Challenges and Prospects for Alpha-1 Antitrypsin Deficiency Gene Therapy

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Alpha-1 antitrypsin (AAT) is a protease inhibitor belonging to the serpin family. A number of identified mutations in the *SERPINA1* gene encoding this protein result in alpha-1 antitrypsin deficiency (AATD). A decrease in AAT serum concentration or reduced biological activity causes considerable risk of chronic respiratory and liver disorders. As a monogenic disease, AATD appears to be an attractive target for gene therapy, particularly for patients with pulmonary dysfunction, where augmentation of functional AAT levels in plasma might slow down respiratory disease development. The short AAT coding sequence and its activity in the extracellular matrix would enable an increase in systemic serum AAT production by cellular secretion. *In vitro* and *in vivo* experimental AAT gene transfer with gamma-retroviral, lentiviral, adenoviral, and adeno-associated viral (AAV) vectors has resulted in enhanced AAT serum levels and a promising safety profile. Human clinical trials using intramuscular viral transfer with AAV1 and AAV2 vectors of the AAT gene demonstrated its safety, but did not achieve a protective level of AAT >11 μM in serum. This review provides an in-depth critical analysis of current progress in AATD gene therapy based on viral gene transfer. The factors affecting transgene expression levels, such as site of administration, dose and type of vector, and activity of the immune system, are discussed further as crucial variables for optimizing the clinical effectiveness of gene therapy in AATD subjects.

INTRODUCTION

ALPHA-1 ANTITRYPSIN (AAT) IS A 52 kDa acute-phase alpha-1-globulin that consists of 394 amino acids.^{1,2} AAT is one of the strongest serum serine protease inhibitors belonging to the serpin superfamily.² It is expressed primarily in hepatocytes and to a far lesser extent in intestinal and airway epithelial cells, neutrophils, and alveolar macrophages.^{3–7} AAT exhibits considerable inhibitory activity against a variety of enzymes and proinflammatory mediators, including neutrophil elastase released during the inflammatory response, as well as cathepsins, alpha-defensins, proteinase-3, and caspase-3.²

Alpha-1 antitrypsin deficiency (AATD) is a monogenic genetic disorder that was first described in 1963 by Carl-Bertil Laurell and Sten Eriksson.^{8,9} AATD is associated with a considerable risk of chronic liver and respiratory disorders, including early onset pulmonary emphysema, bronchiectasis,

bronchial asthma, and vasculitis.^{10–12} The AAT serum concentration in healthy individuals varies from 15–30 μM ,² while an AAT level below the protective threshold of 11 μM increases the risk of lung disease manifestations because of unrestrained degradation of local connective tissue.^{1,2}

Supplementary gene therapy is an attractive approach in the treatment of AATD and may increase AAT levels in serum. There are many experimental opportunities available, because of relatively short AAT coding sequence and its subsequent secretion into the extracellular matrix by many types of cells. Several experimental therapeutic strategies have been proposed, including viral (adenoviral, gamma-retroviral, and lentiviral vectors) and nonviral gene transfer. Unfortunately, only a few have progressed to clinical trials.^{13–16} Lately, the novel concept of combining supplementary and suppressive gene therapy has been proposed, because its dual impact on AAT synthesis and systemic concentration, as

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well as abnormal AAT intrahepatocellular accumulation, might prove particularly effective in patients with liver symptoms resulting from AATD.⁹ This article presents and critically analyzes current developments and prospects for AATD gene therapy.

GENETIC PATTERN OF AATD

The gene encoding AAT (*SERPINA1*) has been localized to the q32.1 region of chromosome 14. The coding region extends over 12,000 bp and includes seven exons separated by six introns. The *SERPINA1* locus is characterized by considerable polymorphism resulting from more than 130 different AAT variations.¹⁷ Mutations in the amino acid sequence directly affect the net charge of AAT variants. Therefore, they are classified according to electrophoretic mobility as assessed by isoelectric focusing in a polyacrylamide gel at pH 4–5. Variants characterized by a higher isoelectric point and rapid gel migration are marked by letters before M, while slower moving proteins are denoted by letters after M (low isoelectric point). The pathological and also normal MAAT alleles have been listed by Cox and Crystal.^{18,19}

