EXPERT OPINION ON THERAPEUTIC PATENTS, 2016
http://dx.doi.org/10.1517/13543776.2016.1165210

Taylor & Francis
Taylor & Francis Group

REVIEW

Therapeutic compositions and uses of alpha1-antitrypsin: a patent review (2012 – 2015)

Yotam Lior[a], Assaf Geyra[b] and Eli C Lewis[a]

[a]Department of Clinical Biochemistry & Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel; [b]Geyra Gassner IP Law, Tel-Aviv Yaffo, Israel

ABSTRACT

Introduction: Identified as a circulating serine-protease inhibitor, the genetic deficiency of which predisposes to the development of lung emphysema, alpha1-antitrypsin (AAT) has recently been found to possess various anti-inflammatory and immunomodulatory activities outside the biochemical inhibition of serine-proteases. AAT is presently extracted from human plasma to supply life-long infusions to patients with genetic AAT deficiency. However, its newly appreciated functions point to extended therapeutic uses; these, alongside modified production attempts, represent a novel and dynamic niche of drug repurposing, set apart from addressing lung emphysema in AAT-deficient individuals.

Areas Covered: The review provides a comprehensive summary of patent-protected inventions in the field of novel clinical indications for AAT and innovations in AAT production.

Expert Opinion: A molecule no longer patentable per se, presents with novel clinical applications; its mechanism still unfolding. While modified protein sequences are patentable and potentially superior, they are burdened by regulatory setbacks. Thus, recent approaches in the context of AAT appear in patents that describe combinations with other drugs, redefined clinical subclasses, and unique recombinant entities, carefully skirting saturated areas of AAT patentology. It will be fascinating to follow technologies and creative patenting as AAT navigates the trying trades of pharmaceutical industry towards an increasing lineup of unmet clinical needs.

ARTICLE HISTORY
Received 22 January 2016
Accepted 4 March 2016
Published online
25 March 2016

KEYWORDS
Acute myocardial infarction; allograft rejection; alpha1 proteinase inhibitor; diabetes; graft versus host disease; immunomodulation; inflammation; SERPINA1; xenoimmune response

1. Introduction

1.1. Purification of clinical-grade AAT

Reduced levels of circulating alpha1-antitrypsin (AAT), as in the case of genetic AAT deficiency (AATD), was first identified in 1963 to be the outcome of a variety of mutations in a single gene encoding the human glycoprotein, AAT.[1] The condition is rare (~1 in 5000–10,000 individuals) and is associated most commonly with pulmonary emphysema, as well as with cirrhosis, systemic vasculitis (particularly anti-neutrophil cytoplasmic antibody [ANCA] positive) and neutrophilic panniculitis.[2,3] In 1981, a treatment for AATD has been developed: augmentation (replacement) therapy that consists a life-long once-a-week slow-drip infusions of plasma-derived affinity-purified human AAT. Using this approach, AAT serum levels are typically restored to the desired >11 μM and the risk of emphysema is reduced.[2–6]

Clinical-grade AAT is extracted from pooled human plasma using AAT-enriched plasma preparations, as initially described by Gadek et al.[7] To date, at least four manufacturers are licensed by the US Food and Drug Administration (FDA) to produce clinical-grade AAT: Grifols Ltd. produce *Prolastin*, Baxter Therapeutics Ltd. produce *Aralast*, CSL-Behring Ltd. produce *Zemaira*, and Kamada Ltd. produce *Glassia*. All four companies apply mild modifications to Cohn's cold ethanol fractionation [8] as the fundamental step in AAT purification, obtaining mostly human serum albumin and AAT from fractions II, III, and IV. At this point of the process, the fractions also contain a moiety of blood factors, such as AAT-associated apolipoprotein 1-A.[9,10] Thus, they are further processed by chromatographic or precipitation purification methods in order to separate the molecule from the remainder of solution content. In order to minimize the risk of bloodborne pathogens, all companies include nanofiltration alongside solvent/detergent or pasteurization processes. As a final release stage, the product is then tested for its capacity to inhibit neutrophil–elastase. To this end, Kamada Ltd. manufactures a solution of AAT, the other companies produce lyophilized AAT.

The different purification methods give rise to mild variations in purity, as well as in the tendency of the purified protein to form aggregates under particular conditions. Variations have also been described in the degree of specific activity, as well as inclusion of multiple isoforms of human AAT, as described by Cowden and colleagues.[2] The various purification methods also yield AAT with some alterations in both structure and glycosylation patterns, rendering the clinical-grade material moderately different from the circulating native protein.[11] The implications of such variations are presently studied. Nonetheless, all present methods for obtaining clinical-grade human AAT are satisfactory; safety, quality,

CONTACT Eli C Lewis lewis@bgu.ac.il Department of Clinical Biochemistry & Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, POB 653, 84105 Beer Sheva, Israel

Article highlights

- Alpha1-antitrypsin (AAT) is a serine–protease inhibitor glycoprotein housing a multitude of immunoregulatory and immunomodulatory activities.
- To date AAT is purified from pooled human plasma, a process still being perfects by recent patents.
- Currently, the sole clinical indication for AAT transfusion remains AAT deficiency, a one gene disease usually characterized by early onset emphysema and liver disease.
- Patents published in recent years depict the clinical use of AAT in conditions varying from treatment of diabetes, Graft vs Host disease, graft protection, bacterial infections and even ameliorating radiation exposure adverse effects.
- The need for increased production rates has raised attention to recombinant production of AAT, alongside the modification options this method harbors.

This box summarizes the key points contained in the article.

quantity, and efficacy are well in the desired range, and all products inhibit neutrophil–elastase.

