 weeks, the latest point of the study (Figure 4A). The high serum AAT levels translated to similar therapeutic levels in lung ELF, demonstrating biochemical efficacy (Figure 4B). Importantly, the AAVrh.10 vector-produced AAT was functional in the inhibition of neutrophil elastase. In the context that AAV vectors are less potent in female mice, administration of AAVrh.10 vector (10^{11} gc) via the intrapleural route produced greater than therapeutic levels of AAT not just in male mice but also in female mice. The AAVrh.10 vector is derived from rhesus macaque, and therefore, another potential advantage is that pre-existing anti-vector immunity in humans is minimal.[107] To assess whether the AAVrh.10 vector is functional in the presence of pre-existing immunity against the common human serotypes AAV2 and AAV5, administration of the AAVrh.10hAAT to AAV2- and AAV5-preimmune mice showed high level expression of AAT compared to nonimmune mice, thus demonstrating that AAVrh.10 is capable of circumventing common human immunity to AAV.[78]

AAVrh.10hAAT Safety and Toxicology Study

Based on the promising efficacy data with AAVrh.10hAAT in mice, the novel intrapleural delivery approach advanced to a safety and toxicology study.[74] This study included 280 mice and 36 NHPs. The AAVrh.10 vector was administered via the intrapleural route at 2 doses in each species (10^{10} and 10^{11} gc in mice, 10^{12} and 10^{13} gc in NHPs). The safety of the intrapleural vector delivery assessment parameters included hematology, serum chemistry and histopathology. Additionally, vector genome biodistribution and transgene expression were evaluated at multiple time points over 6 months.

The mouse toxicology study involved 2 parts. For the primary study, 120 male and 120 female mice were administered either PBS or AAVrh.10hAAT (10^{10} gc or 10^{11} gc) by the intrapleural route, with assessment of: (1) safety following vector intrapleural administration; (2) biodistribution of the vector; and (3) hAAT mRNA expression in chest cavity organs over a course of 6 months. The second study assessed the potential toxicity of direct injection of PBS (control) or 10^{11} gc AAVrh.10hAAT into lung parenchyma in 20 male and 20 female mice, as a worse case scenario model for misplaced dosing during intrapleural administration over the course of a month. Overall, the AAVrh.10hAAT vector was well tolerated without any vector-related morbidity or mortality in either group, except a few surgical procedure-related deaths in the group receiving vector via intrapleural administration (n=8 of 240). For both studies, the assessment of hematology, serum chemistry and histopathology showed the therapy to be safe. In the intrapleural study, all of the vector-administered groups of mice developed dose-dependent AAVrh.10 neutralizing antibodies. Assessment of vector DNA in various organs showed high levels of transduction of the liver (>10^6 copies/μg total DNA at the high dose and >10^4 copies/μg total DNA at the low dose) followed by the diaphragm and lungs, while other organs had low vector DNA levels, detectable only in the higher dose group. To assess whether intrapleural delivery of the vector mediated sufficient transgene expression in the chest cavity, AAT mRNA levels were quantified in chest cavity organs. The diaphragm had the highest levels of mRNA (>10^6 copies/μg total RNA at the high dose and >10^4 copies/μg total RNA at the low dose), and these levels were sustained up to 182 days, the latest time point of the study. Similar high levels of AAT mRNA were detectable in other chest cavity organs including pleura, left lung and right lung, demonstrating efficient transgene delivery to the proximity of the lung and hence availability to protect the lung from proteolytic damage.

