We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Recent Advances in the Research and Development of Alpha-1 Proteinase Inhibitor for Therapeutic Use

Elena Karnaukhova*

Division of Hematology,

Center for Biologics Evaluation and Research

Food and Drug Administration

USA

1. Introduction

Human alpha-1-proteinase inhibitor (α_1 -PI) is a well-characterized multifunctional protease inhibitor, the major physiological role of which is inhibition of neutrophil elastase (NE) in the lungs. The importance of α_1 -PI is underlined by its deficiency which is characterized by low levels of α_1 -PI in the circulation. Under such conditions, lower levels of α_1 -PI are transported to tissues, including the fragile alveoli of the lungs. α_1 -PI deficiency (with levels of α_1 -PI in blood below 11 μ M, insufficient for inhibition of proteolytic enzymes in the lungs) is a common genetic condition predisposing α_1 -PI-deficient individuals to the development of chronic obstructive pulmonary disease (COPD). Hereditary α_1 -PI deficiency is classically associated with the development of premature, ultimately fatal, panacinar emphysema. To slow down the progression of emphysema, several licensed α_1 -PI concentrate preparations derived from pooled human plasma are currently available for intravenous augmentation therapy for patients with congenital α_1 -PI deficiency and clinically evident emphysema. In addition, and as an alternative to the plasma-derived α_1 -PI products, multiple efforts have been made to develop recombinant versions of human α_1 -PI over the last three decades. This review describes the recent advances in the research and development of human α_1 -PI for therapeutic use and covers the following: characterization of human α_1 -PI; epidemiology of α_1 -PI deficiency and currently licensed treatment; summary of the manufacturing and recent quality improvements of the α_1 -PI plasma-derived products; safety and efficacy of α_1 -PI intravenous augmentation and alternative routes; development of recombinant versions of human α_1 -PI; conditions other than emphysema that are associated with α_1 -PI; and some other aspects related to the research and development of α_1 -PI for therapeutic use.

* The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy

2. Human α_1 -PI and α_1 -PI deficiency

2.1 Structure and function of α_1 -PI

Human alpha-1-proteinase inhibitor (α_1 -PI), also known as alpha-1-antitrypsin, is the most abundant inhibitor of serine proteases in plasma. It is predominantly synthesized in hepatocytes, but is also produced, to a lower extent, by alveolar macrophages, neutrophils, and some other cells (White et al., 1981; Carlson et al., 1988; Paakko et al., 1996). In healthy individuals, the concentration of α_1 -PI in blood normally varies from 20 μ M to 53 μ M (1.04–2.76 g/L) (Brantly et al., 1988; Brantly et al., 1991) with a half-life in the circulation of about 3–5 days (Crystal, 1989; Kalsheker et al., 2002). Though α_1 -PI has a wide range of inhibitory activities, its main physiological role is known to be the inhibition of polymorphonuclear leukocyte (neutrophil) elastase (NE) in the lungs (Travis, 1988). In the lower respiratory tract of healthy lungs, α_1 -PI provides more than 90% of the anti-neutrophil elastase protection (Crystal, 1991; Crystal et al., 1989). Hereditary α_1 -PI deficiency (with levels of α_1 -PI in blood below 11 μ M, insufficient for inhibition of NE) is classically associated with development of early-onset pulmonary emphysema, a hallmark of α_1 -PI deficiency (Crystal et al., 1989; Snider, 1992). Smoking is known to be the biggest risk factor for developing emphysema; in smokers with α_1 -PI deficiency a severe lung impairment is usually observed in their fourth decade of life.

α_1 -PI is encoded by a single 12.2 kb gene (Pi) located on the long arm of chromosome 14 (Long et al., 1984; Rabin et al., 1986). Over 120 alleles of α_1 -PI have been identified with approximately 35 of them being associated with α_1 -PI deficiency, including Z-allele, which is the most common cause of the deficiency when inherited in a homozygous fashion. Due to a single mutation in the mobile domain (Glu342Lys), the α_1 -PI Z-mutant undergoes aberrant conformational transitions that prompts the protein to aggregate. This results in retention of polymerized α_1 -PI Z mutant within hepatocytes, thus inducing disease conditions in the liver and causing α_1 -PI deficiency in the circulation (Ekeowa et al., 2011; Lomas, 2005; Volpert et al., 2000). The prevalence of three major α_1 -PI variants (PiM, PiS, and PiZ) defines the number of carriers (PiMZ and PiMS) and individuals with deficiency phenotypes (PiZZ, PiSZ, and PiSS). The epidemiology of α_1 -PI deficiency and its clinical manifestations, including lung diseases and liver diseases, has been described in detail (Ekeowa et al., 2011; Luisetti & Seersholm, 2004; Needham & Stockley, 2004; Gooptu & Lomas, 2009). Based on the α_1 -PI serum concentration, a common classification to define α_1 -PI deficiency includes the four major categories: (1) normal (with α_1 -PI serum levels not lower than 20 μ M); (2) deficient (with α_1 -PI concentrations in serum lower than 20 μ M); (3) dysfunctional (with normal α_1 -PI level, but lost or lower inhibitory activity); and (4) null (with α_1 -PI serum concentrations below the detectable level).

α_1 -PI is a 52 kDa glycoprotein belonging to the serine protease inhibitor (serpin) superfamily, which in addition to α_1 -PI also includes α_1 -antichymotrypsin, antithrombin, plasminogen activator inhibitor, C1 esterase inhibitor, and many others (Stein & Carrell, 1995; Silverman et al., 2001). A single polypeptide chain of α_1 -PI is comprised of 394 amino acid residues, including one cysteine, 2 tryptophanes, and 9 methionine residues (Carp et al., 1982; Johnson & Travis, 1979). Three N-linked glycans attached to asparagine residues 46, 83, and 247 represent ~12% of α_1 -PI by molecular weight (Mega et al., 1980a,b; Carrell et al., 1981, 1982). The carbohydrate moiety is comprised of biantennary N-glycans, but also triantennary and traces of tetraantennary structures grounded on the mannose fork core and containing N-acetyl glucosamine, galactose, and terminal negatively-charged sialic

(N-acetylneuraminic) acid (Mega et al., 1980b; Travis & Salvesen, 1983; Kolarich et al., 2006a). The glycosylation pattern is a major cause of the iso-electric focusing (IEF) pattern typical for α_1 -PI with major isoforms M2, M4, M6, and also M7 and M8 due to the N-terminal truncation (Jeppsson et al., 1985; Kolarich et al., 2006a,b). Some characteristics of human α_1 -PI are listed in Table 1. Like the majority of other native glycoproteins, α_1 -PI is intrinsically a highly heterogeneous moiety, mainly due to variably trimmed glycosylation and an N-terminal pentapeptide that can be absent (Hercz, 1985; Krasnewich et al., 1995; Vaughan et al., 1982).

Characteristics	Description
Synonyms	alpha-1-proteinase inhibitor, alpha-1-antitrypsin
Common abbreviations	α_1 -PI, alpha-1-PI, α_1 -AT, alpha-1-AT, A1AT, ATT, AT
Classification	Serine proteinase inhibitor (serpin)
Substrates	Neutrophil elastase, trypsin, chymotrypsin
Molecular weight	52,000 Da (50,300 Da by mass spectrometric analysis)
Glycosylation	Three N-attached carbohydrates (12% w/w)
Polypeptide	Single polypeptide chain of 394 amino acid residues
Heterogeneity	Highly heterogeneous protein
Major isoforms	M2, M4, M6, M7 and M8
Half-life in circulation	3-5 days (for native plasma α_1 -PI)
Concentration in blood	Acute-phase plasma protein, concentration normally varies from 20 μ M to 53 μ M (1.04-2.76 g/L)
Major biological activities	Inhibitory anti-serine proteinase activity Multiple non-inhibitory activities
Aggregation	α_1 -PI Z mutant is naturally prone to aggregation α_1 -PI S mutant aggregates to a lower degree
Physiologically important phenotypes	PiMM (normal); PiSS, PiSZ & PiZZ (deficiency phenotypes); PiZZ, PiSS & PiNull (the most abnormal)
Diagnostic α_1 -PI variants (serum concentrations)	Normal (NLT ^a 20 μ M); Deficient (lower than 20 μ M; Dysfunctional (NLT 20 μ M, inactive); Null (n.d. ^b level)
Diseases related to α_1 -PI deficiency and aggregation	Pulmonary and liver diseases Other rare diseases (putative) ^c

^a NLT, not lower than; ^b n.d., non-detectable; ^c See Table 3

Table 1. Characteristics of human α_1 -PI

Figure 1 shows a crystal structure of α_1 -PI, typical for serpins, which features 9 α -helices, 3 β -sheets (A, B, and C), and a mobile 15-residue reactive center loop (RCL) exposed for interaction with the target serine protease (Johnson & Travis, 1979; Lomas, 2005). Protease attack of the RCL results in cleavage at Met358-Ser359, formation of a covalent α_1 -PI-protease complex with the amino-terminal polypeptide inserted into the A β -sheet, and an overall dramatic conformational change (Huntington et al., 2000; Ludeman et al., 2001; Stratikos & Gettins, 1999; Wilczynska et al., 1997).

Unlike the majority of proteins, α_1 -PI is naturally folded in a metastable structure which is essential for its function. This is not the most thermodynamically stable form, and thus, α_1 -PI is prone to a variety of conformational transitions and modifications (Lomas, 2005; Lomas

et al., 1995). Much like other serpins, α_1 -PI can intramolecularly convert into a more stable latent form, which is inactive, but the biological activity can be restored via denaturation and refolding (Lomas et al., 1995; Silverman et al., 2001).

Fig. 1. Crystal structure of α_1 -PI (PDB 1HP7) in two projections. (A) Front view at the α_1 -PI structure in respect to β -sheet A, and (B) Side view obtained by 90° clockwise rotation of the molecule. The images were obtained using PyMOL (the PyMOL Molecular Graphics System, Version 1.1r1, Schrödinger, LLC).

In addition to its inhibitory antiprotease function, α_1 -PI exhibits a broad spectrum of non-inhibitory activities (Brantly, 2002; Janciauskiene et al., 2011; Nita et al., 2005). Because of the nine methionine residues in α_1 -PI molecule, its plausible role as a putative antioxidant has been suggested (e.g., Levine et al., 1999, 2000).