1.2. Recombinant AAT and the impediment of protein glycosylation

Costly production processes and treatment protocols (estimated at $14,000 per patient per year in the United States alone [12]), as well as the constant concern regarding infusion of blood products, combined with finite amounts of global plasma pools, have all given rise to increased interest in recombinant production of AAT. Recombinant human AAT has been generated in multiple systems, including bacteria, yeast, insect cells, mammalian cells, and entire transgenic plants and animals. However, none of these attempts was approved for clinical use.[6]

One of the leading challenges facing recombinant production of AAT is the complexity of its post-translational processing, namely, a distinctive glycosylation process. Being a 394-amino-acid-long molecule, AAT measures at 46 kDa; yet with its added glycosylations it grows to become a 52-kDa glycoprotein. Its three *N*-glycosylation sites act as the docking point of glycation chains that may comprise up to 16% of its weight (positions 46, 83, and 247). The composition and pattern of these extensions slightly differ between humans, giving rise to eight major isoforms (M1–M8). Changes in the glycosylation pattern of AAT affect primarily the rate of protein degradation both in the endoplasmic reticulum and in the plasma, as well as the rate by which the protein aggregates. These factors have a direct effect on the plasma half-life: a fully glycosylated AAT has a 3–4-day half-life, a non-glycosylated AAT has a 7-min-long half-life.[13–16] Constructing these particular glycosylated structures on the surface of AAT is highly diverse between cell types, species and without exception, plants, yeast cells, and bacteria.

1.3. AAT modulates inflammation and immune responses

Although known primarily for its anti-protease and anti-inflammatory activities, studies conducted over the past decade have cumulatively demonstrated that AAT is also an immune-modulator and a cytoprotective molecule. As such, microenvironments enriched with AAT were shown to contain reduced levels of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α,[17] and increased levels of anti-inflammatory mediators such as IL-1 receptor antagonist and IL-10.[18] This phenomenon was also depicted in human PBMC *in vitro* studies,[19,20] as well as in samples obtained from cystic fibrosis (CF) patients who received inhaled AAT.[21] Concomitantly, AAT has been demonstrated to directly bind IL-8,[22] and danger-associated molecular pattern molecules (DAMPs), such as extracellular heat shock protein (HSP) 70 [23] and gp96, [24] which otherwise act as adjuvants to the associated immune responses.[25,26]

Surprisingly, the anti-inflammatory qualities of AAT still allow innate immune cells to respond to authentic threats; macrophages readily phagocytose bacteria, neutrophils decontaminate infected sites,[27] and antigen-loaded dendritic cells migrate to draining lymph nodes.[18] T lymphocytes, on the other hand, respond to AAT-rich environment indirectly, pending stimulation from innate immunocytes. For example, AAT has been shown to elicit semi-mature antigen presenting cells that favor the expansion of protective regulatory T cell.[18,25,28–30] B lymphocytes, which belong to both the innate and adaptive immune system, appear to exert a modified response in the presence of AAT, in the form of diminished isotype switching which results in enhanced protective IgM production.[25,30] Indeed, it has been suggested that the reduction in bacterial burden, as depicted in CF patients for instance,[31] during treatment with AAT may relate to enhanced anti-pathogen immune responses; at the same time, local tissues are spared from inappropriate excessive injury that might promote deleterious adaptive responses by elevating the levels of local DAMPs.[27,32,33]

1.4. The biochemical activities of AAT extend beyond serine–protease inhibition

As our appreciation of these multiple functions of AAT grew, it became apparent that we may have to consider the possibility that AAT exerts at least some of its attributes irrespective of its capacity to inhibit serine–proteases. In fact, treatment with several forms of other elastase inhibitors (e.g. aprotinin) does not overlap the favorable outcomes of AAT therapy in multiple preclinical studies. Thus, studies have begun to surface in the past decade to describe unexpected binding partners and an extended anti-proteolytic repertoire outside the prototypic elastase-binding site. AAT was found to inhibit proteases outside of the serine–protease family, such as metalloproteinase (MMP) 9 and caspase-3.[25,30] There is evidence for AAT binding to lipids and carried in the blood in association with high density lipoprotein (HDL),[22,34–37] apparently granting the lipoprotein particle added anti-inflammatory attributes. Concomitantly, AAT has been demonstrated to prevent apoptosis in various systems, presumably by reducing the activity of caspase-3,[29,38–41] as well as preventing necrotic cells death under various triggers.[42] Based on these findings, a patent application was published in 2011 in which the inventors propose that AAT interferes with the process of necrosis in several clinical situations.[42] Indeed, AAT has been shown

to induce a tissue protective effect in the form of improved function of stress-inflicted cells residing that surround a point of immediate pathological impact in neurologic, cardiogenic, and pancreatic models.[25]

Being unrelated to the binding site of neutrophil–elastase within the protein sequence of AAT, some studies have found that these properties of AAT may explain consistent anti-inflammatory outcomes in platforms that use modified forms of AAT that *lack* elastase-inhibiting capacity. A head-to-head comparison was held between commercial plasma-purified AAT and the recombinant version that lacks anti-elastase property [43]; in that study, neutrophil infiltration and inflammatory cytokines and chemokines were all downregulated both *in vitro* and *in vivo*. Interestingly, the concentrations of recombinant AAT that were required to elicit an anti-inflammatory environment were 40–100-fold lower than those required of the plasma-derived protein. In another study, the use of AAT-Fc fusion molecule, which also lacks elastase inhibition capacity most probably due to structural rigidity, had induced an anti-inflammatory effect as well a cytoprotective effect in an animal model.[44]

Indeed, it appears that elastase inhibition, the sole activity most commonly associated with AAT and the only functional release-criteria for commercial plasma-purified AAT, constitutes only a fraction of its clinically favorable mechanism of action, and represents an activity that might actually render the molecule *susceptible* to irreversible proteolytic inactivation.