There are many variants of *SERPINA1* encoding the normal form of AAT. Variants of the M1–M4 allele occur with the highest incidence, in 95% of healthy individuals. The occurrence of these four normal variants is associated with changes in the amino acid sequence at positions 213, 101, and 376. However, these changes do not affect the tertiary structure of the protein or its function.²⁰ Incorrect variants are responsible for the occurrence of abnormal AAT forms. The most common alleles in this group are S and Z alleles, which are particularly prevalent in the European population.²¹ Defective AAT proteins result in a qualitative deficiency, which is manifested by normal AAT levels but a lack of enzymatic activity, or a quantitative deficiency, which is characterized by low levels of AAT in serum and subsequent deficits in protective protein functions. Therefore, AAT alleles are classified as having normal AAT expression (normal), deficient expression (serum AAT <35% of normal), dysfunctional expression (the AAT protein with abnormal enzymatic activity), or null expression (no AAT expression).²²

Deficient alleles result in a significant reduction in circulating AAT levels, while AAT enzyme activity remains fully or partially preserved. In this group, the two most common alleles are PiS and PiZ. The simultaneous occurrence of the two PiZ alleles because of ZZ homozygosity is characterized by an average decrease in circulating AAT to <15%

of normal. In SS subjects, AAT levels are 40% of levels observed in MM homozygotes. The Z allele is associated with a Glu342Lys mutation that produces the Z protein prone to polymerization and accumulation in hepatocytes, which results in symptomatic liver disease. Interestingly, the Z mutation also diminishes enzymatic activity. Therefore, Z variants are not only deficient as a result of increased intracellular degradation but also dysfunctional because of the partial loss of inhibitory ability. The S allele contains the point mutation Glu264Val, which determines the unstable protein variant susceptible to degradation at the site of the synthesis. As a consequence, the protease–antiprotease balance is affected and the risk of respiratory disorders increases considerably.²⁰

Dysfunctional alleles cause a qualitative deficiency because of reduced AAT inhibitory activity, usually without affecting serum protein concentrations. For example, the AAT protein encoded by the PiF allele exhibits significantly diminished binding affinity to neutrophil elastase, which affects the balance between proteases and anti-proteases and increases the risk of emphysema. The serum AAT level in PiF individuals is within the normal range; thus, only genotyping or phenotyping allows for a correct AATD diagnosis.²³

The null allele (Q) results from single or multiple parallel disturbances at the molecular level, such as frame shift mutations, premature translation–termination codons, splicing mutations, and deletions in coding regions of the gene. The effect of these mutations is a total absence of AAT in serum and a significant increase in the risk of lung injury.

The clinical presentation of AATD has been discussed in detail by several authors and is not the subject of this review.^{11,24} In Tables 1 and 2, detailed descriptions of described genetic variants are presented.

CONVENTIONAL THERAPIES: WHY THEY ARE INSUFFICIENT

Commonly used therapy for AATD is similar to chronic obstructive pulmonary disease (COPD) and emphysema treatment: abstinence from smoking, long-acting bronchodilators, antibiotics, inhalations of corticosteroids, and long-acting beta-agonists are recommended. However, application of such solutions does not increase the functional AAT level in serum.

The only currently approved and dedicated treatment for AATD is the augmentation therapy, that is, weekly intravenous supplementation with the AAT protein purified from pooled healthy donors' plasma

Table 1. The most frequent normal and abnormal alleles causing alpha-1 antitrypsin deficiency