Figure 3. Transgene Expression Organ Distribution After Adeno-Associated Virus Vector rh.10 Intrapleural Administration



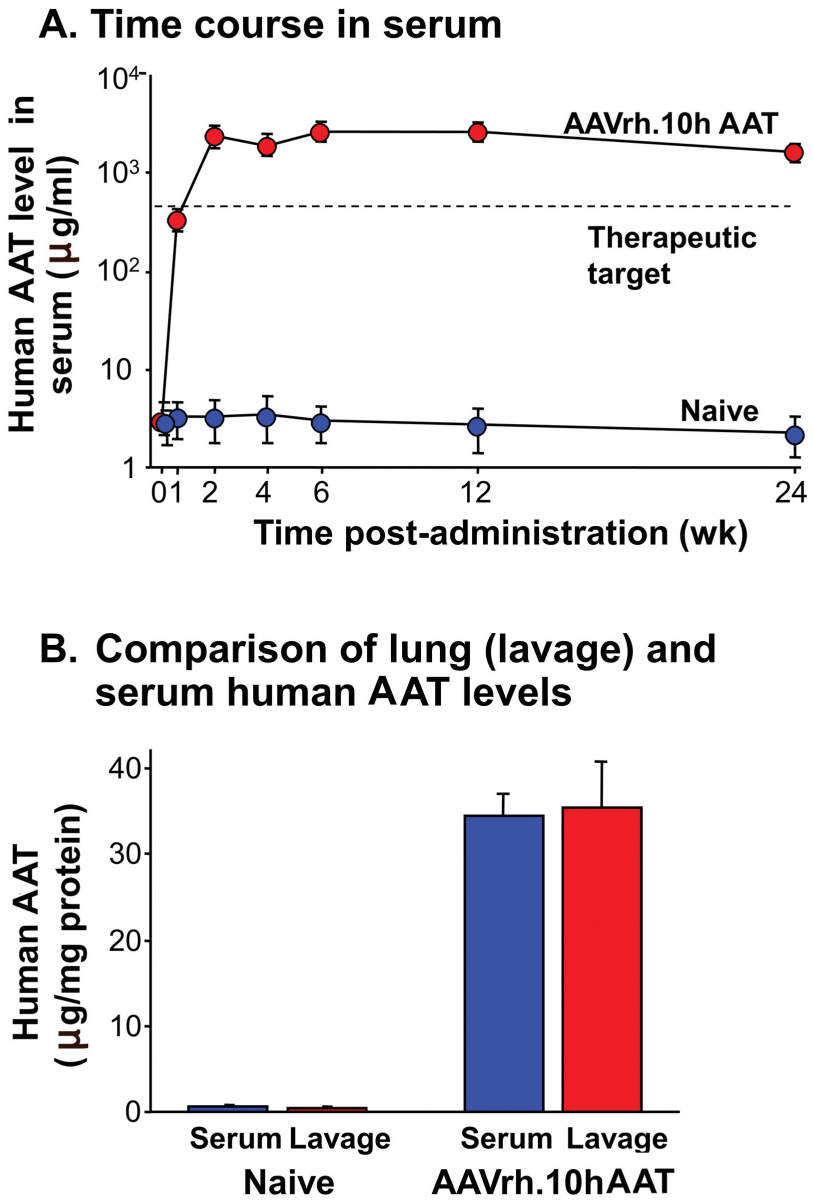
The distribution of luciferase expressed from an AAVrh.10 vector administered by intrapleural administration was assessed in various organs. The luciferase activity/ mg protein (upper panel) and total amount per organ (lower panel) are shown.

AAV=adeno-associated virus

The NHP study included 18 male and 18 female African green monkeys. Overall safety and vector-mediated expression of AAT in the chest cavity was assessed. All animals remained healthy with normal weight gain, heart rate and respiratory rate. Overall hematology parameters and serum chemistry data were normal, with only a few sporadic changes in individual animals that were not statistically significant. Pathology and organ weights measured at necropsy showed that there were no vector-related gross anatomic changes and organ-to-body and organ-to-brain weights remained normal. No vector-related significant histopathological changes were observed. Assessment of AAVrh.10 neutralizing antibody levels in serum showed dose-dependent AAVrh.10 neutralizing antibody titers, persisting up to 360 days, the latest time point of the study. Finally, quantification of human AAT mRNA in the chest cavity tissues demonstrated high levels of human AAT mRNA localized to the proximity of the lung with the mRNA levels persisting up to 360 days at $>10^4$ copies/µg total RNA in chest wall pleura, diaphragm, and diaphragm pleura.