1.5. Clinical indications for AAT treatment to non-deficient individuals

Currently, the only approved clinical indication for the initiation of intravenous AAT augmentation therapy is AATD. Upon diagnosis, these patients receive 60 mg/kg AAT at the slow rate of 0.08 ml/kg/min, once a week, for life. Based on this population of patients, it has been concluded that the use of AAT is safe for a wide range of ages, including outcomes from long-term studies that extended for up to 7 and 13 years of weekly infusion sessions.[4]

Based on the safety of AAT and its newly appreciated biological activities, expanding its clinical indications became an attractive possibility and one of fast-track approval by regulatory authorities. In fact, there is no requirement for a basic toxicity trial of infusing AAT to individuals that are not AAT deficient. Three clinical trials have been completed and published in the past decade: two reported AAT treatment for recently-diagnosed type 1 diabetes [45,46] and one reported outcomes of AAT treatment for acute myocardial infarction. [47] The diabetes trials are of particular importance as they are the first to report safety of administration of AAT to pediatric and adolescent individuals. Importantly, these patients displayed production of endogenous insulin, as determined by serial measurements of c-peptide, which reflected a positive trend of either slowed-deterioration or in some cases improved production. Table 1 presents a list of active clinical trials listed in the national institutes of health (NIH) registry and that are unrelated to AATD. Two such studies attempt to use AAT to treat treatment-resistant graft versus host disease (GvHD), a potentially life-threatening condition in which transplant-residing immunocytes attack host cells and tissues. Three recent studies attempt to assess the effect of AAT therapy for recently-diagnosed type-1 diabetes; two studies examine AAT treatment for improving pancreatic islet transplant survival and one study examines the efficacy of AAT treatment on lung transplantation. One study is investigating treatment with AAT for relapsing neuromyelitis optica, a condition involving inflammation and demyelination of the optic nerve and spinal cord, and one additional study assesses treatment with AAT for the amelioration of organ injury after cardiac surgery.

Table 1. Active clinical trials involving AAT for non AATD patients.

Registry	Subject	Start date	Estimated completion
NCT01523821	Alpha-1 antitrypsin (AAT) in treating patients with acute graft versus host disease (GvHD)	November 2013	June 2016
NCT01700036	A pilot study of AAT in steroid refractory acute GvHD	July 2013	July 2017
NCT02005848	Phase II–III study to evaluate the efficacy and safety of Glassia® in type-1 diabetes	April 2014	April 2016
NCT01319331	The effects of AAT on the progression of type 1 diabetes	October 2010	October 2015
NCT02093221	Study of human plasma-derived alpha1-proteinase inhibitor in subjects with new-onset type 1 diabetes mellitus	March 2014	May 2016
NCT02520076	Aralast NP in islet transplant	August 2015	February 2017
NCT02464878	Multicenter trial of the effect of AAT on islet transplant engraftment and durability after renal transplant	May 2016	January 2018
NCT02614872	Study to evaluate the safety and efficacy of intravenous Glassia® treatment in lung transplantation	January 2016	January 2020
NCT02087813	Pilot study of AAT to treat neuromyelitis optica relapses (A1AT for NMO)	March 2014	March 2016
NCT02191839	Single dose administration of AAT for the amelioration of organ injury in patients undergoing cardiac surgery	July 2014	July 2015

In the present review, we provide a comprehensive summary of recent patent-protected developments in the field of novel clinical indications for AAT and innovations in AAT production.

2. Results

2.1. Patent database search

On 28 December 2015 an online search was held on both the US Patent Collection database and the AppFT database. Patents were obtained using the restrictions of dates from 1 January 2012 to 31 December 2015 and the search terms: 'AAT' alongside claims for 'AAT', 'treatment or treating' and 'method'. The search held on the US Patent Collection database yielded 51 results and the search in AppFT database yielded an additional 247 results. Search results were meticulously filtered for duplicates and non-therapeutic-related results, yielding a final count of 12 patent applications and 7

patents relevant for the scope of the present review. Patents published under the same title but containing different claims were grouped.

2.2. Patents on AAT production

To date, clinical grade AAT for augmentation therapy is supplied by a process of extraction and affinity-purification of the protein from human plasma, a procedure that has been described and patented about three decades ago. The mildly varying, initial crude processes have undergone multiple minor alterations until the time of the preset review, cumulatively reaching a fairly satisfactory present-day protocol.

Interestingly, in the past few years, some modifications to these protocols have surfaced in the patent database that focus on either modified purification of the material, or its synthesis as a recombinant protein. In a patent dating 2013, metal chelate chromatography is applied to the process of AAT purification from a solution containing primarily AAT and albumin, i.e. Cohn's cold ethanol fractions II, III, or 1-IV (Table 2). In another series of patents and a patent application spanning 2013–2015, AAT is produced by either chemical precipitation, pH modification and fractioning, or a disulfide-reducing agent and protein adsorbing material; as in the former, chromatography is used in order to depict the final product. The major incentive for these added purification parameters relates to an attempt to separate Apolipoprotein 1A from the solution.

With the capacity to avoid human products altogether, recombinant technology has been applied for AAT production as early as 1983. Being that the condition of AATD is a so-called one-gene-disease, the obvious need for replacing the lack of sufficient AAT with recombinant AAT has enjoyed impressive advances in multiple platforms, albeit, using the native sequence for human AAT. As such, the actual sequence has precluded patentability of the produced material, and was focused more on the post-translational composition of the protein. In a 2012 patent application ('recombinant human AAT',[6]) the production of recombinant AAT comprising of N-linked glycans wherein at least 10% of the glycans are tetra-antennary and the degree of capping with sialic acid is at least 50%, resulting in a product that is superior in similarity to native circulating unpurified AAT. The inventors in that patent expressed AAT in PER.C6® cells, immortalized human retinal derived cells, as opposed to other common protein-expressing cellular platforms that while may supply large amounts of recombinant protein, fall short in providing a form of AAT that bares the desired post-translational glycosylation pattern.