Due to the abundance of α_1 -PI in human plasma and its conservative tertiary structure with hydrophobic cavities (Elliott et al., 2000; Lee et al., 2001; Parfrey et al., 2003), α_1 -PI has the capacity to bind small hydrophobic molecules. This property has been explored mainly with respect to the peptides and small molecules that may prevent the aggregation of the α_1 -PI Z mutant (Mahadeva et al. 2002; Mallya et al., 2007; Chang et al. 2009).

2.2 The α_1 -PI deficiency and α_1 -PI replacement therapy

There are approximately 60,000-100,000 severely deficient individuals in the United States which define α_1 -PI deficiency as a rare disease. However, according to several publications, α_1 -PI deficiency is widely under- and mis-diagnosed (e.g., de Serres, 2003; Bals et al., 2007). As reported by the World Health Organization (WHO, 1997), only 4% of the individuals with α_1 -PI deficiency cases are identified, and only a portion of them are receiving treatment. Currently licensed treatment of the patients with α_1 -PI deficiency and manifestation of pulmonary emphysema involves intravenous infusion of plasma-derived α_1 -PI preparations with the recommended dose of 60 mg of active α_1 -PI per kg of body weight administered once weekly. To maintain a threshold level of α_1 -PI (11 μ M), α_1 -PI-deficient patients should receive augmentation therapy for the duration of their lives, to slow the progression of emphysema. This nadir level has been determined based on α_1 -PI

levels observed in the plasma of individuals who are heterozygous for Z-mutant α_1 -PI and who do not develop emphysema. Evaluation of the efficacy of α_1 -PI products used in clinical studies is based on surrogate markers: the infusion of α_1 -PI must elevate the circulating serum level of α_1 -PI above an epidemiologically established 'protective threshold' and the protein must be detectable in bronchoalveolar lavage fluid (Juvelekian & Stoller, 2004; Sandhaus, 2009). However, the ability of α_1 -PI augmentation therapy to reduce the progression of emphysema still remains to be proven. Safety and efficacy of intravenous α_1 -PI augmentation are considered in section 3.3.1. For other disease conditions that may possibly benefit from α_1 -PI therapy see section 3.3.3.

3. Research and development of α_1 -PI for therapeutic use

3.1 Plasma-derived α_1 -PI products

3.1.1 Currently approved α_1 -PI products

Currently there are six commercial plasma-derived α_1 -PI products (Table 2) licensed by the US FDA for intravenous treatment of patients with hereditary α_1 -PI deficiency who show evidence of emphysema. Prolastin® (registered trade name of Bayer Corporation since 1987) was the first α_1 -PI product to be approved. Since 2005, when Bayer Corporation was acquired by Talecris Biotherapeutics (Research Triangle Park, NC, USA; www.talecris.com), the product has been manufactured by Talecris. Aralast® (initially registered trademark of Alpha Therapeutic Corporation) was approved in 2003, and has been manufactured under the direction of Baxter Healthcare Corporation since then (Baxter, Westlake Village, CA, USA www.baxter.com). Zemaira® (registered trade name of Aventis Behring since 2003), another available product, is now manufactured by CSL Behring LLC (Kankakee, IL, USA; www.cslbehring-us.com). In 2007, the US FDA approved another of Baxter's preparations of α_1 -PI concentrate - Aralast NP® - that has the same formulation as its predecessor, but differs from the earlier approved product by having a significantly lower content of C-terminal lysine-truncated α_1 -PI (approximately 2% vs. 67%). In 2009, the US FDA approved Prolastin C®, the updated version of the earlier Talecris product that had been on the market for more than two decades. Due to more sophisticated purification and pathogen reduction steps, including two dedicated viral inactivation steps instead of heat treatment, the specific activity of Prolastin C® (above 0.7 mg of functional α_1 -PI per mg of total protein) is twice higher than that of Prolastin®, which means that lower volumes and shorter transfusion time are needed. Most recently, in July 2010, the FDA approved Glassia™ (formerly Respira), a product manufactured by Kamada (Weizmann Science Park, Ness Ziona, Israel; www.kamada.com) and commercially launched by Baxter in the United States and some other countries. Glassia™ is another highly purified α_1 -PI (with specific activity above 0.7 mg of active α_1 -PI per mg of total protein) and the only α_1 -PI product that is available in a ready-to-use liquid form with a shelf-life stability of two years under refrigerated conditions.

α_1 -PI products are manufactured as part of a complex plasma fractionation scheme which was originally developed for large-scale production of albumin, but now also yields many other plasma therapeutics*. Since products are made from pooled human plasma, they may

* The US FDA product approval information is available at <http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/default.htm>

Drug product	Manufacturer	Date of licensure	Product form	Major steps of viral inactivation/removal
Prolastin®	Talecris Biotherapeutics	12/2/1987	Lyophilized powder ^b	Depth Filtration Heat Treatment
Aralast® ^c	Baxter Healthcare Co.	3/21/2003	Lyophilized powder	Solvent/Detergent & Nanofiltration
Zemaira®	CSL Behring	7/8/2003	Lyophilized powder	Heat Treatment & Ultrafiltration
Aralast NP® ^d	Baxter Healthcare Co.	5/4/2007	Lyophilized powder	Solvent/Detergent & Nanofiltration
Prolastin C®	Talecris Biotherapeutics	10/16/2009	Lyophilized powder	Solvent/Detergent & Nanofiltration
Glassia™	Kamada	7/1/2010	Ready-to-use liquid	Solvent/Detergent & Nanofiltration

^a Based on recent publications including (Stockley, 2010; Tonelli & Brantly, 2010)
^b Reconstitution using Sterile Water for Injection is required
^c Aralast®, previously known as Respitin, contains approximately 67% of α_1 -PI with the truncated C-terminal lysine (Lys394)
^d Aralast NP® contains approximately 2% of α_1 -PI with truncation of C-terminal lysine residue

Table 2. The plasma-derived α_1 -PI therapeutic products approved by the US FDA for chronic augmentation and maintenance therapy in adults with congenital α_1 -PI deficiency and clinically evident emphysema^a

carry the risk of transmitting human infectious agents, *e.g.*, some viruses, and theoretically, the Creutzfeldt-Jakob disease (CJD) agent or disease variant agents, as well as emerging or unknown infectious agents. To reduce the potential risk of transmitting infectious agents, the α_1 -PI preparations are manufactured using a number of viral inactivation and removal steps. Currently approved α_1 -PI products differ in the procedures used for pathogen reduction. For instance, the heat treatment procedure used in manufacturing of Prolastin was one of the reasons for higher content of inactive and aggregated α_1 -PI in the product. The manufacturing procedures of the later products (Table 2) include two dedicated steps that are specifically designed for inactivation and removal of viruses (Hotta et al., 2010). Thus, solvent/detergent treatment and nanofiltration are used as the dedicated pathogen reduction steps in the manufacturing of recently approved Prolastin C®, Glassia™ and both Aralast products. Overall, the manufacturing history of plasma-derived α_1 -PI therapeutic products reflects a trend of continuous improvement of product quality.

3.1.2 Heterogeneity of α_1 -PI products

Heterogeneity of α_1 -PI therapeutic preparations is a complex phenomenon. First of all, heterogeneous nature of plasma α_1 -PI is an intrinsic property of the native glycoprotein (see 2.1). Second, the presence of variously processed α_1 -PI forms including latent, cleaved, complexed or aggregated α_1 -PI species, is barely avoidable. However, it must be kept minimal as the inactive protein species have a direct influence on the product’s specific activity. Third, α_1 -PI products purified from pooled human plasma contain certain impurities of other plasma proteins, including albumin, haptoglobin, α_1 -antichymotrypsin, α_1 -lipoprotein, antithrombin III, C1-esterase inhibitor, etc. The human origin of these

impurities ensures their tolerability, however, the level of these plasma proteins in α_1 -PI concentrate may significantly increase the non-therapeutic protein load in the α_1 -PI preparation intended for transfusion. In addition to all that, multistep manufacturing procedures are known to induce various protein alterations, such as aggregation and chemical modifications (*e.g.*, deamidation, cysteinylolation, and C-terminal truncation). Some modifications can be observed by IEF and other techniques (Cowden et al., 2005; Kolarich et al., 2006a, 2006b) and reflected in the product specifications. Currently there are no data that would demonstrate whether these alterations affect the *in vivo* activity, safety, efficacy or immunogenicity of α_1 -PI therapeutic preparations. In general, commercial plasma-derived α_1 -PI products differ in terms of their purity, specific activity, modifications, and excipients (Lomas et al., 1997; Cowden et al., 2005; Stockley, 2010; Tonelli & Brantly, 2010).

3.2 Research and development of the recombinant versions of human α_1 -PI

3.2.1 Advances in the development of recombinant α_1 -PI

The plasma supply *per se* is a limited source and appears to be insufficient to meet anticipated clinical demand. Moreover, despite effective viral inactivation/removal steps in the manufacturing of plasma proteins (Cai et al., 2005; Hotta et al., 2010), the risk of contamination with new and unknown pathogens may still exist. Therefore, recombinant technology has been widely explored as an alternative approach for the production of human α_1 -PI since the pioneering works of the early 1980s (Bollen et al., 1983; Cabezon et al., 1984; Rosenberg et al., 1984). As evident from numerous reports, both from academic research and industry, the human gene for α_1 -PI has been expressed in virtually all available hosts (*E. coli*, various yeasts, fungi, insect cells, CHO cells, human neuronal cells, and produced in transgenic plants and animals). For more details on research and development of recombinant α_1 -PI (r- α_1 -PI) in different systems and advances and limitations of the recombinant approach for production of stable and biologically active α_1 -PI, see our comprehensive 2006 review (Karnaukhova et al., 2006). More recently, the human gene for α_1 -PI has been expressed in filamentous fungi (Chill et al., 2009; Karnaukhova et al., 2007), transgenic tomato plants (Agarwal et al., 2009), tobacco cell cultures (Huang et al., 2009; Nadai et al., 2009), and human neuronal cell lines (Blanchard et al., 2011). Nevertheless, no r- α_1 -PI is available as a licensed therapeutic treatment. In general, the essential criteria for the development of therapeutics for human use are safety, optimal clinical efficacy, and maximum cost-effectiveness. Among many efforts to develop r- α_1 -PI of therapeutic quality (see Karnaukhova et al., 2006), there appear to be only two examples of the r- α_1 -PIs for which development went far enough to get to clinical trials. The first was r- α_1 -PI produced in the yeasts *Saccharomyces cerevisiae* and manufactured by Arriva Pharmaceuticals Inc. (Arriva) for several indications. A nebulized formulation of this non-glycosylated r- α_1 -PI preparation has been intended for the treatment of respiratory disorders including emphysema and COPD (phase II clinical trials), and asthma (pre-clinical studies) (Brown, 2006a). Although animal studies have been considered to be successful (Pemberton et al., 2006), human trials have not been recommended (see review by Stockley, 2010). A topical gel formulation of r- α_1 -PI has been intended for the treatment of dermatitis and other severe dermatological disorders in phase II clinical trials (see Brown, 2006b).