Allele nature	Allele name	Site of mutation (exon)	Change in sequence	Effect at the cellular level	Clinical symptoms
Normal	M1 (Ala213)	III	—	—	—
	M1 (Val213)	III	—	—	—
	M2	II	Arg101His (relative to M3)	—	—
	M3	V	Glu376Asp (relative to M1 Val213)	—	—
	M4	II	Arg101His (relative to M1 Val213)	—	—
Deficient	Z	V	Glu342Lys (relative to M1 Ala213)	Intracellular aggregation	Emphysema, liver disease
	S	III	Glu264Val (relative to M1 Val213)	Intracellular aggregation	Emphysema
Dysfunctional	Z	V	Glu342Lys (relative to M1 Ala213)	Impairments in inhibitory ability	Emphysema, liver disease
	F	III	Arg223Cys (relative to M1 Val213)	Impairments in inhibitory ability	Emphysema
Null	Q	II-V	Various types of mutations: deletions, insertions, premature STOP codon	Degradation or intracellular aggregation, no mRNA	Emphysema, respiratory tract infections

Approximately 30 variants of alpha-1-antitrypsin had been described and named according to the electrophoretic mobility in 1981.²⁵ Seventy-five alleles have been identified at the protein and gene level, including 10 normal AAT alleles.²⁶ The M1 (Val213) variant is the most common (44–49%). Other alleles are, respectively, M1 (Ala213) (20–23%), M2 (14–19%), and M3 (10–11%).²⁷ Crystal et al. also divided AAT alleles “at risk” into “deficiency” alleles and “null.”²⁶ The most common AAT deficiency allele is the Z allele that is associated with a high risk of emphysema and also liver disease. Another common alpha-1 antitrypsin deficiency alleles is an S variant that is associated with emphysema.^{28,29} Garver et al. found also null alleles, which are rare.³⁰ To sum up, according to the current literature, many different alleles encoding both normal and the mutated AAT protein exist. Their incidence is also varied, as well as consequences they cause. Many existing AAT variants, their incidence, and effects of their occurrence, at the cellular and also clinical manifestations level, have been discussed in detail in many articles.^{23,26,31–41} AAT, alpha-1 antitrypsin.

that has become available since the end of the 1980s.^{24,44} It is based on the administration of the purified human AAT at a dose of 60 mg/kg per week in order to achieve the protective level of AAT in serum.^{45,46} It is recommended for patients with severe form of AATD (PiZZ, PiSZ, PiQ0), serum AAT level below 80 mg/dl ($\leq 11 \mu\text{M}$), lung emphysema, and moderate–severe impairment (postbronchodilator FEV₁ 35–60% predicted) or rapid decline of lung function ($\Delta\text{FEV}_1 > 100 \text{ ml/year}$). Therapy is aimed at ensuring the AAT level in the blood above the generally accepted minimum protective level of 80 mg/dl ($\leq 11 \mu\text{M}$), arbitrarily set on the basis of epidemiological data. It has been suggested that continuous maintenance of the protective AAT levels in serum ($> 80 \text{ mg/dl}$) results in restoration of the protease–antiprotease balance and reduction of airway inflammation. While the protective effect of augmentation therapy on FEV₁ decline in lung emphysema because of AATD has been confirmed only recently,⁴⁷ previous clinical trials hinted at the possible protective effect on lung density, as well as demonstrated reduction in number and severity of COPD exacerbations.^{48,49} Yet, no significant effect on patients

mortality has ever been reported, possibly because of the well-known limitations of AATD *per se* like low disease incidence and lack of adequately sensitive tools to monitor lung structure deterioration. Consequently, current clinical guidelines do not recommend augmentation therapy for patients with very severe lung function loss (FEV₁ $< 30\%$ predicted) as its effectiveness has not been confirmed in this subgroup. Similarly, according to data from the National Heart, Lung, and Blood Institute (NHLBI) registry, augmentation therapy did not affect yearly decline of FEV₁ in AATD patients with bronchial asthma.⁵⁰

Commercial drug of human AAT, Prolastin (Bayer Inc. and Talecris Biotherapeutics), is one of the most frequently used medicament of augmentation therapy and so far has been approved or registered in selected countries in Europe and South America.⁴⁵ Augmentation therapy includes also two more medications: Aralast/Glassia (Baxter) and Zemira (CSL Behring). Both of them, as well as Prolastin, consist of purified protein intravenous formulations obtained from pooled plasma of human.⁵¹