Other recombinant AAT protocols have had AAT fused to various protein carriers. In 2013, such a patent application was issued describing the production of Fc-fusion recombinant AAT molecule, later also mentioned to have superior serum half-life to the wild-type version of the protein, while still retaining its anti-inflammatory properties.[54]

Table 2. Patents on the production and purification of AAT (2012–2015).

Inventor(s)	Importance	Reference
Brinkman et al.	Production of recombinant AAT comprising of N-linked glycans wherein at least 10% of the glycans are tetra-antennary and the degree of capping with sialic acid is at least 50%	[6]
Brinkman et el.	AAT separation and purification from a fraction of human blood plasma using (1) ethanol and precipitation, (2) fumed silica or (3) dithiotheitol, fumed silica, and exchange/ hydrophobic chromatography	(1) [48] (2) [49] (3) [50]
Dinarello et al.	Production of AAT-Fc fusion molecule	[51]
Kee et al.	A streamlined method for purifying AAT from AAT-containing protein mixture by cleavage of disulfide bonds with a reducing reagent which does not affect AAT, solid protein adsorbing material and chromatographic purification	[52]
Kumpalume et al.	Separation of AAT from a solution containing AAT and albumin by metal chelate chromatography	[53]

2.3. Patents on therapeutic uses of AAT

Between 2012 and 2015, the possibility of treating non-deficient individuals with AAT has given rise to clinical approaches that involve the *functions* of AAT rather than its mere circulating levels. Preclinical evidence pointed at a profound effect on immune responses in a manner which appears to allow an intact immune system to combat pathogens rather than innocent mammalian cells. Nonetheless, previous patents precluded obvious extensions of the use of AAT and required new patents to focus on superior novelty and narrower clinical vignettes.

2.3.1. Patents on allograft and xenograft protection

These patents address the potential clinical benefit of applying AAT as a treatment for the prevention of graft rejection. Currently, post-transplantation treatment protocols are comprised of long-term supplementation of immunosuppressive drugs aimed to keep the balance between graft protection and a functioning immune system capable of defending the host from pathogens. Unfortunately, in several cases, such as in the case of pancreatic islet transplant for type 1 diabetes individuals, the immunosuppressive treatment itself was found to be toxic for the grafted cells.[55,56]

The cumulative data regarding the immunomodulatory capabilities, which AAT harbors was applied repeatedly in recent years as the background for several groundbreaking studies in the field of graft transplantation and protection [28,30,57,58] proving that the immune system can be re-educated so as to 'tolerate' the differences between the graft and the host, even without immunosuppression. In a patent application published in 2012, Lewis et al. describe a treatment method aimed at reducing the risk of transplant rejection by immune-tolerance therapy using commercial AAT. The notion of using AAT as an immune-tolerogenic drug was further advanced in a patent application published in 2015, which holds several novelties: a supplementation of AAT treatment with temporal T-cell depletion using anti-CD4 antibodies, anti-CD8 antibodies or their combination, and the possibility of applying the combination treatment in xenotransplants, i.e. animals and humans.

2.3.2. Patents on protection from GvHD

Addressing the clinical entity of an injurious unwanted immune response following bone-marrow or organ grafting, two 2014 patent applications were published describing the use of AAT in order to modulate the response of a grafted immune system in a recipient individual, commonly termed GvHD. While the reduction of risk for GvHD was already described in the patent from 2012, this is the first time AAT is described as a treatment option other than immunosuppression, for existing ongoing GvHD.

2.3.3. Patents on type 1 autoimmune diabetes

Several patents describe the use of AAT as a therapeutic for recent onset autoimmune diabetes. Based on a large volume of preclinical data, the use of AAT for altering the course of type 1 autoimmune diabetes has been extensively studied in the past decade. Accordingly, several patents regarding this approach have been issued in the year 2012 with mild differences between patent contents (Table 3). The use of AAT for diabetes has been suggested in a prior patent dating back to 1999 [31] with regards to protection of cells from apoptotic death and to 2008 with specific regards to diabetes.[59] In 2012, two patents applications addressed the therapeutic use of AAT in diabetes. The novelty of these patents was in their targeted populations; the first aims for a population with active insulin-secreting beta cells as evident by serum c-peptide levels while the second aims for a population in which the disease diagnosis is established upon the presence of apoptotic beta cells. Importantly, of all the patents in the past in which AAT has been considered for therapy and that were a generalized approach to lists of diseases, these are the first that appear at a date that has passed the publication of the first clinical trial to administer AAT to non-deficient individuals.[46]

2.3.4. Patents on reduction of bacterial burden

Using AAT as an agent that may reduce bacterial burden is detailed in a few recent patents. Accumulating evidence of AAT's inflammation reducing capabilities has risen concerns that opportunistic bacterial infections might become a serious threat to the life-long users of AAT augmentation therapy. However, clinical studies show that patients under AAT augmentation therapy not only do not suffer from opportunistic infection but rather seem to have reduced lung infection rates.[70–72] This apparent contradiction was further explored in several pulmonary and extra-pulmonary animal models, all of which suggested an anti-bacterial property of AAT *in vivo*.[27,31,73]

The first patent to describe the use of AAT for the treatment of bacterial infection was published in 2005, [74] describing the administration of AAT as a means of protection from and treatment of mycobacterial infection. On this ground, one patent application was published in 2012 and two in 2014, all of which describe the construct of an AAT-Fc fusion molecule, already known to be anti-inflammatory and cytoprotective, [43,44,54] to treat and prevent mycobacterial infection.

2.3.5. Patents on cytoprotection during radiation therapy

The cytoprotective properties of AAT and its ability to diminish the rate of apoptosis, necrosis and other forms of cellular death have gained solid evidence in the past decade, both *in vitro* and *in vivo*.[19,24–27] These qualities are harnessed in a 2011 patent that describes the therapeutic use of AAT as an anti-necrotic agent.[42] Another patent was published in 2015, describing the administration of AAT to subjects exposed to or scheduled to be exposed to radiation in order to prevent or minimize cellular death related to radiation-induced cellular damage.