The second example of the advanced development of recombinant human α_1 -PI is large scale production performed in transgenic dairy animals (t- α_1 -PI): sheep [by PPL Therapeutics (UK) in partnership with Bayer Biologicals (USA), (Dalrymple & Garner, 1998;

Wright et al., 1991)], and goats [by Genzyme Transgenics Corporation (USA), (Ziomek, 1998)]. The transgenic α_1 -PI recovered from sheep milk was purified to 99.9% purity. Even so, sheep native α_1 -PI and sheep α_1 -antichymotrypsin were major impurities, at 6.7-18.7 mg/L and 60.3-75.8 parts per million, respectively. Two sequential clinical studies were performed to evaluate the safety and immunogenicity of aerosolized transgenic human α_1 -PI. None of the subjects had an antibody response to human t- α_1 -PI (Tebbutt, 2000; Spencer et al., 2005); however, antibody responses were observed to sheep α_1 -PI and to sheep α_1 -antichymotrypsin (Spencer et al., 2005). Four patients withdrew from the study due to the development of dyspnea and a decline in lung function, and the later product development was terminated.

3.2.2 Pitfalls in the development of r- α_1 -PI for therapeutic use

The general regulatory requirements for biologicals intended for therapeutic use, including r- α_1 -PI, are purity, safety, and efficacy. In order to be effective, therapeutic proteins have to be stable *in vivo* and *in vitro* (Karnaukhova et al., 2006). Reviewing the work performed over the last two decades to produce stable and biologically active r- α_1 -PI of therapeutic quality, one can see basically two major factors that were impeding the progress: (1) impurities that could induce antibody responses and cause adverse reactions in patients, and (2) lower stability than that of plasma counterpart, mainly caused by the lack of glycosylation or non-human type of glycosylation (the latter may also induce immune responses). Although presently the first reason can be technically better solved, removal of trace amounts of non-human native proteins derived from the host, *e.g.*, sheep α_1 -PI, from the human r- α_1 -PI to exclude further adverse reactions, requires a much higher level of purification than was possible at the time of that development. As for the second reason, indeed, glycosylation is considered to be a cause of rapid clearance of r- α_1 -PI from the circulation (Casolaro et al., 1987; Cantin et al., 2002a). Aberrant glycosylation (or lack of glycans) does not necessarily affect biological activity of the recombinant protein, but it is important for its stability. According to recently published data, glycosylation of α_1 -PI does not interfere with the serpin native state flexibility (or instability) essential for its efficient function, though it may confer resistance to degradation by proteases and thus extend its half-life in the circulation (Sarkar & Wintrode, 2011). Extensive work performed over decades for the development of viable r- α_1 -PI of therapeutic quality and lessons learned from these experiences truly paved the way for other protein therapeutics. It is worthwhile to mention two serpins produced in transgenic animals that were recently approved. In 2009, the US FDA approved recombinant antithrombin (ATryn®) produced in the milk of transgenic goats (Fyfe & Tait, 2009). In 2010, another serpin, recombinant human C1-esterase inhibitor (Ruconest®) produced in the milk of transgenic rabbits was granted European marketing authorization (Varga & Farkas, 2011). Both pharmaceutical proteins show a faster clearance, yet it may not be an issue depending on the intended use. For instance, Ruconest® was approved for the treatment of acute attacks of hereditary angioedema, and therefore there is no need to maintain its higher level in blood longer than its action is required. Given a shorter *in vivo* half-life of recombinant α_1 -PI, it has been considered for other administration routes and applications, such as inhalation for the treatment of emphysematous condition, and topical application for various skin diseases. However, a convincing proof of the recombinant product efficacy and safety in appropriate clinical trials is as problematic as it is for plasma-derived α_1 -PI; large clinical trials in the cases of rare diseases are difficult to perform because of small geographically dispersed patient populations. In addition, a limited population means a

limited market, which is less attractive for large investments. No doubt, these reasons markedly slow down the development of r- α_1 -PI.

3.3 α_1 -PI –based therapies

3.3.1 Safety and efficacy of intravenous α_1 -PI augmentation

The intravenous augmentation of α_1 -PI was shown to be safe and well tolerated over a long history of the replacement therapy. However, its impact on disease progression and mortality still remains to be convincingly proven. α_1 -PI augmentation is assumed to slow down the rate of emphysema development and progression and, thus, to improve the life quality and duration of α_1 -PI deficient patients, yet the essential proof of efficacy is missing. According to Hubbard & Crystal (1990), only approximately 2-3% of infused α_1 -PI actually reaches the lungs; and the effectiveness of α_1 -PI replacement therapy has been evaluated mainly on the bases of biochemical (not clinical) criteria (Tonelli & Brantly, 2010). For recently approved α_1 -PI products, their pharmacokinetic equivalence and comparable safety profile to Prolastin were demonstrated (e.g., Stocks et al., 2010). α_1 -PI therapy is a life-long and very expensive treatment that may cost up to \$150,000 (Silverman, 2009) in the United States. Whether this therapy decreases mortality also remains unknown, as there are no reliable data on mortality, as well as morbidity and survival (Gøtzsche & Johansen, 2010a). Some observational studies support the idea that augmentation therapy may help to slow the decline in lung function (Seersholm et al., 1997; Wencker et al., 2001; Kueppers, 2011). But there are also more critical evaluations including the opinion that α_1 -PI augmentation therapy cannot be recommended due to lack of evidence of clinical benefit and the cost of treatment (Gøtzsche & Johansen, 2010a, 2010b). It is currently widely admitted that the efficacy of α_1 -PI augmentation therapy has never been persuasively demonstrated and must be proven in a proper clinical trial. Due to the widespread and small clusters of patients all over the country, conducting a prospective, randomized, placebo-controlled clinical trial is challenging. In addition, the development of emphysema proceeds slowly, creating the additional difficulties of monitoring lung function decline and mortality data (Hutchinson & Hughes, 1997; Schluchter et al., 2000).

3.3.2 Alternative routes of administration of α_1 -PI products

Due to the inconvenience of life-time intravenous augmentation therapy and low levels of α_1 -PI reaching lungs, the inhalation of aerosolized α_1 -PI has been suggested as a less invasive and more efficient way to deliver large amounts of α_1 -PI directly to the lungs where it is most needed (Hubbard et al., 1989; McElvaney et al., 1991; Cockett, 1999). Although strategies for aerosol therapy of α_1 -PI deficiency has been proposed two decades ago (Hubbard et al., 1989; Hubbard & Crystal, 1990), there is still no α_1 -PI aerosolized treatment approved. Several studies examined efficiency of the α_1 -PI inhalation therapy in animals and in humans (Kropp et al., 2001; Siekmeier, 2010). It was demonstrated (Kropp et al., 2001) that significantly more α_1 -PI was deposited in the lungs through the inhalational route than via intravenous infusion (14.6% vs. 2%). Although the inhalation route seems attractive, nevertheless, enabling the inhaled material to reach the lung interstitium, the most important to the emphysematous process region, is still problematic. With regards to recombinant versions of α_1 -PI, it is generally assumed that products directly delivered to the lungs may not require the same degree of stability as α_1 -PI given intravenously. However, as mentioned above, human studies using r- α_1 -PI from transgenic sheep were associated

with adverse reactions due to impurities derived from the host (Spenser et al., 2005). Thus, higher levels of purification and more clinical studies are required.

3.3.3 Other α_1 -PI applications

Currently, α_1 -PI therapeutic preparations are licensed exclusively for one indication, *i.e.*, chronic augmentation and maintenance therapy in individuals with emphysema due to congenital α_1 -PI deficiency. Previously unrecognized inherited disorder, α_1 -PI deficiency was first described in 1963 (Laurell & Eriksson, 1963) based on the serum electrophoretic analysis that revealed five individuals deficient of α_1 -fraction; three of those patients had developed emphysematous conditions. Six years later, in 1969, cirrosis associated with α_1 -PI deficiency was described (Sharp et al., 1969). These findings initiated a concept of linkage between α_1 -PI deficiency and pulmonary and liver diseases. As evident from the available literature, due to the multiple biological activities of α_1 -PI, it has been associated with other lung diseases (first of all, cystic fibrosis) and many non-pulmonary diseases (Table 3). Some of these conditions may possibly benefit from α_1 -PI augmentation therapy (see recent reviews by Blanco et al., 2011 and Janciauskiene et al., 2011).

According to Blanco et al. (2011), α_1 -PI therapy has proven remarkable efficacy in small cohorts of α_1 -PI-deficient patients who also suffer from fibromyalgia, systemic vasculitis, relapsing panniculitis and bronchial asthma. Although the putative benefits of α_1 -PI therapy for treatment of additional rare diseases (some are listed in Table 3) requires much more clinical data than are currently available to support clinical efficacy and safety of α_1 -PI treatment, in general it indicates a clear potential for additional α_1 -PI supply to satisfy the anticipated clinical demand in near future. Because of controversy related to the additional clinical implications of α_1 -PI deficiency, more clinical data are needed to verify whether the reported links between α_1 -PI deficiency and other rare diseases are real or accidental.