Unfortunately, augmentation therapy is suitable only for individuals with respiratory disease without very severe lung function loss and not subjects with liver disorders resulting from the accumulation of the incorrectly folded AAT protein in hepatocytes, for which there is no specific treatment available, except liver transplantation.^{46,50,52,53} Furthermore, the significant cost of augmentation therapy (total healthcare cost range from \$36,471 to \$46,114), its short-term effect, and the lack of non-invasive therapeutic options for AATD patients

Table 2. Serum AAT concentrations according to protein phenotype

AAT concentration (serum)	Phenotype						
	MM	MS	MZ	SZ	SS	ZZ	Null
μM	20–53	18–52	15–42	10–23	20–48	3.4–7	0
mg/dl	102–254	86–218	62–151	33–108	43–154	29–52	0

Adapted from Brantly et al.⁴² and de Serres et al.⁴³

with liver disease provide strong motivation to look for novel and more effective ways of treating AATD.^{46,53} Thus, gene therapy is expected to be a promising alternative for AATD subjects.

GENE THERAPY STRATEGIES IN AATD TREATMENT

AATD gene therapy relies on the application of recombinant viral vectors for nucleic acid transfer into target cells. Most experimental studies have been performed using recombinant adeno-associated viral (rAAV) vectors, although several studies have also evaluated retroviral, lentiviral, and adenoviral vectors.

Increased risk of mutagenesis with retroviral vectors

Primary *in vitro* studies about the upregulation of AAT level in AATD were initiated in 1986, when Ledley and Woo described the retroviral model based on human alpha-1 antitrypsin (hAAT) gene transfer. They demonstrated hAAT expression in a transduced murine fibroblast cell line (NIH3T3) *in vitro*, thereby establishing the prospect of future replacement retroviral therapy.⁵⁴ In consequence, one year later, Garver et al. showed that hAAT gene transfer with the pN2-FAT retroviral vector resulted in the production of the glycosylated protein capable of inhibiting neutrophil elastase in a ψ 2/FAT murine cell line.⁵⁵ Other authors have suggested the utility of this method for bone marrow progenitor cell modification followed by autologous transplantation to repair the genetic defect.⁵⁶ In contrast, retroviral AAT gene transfer conducted *in vivo* in dog models proved to be only partially effective because of short-lived AAT expression and therapeutic side effects, such as lethargy, hypotension, hematemeses, and most importantly death.⁵⁷ Likewise, others observed severe side effects in severe combined immunodeficiency (SCID)-X1 animal models of retroviral gene transfer, primarily caused by insertional mutagenesis, which results in neoplastic transformation⁵⁸ and is currently the main limiting factor for retroviral vectors' usage.

Severe immune response with adenoviral vectors

The threatening side effect of retroviral vectors, used in the studies mentioned above, includes high risk of insertional mutagenesis. Lemarchand et al. proposed to solve this problem using adenoviral vectors that, as it is well known, do not integrate into the genome. They demonstrated that AAT

gene transfer with replication-defective adenovirus type 5 to intact human umbilical veins *ex vivo* resulted in AAT production that was confirmed by Northern blot and enzyme-linked immunosorbent assay, with AAT levels of 13 μ g/ml in the perfusing medium after gene transfer into the vein.⁵⁹ The possibility of using endothelial cells for AATD gene therapy is of particular interest because of the significant numbers of these cells in the human body, which could allow for increased AAT synthesis. Efficient AAT expression after adenoviral vector administration has been confirmed by several other reports, including *in vivo* models. Rosenfeld et al. showed AAT mRNA in the respiratory epithelium after intratracheal administration in rats and noticed that it was secreted and also synthesized by lung tissue.⁶⁰ Moreover, Kay et al. successfully used adenoviral vectors that generated transient expression of hAAT in a murine model and achieve a therapeutic level up to 700 μ l/ml of AAT.⁶¹

However, one of the major disadvantages of adenoviral vectors is probably their inability to integrate into the genome of target cells, which results in intransient protein expression and forces the multiple vector administration. Furthermore, the adenoviruses and adenovirus-based vectors have proven highly immunogenic.⁶²