2.3.6. Patent on the treatment of chronic fatigue syndrome

Use of AAT as a therapeutic for the possible amelioration of CFS is represented in a 2012 patent. CFS is a medical condition affecting multiple bodily systems with a yet unclear etiology. [75] One of the possible triggers leading to the condition is the chronic activation of the immune system as a result of viral infections or metabolic abnormalities.[76–78] Quintana et al. were first to describe the use of AAT infusions as a possible treatment for CFS.[64] The authors of that patent attribute the alleviation of symptoms and improvement in clinical measurements to the role of AAT in the regulation of gene expression related to the immune system as well as to the anti-inflammatory effect on immunocytes associated with CFS. Whether AAT enters the brain and modifies *local* cell responses is highly unlikely in the healthy brain; yet evidence of AAT within the central nervous system has been documented in cases of a compromised blood brain barrier: ischemic stroke, [1] Parkinson's disease, [2] Alzheimer's disease, [3] and multiple sclerosis.[4]

Table 3. Patents on novel clinical entities to be treated by AAT (2012–2015).

Inventor(s)	Importance	Reference
Crapo et al.	Use of AAT to ameliorate the adverse effects of radiation exposure in patients	[60]
Lewis et al.	Use of AAT for the treatment of type 1 diabetes	[61]
Lewis et al.	Use of AAT for the promotion of graft survival and the treatment and prevention of GvHD	[62]
Lewis et al.	Use of AAT and temporal T-cell depletion for the prevention of allograft and xenograft rejection	[63]
Quintana et al.	Use of AAT for the treatment of cognitive impairment associated with chronic fatigue syndrome (CFS)	[64]
Shapiro	Use of AAT Fc fusion molecule for the treatment and prevention of bacterial and mycobacterial infections, including *B. anthracis*	[65] [66]
Shapiro	Use of AAT for the treatment diabetes.	[67]
Shapiro et al.	Use of AAT for the treatment of GvHD	[68] [69]

3. Conclusion

In this review, we describe patented production and therapeutic uses of AAT between 2012 and 2015. Five patents address production of AAT and eight patents include novel clinical indications. Collectively, these patents accurately depict a watershed moment in the predicted therapeutic role of AAT in medicine. The diseases addressed in these patents are all directed at patients that exhibit *normal* levels of circulating AAT: type 1 autoimmune diabetes, GvHD, acute myocardial infarction, transplant candidates, patients with CFS, patients with a mycobacterial infection, and patients undergoing radiation therapy. Production techniques aim at fine-tuning some aspects of the classic plasma-derived product, and the molecule itself is represented in some patents as a

recombinant entity. Taken together, a rather stirring profile after relatively uneventful 30 years of applying plasma-purified AAT to AAT deficient individuals.

4. Expert opinion

Based on long-term and well-established treatment protocol as well as an outstanding safety record, recent studies appear to propel AAT into the highway of mainstream therapeutics that target inflammation and other unwanted immune responses. However, being visible for such a lengthy period of time, AAT appears to challenge the process of patenting, necessitating a significant distance from the previously described properties of the molecule toward unique biologic aspects of it, and from a monotonous production practice toward an effervescent niche of novel therapeutic forms of AAT. This snapshot of the role of AAT in contemporary medicine, as evident through patented articles, is probably the tip of the iceberg that is expected to rapidly emerge as the understanding of the functions of this molecule and the molecular engineering capabilities grow side-by-side, toward an era of medicine in which preference is conferred to the harnessing of native biological pathways and native, or at least native-based, molecules.

The capacity to apply AAT infusion sessions for non-deficient individuals is presently achievable in the form of off-label use. This gives the physicians the opportunity to treat patients that are otherwise not eligible for AAT infusions or related clinical trials, establishing the treatment protocol based on literature with profound safety in mind. At times, off-label treatment proposals may be supported by an added biological basis, such as the established phenomenon of an inactivated form of circulating AAT in diabetic patients, or the lack of appropriate inducible levels of AAT in both the third trimester of pregnancy and during inflammatory acute phase responses.

The emergence of unsatisfactory levels or function of AAT is a relatively young niche that may encompass a myriad of medical conditions, including as a preventative measure, such as in the case of elderly patients that are susceptible to bacterial pneumonia, any patients during prolonged hospitalization periods and patients receiving elective immunosuppression or chemotherapy. The field is open for clinical use, yet is exceptionally difficult to patent for so-called novel clinical indications, as the information that is already in the public domain is quite vast. Nonetheless, novelty in the patent profile of therapeutic uses for AAT has taken an interesting turn in 2012–2015.

There is little mention of AAT as monotherapy. Combinatory approaches render its use patentable, so long as the choice of drug combination is not obvious. There is practically no reliance on its anti-proteolytic biochemical property as mechanism of action, and there are traces of mention regarding uses of the presently available clinical-grade AAT and its common purification protocols. These all aim at distancing their content from the obvious use of AAT in the context of unwanted immune, inflammatory, and tissue injurious conditions. Thus, the trickle of patent-protected novel clinical applications of AAT is bound to continue to narrow toward an engineered molecule that, ideally, can no longer be called AAT.

Among the greatest challenges facing the application of newly gained understandings regarding the functions of AAT in both disease and health, is the limited availability of clinical-grade AAT. With emerging evidence for superiority of some recombinant forms of AAT, attention is drawn toward *uniquely modified* recombinant formulations. Such a path holds several obstacles, some of which are biological, such as the requirement for a highly defined glycosylation pattern, and some are practical, such as the setback of a standard regulatory process for approval of non-native molecules. However, advancements in the field of recombinant protein design, alongside with better understanding of the post-translational modification processes and their importance to the functions of AAT, appear more tangible today than in previous decades. These technologies provide not only a drastic drop in cost and the flexibility for a scale-up in the production of the product, but also an opportunity to manipulate various protein domains within AAT and possibly develop modified, superior and perhaps disease-oriented formulation based on AAT. For example, controlling half-life and pharmacokinetic distribution may allow for further development of diverse routes of administration of AAT and tissue targeting. Such developments are of importance as weekly infusion sessions of AAT are known to hold low patient compliance rates, and are inapt in some of the suggested clinical applications of AAT, such as the rapidity required for addressing acute myocardial infarction, or intestinal localization for gut GvHD and inflammatory bowel diseases.