The combined data from these pivotal toxicology studies in 2 species (mice and NHPs) demonstrated that the approach of delivering AAVrh.10hAAT by the intrapleural route is both efficient and safe with no toxicity issues. This study provided the groundwork for the planned initiation of a clinical trial of intrapleural human AAVrh.10hAAT for the treatment of AAT deficiency.

Figure 4. Intrapleural Administration of Adeno-Associated Virus Vector Coding for Human Alpha-1 Antitrypsin



A. Persistent AAT levels in serum after AAVrh.10hAAT intrapleural administration (10^{11} gc) male C57Bl/6 mice (n=4/group). Serum human AAT levels were assayed by ELISA. B. Human AAT levels in bronchoalveolar lavage fluid compared to serum at 8 wk after intrapleural administration of 10^{11} gc AAVrh.10h AAT (C57Bl/6 mice, n=4).

AAV=adeno-associated virus; AAT=alpha-1 antitrypsin; AAT=alpha-1 antitrypsin

Design of the Clinical Trial

On the basis of the above described efficacy and safety/toxicology studies of intrapleural administration of a serotype rh.10 replication-deficient AAV gene transfer vector expressing the human AAT cDNA in mice and in NHPs, an investigational new drug (IND) application was submitted to the FDA. This IND has been granted by the FDA (BBIND 16008) and has been licensed to Adverum Biotechnologies. The allowed clinical study (Protocol No. 1401014659, Clinical Trial ID: NCT02168686) is a gene transfer strategy designed to provide persistent high levels of human AAT. The study design has been previously published[108] and is

summarized here.

This study is designed as a phase I/II clinical trial to assess the safety and determine the preliminary efficacy of AAVrh.10hAAT (expressing the normal M1-type AAT) in humans with AATD. It is a 2-dose, open label study with n=5 individuals in each dose cohort ($8x10^{12}$ and $8\ x10^{13}$ gc) receiving the vector by the intrapleural delivery route. In addition, as a comparison to the intrapleural route, n=5 AAT-deficient individuals at each dose level will instead be administered the AAVrh.10h AAT vector by the intravenous route. A total of n=20 individuals with a genotype of ZZ or Z Null, with serum AAT levels of <11μM will be recruited to participate in this study. All participants will be monitored before and after vector administration with a variety of safety measures. In addition, biologic efficacy parameters will be assessed to generate preliminary assessment of the therapeutic impact of this intervention including serum and ELF AAT levels and function. The goal for the treatment to be considered efficacious and therapeutic is for the serum AAT levels to be >11 μM and AAT levels in the lung ELF to be >1.2 μM, the levels considered to be the "protective level" for AAT.[63]

Intrapleural AAT Gene Therapy–Moving Forward

Information gleaned from previous AAT clinical trials combined with the data from the preclinical studies suggest that intrapleural delivery of an AAVrh.10hAAT is a promising treatment for AATD. The upcoming clinical trials will help to determine whether administration of this vector by the intrapleural route will finally allow gene therapy to achieve the target protective level for biochemical efficacy. Achieving this primary end goal still opens the door for further questions to be evaluated. The next step after a demonstration of biochemical efficacy would be to evaluate clinical efficacy by monitoring stabilization of lung function or employing computer tomography lung density scans at total lung capacity to evaluate the rate of progression of lung destruction, as recently shown for AAT protein supplementation therapy.[64,65] The advantage of AAT gene therapy over the current protein supplementation therapy is the potential for a single administration of the treatment. AAV vectors have been shown to drive persistent AAT expression out to at least 5 years with little diminution in the level of expression.[90,91] However, the total longevity of expression is not yet known, and this leaves the possibility that readministration of the AAT gene therapy may be necessary during the lifetime of the patient. Future studies would be needed to help determine if an alteration in AAV serotype or the use of an immunodepressant regimen might be required for readministration or whether additional enhancements to current vectors might further improve gene expression stability.

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Declaraton of Interest

Adverum Biotechnologies has licensed the AAVrh.10hAAT intrapleural technology and investigational new drug from Cornell University. RGC is a consultant and holds equity in Adverum. RGC, DS, SK share licensing fees paid to Cornell University.

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