AAV vectors in AATD gene therapy

Studies conducted on AAV were developed in order to overcome problems associated with the retrovirus and adenovirus vectors. AAV is a single-stranded DNA virus from the Parvoviridae family.⁶³ The key advantage of AAV is a slight pathogenicity.⁶⁴ Unlike retroviruses, AAV recombinant vectors (rAAV) do not integrate into the genome of the target cell, which eliminates the risk of insertional oncogenesis.^{58,65} In addition, rAAVs have the ability to infect and persist for a long time in both dividing and nondividing cells.^{66,67} They induce only some humoral immune response with the formation of neutralizing antibodies but, unlike adenoviral vectors, they do not seem to activate T cytotoxic cells.^{68,69} Consequently, rAAVs are currently considered the most effective and harmless gene therapy solution for AATD.

Most researchers are focusing on the optimization of rAAVs' transgene expression, which includes the selection of an AAV vector serotype to avoid the neutralizing antibodies resulting from previous exposure to a specific type of the AAV virus. Other modifications have focused on the promoter, method, and optimal application site to supply a high level of transgene expression.^{66,67,70-77}

Efficiency of AAT expression at the rAAV application site. The first study using rAAVs in AATD was conducted in 1998. Song et al. succeeded in delivering AAV-based recombinant plasmid encoding hAAT gene to murine skeletal muscle under the control of the cytomegalovirus or human elongation factor 1- α promoter. Satisfactory expression of the transgene was observed after 4 weeks, yet a large number of viral particles (5×10^{10}) had to be administered.⁷⁰ The difference in the size of human and murine bodies forced a reduction in the amount of viral particles to avoid a potential immune response. The optimization of this method relied on a 10-fold decrease in the vector dose and administration into the portal vein to transduce murine hepatocytes.⁷¹ This approach achieved AAT concentrations in serum similar to levels obtained during transduction of murine muscle cells, with no side effects.

Similar to AATD augmentation therapy, only a small percentage (<3%) of AAT synthesized by modified cells reaches its target destination in the lungs because only small fraction of serum AAT could cross through the alveolar capillary endothelial and also epithelial barrier into the interstitium and alveolar spaces.⁶⁶ Furthermore, administering a considerable number of AAVs into the portal vein may increase the risk of hepatitis, as previously reported for AAV2 in hemophilia B gene therapy trials.⁷² Consequently, novel methods that allow direct introduction of AAV vectors into the lungs have been developed.⁷³ Flotte reported that administration of a vector encoding hAAT, a hybrid construct consisting of the cytomegalovirus and beta-actin promoter based on the AAV2 strain, was effective after intratracheal administration in mice.⁷⁴ Transfection of the macrophage cell line J774A.1 with the pAAV-CB-AAT vector encoding hAAT and subsequent intratracheal injection resulted in therapeutic hAAT concentrations in murine lungs.^{66,76}

Another possible administration site is the pleural cavity. Introduction of the AAV5 serotype vector via this route in mice resulted in 10-fold higher levels of AAT in serum and bronchoalveolar lavage compared with an AAV2 vector. This method allowed for a 1.6-fold higher AAT concentration than the accepted therapeutic level of 570 $\mu\text{g/ml}$, and long-lived protein expression over 40 weeks.⁷³ Therefore, intrapleural administration was associated with the high availability of therapeutic protein in the respiratory system, lower vector dosage compared with intravenous injection, and consequently a lower risk of side effects, including inflammatory response.⁷⁵

Efficiency of AAT expression and production of host neutralizing antibodies. Another important issue in AATD gene therapy concerns the immune response. Halbert et al. have shown that some AAV vectors (AAV6) used in AATD gene therapy induce an acute immune response and diminish or abolish their therapeutic effect even after immunosuppression.⁷⁸ Moreover, AAV-neutralizing antibodies directed against the AAV2 and AAV5 serotypes most frequently used in animal models of gene therapy are present in a relatively large proportion of the human population.⁷⁹ Limberis and Wilson constructed an AAV2/9 vector that was able to transduce murine respiratory epithelial cells and synthesize hAAT without producing neutralizing antibodies. The AAV2/9 vector generated 60-fold higher AAT serum levels in mice than the AAV5 vector. The vector resulted in stable and prolonged 9-month expression of AAT, proving its ability to transduce progenitor cells within the respiratory system. Moreover, neutralizing antibodies were not found in bronchoalveolar lavage fluid harvested from experimental animals.⁶⁷