As a potent anti-inflammatory agent, α_1 -PI has been investigated in clinical studies for treatment of cystic fibrosis (Jones & Helm, 2009). Whereas patients with emphysematous conditions suffer from the hereditary α_1 -PI deficiency and, thus, insufficient levels of the protease inhibitor in the lungs due to impaired α_1 -PI synthesis in hepatocytes, patients with cystic fibrosis may have normal synthesis of α_1 -PI and suffer from severe pulmonary inflammation due to high excess of NE in the lungs, leading to a progressive loss of lung function (Allen, 1996; Siekmeier, 2010). Therefore, it has been proposed that both groups of patients may benefit from α_1 -PI augmentation therapy to prevent the deleterious effect of free protease (Allen, 1996; Birrer, 1995; Birrer et al, 1996). However, intravenous administration of α_1 -PI did not result in a suppression of the respiratory neutrophil elastase burden (McElvaney et al, 1991). Several studies have been conducted using inhalation of an aerosolized α_1 -PI in cystic fibrosis and α_1 -PI deficiency (Hubbard et al., 1989; Griesse et al, 2001, 2007; Martin et al, 2006; Brand et al, 2009).

Whereas several studies that investigated the efficacy of treatment with an aerosolized α_1 -PI both in patients with cystic fibrosis and in those with α_1 -PI deficiency came to positive conclusions regarding deposition of inhaled α_1 -PI in the lungs and its anti-elastase activity (see review by Siekmeier, 2010), the conclusion from other studies was that treatment with α_1 -PI did not demonstrate any clinical improvements (Martin, 2006). If further clinical studies support the safety and efficacy of an aerosolized α_1 -PI, and it is approved for treatment of cystic fibrosis, the demand for therapeutic α_1 -PI preparations could be significantly increased.

Disease	References
Vasculitis	Dowd et al., 1995; Esnault, 1997; Griffith et al., 1996
Panniculitis	Chowdhury et al., 2002; Gross et al., 2009; Kjus et al., 2002; Smith et al., 1987; Valverde et al., 2008
Fibromyalgia	Ablin et al., 2009; Blanco et al., 2004; Blanco et al., 2010
Asthma	Blanco et al., 2008; Blanco et al., 2011; Eden et al., 1997
Pancreatitis	Rabassa et al., 1995; Needlham & Stockley, 2004
Renal	Szönyi et al., 2006; Ting et., 2008
Diabetes	Kalis et al., 2010; Lisowska-Myjak et al., 2006;
Cancer	Li et al., 2011; Lindor et al., 2010; Topic et al., 2011
Rheumatoid arthritis	Grimstein et al., 2010; Grimstein et al., 2011
Atherosclerosis	Stakisaitis et al., 2001; Talmud et al., 2003;
Acute anterior uveitis	Fearnley et al., 1988; Saari et al., 1986
Chronic rhinosinusitis	Kilty et al., 2008, 2010; Maune et al., 1995

Table 3. Conditions other than emphysema and liver disease possibly associated with α_1 -PI

3.3.4 Research toward the enhancement of α_1 -PI-therapies

During last decade various approaches have been considered for the enhancement of α_1 -PI-based therapies. For instance, to prolong a short half-life of r- α_1 -PI in the circulation, Cantin and co-workers hypothesized that conjugation of r- α_1 -PI with polyethylene glycol (PEG) at Cys²³² could extend the *in vivo* half-life of recombinant protein in blood and lung (Cantin et al., 2002b). According to their data, the site-specific conjugation with either 20 or 40 kD PEG at Cys²³² of nonglycosylated r- α_1 -PI (human) results in an active inhibitor with extended *in vivo* stability. Moreover, 72 h later after airway instillation, the PEG-r- α_1 -PI seemed to be significantly better than glycosylated α_1 -PI at protecting the lung against elastase-induced lung hemorrhage. As an example of the *in vitro* biochemical evaluation of the concept, α_1 -PI has been considered for its affinity to various small ligands and drugs for different reasons. Mainly this approach has been explored with respect to the peptides and small molecules in order to prevent the aggregation of Z mutant (*e.g.*, Mallya et al., 2007; Chang et al. 2009). In the meantime, the protein’s potential for binding small ligands of pharmaceutical interest has been proposed as a promising approach that is directed at, and may ultimately enhance, currently existing α_1 -PI therapies (Karnaukhova et al., 2010). For instance, α_1 -PI’s affinity to retinoic acid, which is known for a wide range of physiological activities including alveolar repair and regrowth (Roche clinical studies, see Stockley, 2010; Massaro & Massaro, 1996, 1997) and tissue rejuvenation in various dermatologic diseases, has been convincingly demonstrated in biochemical experiments *in vitro* (Karnaukhova et al., 2010). As α_1 -PI augmentation therapy cannot cure, but may only slow down, the progression of emphysema, its complexation with retinoic acid could be more efficient for treatment than α_1 -PI alone. It is noteworthy that the interactions of α_1 -PI with several other physiologically active ligands (including porphyrins) may reveal additional properties of this multifunctional serpin.

4. Conclusions

Since α_1 -PI deficiency was first described by Carl-Bertil Laurell and Sten Eriksson (Laurell & Eriksson, 1963) as a condition that could lead to the development of severe obstructive

pulmonary disease, our knowledge about α_1 -PI structure-function relationships and clinical manifestations of α_1 -PI deficiency has increased tremendously. Moreover, multi-disciplinary research efforts prompted the development of α_1 -PI-based augmentation therapy to maintain the inhibitor level above the protective threshold. Since 1987, several α_1 -PI products derived from pooled human plasma have been approved and are currently available to slow down the progression of emphysematous conditions in α_1 -PI-deficient patients. In addition, due to its multiple physiological activities, α_1 -PI has been identified for its putative involvement in several other rare diseases, the treatment of which may possibly benefit from α_1 -PI-based therapies. As an alternative to intravenous administration that may improve the efficacy of α_1 -PI treatment, the inhalation of aerosolized α_1 -PI preparations has been in clinical trials. Recombinant versions of human α_1 -PI have been produced in all available hosts and in several transgenic animals. These efforts made a remarkable impact on the research realm of recombinant protein therapeutics, but did not yet bring any viable version of recombinant α_1 -PI to the treatment. In regards to therapeutic preparations and their use, there are several questions to be addressed when looking to the future. Keeping in mind the long history of replacement therapy using currently approved plasma-derived α_1 -PI products, it is essential that the efficacy of α_1 -PI replacement therapy be clearly demonstrated in prospective, randomized, placebo-controlled trials. Will the efficacy of inhalation therapy using aerosolized α_1 -PI preparations be proven to be superior to that of the intravenous route? Will the recombinant/transgenic versions of human α_1 -PI be optimized to meet the requirements for protein therapeutics? Will other rare diseases currently implicated in association with α_1 -PI and α_1 -PI deficiency be clearly proven to benefit from α_1 -PI treatment? From the standpoint of product quality, safety and efficacy, the current state of research and development of α_1 -PI for therapeutic use demonstrates a symbiosis of the recent achievements and controversies, hopefully typical of our progress.

5. References

- Ablin, J.N., Bar-Shira, A., Yaron, M. & Orr-Urtreger, A. (2009). Candidate-gene approach in fibromyalgia syndrome: association analysis of the genes encoding substance P receptor, dopamine transporter and alpha1-antitrypsin. *Clin Exp Rheumatol*, Vol. 27, No.5 Suppl 56, (September-October 2009), pp. S33-S38, ISSN 0392-856X
- Agarwal, S., Jha, S., Sanyal, I. & Amla, D.V. (2009). Effect of point mutations in translation initiation context on the expression of recombinant human alpha (1)- proteinase inhibitor in transgenic tomato plants. *Plant Cell Rep*, Vol.28, No.12, (December 2009), pp. 1791-1798, ISSN: 0721-7714
- Allen, E.D. (1996). Opportunities for the use of aerosolized α_1 -antitrypsin for the treatment of cystic fibrosis. *Chest*, Vol.110, Supl.6, (December 1996), pp. 256S-260S, ISSN 0012-3692
- Bals, R., Koczulla, R., Kotke, V., Andress, J., et al. (2007). Identification of individuals with alpha-1-antitrypsin deficiency by a targeted screening program. *Respir Med*, Vol. 101, No.8, (August 2007), pp. 1708-1714, ISSN 0954-6111
- Birrer, P. (1995). Proteases and antiproteases in cystic fibrosis: pathogenetic considerations and therapeutic strategies. *Respiration* Vol. 62, Suppl.1, pp. 25S-28S, ISSN 0025-7931