In addition to optimizing the production of the native protein sequence of AAT, some segments in the protein may be targeted for mutational optimization, as commonly applied in peptide technology. In the case of AAT, these may afford enhanced functions in the fields of anti-bacterial, anti-inflammatory, and immunomodulatory activities that are presently suggested in preclinical studies.

Taking into account that human AAT is a naturally occurring protein, alongside the fact that it rises during inflammatory responses and during the last trimester of healthy pregnancies, and has been injected intravenously to AATD patients in the past three decades with little to no side effects, it is reasonable to deduce that in the coming few years AAT therapy will enter the clinical realm as a therapeutic directed toward indications outside AATD. Yet, there are some major milestones on the way to applicability of this trend. For one, each clinical indication will require a specifically tailored clinical study for, at the least, efficacy, and dosing. Several of such trials are already in progress, including recently diagnosed type 1 diabetes, treatment-resistant GvHD, and acute myocardial infarction. In these examples, even though pediatric patients were included, there was no requirement for a phase I toxicology study, granting a fast-track process of ethical approval for trial design. The second, or rather parallel milestone would be the capacity to generate a recombinant form of bioactive AAT for the mere reason of the anticipated large size of target population. As this step in and of its own

will require a more stringent regulatory path, it may well be the case that native AAT will already be approved for novel clinical indications, while the development of a recombinant form of the drug will complete toxicology requirements ideally at the same time. Being cheaper to manufacture at a larger scale than the classic product, this specific avenue might be limited only by the degree of patentability.

The amount of data published regarding AAT as a target of research has been growing steadily in recent years, reaching 120 papers published in the year 2015 alone; each study exposes yet another piece of the ever growing spectrum of activities that this glycoprotein seems to possess. It is believed that as our understanding of the functions held within this unique molecule will deepen, so will our ability to apply it in the form of a better-tailored treatment for particular aspects of unmet clinical needs. For example, in the context of xenogeneic organ and cell transplants, a benefit over present options was found only in the use of AAT as an adjunct therapy; nonetheless, parameters such as timing of treatment, distribution of doses, accompanying therapeutics and other particularities for each and every clinical subset of these procedures, are still at large. The same lack of information exists with regards to the various types of autoimmune conditions that are on the pipeline for incorporating AAT into their therapeutic course. Based on preclinical data these would include multiple sclerosis, inflammatory bowel diseases, rheumatoid arthritis and systemic lupus erythematosus. Evidence for cytoprotective properties may favor the incorporation of AAT as a means of minimizing the occurrence of cell injury during cytotoxic treatments, including radiation therapy, and immunosuppressive regimens with known organ-specific toxicities. The ultimate goal would be based on these attractive directions and the increasing knowledge base regarding structure–function attributes of AAT, that a formulation be developed *specifically* for each condition, and routes of administration extend beyond the present infusion protocol toward optimized drug half-life and desired outcomes.

It may be concluded that due to the complexity and multitude of functions of AAT, a multi-disciplinary scientific approach, combined with academic–industrial collaborations world-wide will reveal the outstanding potential of AAT and help in its transition toward a biologic, aimed at multiple unmet, often treatment-resistant and often lethal medical needs.

Declaration of interest

EC Lewis is a scientific advisor to Kamada Ltd., Israel. 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 apart from those disclosed.

References

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

1. Laurell CB, Eriksson S. The electrophoretic alpha1-globulin pattern of serum in alpha1-antitrypsin deficiency. 1963. Copd. 2013;10 (Suppl 1):3–8.
2. Cowden DI, Fisher GE, Weeks RL. A pilot study comparing the purity, functionality and isoform composition of alpha-1-proteinase inhibitor (human) products. Curr Med Res Opin. 2005;21:877–883.
3. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med. 2009;360:2749–2757.
4. Teschler H. Long-term experience in the treatment of alpha1-antitrypsin deficiency: 25 years of augmentation therapy. Eur Respir Rev. 2015;24:46–51.
5. Hubbard RC, Crystal RG. Augmentation therapy of alpha 1-antitrypsin deficiency. Eur Respir J Suppl. 1990;9:44s–52s.
6. Brinkman EC, Hack CE, Van Den Nieuwenhof I, Recombinant human alpha1-antitrypsin. US20120214747A1. 2013.
7. 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. J Clin Invest. 1981;68:1158–1165.
8. Cohn EJ, Strong LE, et al. Preparation and properties of serum and plasma proteins; a system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc. 1946;68:459–475.
9. Janciauskiene S, Eriksson S. In vitro complex formation between cholesterol and alpha 1-proteinase inhibitor. FEBS Lett. 1993;316:269–272.
10. Karlsson H, Leanderson P, Tagesson C, et al. Lipoproteomics II: mapping of proteins in high-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. Proteomics. 2005;5:1431–1445.
11. Kolarich D, Turecek PL, Weber A, et al. Biochemical, molecular characterization, and glycoproteomic analyses of alpha(1)-proteinase inhibitor products used for replacement therapy. Transfusion. 2006;46:1959–1977.
12. Alkins SA, O'Malley P. Should health-care systems pay for replacement therapy in patients with alpha(1)-antitrypsin deficiency? A critical review and cost-effectiveness analysis. Chest. 2000;117:875–880.
13. Hosokawa N, Tremblay LO, You Z, et al. Enhancement of endoplasmic reticulum (ER) degradation of misfolded Null Hong Kong alpha1-antitrypsin by human ER mannosidase I. J Biol Chem. 2003;278:26287–26294.
14. Wu Y, Swulius MT, Moremen KW, et al. Elucidation of the molecular logic by which misfolded alpha 1-antitrypsin is preferentially selected for degradation. Proc Natl Acad Sci U S A. 2003;100 (p):8229–8234.
15. Kwon KS, Yu MH. Effect of glycosylation on the stability of alpha1-antitrypsin toward urea denaturation and thermal deactivation. Biochim Biophys Acta. 1997;1335:265–272.
16. Casolaro MA, Fells G, Wewers M, et al. Augmentation of lung antineutrophil elastase capacity with recombinant human alpha-1-antitrypsin. J Appl Physiol (1985). 1987;63:2015–2023.
17. Stockley RA. The multiple facets of alpha-1-antitrypsin. Ann Transl Med. 2015;3:130.
    - **A well-written paper, describing the various merits of AAT, which exist alongside its anti-protease quality**.
18. Ozeri E, Mizrahi M, Shahaf G, et al. alpha-1 antitrypsin promotes semimature, IL-10-producing and readily migrating tolerogenic dendritic cells. J Immunol. 2012;189:146–153.
19. Pott GB, Chan ED, Dinarello CA, et al. Alpha-1-antitrypsin is an endogenous inhibitor of proinflammatory cytokine production in whole blood. J Leukoc Biol. 2009;85:886–895.
20. Tilg H, Vannier E, Vachino G, et al. Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells. J Exp Med. 1993;178:1629–1636.
21. Martin SL, Downey D, Bilton D, et al. Safety and efficacy of recombinant alpha(1)-antitrypsin therapy in cystic fibrosis. Pediatr Pulmonol. 2006;41:177–183.
22. Bergin DA, Reeves EP, Meleady P, et al. alpha-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. J Clin Invest. 2010;120:4236–4250.