To prevent the influence of antibodies produced before vector application, De et al. screened 25 known AAV viruses to establish which occur most infrequently in humans. They identified the AAVrh.10 serotype, which has since been considered as extremely powerful expressive tool. The vector resulted in almost 300% higher expression of hAAT than conventionally used AAV5-based vectors.⁷⁶ The preclinical study demonstrated that intrapleural administration of the carrier allowed for the detection of AAT mRNA for 6 months in 280 mice and 1 year in 36 nonhuman primates, with no side effects.⁷⁷

AAVs in AATD experimental treatment: summary. Numerous studies have demonstrated that the therapeutic effect depends on multiple factors, such as AAV serotype, prior exposure to virus, and administration site. In contrast to adenoviral, AAV vectors are associated with a number of advantages, the most important being their low immunogenicity and pathogenicity. Similarly, the administration site has proven vital with direct application into the portal vein, as well as an intratracheal or intrapleural route, providing the most efficient transgene expression. In addition, an intratracheal or intrapleural route generated fewer side effects than other methods.

In summary, AAV vectors are currently considered the most appropriate for effective, long-term, and safe *in vivo* transduction.

Suppressive gene therapy for liver disease of AATD

AATD experimental gene therapy trials, described above, have aimed to achieve a protective AAT threshold in lungs. Another primary goal of gene therapy approaches of AATD-associated liver diseases is the elimination of the accumulated AAT protein from hepatocytes.^{80–84} Several reports have indicated the utility of RNA interference and ribozymes in posttranscriptional silencing of the defective genes expressed in ZZ homozygotes. Some studies have examined the possibility of intracellular vector administration, simultaneously carrying coding sequences of fully active AAT and molecules silencing the expression of the Z form of the protein, which would allow for recovery of protective serum AAT levels.^{81–84}

Cruz et al. applied a recombinant vector, AAV8-3X-siRNA, encoding three different siRNAs directed against small regions of the second, third, and fourth mRNA exons of Z-ATT, into the portal vein of transgenic mice expressing Z-AAT. This resulted in an almost threefold decrease in circulating serum Z-AAT levels. Furthermore, the intrahepatocellular accumulation of abnormal Z protein monomers was significantly diminished.⁸¹ Li et al. achieved normal serum AAT levels using an AAV8-shRNA-AAT-opt vector simultaneously encoding shRNA sequences silencing the Z allele and fully active optimized M-AAT. With this method, almost 90% decrease in Z-AAT expression and a 13–30-fold increase in circulating AAT levels were achieved. This dual approach seems promising, since it might enable successful treatment of liver pathology with parallel correction of AAT concentrations in peripheral blood.⁸² To avoid any side effects from siRNA and shRNA therapy, Mueller et al. proposed the use of microRNAs, which have proven effective in posttranscriptional silencing of defective genes.^{85,86}

The retroviral vector encoding ribozyme specific for Z-AAT, and modified M-AAT mRNA not susceptible to its activity, allowed for simultaneous inhibition of Z-AAT expression and an increase in functional protein concentrations in an established liver cancer cell line, similar to vectors that induce RNA interference.⁸³ Zern et al. proposed a model based on a modified simian viral vector (SV40) regarded as more efficient than retrovirus because of the ability to express encoded genes in many cell types, including nondividing cells. The ribozyme was expressed in an established human hepatoma-derived cell line under the control of the tRNA promoter, resulting in a decrease in production of the deficient Z-AAT variant by nearly 75%.⁸⁰ Si-

milar results were observed in murine models, with a significant 50% decrease of Z-AAT in serum.⁸⁴

In conclusion, there are a few reports describing suppressive AATD gene therapy. Vectors encoding miRNA, which are endogenous silencing regulators of gene expression, seem to be an interesting prospect. However, information about the safety profile of these attempts is insufficient and further examinations are necessary.