- Birrer, P., McElvaney, N.G., Rudeberg, A., et al. (1994). Protease-antiprotease imbalance in the lungs of children with cystic fibrosis. *Am J Respir Crit Care Med*, Vol.150, (July 1994), pp. 207-213, ISSN 1073-449X
- Blanchard, V., Liu, X., Eigel, S., Kaup, M., Rieck, S., Janciauskiene, S., Sandig, V., Marx, U., Walden, P., Tauber, R. & Berger, M. (2011). N-Glycosylation and biological activity of recombinant human alpha1-antitrypsin expressed in a novel human neuronal cell line. *Biotechnol Bioeng*, Vol.108, No.9, (September 2011), pp. 2118-2128, ISSN 0006-3592
- Blanco, I., Canto, H., de Serres, F.J., Bustillo, E.F. & Rodríguez, M.C. (2004). Alpha-1-antitrypsin replacement therapy efficiently controls fibromyalgia symptoms in two PI ZZ alpha-1-antitrypsin deficiency patients. *J Rheumatol*, Vol.31, No.10, (October 2004), pp. 2082-2085, ISSN 0315-162X
- Blanco, I., Canto, H., Flóres, J., Camblor, C., Cárcaba, V., de Serres, F.J., Janciauskiene, S. & Bustillo, E.F. (2008). Long-term augmentation therapy with alpha-1 antitrypsin in an MZ-AAT severe persistent asthma. *Monaldi Arch Chest Dis*, Vol.69, No.4, (December 2008) pp. 178-82, ISSN 1122-0643
- Blanco, I., Astudillo, A., Domínguez, F., Janciauskiene, S., Cárcaba, V., Gallo, C., Canto, H., de Serres, F.J. & Bustillo, E.F. (2010). Intravenous infusions of purified alpha 1-antitrypsin effectively controls symptoms and reverts muscle biopsy changes in an MZ-alpha-1 antitrypsin deficiency and fibromyalgia syndrome patient. *J Musculoskel Pain*, Vol.18, No.2, (June 2010), pp. 167-172, ISSN 1058-2452
- Blanco, I., Lara, B. & de Serres, F. (2011). Efficacy of alpha1-antitrypsin augmentation therapy in conditions other than pulmonary emphysema. *Orphanet J Rare Dis*, Vol.6, No.14, doi:10.1186/1750-1172-6-14, (April 2011), ISSN 1750-1172
- Bollen, A., Herzog, A., Cravador, A., Herion, P. et al. (1983). Cloning and expression in *E. coli* of full length DNA coding for human A₁AT. *DNA* Vol.4, No.4, pp.255-264, ISSN 0198-0238
- Brand, P., Schulte, M., Wencker, M., Herpich, C.H., Klein, G., Hanna, K. & Meyer, T. (2009). Lung deposition of inhaled α_1 -proteinase inhibitor in cystic fibrosis and α_1 -antitrypsin deficiency. *Eur Respir J*, Vol.34, (August 2009), pp. 354-360, ISSN 0903-1936
- Brantly, M. (2002). α_1 -Antitrypsin: Not just an antiprotease: Extending the half-life of a natural anti-inflammatory molecule by conjugation with polyethylene glycol. *Am J Respir Cell Mol Biol*, Vol.27, No.6, (December 2002), 652-654, ISSN 1044-1549
- Brantly, M., Nukiwa, Y. & Crystal, R.G. (1988). Molecular basis of α_1 -antitrypsin deficiency. *Am J Med*, Vol.84, No. 6A, (June 1988), pp. 13-31, ISSN 0002-9343
- Brantly, M.L., Wittes, J.T., Vogelmeier, C.F., Hubbard R.C., et al. (1991). Use of highly purified α_1 -antitrypsin standard to establish ranges for the common normal and deficient α_1 - antitrypsin phenotypes. *Chest*, Vol.100, No.3, (September 1991), pp. 703-708, ISSN 0012-3692
- Brown, W.M. (2006b). rAA_t (dermatological) Arriva/ProMetric. *Curr Opin Mol Ther*, Vol.8, No.1, (February 2006), pp. 69-75, ISSN 1464-8431
- Brown, W.M. (2006a). rAA_t (inhaled) Arriva/Hyland Immuno. *Curr Opin Mol Ther*, Vol.8, No.1, (February 2006), pp. 76-82, ISSN 1464-8431

- Cabezón, T., De Wilde, M., Herion, P., Lorian, R. & Bollen, A. (1984). Expression of human α_1 -antitrypsin cDNA in the yeast *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA*, Vol.81, No.21, (November 1984), pp. 6594–6598, ISSN 0027-8424
- Cai, K., Gierman, T.M., Hotta, J., Stenland, C.J., Lee, D.C., Pifat, D.Y. & Petteway, Jr. S.R. (2005). Ensuring the biologic safety of plasma-derived therapeutic proteins. Detection, inactivation and removal of pathogens. *Biodrugs*, Vol.19, No.2, pp. 79-96, ISSN 1173-8804
- Cantin, A., Woods, D.E., Cloutier, D., Heroux, J., Dufour, E.K. & Ledoc, R. (2002a). Leukocyte elastase inhibition therapy in cystic fibrosis: role of glycosylation on the distribution of alpha-1-proteinase inhibitor in blood versus lung. *J Aerosol Med*, Vol.15, No.2, (Summer 2002), pp. 141-148, ISSN 0894-2684
- Cantin, A.M., Woods, D.E., Cloutier, D., Dufour, E.K. & Leduc, R. (2002b). Polyethylene glycol conjugation at Cys232 prolongs the half life α_1 proteinase inhibitor. *Am J Respir Cell Mol Biol*, Vol.27, No.6, (December 2002), pp. 659–665, ISSN 1044-1549
- Carlson, J.A., Rogers, B.B., Sifers, R.N., et al. (1988). Multiple tissues express alpha 1-antitrypsin in transgenic mice and man. *J Clin Invest*, Vol.82, No.1 (April 1988), pp. 26–36, ISSN 0021-9738
- Carp, H., Miller, F., Hoidal, J.R. & Janoff, A. (1982). Potential mechanism of emphysema: alpha 1-proteinase inhibitor recovered from lungs of cigarette smokers contains oxidized methionine and has decreased elastase inhibitory capacity. *Proc Natl Acad Sci USA*, Vol.79, No.6, (March 1982), pp. 2041-2045, ISSN 0027-8424
- Carrell, R.W., Jeppson, J.O., Vaughan, L., Brennan, S.O., Owen, M.C. & Boswell, D.R. (1981). Human α_1 -antitrypsin: carbohydrate attachment and sequence homology. *FEBS Lett*, Vol.135, No.2, (December 1981), pp. 301-303, ISSN 0014-5793
- Carrell, R.W., Jeppson, J.O., Laurell, C.B., Brennan, S.O., Owen, M.C., Vaughan, L. & Boswell, D.R. (1982). Structure and variation of human α_1 -antitrypsin. *Nature* (London), Vol.298, No.5872, (July 1982), pp. 329-334, ISSN 0028-0836
- Casolaro, M.A., Fells, G., Wewers, M., et al. (1987). Augmentation of lung antineutrophil elastase capacity with recombinant human alpha-1-antitrypsin. *J Appl Physiol*, Vol.63, No.5, (November 1987), pp. 2015-2023, ISSN 8750-7587
- Chang, Y.P., Mahadeva, R., Chang, W.S., Lin, S.C. & Chu, Y.H. (2009). Small-molecule peptides inhibit Z alpha(1)-antitrypsin polymerization. *J Cell Mol Med*, Vol.13, No.8B, (August 2009), pp. 2304-2316, ISSN 1582-1838
- Chill, L., Trinh, L., Azadi, P., Ishihara, M., Sonon, R., Karnaukhova, E., Ophir, Y., Golding, B. & Shiloach, J. Production, purification, and characterization of human α_1 -proteinase inhibitor from *Aspergillus niger*. *Biotechnol Bioeng*, Vol.102, No.3, (February 2009), pp. 828-844, ISSN 0006-3592
- Chowdhury, M.M., Williams, E.J., Morris, J.S., Ferguson, B.J., McGregor, A.D., Hedges, A.R., Stamatakis, J.D. & Pope, F.M. (2002). Severe panniculitis caused by homozygous ZZ alpha1- antitrypsin deficiency treated successfully with human purified enzyme (Prolastin). *Br J Dermatol*, Vol.147, No.6, (December 2002), pp. 1258-1261, ISSN 0007-0963
- Cockett, M.I. (1999). Technology evaluation: cystic fibrosis therapy, Genzyme. *Curr Opin Mol Ther* Vol.1, No.2, (April 1999), pp. 279-283, ISSN 1464-8431
- Cowden, D.I., Fisher, G.E. & Weeks, R.L. (2005). A pilot study comparing the purity, functionality and isoform composition of alpha-1-proteinase inhibitor (human)

- products. *Curr Med Research Opinion* Vol.21, No.6 (June 2005), pp. 877-883, ISSN 0300-7995
- Crystal, R.G. (1989). The α 1-antitrypsin gene and its deficiency states. *Trends Genet*, Vol. 5, No.12, (December 1989), pp. 411-417, ISSN 0168-9525
- Crystal, R.G., Brantly, M.L., Hubbard, R.C., Curiel, D.T., States, D.J., & Holmes, M.D. (1989). The alpha 1-antitrypsin gene and its mutations. Clinical consequences and strategies for therapy. *Chest*, Vol.95, No.1, (January 1989), pp. 196-208, ISSN 0012-3692
- Crystal, R.G. (1991). α 1-Antitrypsin deficiency: pathogenesis and treatment. *Hospital Practice*, Vol.15, (February 1991), pp. 81-94, ISSN 8750-2836
- Dalrymple, M.A. & Garner, I. (1998). Genetically modified livestock for the production of human proteins in milk. *Biotechnol Genet Eng Rev*, Vol.15, pp. 33-49, ISSN 0264-8725
- de Serres, F.J. (2003). Alpha-1 Antitrypsin deficiency is not a rare disease but a disease that is rarely diagnosed. *Environ Health Perspect*, Vol.111, No.16, (December 2003), pp. 1851-1854, ISSN 0091-6765
- Dowd, S.K., Rodgers, G.C. & Callen, J.P. (1995). Effective treatment with alpha 1-protease inhibitor of chronic cutaneous vasculitis associated with alpha 1-antitrypsin deficiency. *J Am Acad Dermatol*, Vol.33, No.5, Pt. 2, (November 1995), Pt. 2, pp. 913-916, ISSN 0190-9622
- Eden, E., Mitchell, D., Mehlman, B. et al. (1997). Atopy, asthma, and emphysema in patients with severe alpha-1-antitrypsin deficiency. *Am J Respir Crit Care Med*, Vol.156, (July 1997), pp. 68-74, ISSN 1073-449X
- Ekeowa, U.I., Marciniak, S.J. & Lomas, D.A. (2011). α (1)-antitrypsin deficiency and inflammation. *Expert Rev Clin Immunol*, Vol.7, No.2, (March 2011), pp. 243-252, ISSN 1744-666X
- Elliott, P.R., Pei, X.Y., Dafforn, T.R., Lomas, D.A. (2000). Topography of a 2.0 Å structure of α 1-antitrypsin reveals targets for rational drug design to prevent conformational disease. *Protein Sci*, Vol.9, No.7, (July 2000), pp. 1274-1281, ISSN 0961-8368
- Esnault, V.L. (1997). ANCA-positive vasculitis and alpha 1-antitrypsin deficiency: could free ANCA antigens released by neutrophils mediate vasculitic lesions? *Nephro Dial Transplant*, Vol.12, (February 1997), pp. 249-251, ISSN 0931-0509
- Fearnley, I.R., Spalton, D.J., Ward, A.M., Slavin, B. & Muncey, F. (1988). Alpha 1- antitrypsin phenotypes in acute anterior uveitis. *Br J Ophthalmol*, Vol.72, (August 1988), pp. 636-639, ISSN 0007-1161
- Fyfe, A. & Tait, R.C. (2009). Antithrombin- α for the prophylaxis of venous thrombosis in congenital antithrombin deficiency. *Expert Rev Hematol*, Vol.2, No.5, (October 2009), pp. 499-507, ISSN 1747-4086
- Gooptu, B. & Lomas, D.A. (2009). Conformational pathology of the serpins: themes, variations, and therapeutic strategies. *Annu Rev Biochem*, Vol.78, (July 2009), pp. 147-76, ISSN 0066-4154
- Gøtzsche, P.C. & Johansen, H.K. (2010a). Intravenous alpha-1 antitrypsin augmentation therapy for treating patients with alpha-1 antitrypsin deficiency and lung disease *Cochrane Database of Syst Rev*, Vol. 7, (July 2010), p. CD007851, ISSN 1469-493X
- Gøtzsche, P.C. & Johansen, H.K. (2010b). Intravenous alpha-1 antitrypsin augmentation therapy: systematic review. *Dan Med Bull*, Vol. 57, No.9, (September 2010), p. A4175, ISSN 0907-8916