23. Finotti P, Pagetta A. A heat shock protein70 fusion protein with alpha1-antitrypsin in plasma of type 1 diabetic subjects. Biochem Biophys Res Commun. 2004;315:297–305.
24. Ochayon DE, Mizrahi M, Shahaf G, et al. Human alpha1-antitrypsin binds to heat-shock protein gp96 and protects from endogenous gp96-mediated injury in vivo. Front Immunol. 2013;4:320.
25. Guttman O, Baranovski BM, Schuster R, et al. Acute-phase protein alpha1-anti-trypsin: diverting injurious innate and adaptive immune responses from non-authentic threats. Clin Exp Immunol. 2015;179:161–72.

•• **A comprehensive review describing the anti-inflammatory, immuno-regulatory, and cytoprotective properties of AAT in an easy to follow and understand manner**.

26. Bergin DA, Hurley K, McElvaney NG, et al. Alpha-1 antitrypsin: a potent anti-inflammatory and potential novel therapeutic agent. Arch Immunol Ther Exp (Warsz). 2012;60:81–97.
27. Kaner Z, Ochayon DE, Shahaf G, et al. Acute phase protein alpha1-antitrypsin reduces the bacterial burden in mice by selective modulation of innate cell responses. J Infect Dis. 2015;211:1489–1498.
28. Ehlers MR. Immune-modulating effects of alpha-1 antitrypsin. Biol Chem. 2014;395:1187–1193.
29. Lewis EC, Shapiro L, Bowers OJ, et al. Alpha1-antitrypsin monotherapy prolongs islet allograft survival in mice. Proc Natl Acad Sci U S A. 2005;102:12153–12158.
30. Lewis EC. Expanding the clinical indications for alpha(1)-antitrypsin therapy. Mol Med. 2012;18:957–70.

• **An elegant paper calling for the expansion of clinical indications for the use of AAT beyond the scope of AAT deficiency**.

31. Cantin AM, Woods DE. Aerosolized prolastin suppresses bacterial proliferation in a model of chronic Pseudomonas aeruginosa lung infection. Am J Respir Crit Care Med. 1999;160:1130–1135.
32. Congote LF. Serpin A1 and CD91 as host instruments against HIV-1 infection: are extracellular antiviral peptides acting as intracellular messengers? Virus Res. 2007;125:119–134.
33. Bristow CL, Babayeva MA, LaBrunda M, et al. alpha1Proteinase inhibitor regulates CD4+ lymphocyte levels and is rate limiting in HIV-1 disease. PLoS One. 2012;7:e31383.
34. Moreno JA, Ortega-Gomez A, Rubio-Navarro A, et al. High-density lipoproteins potentiate alpha1-antitrypsin therapy in elastase-induced pulmonary emphysema. Am J Respir Cell Mol Biol. 2014;51:536–549.
35. Subramaniyam D, Zhou H, Liang M, et al. Cholesterol rich lipid raft microdomains are gateway for acute phase protein, SERPINA1. Int J Biochem Cell Biol. 2010;42:1562–1570.
36. Vaisar T, Pennathur S, Green PS, et al. Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL. J Clin Invest. 2007;117:746–756.
37. Talmud PJ, Martin S, Steiner G, et al. Progression of atherosclerosis is associated with variation in the alpha1-antitrypsin gene. Arterioscler Thromb Vasc Biol. 2003;23:644–649.
38. Kalis M, Kumar R, Janciauskiene S, et al. alpha 1-antitrypsin enhances insulin secretion and prevents cytokine-mediated apoptosis in pancreatic beta-cells. Islets. 2010;2:185–189.
39. Petrache I, Fijalkowska I, Medler TR, et al. alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. Am J Pathol. 2006;169:1155–1166.
40. Gao W, Zhao J, Kim H, et al. alpha1-Antitrypsin inhibits ischemia reperfusion-induced lung injury by reducing inflammatory response and cell death. J Heart Lung Transplant. 2014;33:309–315.
41. Zhang B, Lu Y, Campbell-Thompson M, et al. Alpha1-antitrypsin protects beta-cells from apoptosis. Diabetes. 2007;56:1316–1323.
42. Nathan I, Lewis E, Khalfin B, Antinecrotic activity of alpha 1-antitrypsin. US 20110237496A1. 2009.
43. Jonigk D, Al-Omari M, Maegel L, et al. Anti-inflammatory and immunomodulatory properties of alpha1-antitrypsin without inhibition of elastase. Proc Natl Acad Sci U S A. 2013;110:15007–12.