Mature stem cells in AATD gene therapy: lentiviral or AAV vectors?

AATD gene therapy that exploits mature stem cells (MSCs) of various types and origins appears to be a potential therapeutic strategy. Gene modification of stem cells *ex vivo* and their subsequent re-transplantation, performed as autologous transplantation, reduces the risk of rejection, and omits the ethical problem of using embryonic stem cells. Furthermore, the typical problem of transgene delivery to a nonspecific target cells has been solved. In addition, mature genetically modified stem cells are capable of self-renewal and replacement of old and damaged somatic cells, thereby eliminating the necessity of repetitive gene therapy administration.⁸⁷

The potential efficacy of MSCs in AATD gene therapy has been demonstrated by several studies. It enabled the restoration of protective serum AAT levels in the lung. Furthermore, under appropriate conditions, MSCs can replace hepatocytes producing defective Z-AAT.^{64,87,88} Several studies have determined the effectiveness of rAAV and lentiviral vectors in stem cell modifications. Wilson et al. applied a lentiviral vector, which allowed for transduction of hematopoietic cells that resulted in 24 weeks of hAAT expression in the circulatory system. Lentiviral vectors ensure constant expression of the introduced transgene, but have the ability to randomly integrate into the cell genome, similar to retroviruses. Under unfavorable conditions, they might cause the malignant transformation that results from insertional mutagenesis.⁸⁹ Ghaedi et al. also used the lentiviral vector to demonstrate efficient transduction of hepatocytes differentiated from a population of mesenchymal stem cells. It is believed that the proposed system could be an *in vitro* source of cells for transplantation in patients with AATD.⁹⁰

The problem of insertional mutagenesis has been reduced in further studies. Song et al. demonstrated the effectiveness of the rAAV1-CB-AAT vector in transduction of murine oval cells, which are progenitors of hepatocytes.^{64,91} The study showed the ability of modified progenitor cells to differentiate into liver cells, the colonization of the

surrounding area, and the subsequent proliferation and regeneration of precut fragments. Cells were also capable of continuous AAT synthesis. This suggests the integration of the vector into the genome of the cell, because recombinant AAV vectors are lost during cell division.⁶⁴ Li et al. adapted this experimental model, transducing bone marrow cells—a rich source of hematopoietic and mesenchymal stem cells—with rAAV8-CB-hAAT vector. Immunofluorescence staining proved prolonged expression of the AAT in stem cells after reimplantation into murine liver.⁸⁷ In subsequent studies, the same group demonstrated the feasibility of using mesenchymal stem cells derived from adipose tissue. After transduction with rAAV8-CB-hAAT and subsequent transplantation into murine liver, they observed satisfactory AAT expression and no anti-AAT antibody production. Because of significant similarity to bone marrow stem cells and easier extraction, exploitation of mesenchymal stem cells originating from adipose tissue seems to be an attractive alternative, in contrast to previous attempts.⁸⁸

AATD THERAPEUTIC CLINICAL TRIALS CONDUCTED ON THE BASIS OF GENE TRANSFER

There are more than 50 ongoing or completed clinical trials referring to AATD in the U.S. National Institutes of Health registry, but only 2 of them are based on the gene transfer. Both trials evaluated the effectiveness of viral hAAT gene transfer into muscle cells with AAV vectors that had previously demonstrated advantages, justifying their further development into clinical trials. Both studies used different application systems and vector therapeutic doses.^{13–16}