- Griese, M., Latzin, P., Kappler, M., Weckerle, K., Heinzlmaier, T., Bernhardt, T. & Hartl, D. (2007). α_1 -Antitrypsin inhalation reduces airway inflammation in cystic fibrosis patients. *Eur Respir J*, Vol.29, (February 2007), pp. 240-250, ISSN 0903-1936.
- Griese, M., von Bredow, C., Birrer, P., Schams, A. (2001). Inhalation of α_1 -protease inhibitor in cystic fibrosis does not affect surfactant convertase and surface activity. *Pulm Pharmacol Ther*, Vol.14, pp. 461-467, ISSN 1094-5539
- Griffith, M.E., Lovegrove, J.U., Gaskin, G., Whitehouse, D.B. & Pusey, C.D. (1996). C-antineutrophil cytoplasmic antibody positivity in vasculitis patients is associated with the Z allele of alpha-1-antitrypsin, and the P-antineutrophil cytoplasmic antibody positivity with the S allele. *Nephrol Dial Transplant*, Vol.11, (March 1996), pp. 438-443, ISSN 0931-0509
- Grimstein, C., Choi, Y.K., Satoh, M., et al. (2010). Combination of alpha-1 antitrypsin and doxycycline suppresses collagen-induced arthritis. *J Gene Med*, Vol.12, No.1 (January 2010), pp. 35-44, ISSN 1099-498X
- Grimstein, C., Choi, Y.K., Wasserfall, C.H., et al. (2011). Alpha-1 antitrypsin protein and gene therapies decrease autoimmunity and delay arthritis development in mouse model. *J Transl Med*, Vol.9, (February 2011), No.21, pp. 1-13, ISSN 1479-5876
- Gross, B., Grebe, M., Wencker, M., Stoller, J.K., Bjursten, L.M., Janciauskiene, S. (2009). New findings in PiZZ alpha(1)-antitrypsin deficiency-related panniculitis. Demonstration of skin polymers and high dosing requirements of intravenous augmentation therapy. *Dermatology*, Vol.218, No.4, pp. 370-375, ISSN 1018-8665
- Hercz, A. (1985). Proteolytic cleavages in α_1 -antitrypsin and microheterogeneity. *Biochem Biophys Res Commun*, Vol.128, No.1, (April 1985), pp. 199-203, ISSN 0006-291X
- Hotta, J., Chao, S.F., Gall, M., Roth, N.J., Lang, J. & Lee, D. (2010). Effective and robust enveloped virus inactivation by a non-traditional solvent/detergent treatment step. *US Respiratory Disease*, Vol.6, pp. 40-46
- Huang, T.K., Plesha, M.A., Falk, B.W., Dandekar, A.M. & McDonald, K.A. (2009). Bioreactor strategies for improving production yield and functionality of a recombinant human protein in transgenic tobacco cell cultures. *Biotechnol Bioeng*, (February 2009), Vol.102, No.2, pp. 508-520, ISSN 0006-3592
- Hubbard, R.C., Brantly, M.L., Sellers, S.E., et al. (1989). Anti-neutrophil-elastase defenses of the lower respiratory tract in α_1 -antitrypsin deficiency directly augmented with an aerosol of α_1 -antitrypsin. *Ann Intern Med*, Vol.111, (August 1989), pp. 206-212, ISSN 0003-4819
- Hubbard, R.C. & Crystal, R.G. (1990). Strategies for aerosol therapy of alpha1-antitrypsin deficiency by the aerosol route. *Lung*, Vol.168 (Suppl), pp. 565-578, ISSN 0341-2040
- Hutchinson, D.C. & Hughes, M.D. (1997). Alpha-1-antitrypsin replacement therapy: will its efficacy ever be proved? *Eur Respir J*, Vol.10, pp. 2191-2193, ISSN 0903-1936
- Huntington, J.A., Read, R.J. & Carrell, R.W. (2000). Structure of a serpin-protease complex shows inhibition by deformation. *Nature*, Vol.407, No.6806, (October 2000), pp. 923-926, ISSN 0028-0836
- Janciauskiene, S.M., Bals, R., Koszulla, R., Vogelmeier, C., Köhnlein, T. & Welte, T. (2011). The discovery of α_1 -antitrypsin and its role in health and disease. *Respir Med*, Vol.105, (August 2011), pp. 1129-1139, ISSN 0954-6111

- Jeppsson, J. O., Lilja, H. & Johansson, M. (1985). Isolation and characterization of two minor fractions of alpha 1-antitrypsin by high-performance liquid chromatographic chromatofocusing. *J Chromatogr*, (June 1985), Vol.327, pp. 173-177, ISSN 0021-9673
- Jones, A.M. & Helm, J.M. (2009). Emerging treatments in cystic fibrosis. *Drugs*, Vol.69, No.14, (October 2009), pp. 1903-1910, ISSN 0012-6667
- Johnson, D. & Travis, J. (1979). The oxidative inactivation of human alpha-1-proteinase inhibitor. Further evidence for methionine at the reactive center. *J Biol Chem*, Vol.254, No.10, (May 1979), pp. 4022-4026, ISSN 0021-9258
- Juvelekian, G.S. & Stoller, J.K. (2004). Augmentation therapy for α 1-antitrypsin deficiency. *Drugs*, Vol.64, No.16, pp. 1743-1756, ISSN 0012-6667
- Kalis, M., Kumar, R., Janciauskiene, S., Salehi, A. & Cilio, C.M. (2010). α 1-antitrypsin enhances insulin secretion and prevents cytokine-mediated apoptosis in pancreatic β -cells. *Islets*, Vol.2, No.3 (May-June 2010), pp. 185-189, ISSN 1938-2014
- Kalsheker, N., Morley, S. & Morgan, K. (2002). Gene regulation of the serine proteinase inhibitors alpha1-antitrypsin and alpha1- antichymotrypsin. *Biochem Soc Trans*, Vol.30, No.2, (April 2002), pp. 93-98, ISSN 0300-5127
- Karnaukhova, E. (2010). Interactions of alpha1-proteinase inhibitor with small ligands of therapeutic potential: binding with retinoic acid. *Amino Acids*, Vol.38, No.4, (April 2010), pp. 1011-1020, ISSN 0939-2199
- Karnaukhova, E., Ophir, Y., Trinh, L., Dalal, N., Punt, P.J., Golding, B. & Shiloach, J. (2007). Expression of human α 1-proteinase inhibitor in *Aspergillus niger*. *Microbial Cell Factories*, Vol. 6, No.34, (October 2007), pp. 1-10, ISSN 1475-2859
- Karnaukhova, E., Ophir, Y. & Golding, B. (2006). Recombinant human alpha-1 proteinase inhibitor: towards therapeutic use. *Amino Acids*, Vol.30, No.4, (June 2006), pp. 317-332, ISSN 0939-4451
- Kjus, T., Lützow-Holm, C., Christensen, O.B. (2002). Treatment of panniculitis associated with alpha-1-antitrypsin deficiency with alpha-1-protease inhibitor. *Br J Dermatol*, Vol.147, No.6, pp. 1258-1261, ISSN 0007-0963
- Kilty, S.J. & Desrosiers, M.Y. (2008). Chronic sinusitis and alpha1-antitrypsin deficiency: potential role for protease in rhinosinusitis? *J Otolaryngol Head Neck Surg*, Vol.37, No.6, (December 2008), pp. e179-e182, ISSN 1916-0216
- Kilty, S.J., Bossé, Y., Cormier, C., Endam, L.M. & Desrosiers, M.Y. (2010). Polymorphisms in the SERPINA1 (Alpha-1-Antitrypsin) gene are associated with severe chronic rhinosinusitis unresponsive to medical therapy. *Am J Rhinol Allergy*, Vol.24, No.1, (January 2010), pp. e4-e9, ISSN 1945-8924
- Kolarich, D., Weber, A., Turecek, P.L., et al. (2006a). Comprehensive glyco-proteomic analysis of human alpha1-antitrypsin and its charge isoforms. *Proteomics*, Vol.6, No.11, (June 2006), pp. 3369-3380, ISSN 1615-9853
- Kolarich, D., Turecek, P.L., Weber, A., et al. (2006b). Biochemical, molecular characterization, and glycoproteomic analyses of alpha(1)-proteinase inhibitor products used for replacement therapy. *Transfusion*, Vol.46, No.11, (November 2006), pp. 1959-1977, ISSN 0041-1132
- Krasnewich, D.M., Holt, G.D., Brantly, M., Skovby, F., Redwine, J. & Gahl, W.A. (1995). Abnormal synthesis of dolichol-linked oligosaccharides in carbohydrate-deficient glycoprotein syndrome. *Glycobiology*, Vol.5, No.5, (July 1995), pp. 503-510, ISSN 0959-6658