•• **A well-written paper, describing in details a non-dependent relationship between the anti-protease and anti-inflammatory qualities of AAT**.

44. 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.
45. Kamada L, Phase II-III Study to Evaluate the Efficacy and Safety of Glassia® in Type-1 Diabetes. 2013. [cited 2016 Jan 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT02005848
46. University of Colorado, D., The Effects of Alpha-1 Antitrypsin (AAT) on the Progression of Type 1 Diabetes. 2011. [cited 2016 Jan 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT01319331
47. University, V.C., Alpha-1 Anti-Trypsin (AAT) Treatment in Acute Myocardial Infarction (VCU-Alpha1RT). 2013. [cited 2016 Jan 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT01936896
48. Brinkman N, et al. Methods for purification of alpha-1-antitrypsin andapolipoprotein A-1. US8436152B2. 2013.
49. Brinkman N, et al. Methods for purification of alpha-1-antitrypsin and apolipoprotein A-1. US8653245B2. 2014.
50. Brinkman N, Bigler D, Bolli R, et al. Methods for purification of alpha-1-antitrypsin and apolipoprotein A-1. US Patent 8,962,802. 2015.
51. Dinarello C, Crapo JD, Kim S, Compositions, Methods and Uses For Alpha-1 Antitrypsin Fusion Molecules. US Patent 20,140,341,899. 2014.
52. Kee SM, et al. Methods of treatment using alpha-1-antitrypsin compositions. US20150320846A1. 2015.
53. Kumpalume P, Podmore A, Dalton J, Method for the purification of alpha-1-antitrypsin. US8580931B2. 2013.
54. 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. 2015. DOI:10.1136/annrheumdis-2014-206966.
55. Weir MR, Fink JC. Risk for posttransplant Diabetes mellitus with current immunosuppressive medications. Am J Kidney Dis. 1999;34:1–13.
56. Drachenberg CB, Klassen DK, Weir MR, et al. Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. Transplantation. 1999;68:396–402.
57. Tawara I, Sun Y, Lewis EC, et al. Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci U S A. 2012;109:564–569.
58. Mizrahi M, Cal P, Rosenthal M, et al. Human alpha1-antitrypsin modifies B-lymphocyte responses during allograft transplantation. Immunology. 2013;140:362–373.
59. Shapiro L, Methods and compositions for treating diabetes. US8071551B2. 2008.
60. Crapo JD, Marcondes AMQ, Deeg HJ, Method for ameliorating radiation exposure effects with alpha-1 antitrypsin. US8980574B2. 2015.
61. Lewis EC, et al. Compositions, methods and uses for treatment of type 1 diabetes. US20120225813A1. 2012.
62. Lewis EC, Dinarello CA, Shapiro L, Methods and compositions for treatment of graft rejection and promotion of graft survival. US20120045460A1. 2011.
63. Lewis E, Combined therapy of alpha-1-antitrypsin and temporal t-cell depletion for preventing graft rejection. US20150010581A1. 2013.
64. Quintana AG, Martin JA, Use of alpha-1-antitrypsin for the preparation of drugs for the treatment of chronic fatigue syndrome. US9205134B2. 2012.
65. Shapiro L, Compositions of, and methods for, alpha-1 anti-trypsin Fc fusion molecules. US20140370036A1. 2014.
66. Shapiro L, Compositions of, and methods for, Alpha-1 antitrypsin Fc fusion molecules. US8633305B2. 2014.
67. Shapiro L. Methods and compositions for treating diabetes. US20120040913A1. 2012.
68. Leland S, Lewis EC, Dinarello CA, Compositions and methods related to graft versus host disease and treatments thereof. US20140242096A1. 2014.
69. Leland S, Lewis EC, Dinarello CA, Compositions and methods related to graft versus host disease and treatments thereof. US20140127201A1. 2014.

70. Griese M, Latzin P, Kappler M, et al. alpha1-Antitrypsin inhalation reduces airway inflammation in cystic fibrosis patients. Eur Respir J. 2007;29:240–250.
71. Lieberman J. Augmentation therapy reduces frequency of lung infections in antitrypsin deficiency: a new hypothesis with supporting data. Chest. 2000;118:1480–1485.
72. Petrache I, Hajjar J, Campos M. Safety and efficacy of alpha-1-antitrypsin augmentation therapy in the treatment of patients with alpha-1-antitrypsin deficiency. Biologics. 2009;3:193–204.
73. Pott GB, Beard KS, Bryan CL, et al. Alpha-1 antitrypsin reduces severity of pseudomonas pneumonia in mice and inhibits epithelial barrier disruption and pseudomonas invasion of respiratory epithelial cells. Front Public Health. 2013;1:19.
74. Shapiro L, Serine protease inhibitors for treatment of bacterial infections. WO2008142397A1. 2005.
75. Clark JE, Fai Ng W, Watson S, et al. The aetiopathogenesis of fatigue: unpredictable, complex and persistent. Br Med Bull. 2016. DOI:10.1093/bmb/ldv057.
76. Romano GF, Tomassi S, Russell A, et al. Fibromyalgia and chronic fatigue: the underlying biology and related theoretical issues. Adv Psychosom Med. 2015;34:61–77.
77. Afari N, Buchwald D. Chronic fatigue syndrome: a review. Am J Psychiatry. 2003;160:221–236.
78. Morris G, Berk M, Galecki P, et al. The emerging role of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs). Mol Neurobiol. 2014;49:741–756.