The study by Flotte et al. was the first to assess the effectiveness of the rAAV2-CB-hAAT vector in humans. The phase I clinical trial group consisted of 12 adults with inherited AAT deficiency, as PiZZ or compound heterozygote of PiZ, diagnosed with chronic obstructive pulmonary disorder (COPD; FEV₁ >25% of predicted). Patients (4 dose cohorts) received vectors into the deltoid muscle of the nondominant arm (doses of 2.1×10^{12} to 6.9×10^{13} vector genomes/kg; 3 subjects per cohort). The 3.3 ml volume of vector was separated into 3 single injections of 1.1 ml each. Only slight redness, muscle tenderness, or light bruising was noted locally at the injection site. Blood morphology and biochemistry parameters, urinalysis, and lung function parameters were within the normal range throughout the study period. No significant immune response was

observed, with the exception of the anticipated humoral response toward the capsid of AAV2 vector. Likewise, a semen assay for the vector genome was negative. Only 1 out of 12 patients demonstrated an increase in serum M-AAT protein level at day 30 after injection, although the level only slightly exceeded the detection level at 82 nM.^{13,14}

A phase I/II clinical trial evaluating the safety and effective dosage of rAAV1-CB-hAAT vector has been slightly more promising. The study group included 9 patients with AATD, as PiZZ or compound heterozygote of PiZ, diagnosed with COPD (FEV₁ >25% of predicted). Vector was introduced via intramuscular injection in a total volume of 9.9 ml (9 separate injections of 1.1 ml each) in 1 of 3 examined doses of 6.9×10^{12} , 2.2×10^{13} , or 6.0×10^{13} vector genomes per cohort of 3 patients. Therapy was well tolerated with minor side effects at the injection site, such as bruising in 6 subjects, swelling or induration in 3, redness in 2, and warmth and tenderness in 1 case as well. Persistent M-AAT expression has been detected for 12 months in 2 subjects and for 90 days in the 1 subject in the 6.0×10^{13} vector genome dosage group. However, AAT serum concentration rose to only 0.1% of its protective level. No immune response directed against the M-AAT protein was observed, though antibodies against AAV1 capsid were produced.¹⁵

For this reason, the study was stopped and resumed 2 years later in 2011.^{15,16,92} The same vector was applied but this time the herpes simplex virus complementation method was used. Vectors produced in this way showed significantly greater efficiency *in vivo*.^{16,92} Accordingly, augmentation of serum M-AAT was observed in all of nine AATD patients and proved to be dose-dependent. The lowest vector dose (6.0×10^{11} genome particles per kg) resulted in 2-fold higher expression of the M-AAT protein than achieved in the 2009 study (70 vs. 30 nM). The highest dose of vector produced serum M-AAT of 412–694 nM, which was insufficient to achieve the protective threshold of >11 μ M. As shown previously, no major adverse effects were observed.⁹²

Thus, both clinical trials demonstrated that AAV1- and AAV2-based vectors are unfailingly safe. However, further studies and optimizations are needed.

CONCLUSIONS

AATD gene therapy is a promising substitute for currently recommended therapeutic options because viral transfer of the human AAT gene and/or molecules that inhibit the expression of the defective AAT protein variants has shown

encouraging results *in vivo*. Because of the lack of pathogenicity and sporadic integration into the genome of a target cell, recombinant AAV vectors represent a potentially useful tool in experimental supplemental and suppressive AATD gene therapy and in MSCs. However, despite their safety, intramuscular expression of AAT remained lower than the minimum protective concentration. Further studies are needed to optimize the procedures, such as vector dose or site administration, and the applicability of capsids other than AAV1 and AAV2, to avoid the neutralizing effect of the human immune system. In addition, in the face of reports that demonstrate more efficient transgene expression following intravenous, rather than intramuscular application, novel administration methods, such as intra- or subcutaneous application, should be considered and evaluated. Another possibility to improve the quality of treatment could

be production of second-generation antisense oligonucleotide (ASO) targeted against hAAT (AAT-ASO),⁵² or development of vectors encoding protein molecules that prevent retention and polymerization of Z-AAT in liver or even promote its secretion and/or degradation, for instance, by accelerating intracellular proteolysis pathways. Finally, it should not be forgotten that personalized therapies are currently an attractive approach and different drug dosages for each individual patient should be considered.

Taken together, despite the unsatisfactory levels of AAT achieved in clinical trials, the safety profile has been very rewarding, which should encourage research of AATD gene therapy.

AUTHOR DISCLOSURE

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