- Kropp, J., Wencker, M., Hotze, A., et al. (2001). Inhalation of [^{123}I] α_1 -protease inhibitor: toward a new therapeutic concept of α_1 -protease inhibitor deficiency? *J Nucl Med*, Vol.42, No.5, (May 2001), pp. 744–751, ISSN 0161-5505
- Kueppers, F. (2011). The role of augmentation therapy in alpha-1 antitrypsin deficiency. *Curr Med Res Opin*, Vol.27, No.3, (March 2011), pp. 579–588, ISSN 0300-7995
- Laurell, C.B. & Eriksson, S. (1963). The electrophoretic alpha-1-globulin pattern of serum in alpha1-antitrypsin deficiency. *Scan J Clin Lab Invest*, Vol.15, pp. 132–140, ISSN 0036-5513
- Lee, C., Maeng, J.S., Kocher, J.P., Lee, B. & Yu, M.H. (2001). Cavities of α_1 -antitrypsin that play structural and functional role. *Protein Sci*, Vol.10, No.7, (July 2001), pp. 1446–1453, ISSN 0961-8368
- Levine, R.L., Berlett, B.S., Moskovitz, J., Mosoni, L. & Stadtman, E.R. (1999). Methionine residues may protect proteins from critical oxidative damage. *Mech Ageing Dev*, Vol.107, No.3, (March 1999), pp. 323–332, ISSN 0047-6374
- Levine, R.L., Moskovitz, J. & Stadtman, E.R. (2000). Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. *IUBMB Life*, Vol.50, No.4-5, (October-November 2000), pp. 301–307, ISSN 1521-6543
- Li, Y., Krowka, M.J., Qi, Y., et al. (2011). Alpha1-antitrypsin deficiency carriers, serum alpha 1-antitrypsin concentration, and non-small cell lung cancer survival. *J Thorac Oncol*, Vol.6, No.2, (February 2011), pp. 291–295, ISSN 1556-0864
- Lindor, N.M., Yang, P., Evans, I., et al. (2010). Alpha-1-antitrypsin deficiency and smoking as risk factors for mismatch repair deficient colorectal cancer: a study from the colon cancer family registry. *Mol Genet Metab*, Vol.99, (February 2010), pp. 157–159, ISSN 1096-7192
- Lisowska-Myjak, B., Pachecka, J., Kaczyńska, B., Miskurka, G. & Kadziela, K. (2006). Serum protease inhibitor concentrations and total antitrypsin activity in diabetic and non-diabetic children during adolescence. *Acta Diabetol*, Vol. 43, (December 2006), pp. 88–92, ISSN 0940-5429
- Lomas, D.A., Elliott, P.R., Chang, W.S.W., Wardell, M.R. & Carrell, R.W. (1995). Preparation and characterization of latent α_1 -antitrypsin. *J Biol Chem*, Vol.270, No.10, (March 1995), pp. 5282–5288, ISSN 0021-9258
- Lomas, D.A., Elliott, P.R., Carrell, R.W. (1997). Commercial plasma α_1 -antitrypsin (Prolastin®) contains a conformationally inactive, latent component. *Eur Respir J*, Vol. 10, (March 1997), pp. 672–675, ISSN 0903-1936
- Lomas, D. (2005). Molecular mousetraps, α_1 -antitrypsin deficiency and the serpinopathies. *Clin Med*, Vol.5, No.3, (May-June 2005), pp. 249–257, ISSN 1470-2118
- Long, G.L., Chandra, T., Woo, S.L., Davie, E.W. & Kurachi, K. (1984). Complete sequence of the cDNA for human α_1 -antitrypsin and the gene for the S variant. *Biochemistry*, Vol.23, No.21, (October 1984), pp. 4828–4837, ISSN 0006-2960
- Ludeman, J.P., Whisstock, J.C., Hopkins, P.C.R., Le Bonniec, B.F. & Bottomley, S.P. (2001). Structure of a serpin-enzyme complex probed by cysteine substitutions and fluorescence spectroscopy. *Biophys J*, Vol.80, No.1, (May 2001), pp. 491–497, ISSN 0006-3495
- Luisetti, M. & Seersholm, N. (2004). Alpha1-antitrypsin deficiency. 1: epidemiology of alpha1-antitrypsin deficiency. *Thorax*, Vol.59, No.2 (February 2004), pp. 164–169, ISSN 0040-6376

- Mahadeva, R., Dafforn, T.R., Carrell, R.W. & Lomas, D.A. (2002). 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization. Implications for the prevention of Z alpha(1)-antitrypsin-related cirrhosis. *J Biol Chem*, Vol.277, No.9, (March 2002), pp. 6771-6774, ISSN 0021-9258
- Mallya, M., Phillips, R.L., Saldanha, S.A., Gooptu, B., et al. (2007). Small molecules block the polymerization of Z α 1-Antitrypsin and increase the clearance of intracellular aggregates. *J Med Chem*, Vol.50, No.22, (November 2007), pp. 5357-5363, ISSN 0022-2623
- Massaro, G.D. & Massaro, D. (1996). Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats. *Am J Physiol*, Vol.270, No.2 Pt1, (February 1996), pp. L305-L310, ISSN 0002-9513
- Massaro, G.D. & Massaro, D. (1997). Retinoic acid treatment abrogates elastase induced pulmonary emphysema in rats. *Nat Med*, Vol.3, No.6, (June 1997), pp. 675-677, ISSN 1078-8956
- Martin, S.L., Downey, D., Bilton, D., et al. on behalf of the Recombinant AAT CF Study Team (2006). Safety and efficacy of recombinant α ₁-antitrypsin therapy in cystic fibrosis. *Pediatr Pulmonol*, Vol. 41, (February 2006), pp. 177-183, ISSN 8755-6863
- Maune, S., Rath, N.F., Görögh, T. & Steinert, R. (1995). Genetic disposition to chronic polypoid sinusitis and alpha 1-proteinase inhibitor deficiency types. *HNO*, Vol.43, (September 1995), pp. 537-539, ISSN 0017-6192
- McElvaney, N.G., Hubbard, R.C., Birrer, P., Chernick, M.S., Caplan, D.B., Frank, M.M. & Crystal, R.G. (1991). Aerosol α ₁-antitrypsin treatment for cystic fibrosis. *Lancet*, Vol. 337, (February 1991), pp. 392-395, ISSN 0140-6736
- Mega, T., Lujan, E. & Yoshida, A. (1980a). Studies on the oligosaccharide chains of human α ₁-protease inhibitor: I. Isolation of glycopeptides. *J Biol Chem*, Vol.255, No.9, (May 1980), pp. 4053-4056, ISSN 0021-9258
- Mega, T., Lujan E. & Yoshida, A. (1980b) Studies on the oligosaccharide chains of human α ₁-protease inhibitor: II. Structure of oligosaccharides. *J Biol Chem* Vol.255, No.9, (May 1980), pp. 4057-4061, ISSN 0021-9258
- Nadai, M., Bally, J., Vitel, M., Job, C., Tissot, G., Botterman, J. & Dubald, M. (2009). High-level expression of active human alpha1-antitrypsin in transgenic tobacco chloroplasts. *Transgenic Res*, Vol.18, No.2, (April 2009), pp. 173-183, ISSN 0962- 8819
- Needham, M. & Stockley, R.A. (2004). Alpha 1-antitrypsin deficiency. 3: Clinical manifestations and natural history. *Thorax*, Vol. 59, No.5, (May 2004), 441-445, ISSN 0040-6376
- Nita, I., Hollander, C., Westin, U. & Janciauskiene, S.M. (2005). Prolastin, a pharmaceutical preparation of purified human alpha1-antitrypsin, blocks endotoxin-mediated cytokine release. *Respir Res*, Vol.6, No.12, doi:10.1186/1465-9921-6-12; ISSN 1465-9921
- Paakko, P., Kirby, M., du Bois, R.M., et al. (1996). Activated neutrophils secrete stored α ₁-antitrypsin. *Am J Respir Crit Care Med*, Vol.154, No. 6 Pt1, (December 1996), pp. 1829-1833, ISSN 1073-449X
- Parfrey, H., Mahadeva, R., Ravenhill, N.A., et al. (2003). Targeting a surface cavity of alpha 1-antitrypsin to prevent conformational disease. *J Biol Chem*, Vol.278, No.35, (August 2003), pp. 33060-33066, ISSN 0021-9258

- Pemberton, P.A., Kobayashi, D., Wilk, B.J., et al. (2006). Inhaled recombinant alpha 1-antitrypsin ameliorates cigarette smoke-induced emphysema in the mouse. *COPD*, Vol.3, No.2, (June 2006), pp. 101-108, ISSN 1541-2555
- Rabassa, A.A., Schwartz, M.R. & Ertan, A. (1995). Alpha 1-antitrypsin deficiency and chronic pancreatitis. *Dig Dis Sci*, Vol.40, (September 1995), pp. 1997-2001, ISSN 0163-2116
- Rabin, M., Watson, M., Kidd, V., Woo, S.L., Breg, W.R. & Ruddie, F.H. (1986). Regional location of α 1-antichymotrypsin and α 1-antitrypsin genes on human chromosome 14. *Somatic Cell Mol Gen*, Vol.12, No. (March 1986), pp. 209-214, ISSN 0740-7750
- Rosenberg, S., Barr, P.J., Najarian, R.C. & Hallewell, R.A. (1984). Synthesis in yeast of a functional oxidation-resistant mutant of human α 1-antitrypsin. *Nature*, Vol.312, No.5989, (November 1984), pp. 77-80, ISSN 0028-0836
- Saari, K.M., Kaarela, K., Korpela, T., Laippala, P., Frants, R.R. & Eriksson, A.W. (1986). Alpha 1-antitrypsin in acute anterior uveitis and rheumatic diseases. *Acta Ophthalmol*, Vol.64, No.5 (October 1986), pp. 522-529, ISSN 0001-639X
- Sandhaus, R.A. (2009). Augmentation therapy in alpha-1 antitrypsin deficiency. *COPD*, Vol.6, No.3, (June 2009), pp. 147-148, ISSN 1541-2555
- Sarkar, A. & Wintrode, P.L. (2011). Effects of glycosylation on the stability and flexibility of a metastable protein: the human serpin α (1)-antitrypsin. *Int J Mass Spectrom*, Vol. 302, No.1-3, (April 2011), pp. 69-75, ISSN 1387-3806
- Seersholm, N., Wencker, M., Banik, N., et al. (1997). Does alpha1-antitrypsin augmentation therapy slow the annual decline in FEV1 in patients with severe hereditary alpha1-antitrypsin deficiency? Wissenschaftliche Arbeitsgemeinschaft zur Therapie von Lungenerkrankungen (WATL) alpha1-AT study group. *Eur Respir J*, Vol.10, (October 1997), pp. 2260-2263, ISSN 0903-1936
- Sharp, H.L., Bridges, R.A., Krivit, W. & Freier, E.F. (1969). Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. *J Lab Clin Med*, Vol.73, (June 1969), pp. 934-939, ISSN 0022-2143
- Schluchter, M.D., Stoller, J.K., Barker, A.F. et al. (2000). Feasibility of a clinical trial of augmentation therapy for α 1-antitrypsin deficiency. *Am J Respir Crit Care Med*, Vol.161, No.3, (March 2000), pp.796-801, ISSN 1073-449X
- Siekmeier, R. (2010). Lung deposition of inhaled alpha-1-proteinase inhibitor (Alpha₁-PI) – Problems and experience of Alpha₁-PI inhalation therapy in patients with hereditary Alpha₁-PI deficiency and cystic fibrosis. *Eur J Med Res*, Vol.15, Suppl. II, (November 2010), pp. 164-174, ISSN 0949-2321
- Silverman, G.A., Bird, P.I., Carrell, R.W., et al., (2001). The serpins are an expanding superfamily of structurally similar but functionally diverse proteins: evolution, mechanism of inhibition, novel functions, and a revised nomenclature. *J Biol Chem*, Vol.276, No. (September 2001), pp. 33293-33296, ISSN 0021-9258
- Silverman, E.K. & Sandhaus, R.A. (2009). Alpha1-antitrypsin deficiency. *New Engl J Med*, Vol. 360, (June 2009), pp. 2749-2757, ISSN 0028-4793
- Smith, K.C., Pittelkow, M.R. & Su, W.P. (1987). Panniculitis associated with severe alpha 1-antitrypsin deficiency. Treatment and review of the literature. *Arch Dermatol*, Vol.123, No.12, (December 1987), pp. 1655-1661, ISSN 0003-987X
- Snider, G.L. (1992). Emphysema: the first two centuries and beyond: a historical overview, with suggestions for future research. *Am Rev Respir Dis*, Vol.146, No.5 Pt 1, (December 1992), pp. 1334-44 & No.6, pp. 1615-22, ISSN 0003-0805

- Spencer, L.T., Humphries, J.E. & Brantly, M.L. (2005). Transgenic Human Alpha 1-Antitrypsin Study Group. Antibody response to aerosolized transgenic human alpha1-antitrypsin. *N Engl J Med*, Vol.352, No.19, (May 2005), pp. 2030-2031, ISSN 0028-4793
- Stakisaitis, D., Basys, V. & Benetis, R. (2001). Does alpha-1-proteinase inhibitor play a protective role in coronary atherosclerosis? *Med Sci Monit*, Vol.4, No.4, (Jul-Aug, 2001), pp. 701-11, ISSN 1234-1010
- Stein, P.E. & Carrell, R.W. (1995). What do dysfunctional serpins tell us about molecular mobility and disease? *Nat Struct Biol*, Vol.2, No.2, (February 1995), pp. 96-113, ISSN 1072-8368
- Stockley, R.A. (2010). Emerging drugs for alpha-1-antitrypsin deficiency. *Expert Opin Emerg Drugs*, Vol.15, No.4, (December 2010), pp. 685-694, ISSN 1472-8214
- Stocks, J.M., Brantly, M.L., Wang-Smith, L., et al. (2010). Pharmacokinetic comparability of Prolastin®-C to Prolastin® in alpha₁-antitrypsin deficiency: a randomized study. *BMC Clin Pharmacol*, Vol.10, No.13, (September 2010), doi:10.1186/1472-6904-10-13, ISSN 1472-6904
- Stratikos, E. & Gettins P.G.W. (1999). Formation of the covalent serpin-proteinase complex involves translocation of the proteinase by more than 70Å and full insertion of the reactive centre loop into β -sheet A. *Proc Natl Acad Sci USA*, Vol.96, No.9, (April 1999), pp. 4808-4813, ISSN 0027-8424
- Szönyi, L., Dobos, M., Vásárhelyi, B., et al. (2006). Prevalence of alpha1-antitrypsin phenotypes in patients with IgA nephropathy. *Clin Nephrol*, Vol.62, pp. 418-422
- Talmud, P.J., Martin, S., Steiner, G., et al. (2003). Progression of atherosclerosis is associated with variation in the alpha1-antitrypsin gene. *Arterioscler Thromb Vasc Biol*, Vol.23, No.4, (April 2003), pp. 644-649, ISSN: 1524-4636
- Tebbutt, S.J. (2000). Technology evaluation: transgenic alpha-1-antitrypsin (AAT), PPL therapeutics. *Curr Opin Mol Ther*, Vol.2, No.2, (April 2000), pp. 199-204, ISSN 1464-8431
- Ting, S.M., Toth, T. & Caskey, F. (2008). Alpha1-antitrypsin (A1AT) deficiency presenting with IgA nephropathy and nephritic syndrome: is renal involvement caused by A1AT deposition? *Clin Nephrol*, Vol. 70, (August 2008), pp. 159-162, ISSN 0301-0430
- Tonelli, A.R. & Brantly, M.L. (2010). Augmentation therapy in alpha-1 antitrypsin deficiency: advances and controversies. *Ther Adv Respir Dis*, Vol.4, No.5, (October 2010), pp. 289-312, ISSN 1753-4658
- Topic, A., Lujic, M., Nikolic, A., Petrovic-Stanojevic, N., et al. (2011). Alpha-1-antitrypsin phenotypes and neutrophil elastase gene promoter polymorphisms in lung cancer. *Pathol Oncol Res*, Vol.17, No.1, (March 2011), pp. 75-80, ISSN 1219-4956
- Travis, J. (1988). Structure, function, and control of neutrophil proteinases. *Am J Med*, Vol. 84, No.6A, (June 1988), pp. 37-42, ISSN 0002-9343
- Travis, J. & Salvesen, G.S. (1983). Human plasma proteinase inhibitors. *Annu Rev Biochem*, Vol.52, No. (1983), pp. 655-709, ISSN 0066-4154
- Valverde, R., Rosales, B., Ortiz-de Frutos, F.J., Rodriguez-Peralto, J.L., Ortiz-Romero, P.L. (2008). Alpha-1-antitrypsin deficiency panniculitis. *Dermatol Clin*, Vol.26, (October 2008), pp. 447-451, ISSN 0733-8635

- Varga, L. & Farkas, H. (2011). rhC1INH: a new drug for the treatment of attacks in hereditary angioedema caused by C1-inhibitor deficiency. *Expert Rev Clin Immunol*, Vol.7, No.2, (March 2011), pp. 143-153, ISSN 1744-666X
- Vaughan, L., Lorier, M.A. & Carrell, R.W. (1982). α 1-Antitrypsin microheterogeneity: isolation and physiological significance of isoforms. *Biochem Biophys Acta*, Vol.701, No.3, (March 1982), pp. 339-345, ISSN 0006-3002
- Volpert, D., Molleston, J.P. & Perlmutter, D.H. (2000). Alpha1-antitrypsin deficiency-associated liver disease progresses slowly in some children. *J Pediatr Gastroenterol Nutr*, Vol.31, No.3, (September 2000), pp. 258-63, ISSN 0277-2116
- Wencker, M., Fuhrmann, B., Banik, N. & Konietzko, N. (2001). Longitudinal follow-up of patients with alpha1-protease inhibitor deficiency before and during IV alpha1-protease inhibitor, *Chest*, Vol.119, No.3, (March 2001), pp. 737-744, ISSN 0012-3692
- White, R., Lee, D., Habicht, G.S. & Janoff, A. (1981). Secretion of alpha-1-proteinase inhibitor by cultured rat alveolar macrophages. *Am Rev Respir Dis*, Vol.123, No.4, Pt 1, (April 1981), pp. 447-449, ISSN 0003-0805
- WHO (1997). World Health Organization, Human Genetics Programme. Alpha-1-antitrypsin deficiency. Report of WHO meeting held in Geneva on 18-20 March 1996, *Bull World Health Organ*, Vol.75, No.5, pp. 397-415
- Wilczynska, M., Fa, M., Karolin, J., Ohlsson, P.I., Johansson, L.B. & Ny, T. (1997). Structural insights into serpin-protease complexes reveal the inhibitory mechanism of serpins. *Nat Struct Biol*, Vol.4, No.5, (May 1997), pp. 354-357, ISSN 1072-8368
- Wright, G., Carver, A., Cottom, D., Reeves, D., Scott A., Simons, P., Wilmut, I., Gamer, J. & Colman, A. (1991). High level of expression of active human alpha-1-antitrypsin in the milk of transgenic sheep. *Bio/Technology*, Vo.9, No.9, (September 1991), pp. 830-834, ISSN 0733-222X
- Ziomek, C.A. (1998). Commercialization of proteins produced in the mammary gland. *Theriogenology*, Vol.49, No.1, (January 1998), pp. 139-144, ISSN 0093-691X

IntechOpen

Lung Diseases - Selected State of the Art Reviews

Edited by Dr. Elvisgran Malcolm Irusen

ISBN 978-953-51-0180-2

Hard cover, 690 pages

Publisher InTech

Published online 02, March, 2012

Published in print edition March, 2012

The developments in molecular medicine are transforming respiratory medicine. Leading clinicians and scientists in the world have brought their knowledge and experience in their contributions to this book. Clinicians and researchers will learn about the most recent advances in a variety of lung diseases that will better enable them to understand respiratory disorders. This treatise presents state of the art essays on airways disease, neoplastic diseases, and pediatric respiratory conditions. Additionally, aspects of immune regulation, respiratory infections, acute lung injury/ARDS, pulmonary edema, functional evaluation in respiratory disorders, and a variety of other conditions are also discussed. The book will be invaluable to clinicians who keep up with the current concepts, improve their diagnostic skills, and understand potential new therapeutic applications in lung diseases, while scientists can contemplate a plethora of new research avenues for exploration.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Elena Karnaukhova (2012). Recent Advances in the Research and Development of Alpha-1 Proteinase Inhibitor for Therapeutic Use, Lung Diseases - Selected State of the Art Reviews, Dr. Elvisgran Malcolm Irusen (Ed.), ISBN: 978-953-51-0180-2, InTech, Available from: <http://www.intechopen.com/books/lung-diseases-selected-state-of-the-art-reviews/recent-advances-in-the-research-and-development-of-alpha-1-proteinase-inhibitor-for-therapeutic-use>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen