AAT-Fc fusion protein

FIELD OF THE INVENTION

The present invention discloses a fusion protein comprising an immunoglobulin IgG Fc domain and an alpha1-antitrypsin (AAT) polypeptide. The present invention also discloses a method of treating or preventing a disorder or disease comprising administering a fusion protein comprising an immunoglobulin IgG Fc domain and an alpha1-antitrypsin (AAT) polypeptide to a subject.

BACKGROUND OF THE INVENTION

Alpha-1 antitrypsin (AAT) is a protease inhibitor with diverse roles throughout the body. For example, AAT protects the lung by inhibiting neutrophil proteinases, but AAT has many other non-proteolytic functions that are anti-inflammatory, antiviral, and homeostatic. Approximately 1 in 1600 to 1 in 5000 people have the homozygous Z mutation, which causes AAT misfolding, accumulation in (predominantly) liver cells and low circulating levels of AAT, leading to AAT deficiency (AATD). AATD is classically a disease of neutrophilic inflammation with an aggressive and damaging innate immune response contributing to emphysema and other pathologies. AATD is one of the most common genetic disorders but considerably under-recognised (Crossley et al., Alpha-1 Antitrypsin Deficiency and Accelerated Aging: A New Model for an Old Disease?, June 2019, Drugs & Aging 36:823-840). The most clinically advanced therapy for the treatment of AATD is a fusion protein comprising an AAT polypeptide and an IgG4 Fc domain, as described in WO2013/003641.

AAT constructs are also useful in the treatment of diseases or disorders of the nervous system and acquired inflammatory conditions. For example, treatment with plasma-derived AAT has been shown to be effective in the treatment of Charcot-Marie-Tooth disease (CMT) in a mouse model (WO2023247736).

Diseases or disorders of the nervous system are diseases or disorders which can dramatically affect the peripheral nervous system (PNS) and/or central nervous system (CNS). In the last decade, neuroinflammation has become more and more central in our understanding of neurological disorders. Inflammation per se may directly or indirectly trigger the disease but

it does undoubtedly contribute to the pathogenesis of the disease throughout the PNS and CNS. Peripheral diseases like Guillain-Barré Syndrome (GBS) (Chang et al. 2012), Charcot-Marie-Tooth Disease (Hoyle et al. 2015), neuropathic pain, fibromyalgia and other peripheral neuropathies (PNs) (Martin-Aguilar, Pascual-Goni, and Querol 2019) as well as central diseases including Parkinson's (PD) and motor neuron disease (Marogianni et al. 2020), Alzheimer's diseases (AD) (Hampel et al. 2020) and other dementias, Multiple Sclerosis (MS) (Baecher-Allan, Kaskow, and Weiner 2018; Matthews 2019), Amyotrophic Lateral Sclerosis (ALS), ischemia and traumatic brain injuries, depression and autism spectrum disorder have been all linked to mechanisms driven by activated microglia (Skaper et al. 2018).

Hereditary, peripheral neuropathies constitute a highly diverse group of disorders whose most frequent form is collectively known as Charcot-Marie-Tooth disease (CMT), with a world-wide prevalence of 1 in 2500 and a high genetic heterogeneity (>100 different genes involved) (Bird, T. D., 1993, GeneReviews (R)). In this broad range of genetic pattern possibilities, CMT1A is the most common form and accounts for 80% of the CMTs of type 1. It is characterized by intra-chromosomal duplication of the PMP22 gene (Stavrou, Sargiannidou et al. 2021). The major pathogenic component of peripheral neuropathies is damage to the myelin sheath, either after its abnormal development (dysmyelination) in the inherited forms (CMT1A-F and -X) or direct damage in the acquired ones (acute/chronic inflammatory demyelinating polyneuropathy; AIDP/CIDP).

Myelin is produced by Schwann cells (SCs) in the PNS and is crucial for proper transmission of electric impulses in the nerves. In the intricate neuron/glia cross-communication that is required for proper myelin regulation (Rao and Pearse 2016), several diverse signalling pathways are involved. These include growth factors, integrins and cell adhesion molecules but more importantly, the pivotal neuregulin 1 type III (NRG1-III) signal through ERBB2/3 receptors (Taveggia, C., et al., 2005, Neuron 47(5): 681-694.) and its proteolytic sheddase modulator, the tumor necrosis factor-α–converting enzyme, TACE (also known as ADAM-17) (Fleck, D. et al., 2016, J Biol Chem 291(1): 318-333). Although diverse and cutting-edge therapeutic strategies are currently being explored (CRISPR/Cas9 editing, viral-based gene delivery, siRNA nanoparticles), none have successfully completed the phase III in clinical trials and CMTs are left with no actual treatments (Fridman, V. and M. A. Saporta, 2021, Neurotherapeutics 18(4): 2236-2268.). Moreover, very few of these strategies target the NRG1/EBRB2/3/TACE pathway even though inhibition of TACE has been shown to promote

myelination. TACE/ADAM-17, is a transmembrane protein that includes an extracellular zinc-dependent protease domain. In the context of CMT1A, ADAM-17 is known for its inhibitory effect on SCs mediated myelination by cleaving NRG1-III in the epidermal growth factor domain in a ligand independent manner (La Marca, R., 2011, Nat Neurosci 14(7): 857-865.). Conflicting evidence has been reported in the literature with respect to the role of the human protease AAT. Specifically, in 2013, AAT was shown not to interact with TACE/ADAM-17 (van't Wout E. F. et al., 2014, Hum Mol Genet.; 23(4):929-4) in contrast to an earlier report in 2010 that claimed AAT does indeed interact with TACE/ADAM-17 and inhibits its activity in a dose-dependent manner (Bergin, D. A. et al., 2010, J Clin Invest 120(12): 4236-4250.).

The variety of impairments caused by neurological disorders has been increasingly considered as a worldwide public health challenge and its burden is expected to rise in the coming decades.

The common biological trait of many CNS and PNS neurodegenerative diseases is a sustained and acute inflammatory response due to cytokine release orchestrated in feed-forward loops (also called “cytokine storm”). Therefore, reducing the inflammatory reaction stands as a central target of therapeutical strategies. However, the subtleties of inflammatory mechanisms underlying its multiple mediators are not fully understood.

There remains a need for therapeutic AAT-Fc fusion proteins with improved pharmaceutical characteristics.

Furthermore, there is a need for improved therapies for diseases or disorders of the nervous system, as well as genetic diseases and acquired inflammatory conditions.

SUMMARY OF THE INVENTION

The present invention provides a fusion protein comprising an immunoglobulin IgG Fc domain and an alpha1-antitrypsin (AAT) polypeptide, wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain.

The fusion protein further comprises one or more features which reduce the immunogenicity of the fusion protein and/or increase the serum half-life of the fusion protein. For example, the fusion protein may comprise any of the features (a) to (c), or any combination thereof: (a) the Fc domain comprises mutations corresponding to L234A or F234A, L235A,

and P329G ("LALAPG mutation"); (b) the Fc domain comprises mutations corresponding to M252Y, S254T, and T256E ("YTE mutation"); and/or (c) the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

The present inventors have surprisingly found that fusion proteins comprising features (a), (b) and/or (c) have reduced immunogenicity compared with fusion proteins not comprising these features. For example, fusion proteins of the invention may have reduced immunogenicity compared with the fusion protein of SEQ ID NO: 20 or 34 (as described in WO2013/003641), which is currently the most clinically advanced AAT-Fc construct, without any significant loss of ADAM-17 inhibitory activity. Fusion proteins of the invention which comprise the YTE mutation, or a combination of the LALAPG and YTE mutations, have reduced immunogenicity compared to equivalent fusion proteins not comprising these mutations, or fusion proteins comprising the LALAPG mutation alone. In particular, fusion proteins comprising the combination of the LALAPG and YTE mutations have particularly low immunogenicity.

Reduced immunogenicity is advantageous as it reduces the risk of side effects in patients and allows higher doses of the fusion protein to be used safely, thereby increasing the efficacy of the treatment. This result is particularly surprising in the context of fusion proteins of the invention comprising an IgG1 Fc domain, as the literature suggests that the IgG1 Fc domain is more immunogenic than the IgG4 Fc domain (such as the IgG4 Fc domain present in SEQ ID NO: 20 or 34) (see e.g. Wang W, Maliepaard JCL, Damelang T, Vidarsson G, Heck AJR, Reiding KR. Human IgG Subclasses Differ in the Structural Elements of Their N-Glycosylation. ACS Cent Sci. 2024 Oct 10;10(11):2048-2058 and Lai PK, Ghag G, Yu Y, Juan V, Fayadat-Dilman L, Trout BL. Differences in human IgG1 and IgG4 S228P monoclonal antibodies viscosity and self-interactions: Experimental assessment and computational predictions of domain interactions. MAbs. 2021 Jan-Dec;13(1):1991256). In particular, the increased immunogenicity described for the IgG1 Fc domain compared to the IgG4 Fc domain may be due to one or more of the following factors:

- The Fc region of IgG1 binds more strongly to activating Fc receptors (such as FcγRIIIa) compared to IgG4;

- IgG4 binds more effectively to the FcγRIIB inhibitory receptor, which can suppress immune activation. This reduced activation makes IgG4 less immunogenic and less likely to induce inflammatory responses;
- IgG4 is unique in that it has a propensity to undergo "Fab-arm exchange", where the heavy chains of different IgG4 molecules swap, resulting in antibodies that have different antigen-binding arms. This reduces the homogeneity of the Fc region, which might contribute to a lower likelihood of recognition by the immune system as a foreign object. IgG1, on the other hand, has a more stable structure and does not undergo this Fab-arm exchange, making it more recognisable, which can trigger an up-regulation in cytokine response.

Furthermore, the present inventors have surprisingly found that fusion proteins comprising a combination of the LALAPG and YTE mutations have an increased half-life compared to equivalent fusion proteins not comprising this combination of mutations, or compared to fusion proteins comprising only the YTE mutation, and compared to a fusion protein of SEQ ID NO: 20 or 34. Increased half-life enables less frequent and lower dosing, resulting in improved patient compliance and reduced treatment cost.

Finally, the present inventors have surprisingly found that fusion proteins comprising a combination of the LALAPG and YTE mutations, show an increased yield following purification than equivalent proteins not comprising a combination of these mutations.

Accordingly, in one aspect, the present invention provides a fusion protein comprising:

(i) an immunoglobulin IgG Fc domain, and

(ii) an alpha1-antitrypsin (AAT) polypeptide,

wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, and wherein:

(a) the Fc domain comprises mutations corresponding to L234A or F234A, L235A, and P329G;

(b) the Fc domain comprises mutations corresponding to M252Y, S254T, and T256E; and/or

(c) the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally

wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

In a second aspect, the present invention provides a dimer comprising a fusion protein of the invention and a second protein.

In a third aspect, the present invention provides a vector comprising a nucleic acid sequence encoding a fusion protein or a dimer of the invention.

In a fourth aspect, the present invention provides a host cell comprising, or expressing, a fusion protein, a dimer or a vector of the invention.

In a fifth aspect, the present invention provides a pharmaceutical composition comprising a fusion protein, a dimer, a vector, or a host cell of the invention.

In a sixth aspect, the present invention provides a fusion protein, a dimer, a vector, a host cell, or a pharmaceutical composition of the invention for use in a method of treating or preventing a disease or disorder.

In a seventh aspect, the present invention provides a method of treating or preventing a disease or disorder comprising administering a fusion protein, a dimer, a vector, a host cell, or a pharmaceutical composition of the invention to a subject.

In an eighth aspect, the present invention provides the use of a fusion protein, a dimer, a vector, a host cell, or a pharmaceutical composition of the invention in the manufacture of a medicament for use in a method of treating or preventing a disease or disorder.

In a ninth aspect, the present invention provides a method of inhibiting ADAM-17 in a cell, the method comprising contacting the cell with a fusion protein, a dimer, a vector, a host cell, or a pharmaceutical composition of the invention.

In a tenth aspect, the present invention provides a method of promoting remyelination and/ or arresting demyelination of the axons of the PNS in a subject, the method comprising

administering to the subject a fusion protein, a dimer, a vector, a host cell, or a pharmaceutical composition of the invention.

In an eleventh aspect, the present invention provides a method of producing a fusion protein or a dimer of the invention.

In a twelfth aspect, the present invention provides a method of purifying a fusion protein or a dimer of the invention.

The present invention provides a fusion protein for use in the prevention and/or treatment of a disease or disorder of the nervous system, wherein said fusion protein comprises (i) an immunoglobulin IgG Fc domain, a variant or a fragment thereof, and

(ii) an alpha1-antitrypsin (AAT) polypeptide, a variant, an isoform and/or a fragment thereof,

wherein the AAT polypeptide, variant, isoform and/or fragment thereof, is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, variant or fragment thereof.

The present invention also provides a nucleic acid sequence encoding a fusion protein of the invention.

The present invention provides a vector comprising a nucleic acid sequence encoding a fusion protein for use of anyone of the invention.

The present invention also provides a host cell comprising, or expressing, a fusion protein for use of the invention or a vector of the invention.

The present invention also provides a pharmaceutical composition comprising a therapeutically effective amount of i) a fusion protein for use of the invention, ii) a vector of the invention or iii) a host cell of the invention, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

The present invention further provides a method of treatment and/or prevention of a disease or disorder of the nervous system, comprising administering a fusion protein of the invention or a pharmaceutical composition of the invention, to a subject in need thereof.

DESCRIPTION OF THE FIGURES

Figure 1. SDS-PAGE coomassie-stained with AAT from multiple sources

Figure 2. Inhibitory effects of AAT-Fc and of AAT from different sources on the neutrophil elastase activity expressed as percent of control (untreated) enzymatic activity

Figure 3. The graph shows ADAM-17 activity. Plasma-derived AAT (Sigma Aldrich).

Figure 4. Scheme of the experiment. Test plasma-derived AAT and AAT-derived peptides on TNFα stimulated HSC.

Figure 5. NF-kB immunostaining. Blue nuclei (staining with DAPI) and green NF-kB protein. As a result of TNFα stimulation, NF-kB translocates from the cytoplasm to the nucleus. There is some area in which plasma-derived AAT partially avoids total nuclear staining, in which we can see cytoplasmic protein (white arrow). Also, peptides 8 and 14 showed some cytoplasmic staining. Only nuclear staining was seen for peptide 9.

Figure 6. ADAM-17 activity inhibition by AAT-Fc and IC50 values for AAT-Fc ADAM-17 activity inhibition by AAT-Fc and IC50 values for AAT-Fc. In the left graph, ADAM-17 activity is inhibited by AAT-Fc at the concentration tested. As a positive control, plasma-derived AAT was used. The experiment was conducted in technical triplicate. The right graph shows the IC50 value of AAT-Fc based on the percentage of ADAM-17 activity inhibition. According to the results, the IC50 value is 0.25 μM.

Figure 7. MHCII reporter expression in microglia derived from iPSCs. The graph represents the MHCII luciferase expression normalized to cell viability for the indicated treatments. Interferon γ (IFNγ) was used to stimulate MHCII response. The experiment was conducted in technical triplicates.

Figure 8. MHCII reporter expression in microglia derived from iPSCs co-cultured with normal and Parkinson's neurospheres. Interferon γ (IFNγ) was used to stimulate MHCII response. The AAT treatment (50μM, Sigma Aldrich) decreased MHCII reporter activity both

in microglia co-cultured with normal neurospheres (Fig. 8A) and in the Parkinson's model (Fig. 8B).

Figure 9. Symmetric A1AT-FC -Construct 1 (AGX-101SY, corresponding to 2X SEQ ID NO. 10, 2X SEQ ID NO. 16)

Figure 10. Asymmetric A1AT-FC -Construct 2 (AGX-101A, corresponding to SEQ ID NOs. 11 and 12 or SEQ ID NOs. 15 and 12).

Figure 11. Constructs AGX-101A, AGX-101SY, AGX-101S, AGX-101T, AGX-101C, and AGX-101R

Figure 12. ADAM-17 activity inhibition by AAT-Fc derivatives and IC50 values. ADAM-17 activity was measured using a fluorescence-based enzymatic inhibition assay at different concentrations (8, 6, 3, 2.5, 2, 1, 0.5, 0.1 μM). The experiment was conducted in technical triplicates. IC50 values were determined using GraphPad Prism based on percentage of ADAM-17 activity inhibition.

Figure 13. Neutrophil elastase activity inhibition by AAT-Fc derivatives and IC50 values were measured using a neutrophil elastase inhibition assay. Substrate MeOSuc-AAPV-pNA l00 μM, purified human neutrophil elastase (ELA2) 2.2μU/μL and several concentrations of AAT-Fc (100, 30, 10, 5, 3, 2 nM) were incubated together for 30 min. Absorbance was measured every minute in a spectrophotometer reader (A405 nm). Vmax value was determined with the trendline slope during 10 min. The experiment was repeated twice. Error bars indicate standard deviation.

Figure 14. The mRNA levels of pro-inflammatory cytokines (IL-1β, TNFα, IL-6, and IL-4) upon AAT treatment in Schwann cells (SCs). Human SCs were plated in a 24-well plate at a density of 70,000 cells per well. Two days after seeding, AAT-Fc pre-treatment was initiated for 1 day at 3μM. Then, the medium was replaced with fresh medium containing 3μM AAT-Fc for 2 days. Subsequently, the cells were harvested for RNA extraction. Gene expression was analysed by qPCR, with relative expression normalized to a housekeeping gene and visualized using GraphPad Prism.

Figure 15. Plasma concentrations of AGX-101S, AGX-101T and AGX-101R (10 mg/kg SC) following administration to male C57 BL/6 mice.

Figure 16. The mRNA levels of pro-inflammatory cytokines (IL-6, TNFα, and NFκBIA) upon AAT treatment in Schwann cells (SCs) stimulated or not with LPS or TNFα. Human SCs were plated in a 24-well plate at a density of 70 000 cells per well. Two days after seeding, AAT-Fc pre-treatment was initiated for 1 day at 3µM with or without LPS 1 µg/mL or TNFα 10 ng/mL. Then, the medium was replaced with fresh medium containing 3 µM AAT-Fc for 2 days with or without LPS 1 µg/mL or TNFα 10 ng/mL. Subsequently, the cells were harvested for RNA extraction. Gene expression was analysed by qPCR, with relative expression normalized to a housekeeping gene and visualized using GraphPad Prism. Figure 16D represent the 2(DCt) raw data.

DESCRIPTION OF THE INVENTION

AAT-Fc fusion proteins

In one aspect, the present invention provides a fusion protein comprising:

(i) an immunoglobulin IgG Fc domain, and

(ii) an alpha1-antitrypsin (AAT) polypeptide,

wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, and wherein:

(a) the Fc domain comprises mutations corresponding to L234A or F234A, L235A, and P329G;

(b) the Fc domain comprises mutations corresponding to M252Y, S254T, and T256E; and/or

(c) the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

In some embodiments, the fusion protein may have the following features:

(a) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to L234A, L235A, and P329G;

(b) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to M252Y, S254T, and T256E;

(c) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(d) the Fc domain is an IgG4 Fc domain and the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

In some embodiments, the fusion proteins and the dimers of the invention have lower immunogenicity than a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, and P329G have lower immunogenicity than equivalent fusion proteins not comprising mutations corresponding to L234A, L235A, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E have lower immunogenicity than equivalent fusion proteins not comprising mutations corresponding to M252Y, S254T, and T256E. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G have lower immunogenicity than equivalent fusion proteins not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E and comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25 have lower immunogenicity than equivalent fusion proteins not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In the context of the present invention, "immunogenicity" may refer to the inflammation and/or cytokine release triggered by treatment with a fusion protein or a dimer of the invention. Immunogenicity may also refer to expression of pro-inflammatory transcription factors such as NFκBIA. Accordingly, in some embodiments, the fusion proteins and the dimers of the invention trigger a reduced inflammatory response in a cell and/or in a subject compared to a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, and P329G trigger a reduced inflammatory response in a cell and/or in a subject compared to an equivalent fusion proteins not comprising mutations corresponding to L234A, L235A, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E trigger a reduced inflammatory response in a cell and/or in a subject compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A,

M252Y, S254T, T256E, and P329G trigger a reduced inflammatory response in a cell and/or in a subject compared to an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E and comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25 trigger a reduced inflammatory response in a cell and/or in a subject compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the immunogenicity is measured by contacting human Schwann cells with the fusion protein or the dimer. For example, immunogenicity may be measured by contacting the cells with the fusion protein or the dimer, harvesting the cells, extracting RNA, and analysing the expression of at least one cytokine and/or pro-inflammatory transcription factor. In some embodiments, the method may further comprise treating the Schwann cells with pro-inflammatory factors, such as LPS and/or TNFα. In some embodiments, the at least one cytokine is selected from IL-1β, TNFα, IL-6, and/or IL-4. In some embodiments, the at least one cytokine comprises IL-1β. In some embodiments, the at least one cytokine comprises TNFα. In some embodiments, the at least one cytokine comprises IL-6. In some embodiments, the at least one cytokine comprises IL-4. In some embodiments, the at least one cytokine comprises IL-1β, TNFα, IL-4, or IL-6, or any combination thereof. In some embodiments, the pro-inflammatory transcription factor is NFκBIA. In some embodiments, the fusion protein promotes less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine and/or pro-inflammatory transcription factor expression promoted by a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34.

In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, and P329G promote less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine and/or pro-inflammatory transcription factor expression promoted by an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, and P329G. In some embodiments, fusion proteins of the invention comprising

mutations corresponding to M252Y, S254T, and T256E promote less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine and/or pro-inflammatory transcription factor expression promoted by an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G promote less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine and/or pro-inflammatory transcription factor expression promoted by an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E and comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25 promote less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine and/or pro-inflammatory transcription factor expression promoted by an equivalent fusion proteins not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the fusion protein or the dimer can inhibit and/or impair the activity of ADAM metallopeptidase domain 17 (ADAM-17). In some embodiments, inhibition of ADAM-17 is measured by incubating ADAM-17 with increasing concentrations of the fusion protein or the dimer and a fluorogenic ADAM-17 substrate, measuring the fluorescence, and calculating an IC50 value for the fusion protein or the dimer. In some embodiments, the IC50 of the fusion protein or the dimer is less than 10μM, less than 9μM, less than 8μM, less than 7μM, less than 6μM, less than 5μM, less than 4μM, less than 3μM, less than 2μM, or less than 1μM. In some embodiments, the IC50 of the fusion protein or the dimer is between 0.5μM and 10μM. In some embodiments, the IC50 of the fusion protein or the dimer is between 0.5μM and 5μM. In some embodiments, the IC50 of the fusion protein or the dimer is between 0.5μM and 4μM. In some embodiments, the IC50 of the fusion protein or the dimer is between 0.5μM and 2μM. In some embodiments, the IC50 of the fusion protein or the dimer is between 0.5μM and 1μM.

In some embodiments, the fusion protein or the dimer can inhibit and/or impair the activity of neutrophil elastase (NE). In some embodiments, inhibition of NE is measured by incubating NE with the fusion protein or the dimer and a fluorogenic NE substrate, and measuring the fluorescence, and calculating an IC50 value for the fusion protein or the dimer. In some embodiments, the IC50 of the fusion protein or the dimer is less than 100nM, less than 90nM, less than 80nM, less than 70nM, less than 60nM, less than 50nM, less than 40nM, less than 30nM, less than 20nM, or less than 10nM. In some embodiments, the IC50 of the fusion protein or the dimer is between 1nM and 100nM. In some embodiments, the IC50 of the fusion protein or the dimer is between 1nM and 50nM. In some embodiments, the IC50 of the fusion protein or the dimer is between 1nM and 40nM. In some embodiments, the IC50 of the fusion protein or the dimer is between 1nM and 20nM. In some embodiments, the IC50 of the fusion protein or the dimer is between 1nM and 10nM.

In some embodiments, the fusion protein or the dimer has a lower IC50 for NE compared to a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, and P329G have a lower IC50 for NE compared to an equivalent fusion proteins not comprising mutations corresponding to L234A, L235A, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E have a lower IC50 for NE compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G have a lower IC50 for NE compared to an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E and comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25 have a lower IC50 for NE compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the fusion protein or the dimer has increased serum half-life compared to a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34,

or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, and P329G have increased serum half-life compared to an equivalent fusion proteins not comprising mutations corresponding to L234A, L235A, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E have increased serum half-life compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E. In some embodiments, fusion proteins of the invention comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G have increased serum half-life compared to an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G. In some embodiments, fusion proteins of the invention comprising mutations corresponding to M252Y, S254T, and T256E and comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25 have increased serum half-life compared to an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the serum half-life is measured by administering the fusion protein or the dimer to a mouse, taking blood samples from the mouse at various time points (for example at day 1, day 2, day 3, day 4, day 6, day 8, day 10, day 14, day 17, and day 21), and measuring the concentration of the fusion protein or the dimer in the samples using an ELISA. In some embodiments, the fusion proteins and the dimers of the invention have a serum half-life of at least 100 hours, at least 110 hours, at least 120 hours, at least 130 hours, at least 140 hours, at least 150 hours, at least 160 hours, at least 170 hours, at least 180 hours, at least 190 hours, at least 200 hours, at least 210 hours, at least 220 hours, or at least 230 hours.

In some embodiments, the fusion proteins of the invention have:

(a) an IC50 of between 0.5μM and 1μM for ADAM-17;

(b) an IC50 of between 1nM and 10nM for NE; and

(c) a serum half-life of at least 210 hours.

The present invention also contemplates an amino acid sequence as set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID

NO. 13, SEQ ID NO. 14, SEQ ID NO. 15 and SEQ ID NO. 16, a fragment, a variant or a combination of any one thereof.

The fusion protein can form a homodimer or a heterodimer. In one aspect, one AAT-Fc dimerizes with a second AAT-Fc domain thereby forming a homodimer wherein the sequence of the second AAT-Fc is similar to the sequence of the first AAT-Fc (i.e. symmetric homodimer). A non-limiting example of a homodimeric fusion protein is represented in Figure 9 that can be formed by 2X SEQ ID NO. 9, 2X SEQ ID NO. 10, or 2X SEQ ID NO. 16. In one aspect, the two AAT-Fcs can be linked through one or more disulfide bond(s). In some embodiments, the present invention provides a dimer comprising a fusion protein of the invention and a second protein. In some embodiments, the second protein is identical to the fusion protein, i.e. the fusion protein and the second protein comprise or consist of the same amino acid sequence.

In another aspect, one AAT-Fc dimerizes with an other domain thereby forming a heterodimer (i.e. asymmetric heterodimer) wherein the other domain is an Fc domain or an Fc comprising a signal domain. A non-limiting example of a heterodimeric fusion protein is represented in Figure 10 that can be formed by SEQ ID NO. 15 and SEQ ID NO.12 or SEQ ID NO.11 and SEQ ID NO.12. Where the dimer is a heterodimer, the fusion protein and second protein may comprise so called “knob in hole” mutations in their respective Fc domains. For example, the Fc domain of the fusion protein may comprise a mutation corresponding to T366W (according to Kabat numbering of the Fc domain). The Fc domain of the second protein may comprise one or more mutations corresponding to T366S, L368A, Y407V, H435R, and/or Y436F (according to Kabat numbering of the Fc domain). In some embodiments, the second protein does not comprise an AAT polypeptide. In some embodiments, the second protein comprises or consists of an amino acid sequence of SEQ ID NO: 23.

Alpha1-Antitrypsin (hereafter "AAT") is a protein that naturally occurs in the human body. It is derived from blood plasma (pd) by pharmaceutical companies worldwide for the treatment of AAT-deficiency, a hereditary disorder. Preferably, the AAT of the invention is a human sequence, most preferably as set forth in SEQ ID No. 1 (see Table 1) or SEQ ID NO: 28, 35, or 36. AAT protein can be obtained by isolation from blood (e.g. human blood) or can be produced recombinantly.

In some embodiments, the AAT polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36. In some embodiments, the AAT polypeptide comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36.

As used herein, a "fragment" of an AAT protein or Fc protein, peptide or polypeptide of the invention refers to a sequence containing less amino acids in length than the AAT protein, peptide or polypeptide of the invention. This sequence can be used as long as it exhibits the same properties, i.e. is biologically active, as the native AAT sequence from which it derives.

Preferably, the fragment shares about 5 consecutive amino-acids, at least about 7 consecutive amino-acids, at least about 15 consecutive amino-acids, at least about 20 consecutive amino-acids, at least about 25 consecutive amino-acids, at least about 30 consecutive amino-acids, at least about 35 consecutive amino-acids, at least about 40 consecutive amino-acids, at least about 45 consecutive amino-acids, at least about 50 consecutive amino-acids, at least about 55 consecutive amino-acids, at least about 60 consecutive amino-acids, at least about 100 consecutive amino-acids, at least about 150 consecutive amino-acids, at least about 200 consecutive amino-acids, at least about 300 consecutive amino-acids, etc… or more of the native human AAT amino acid sequence.

According to one aspect of the invention, the AAT fragment is a sequence containing less amino acids in length than the C-terminal AAT sequence 374-418 (SEQ ID NO. 2). Alternatively, the AAT fragment comprises, or consists of, SEQ ID NO. 2.

Table 1

Sequence	SEQ ID NO:
MPSSVSWGILLLAGLCCLVPVSLAEDPQGDAAQKTDTSHH DQDHPTFNKITPNLAEFAFS LYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILE GLNFNLTEIPEAQIHEGF	1

QELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKL YHSEAFTVNFGDTEEAKKQ INDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWER PFEVKDTEEEDFHVDQVTTV KVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLP DEGKLQHLENELTHDIITKFL ENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGAD LSGVTEEAPLKLSKAVHKA VLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQN TKSPLFMGKVVNPTQK	
MFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPT QK	2

The term "variant" refers to a protein, peptide or polypeptide having an amino acid sequence that differs to some extent from the native sequence peptide (e.g. AAT or Fc), that is an amino acid sequence that vary from the native sequence by amino acid substitutions, whereby one or more amino acids are substituted by another with same characteristics and conformational roles. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence, e.g. at the N- or C-terminal sequence or within the amino acid sequence. Substitutions can also be conservative, in this case, the conservative amino acid substitutions are herein defined as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly

II. Polar, positively charged residues: His, Arg, Lys

III. Polar, negatively charged residues: and their amides: Asp, Asn, Glu, Gln

IV. Large, aromatic residues: Phe, Tyr, Trp

V. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys.

Non-limiting examples of a variant, e.g. of a variant of an Fc sequence, is shown in SEQ ID NO. 11 (substitution T366W), SEQ ID NO. 12 (substitutions T366S, L368A, Y407V, H435R, and Y436F), SEQ ID NO. 11 / SEQ ID NO. 15 (substitution T366W).

"Homology" refers to the percent identity between two polynucleotide or two polypeptide moieties. Two nucleic acid, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 50% sequence identity, preferably at least about 75% sequence identity, more preferably at least about 80% or at least about 85% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95%-98% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified sequence.

Alternatively, homology can be determined by readily available computer programs or by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single stranded specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art.

The peptidic variants may be linear peptides or cyclic peptides and may be selected from the group comprising short cyclic peptides derived from the C-terminal sequence as set forth in SEQ ID No. 2. Preferably, the short cyclic peptides derived from the C-terminal sequence of Alpha1-Antitrypsin will be selected from the non-limiting group comprising Cyclo-(CPFVFLM)-SH, Cyclo-(CPFVFLE)-SH, Cyclo-(CPFVFLR)-SH, and Cyclo-(CPEVFLM)-SH, or any combination thereof.

As used herein, an "isoform" of an AAT protein, peptide or polypeptide of the invention refers to a splice variant resulting from alternative splicing of the AAT mRNA. Isoforms of AAT are known in the art (see e.g. Matsuda, E., Ishizaki, R., Taira, T., Iguchi-Ariga, S. M., & Ariga, H., 2005, Biological & pharmaceutical bulletin, 28(5), 898–901).

In some aspects, the amino acid sequence of AAT, the variant, isoform or fragment thereof, as described herein, is at least 80% identical to the corresponding amino acid sequence in SEQ ID NO: 1. In some aspects, the amino acid sequence of AAT, the variant, isoform or fragment thereof is 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, or 99% identical to a corresponding amino acid sequence in SEQ ID NO: 1.

As used herein, the molecule that increases or potentiates the capacity of AAT to inhibit a human protease polypeptide, fragment or variant thereof is preferably an Fc domain of an immunoglobulin G (IgG), a fragment or variant of thereof.

In one aspect, the Fc domain of an IgG is preferably a Fc domain of a mouse or a human IgG, most preferably of a human IgG1, IgG2, IgG3 or IgG4, a fragment or a variant thereof.

In one aspect, the Fc domain of a human IgG is selected from the group comprising, or consisting of, an IgG1 Fc (SEQ ID No. 3), an IgG2 Fc (SEQ ID No. 6), an IgG3 Fc (SEQ ID No. 7), and an IgG4 Fc (SEQ ID No. 8), a fragment, a variant, or a combination of one or more of these sequences. In some embodiments, the Fc domain comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NOs: 29 to 33. In some embodiments, the Fc domain comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NOs: 29 to 33.

In some embodiments, the Fc domain comprises a wild-type hinge region. The hinge region is described in, for example, Rispens, T., Huijbers, M.G. The unique properties of IgG4 and its roles in health and disease. Nat Rev Immunol 23, 763–778 (2023). In some embodiments, the Fc domain comprises a truncated hinge region. In some embodiments, (a) the Fc domain is an IgG1 Fc domain, and the IgG1 Fc domain comprises a hinge region, optionally wherein the hinge region comprises or consists of the amino acid sequence of SEQ ID NO: 25; or

(b) the Fc domain is an IgG4 Fc domain, and the IgG4 Fc domain comprises a hinge region, optionally wherein the hinge region comprises or consists of the amino acid sequence of SEQ ID NO: 26 or 27.

A "fragment" of an IgG polypeptide of the invention refers to a sequence containing less amino acids in length than the IgG polypeptide of the invention. This sequence can be used as long as it exhibits the same properties, i.e. is biologically active, as the native IgG sequence from which it derives.

Preferably, the fragment shares about 5 consecutive amino-acids, at least about 7 consecutive amino-acids, at least about 15 consecutive amino-acids, at least about 20 consecutive amino-acids, at least about 25 consecutive amino-acids, at least about 30 consecutive amino-acids, at least about 35 consecutive amino-acids, at least about 40 consecutive amino-acids, at least about 45 consecutive amino-acids, at least about 50 consecutive amino-acids, at least about 55 consecutive amino-acids, at least about 60 consecutive amino-acids, at least about 100 consecutive amino-acids, at least about 150 consecutive amino-acids, at least about 200 consecutive amino-acids, or more of the native human IgG amino acid sequence.

The term "variant" refers to an IgG Fc domain polypeptide having an amino acid sequence that differs to some extent from the Fc domain native sequence peptide, that is an amino acid sequence that varies from the Fc native sequence by amino acid substitutions, whereby one or more amino acids are substituted by another with same characteristics and conformational roles. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence, e.g. at the N- or C-terminal sequence or within the amino acid sequence. Substitutions can also be conservative, in this case, the conservative amino acid substitutions are herein defined as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly

II. Polar, positively charged residues: His, Arg, Lys

III. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln

IV. Large, aromatic residues: Phe, Tyr, Trp

V. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys.

In general, the sequences of such variants will have a high degree of sequence homology or identity to the reference sequence, e.g. sequence homology or identity of more than 25%, generally more than 50% to 70%, even more particularly 80%, or 85% or more, such as at least 90%, or 95% or more, when the two sequences are aligned. Preferably, the reference sequence is the human IgG Fc fragment amino acid sequence as set forth in any one of sequences IgG1 Fc (SEQ ID No. 3), an IgG2 Fc (SEQ ID No. 6), an IgG3 Fc (SEQ ID No. 7), and an IgG4 Fc (SEQ ID No. 8), a fragment or a combination of one or more of these sequences.

Examples of IgG Fc domain variants are known in the art and described, e.g. in Ko S, Park S, Sohn MH, Jo M, Ko BJ, Na JH, Yoo H, Jeong AL, Ha K, Woo JR, Lim C, Shin JH, Lee D,

Choi SY, Jung ST. An Fc variant with two mutations confers prolonged serum half-life and enhanced effector functions on IgG antibodies. Exp Mol Med. 2022 Nov;54(11):1850-1861. In some respects, in particular where the fusion protein is a homo – or heterodimer, the mutations are knob and hole mutations to produce dimerization.

In one aspect, AAT, fragment, or variant thereof, is covalently fused to the N-terminus or the C-terminus of the Fc domain by, or via a linker, e.g. a polypeptide linker. In one aspect, the polypeptide linker consists primarily of stretches of Gly and Ser residues ("GS" linker) or Gly-Gly and Ser residues ("GGS" linker) or Gly-Gly-Gly-Gly and Ser residues ("GGGGS" linker) followed or not by one or more Arg residue ("R" residue). In some embodiments, the linker comprises at least 5 amino acids. In some embodiments, the linker is between 5 and 15 amino acids in length. In some embodiments, the linker comprises at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 amino acids in length. In some embodiments, the linkers comprise 10-30 amino-acids, optionally, 10-25 amino-acids, and further optionally 15-25 amino-acids. Non-limiting examples of GGS and GGGGS linkers are disclosed herein.

Examples of linkers comprise:
SEQ ID NO: 4 – GGS linker
GGSGGSGGSGGSGGSGGSGGS

SEQ ID NO: 5 – GGGGS linker
GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 24 – GGGGS linker
GGGGSGGGGS

In one aspect, one or more signal peptide is fused to the to the N-terminus and/or the C-terminus of the AAT-Fc fusion protein. Usually, the signal peptide comprises 10-30 amino-acids, preferably, 10-25 amino-acids, and more preferably 15-25 amino-acids. In some aspect, the first amino acid of the signal peptide is methionine ("M"). A non-limiting example of a signal peptide is disclosed herein (e.g. SEQ ID NO. 14).

In an alternative aspect, AAT, a fragment, or a variant thereof, is not fused to the Fc domain, fragment, or variant thereof, but both are present in the same composition of the invention and administered simultaneously or staggered in time.

In some embodiments, the fusion protein comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NOs: 17 to 19, 21, or 22. In some embodiments, the fusion protein comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NOs: 17 to 19, 21, or 22.

General definitions

The term "comprise/comprising" is generally used in the sense of include/including, that is to say permitting the presence of one or more features or components. The terms "comprise(s)" and "comprising" also encompass the more restricted ones "consist(s)", "consisting" as well as "consist/consisting essentially of", respectively.

As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

As used herein, "at least one" means "one or more", "two or more", "three or more", etc.

The terms “protein” and “polypeptide” are used interchangeably herein, and are intended to refer to a polymeric chain of amino acids of any length.

The terms “nucleic acid molecule”, “polynucleotide” and “nucleotide sequence” are intended to refer to a polymeric chain of any length of nucleotides, including deoxyribonucleotides, ribonucleotides, or analogues thereof. For example, the nucleic acid molecule, polynucleotide or nucleotide sequence may comprise DNA (deoxyribonucleotides) or RNA (ribonucleotides). The nucleic acid molecule, polynucleotide or nucleotide sequence may consist of DNA. The nucleic acid molecule, polynucleotide or nucleotide sequence may be mRNA. Since the nucleic acid molecule, polynucleotide or nucleotide sequence may comprise RNA or DNA, all references to T (thymine) nucleotides may be replaced with U (uracil).

For the purpose of this invention, in order to determine the percent identity of two sequences (such as two polynucleotide or two polypeptide sequences), the sequences are

aligned for optimal comparison purposes (e.g. gaps can be introduced in a first sequence for optimal alignment with a second sequence). The nucleotide or amino acid residues at each position are then compared. When a position in the first sequence is occupied by the same amino acid as the corresponding position in the second sequence, then the amino acids are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e. % identity = number of identical positions /total number of positions in the reference sequence x 100).

Typically, the sequence comparison is carried out over the length of the reference sequence. For example, if the user wished to determine whether a given ("test") sequence has at least 90% identity to SEQ ID NO: 19, SEQ ID NO: 19 would be the reference sequence. To assess whether a sequence has at least 90% identity to SEQ ID NO: 19 (an example of a reference sequence), the skilled person would carry out an alignment over the length of SEQ ID NO: 19, and identify how many positions in the test sequence were identical to those of SEQ ID NO: 19. If at least 90% of the positions are identical, the test sequence is at least 90% identical to SEQ ID NO: 19. If the sequence is shorter than SEQ ID NO: 19, the gaps or missing positions should be considered to be non-identical positions.

The skilled person is aware of different computer programs that are available to perform an alignment between two sequences. An alignment between two sequences can be accomplished using a mathematical algorithm. For example, an alignment may be performed using the Needleman and Wunsch algorithm (Needleman and Wunsch, 1970, J Mol Biol.;48(3):443- 53) which aligns the sequences optimally over the entire length). Sequences of substantially different lengths may alternatively be aligned using a local alignment algorithm (e.g. 15 Smith and Waterman algorithm (Smith and Waterman, 1981, J Theor Biol. ;91 (2):379-80) or Altschul algorithm (Altschul SF et al., 1997, Nucleic Acids Res.;25(17):3389-402,; Altschul SF et al., 2005, Bioinformatics.;21(8):1451-6). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

Fusion proteins of the invention may comprise mutations in the Fc domain "corresponding to" mutations at specified positions. In these embodiments, the residues are typically numbered according to the EU index of Kabat. For example, an Fc domain comprising a mutation corresponding to L234A refers to an Fc domain with an A at the position that best aligns to L234 in a wild-type Fc domain wherein the residues are numbered according to the

EU index of Kabat. It is within the capabilities of the person skilled in the art to determine which amino acids in an alternative amino acid sequence "correspond to" a given residue in a wild-type Fc domain numbered according to the EU index of Kabat. For example, the person skilled in the art merely needs to perform a sequence alignment of the alternative amino acid sequence with a wild-type Fc domain numbered according to the EU index of Kabat using a suitable alignment algorithm such as that of Needleman and Wunsch described above, and determine which region of the alternative amino acid sequence best aligns to the specified residues in the wild-type Fc domain numbered according to the EU index of Kabat. For example, the skilled person is able to align the alternative amino acid sequence with SEQ ID NO: 3 and determine which amino acid best aligns, and therefore corresponds to, e.g. residue L234 of SEQ ID NO: 3 numbered according to the EU index of Kabat. If the amino acid corresponding to L234 is an A residue, then the amino acid sequence comprises a mutation corresponding to L234A.

Methods of treatment

According to one aspect of the invention, the invention provides a fusion protein for use in the prevention and/or treatment of a disease or disorder of the nervous system, wherein said fusion protein comprises

(i) an immunoglobulin IgG Fc domain, a variant or a fragment thereof, and

(ii) an alpha1-antitrypsin (AAT) polypeptide, a variant, an isoform and/or a fragment thereof,

wherein the AAT polypeptide, variant, isoform and/or fragment thereof, is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, variant or fragment thereof.

The term "treatment" or "treating" means any administration of a composition, pharmaceutical composition, therapeutic agent, compound, etc… of the disclosure to a subject for the purpose of:

(i) inhibiting the disease, that is, arresting the development of clinical symptoms described herein; and/or

(ii) relieving the disease, that is, causing the regression of clinical symptoms described herein.

As used herein, the term “prevention” or “preventing” means any administration of a composition, pharmaceutical composition, therapeutic agent, compound, etc. of the disclosure to a subject for the purpose of preventing a disease or disorder, for example a disease or disorder of the peripheral nervous system (PNS), more preferably a demyelinating and/or dysmyelinating PNS disease or associated disorder, that is, causing the clinical symptoms of the disease not to develop.

In some embodiments, the disease or disorder is selected from the group consisting of alpha-1 antitrypsin deficiency (AATD), chronic obstructive pulmonary disease (COPD), Charcot-Marie-Tooth disease (CMT), Guillain-Barré Syndrome (GBS), Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), neuropathic pain, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), amyloid polyneuropathy (Transthyretin-Related Familial Amyloid Polyneuropathy), panniculitis, vasculitis, anti-proteinase-3 (PR3)-related vasculitis, inflammatory bowel disease (IBD), ulcerative colitis, hypothyroidism, Graft-versus-Host disease (GVHD), Type 1 diabetes mellitus, Type 2 diabetes mellitus, diabetic neuropathies, cardiovascular disease, arterial stiffness, hypertension, osteoporosis, sarcopenia, Alzheimer’s disease, neurodegenerative conditions, viral infection such as HIV-1 infection, coronavirus infection, and microbial infection such as Pseudomonas aeruginosa infection.

In the context of the present invention, the disease may be a disease or disorder of the peripheral nervous system (PNS), more preferably a demyelinating and/or dysmyelinating PNS disease or associated disorder.

As used herein the terms "subject"/"patient", are well-recognized in the art, and are used interchangeably herein to refer to a mammal, including dog, cat, rat, mouse, monkey, cow, horse, goat, sheep, pig, camel, and, most preferably, a human. In some cases, the subject is a subject in need of treatment or a subject with a disease or disorder. However, in other aspects, the subject can be a normal subject. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered. Preferably, the subject is a human, most preferably a human patient having a disease or disorder of the nervous system, preferably a disease or disorder of the peripheral nervous system (PNS), more preferably a demyelinating and/or dysmyelinating PNS disease or associated disorder.

The term "disease or disorder of the nervous system", as used herein, refers to a group of disease or disorders, wherein the pathology involves the nervous system. In some aspects, the disease or disorder of the nervous system described herein is a demyelinating and/or dysmyelinating disease or disorder of the peripheral nervous system (PNS).

Demyelinating diseases are common neurological disorders that affect the central nervous system (CNS) and peripheral nervous system (PNS). They cause substantial disability, and some are associated with a high mortality rate if not treated promptly. Peripheral nerve demyelinating diseases discussed herein are Guillain–Barré syndrome, chronic inflammatory demyelinating neuropathy, anti-myelin-associated glycoprotein neuropathy, and POEMS syndrome.

ADAM-17 activity modulation is involved in myelin regulation and as an inflammation hallmark of chronic inflammatory demyelinating polyneuropathy. Inhibiting and/or impairing the activity of ADAM-17 by a compound, a molecule, a method or a composition of the invention will be beneficial for a subject in need thereof, i.e. suffering from a demyelinating and/or dysmyelinating PNS disease or associated disorder.

Dysmyelinating diseases and disorders affect both the central nervous system (CNS) and peripheral nervous system (PNS) and refer to diseases or disorders characterized by a failure to form myelin normally thus leading to hypomyelination.

According to one aspect of the invention, the demyelinating and/or dysmyelinating disease or disorder of the PNS is selected from the group comprising Charcot–Marie–Tooth (CMT), Guillain-Barré Syndrome (GBS), neuropathic pain and chronic inflammatory demyelinating polyneuropathy (CIDP).

Charcot-Marie-Tooth (CMT) is the most prevalent category of demyelinating and/or dysmyelinating disease or disorder of the PNS. It is an inherited neuropathy with autosomal dominant inheritance pattern being the most common, though there also are X-linked and autosomal recessive subtypes. In addition to a variety of inheritance patterns, there are a myriad of genes associated with CMT, reflecting the heterogeneity of this disorder. Currently, more than 90 distinct genetic variations have been implicated in causing or contributing to the

clinical picture of these neuropathies. The most encountered genetic mutations affect the PMP22, GJB1, MPZ, and MFN2 genes.

Conduction velocity parameters, as well as the pattern of inheritance help stratify CMT into the following major categories: CMT1 as demyelinating subtype (autosomal dominant); CMT2 as axonal subtype (autosomal dominant or recessive CMTA2A); CMTX with intermediate conduction velocities (most classically X-linked, though there are autosomal dominant and recessive intermediate variants); CMT3 also known as Dejerine-Sottas disease and CMT4.

CMT1 can be further subcategorized as: CMT1A, caused by a 1.4 Mb duplication of the PMP22 gene; CMTX1, a common form of hereditary motor and sensory neuropathy that has an X-linked dominant inheritance pattern; CMT1B, caused by mutations in the MPZ gene (1q22).

Guillain-Barré Syndrom (GBS) (including its variant, Miller Fisher syndrome (MFS)), is the most common form of neuromuscular paralysis. It is an acute inflammatory demyelinating polyradiculoneuropathy which is characterized by rapidly progressive proximal and distal symmetric weakness, sensory loss, and depressed reflexes. GBS mostly affects young people and can cause long-term residual disability.

Chronic inflammatory demyelinating polyneuropathy (CIDP), also known as chronic inflammatory demyelinating polyradiculoneuropathy), is an acquired, immune-mediated neuropathy affecting peripheral nerves and nerve roots, typically characterized by a relapsing-remitting or progressive course of symmetric weakness of proximal and distal muscles.

The inventors have discovered that, in contrast to what has been described in previous articles (see e.g. Lee S, Lee Y, Hong K, Hong J, Bae S, Choi J, Jhun H, Kwak A, Kim E, Jo S, Dinarello CA, Kim S. Effect of recombinant α1-antitrypsin Fc-fused (AAT-Fc) protein on the inhibition of inflammatory cytokine production and streptozotocin-induced diabetes. Mol Med. 2013 May 20;19(1):65-71), AAT, when fused to an Fc fragment, possessed a more potent capacity of inhibiting a human protease (i.e. Neutrophil Elastase (NE) or ADAM-17) than plasma derived AAT.

As used herein, Metalloprotease 17 (ADAM-17), also called TACE (tumor necrosis factor-α-converting enzyme), refers to a 70 kDa enzyme that belongs to the ADAM protein family of disintegrins and metalloproteases.

In some embodiments, the disease or disorder is AAT deficiency (AATD) or Chronic Obstructive Pulmonary Disease (COPD).

AATD is a genetic disease characterized by insufficient levels of AAT which may cause lung disease such as emphysema, loss of lung function, and decreased life expectancy. COPD is a chronic lung condition characterized by inflammation in the airways of the lungs, resulting in reduced airflow and breathing difficulties.

Nucleic acids

Further provided is a nucleic acid sequence encoding one or more recombinant constructs of the invention, such as e.g., the fusion proteins of the invention.

Non-limiting examples of an nucleic acid sequence encoding one or more recombinant constructs of the invention comprise a nucleic acid encoding an amino acid sequence as set forth in SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 12, SEQ ID NO. 13, SEQ ID NO. 14, SEQ ID NO. 15 and SEQ ID NO. 16, or any variant, fragment of combination thereof.

The terms "nucleic acid", "polynucleotide," and "oligonucleotide" are used interchangeably and refer to any kind of deoxyribonucleotide (e.g. DNA, cDNA, ...) or ribonucleotide (e.g. RNA, mRNA, ...) polymer or a combination of deoxyribonucleotide and ribonucleotide (e.g. DNA/RNA) polymer, in linear or circular conformation, and in either single or double stranded form. These terms are not to be construed as limiting with respect to the length of a polymer and can encompass known analogues of natural nucleotides, as well as nucleotides that are modified in the base, sugar and/or phosphate moieties (e.g. phosphorothioate backbones). In general, an analogue of a particular nucleotide has the same base-pairing specificity, i.e. an analogue of A will base-pair with T.

Vectors, host cells and compositions

Further contemplated is a plasmid or a vector comprising a nucleic acid sequence of the invention.

The term "vector", as used herein, refers to a viral vector or to a nucleic acid (DNA or RNA) molecule such as e.g. a plasmid or other vehicle, which contains one or more heterologous nucleic acid sequence(s) of the invention and, preferably, is designed for transfer between different host cells. The terms "expression vector", "gene delivery vector" and "gene therapy vector" refer to any vector that is effective to incorporate and express one or more nucleic acid(s) of the invention, in a cell, preferably under the regulation of a promoter. A cloning or expression vector may comprise additional elements, for example, regulatory and/or post-transcriptional regulatory elements in addition to a promoter.

For cloning of polynucleotides of the invention, the vector may be introduced into a host cell (autologous, allogeneic or heterologous) to allow replication of the vector itself and thereby amplify the copies of the polynucleotide contained therein. The cloning vectors of the invention may contain, without limitation, an origin of replication, promoter sequences, transcription initiation sequences, enhancer sequences, and selectable markers. These elements may be selected as appropriate by a person of ordinary skill in the art. For example, the origin of replication may be selected to promote autonomous replication of the vector in the host cell.

In certain aspects, the present disclosure provides isolated host cells, or population of cells, comprising, or expressing, the fusion protein, the vector or plasmid provided herein. The host cells, or population of cells, containing the vector or plasmid may be useful in expression or cloning of the polynucleotide contained in the vector. Suitable host cells can include, without limitation, prokaryotic cells, fungal cells, yeast cells, or higher eukaryotic cells such as mammalian cells. Suitable prokaryotic cells for this purpose include, without limitation, eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans, and Shigella, as well as Bacilli such as B. subtilis and B. licheniformis, Pseudomonas such as P. aeruginosa, and Streptomyces.

The vector or plasmid can be introduced to the host cell, or population of cells, using any suitable methods known in the art, including, without limitation, DEAE-dextran mediated delivery, calcium phosphate precipitation method, cationic lipids mediated delivery, liposome mediated transfection, electroporation, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. Standard methods for transfection and transformation of cells for expression of a vector or plasmid of interest are well known in the art.

Where the host cell is intended to be administered as a cell therapy to the subject in need thereof, the host cell (autologous, allogeneic or heterologous) will preferably be selected from the group comprising a glial cell, a neuronal cell, a fibroblast cell, a cell line, a stem cell or a progenitor of any one of these cells.

The present invention also contemplates compositions as well as pharmaceutical compositions.

In an aspect of the invention, the pharmaceutical composition of the invention comprises a therapeutically effective amount of i) a fusion protein for use of the invention, ii) a vector of the invention or iii) a host cell of the invention, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

In an aspect of the invention, the pharmaceutical composition of the invention comprises a therapeutically effective amount of a fusion protein described herein, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

These constructs can be used as a dimer or as a monomeric form in the composition disclosed herein. In accordance with these aspects, a pharmaceutical composition can include a dimer of AAT-Fc (symmetric or asymmetric) or a monomer of AAT-Fc or AAT cleaved from the Fc or combinations thereof.

In an aspect of the invention, the pharmaceutical composition of the invention comprises a therapeutically effective amount of a plasmid or a vector described herein, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

In an aspect of the invention, the pharmaceutical composition of the invention comprises a therapeutically effective amount of an isolated host cell, or population of cells described herein, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

The pharmaceutical composition described above can further comprise, or be administered in combination with, another therapy.

As used herein, a "therapeutically effective amount" of an agent, (such as e.g., a fusion protein, a plasmid or a vector, an isolated host cell or a population of cells) refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. Furthermore, the effective amount may depend on the individual patient's history, age, weight, family history, genetic makeup, stage of the thyroid-related autoimmune disease, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

In some cases, a therapeutically effective amount of the pharmaceutical composition of the invention can be any amount that reduces the severity, or occurrence, of symptoms of the disease, disorder and/or condition to be treated without producing significant toxicity to the subject. In some cases, an effective amount of the pharmaceutical composition of the invention can be any amount that reduces the number of diseased cells (e.g. dysregulated immune cells), autoantibodies, and/or other disease markers (e.g. cytokines) without producing significant toxicity to the subject.

The therapeutically effective amount of the pharmaceutical composition of the invention (and any additional therapeutic agent) can remain constant or can be adjusted as a sliding scale or variable dose depending on the subject´s response to treatment. In some cases, the frequency of administration can be any frequency that reduces the severity, or occurrence, of symptoms of the disease, disorder and/or condition to be treated without producing significant toxicity to the subject. Various factors can influence the actual effective amount used for a particular application. For example, the frequency of administration, duration of treatment, use of multiple treatment agents, route of administration, and severity of the disease, disorder and/or condition may require an increase or decrease in the actual effective amount administered.

The pharmaceutical compositions of the present invention may be administered to a subject by different routes including oral, parenteral, sublingual, transdermal, rectal, transmucosal, topical, via inhalation, via buccal administration, intrapleural, intravenous,

intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal, intrathecal, and intraarticular or combinations thereof. For human use, the composition may be administered as a suitably acceptable formulation in accordance with normal human practice. The skilled artisan will readily determine the dosing regimen and route of administration that is most appropriate for a particular patient. The compositions of the invention may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The compositions of the invention may be administered alone or in combination with other therapies.

The pharmaceutical compositions of the present invention may also be delivered to the patient, by several technologies including DNA injection of nucleic acid encoding the fusion protein, a variant, an isoform and/or a fragment thereof of the invention (also referred to as DNA vaccination) with and without in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant lentivirus, recombinant adenovirus, and recombinant adeno-associated virus (AAV) as described herein.

Methods of producing and purifying fusion proteins

In some embodiments, the present invention provides a method of producing a fusion protein or a dimer according to the invention. In some embodiments, the method comprises expressing the fusion protein or the dimer in cells, for example CHO HK1 cells. In some embodiments, expressing the fusion protein or the dimer comprises transfecting a cell with a nucleic acid or vector encoding the fusion protein or the dimer.

In some embodiments, the present invention provides a method of purifying a fusion protein according to the invention. In some embodiments, the method comprises a step of affinity chromatography. In some embodiments, the step of affinity chromatography uses a column comprising protein A. In some embodiments, the protein A comprises a Z domain.

The present inventors have surprisingly found that fusion proteins comprising an immunoglobulin IgG Fc domain comprising:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E and a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25,

and a second polypeptide domain have an increased yield compared to equivalent fusion proteins not comprising features (i) to (iii). In some embodiments, the Fc domain is an IgG1 Fc domain.

Without wishing to be bound by theory, it is believed that this increased yield is due to increased binding affinity for modified protein A during a step of affinity chromatography. Fc affinity columns, for example the MabSelect SuRe™ column from Cytiva, comprise modified protein A which comprises a modified Fc-binding domain known as the Z domain (see Yu F, Järver P, Nygren PÅ (2013) Tailor-Making a Protein A-Derived Domain for Efficient Site-Specific Photocoupling to Fc of Mouse IgG1. PLOS ONE 8(2)). The Z domain is known to contact the Fc domain of IgG1 at positions 252-254. Introducing mutations M252Y and S254T into the IgG1 Fc domain appears to increase the affinity of the fusion protein for the Z domain, thereby increasing the yield of the affinity chromatography step.

Accordingly, the present invention provides a fusion protein comprising an immunoglobulin IgG Fc domain comprising:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E and a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25,

and a second polypeptide domain.

In some embodiments, the Fc domain is an IgG1 Fc domain. In some embodiments, the fusion protein has increased affinity for protein A compared to an equivalent fusion protein not comprising features (i) to (iii). In some embodiments, the fusion protein has an increased affinity for the Z domain of protein A. In some embodiments, the fusion protein has increased

yield during a step of affinity chromatography using a column comprising protein A comprising a Z domain compared to an equivalent fusion protein not comprising features (i) to (iii).

In some embodiments, the present invention provides a method of purifying a fusion protein comprising an immunoglobulin IgG Fc domain comprising:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E and a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25,

and a second polypeptide domain.

In some embodiments, the method comprises a step of affinity chromatography. In some embodiments, the step of affinity chromatography uses a column comprising protein A. In some embodiments, the protein A comprises a Z domain. In some embodiments, the method has a higher yield than an equivalent method carried out on a fusion protein not comprising features (i) to (iii).

In some embodiments, the present invention provides a method of increasing the yield of a fusion protein comprising an immunoglobulin IgG Fc domain, the method comprising introducing into the Fc domain:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E wherein the Fc domain comprises a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the method comprises a step of affinity chromatography using a column comprising protein A comprising a Z domain.

In some embodiments, the present invention provides the use of a fusion protein comprising an immunoglobulin IgG Fc domain comprising:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E and a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25,

and a second polypeptide domain for improving the yield of the fusion protein.

Typically, the yield of the fusion protein is the yield of a purification method.

In some embodiments, the purification method comprises a step of affinity chromatography using a column comprising protein A comprising a Z domain. In some embodiments, the yield is improved compared to an equivalent purification method performed on a fusion protein not comprising features (i) to (iii).

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. Priority applications EP24160663.1, EP 24166209.7, and EP24196583.9 are incorporated by reference in their entirety. The publications and applications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting.

In the case of conflict, the present specification, including definitions, will control. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject matter herein belongs. As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood

that the invention includes all such variations and modifications without departing from the spirit or essential characteristics thereof. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features. The present disclosure is therefore to be considered as in all aspects illustrated and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein. Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety. The foregoing description will be more fully understood with reference to the following Examples.

SEQUENCE LISTING

SEQ ID NO: 1 – human AAT (with signal peptide)

MPSSVSWGILLLAGLCCLVPVSLAEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEF
AFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIH
EGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDT
EEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEED
FHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHL
ENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVT
EEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTK
SPLFMGKVVNPTQK

SEQ ID NO: 2 – human AAT fragment (C-terminal sequence 374-418)

MFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

SEQ ID NO: 3 – IgG1 Fc

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO: 4 – GGS linker

GGSGGSGGSGGSGGSGGSGGS

SEQ ID NO: 5 – $(GGGGS)_4$ linker

GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 6 – human IgG2 Fc

ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNW
YVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIE

KTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 7 – IgG3 Fc

ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPR
CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVH
NAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQ
PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDS
DGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

SEQ ID NO: 8 – IgG4 Fc

ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 9 – human AAT-IgG1 Fc

MPSSVSWGILLLAGLCCLVPVSLAEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEF
AFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIH
EGFQELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDT
EEAKKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEED
FHVDQVTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHL
ENELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVT
EEAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTK
SPLFMGKVVNPTQKEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV
TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGK

SEQ ID NO: 10 – human AAT-IgG4 Fc

MGWSCIILFLVATATGVHSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLY
RQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQE
LLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKK
QINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQ
VTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELT
HDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPL
KLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFM
GKVVNPTQKGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

Signal peptide (in italic)
Linker (underlined)

SEQ ID NO: 11 – human AAT-IgG4 Fc Chain 1: Knob (T366W)

MGWSCIILFLVATATGVHSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLY
RQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQE
LLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKK
QINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQ
VTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELT
HDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPL
KLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFM
GKVVNPTQKGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTP
EVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLV
KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
VMHEALHNHYTQKSLSLSLGK

Signal peptide (in italic)

Linker (underlined)

Mutation (framed)

SEQ ID NO: 12 –IgG4 Fc Chain 2: Hole (T366S, L368A, Y407V), H435R, Y436F))

MGWSCIILFLVATATGVHSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPE
VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLSCAVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSV
MHEALHNRFTQKSLSLSLGK

Signal peptide (in italic)

Mutations (framed)

SEQ ID NO: 13 – $(GGGGS)_2$ linker

GGGGSGGGGS

SEQ ID NO: 14 – signal peptide

MGWSCIILFLVATATGVHS

SEQ ID NO: 15 – human AAT-IgG4 Fc Chain 1: Knob (T366W)

MGWSCIILFLVATATGVHSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLY
RQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQE
LLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKK
QINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQ
VTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELT
HDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPL
KLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFM
GKVVNPTQKGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGK

Signal peptide (in italic)

Linker (underlined)

Mutation (framed)

SEQ ID NO: 16 – human AAT-IgG4 Fc

MGWSCIILFLVATATGVHSEDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLY
RQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQE
LLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKK
QINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQ
VTTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELT
HDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPL
KLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFM
GKVVNPTQKGGGGSGGGGSGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

Signal peptide (in italic)

Linker (underlined)

SEQ ID NO: 17 – human AAT-IgG1 Fc, with LALAPG mutations (101C)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKEPKSCDKTHT
CPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**G**APIEKTISK

AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 18 – human AAT-IgG1 Fc with YTE mutations (101T)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKTRTCPPCPAP
ELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 19 – human AAT-IgG1 Fc with LALAPG-YTE mutations (101R)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKEPKSCDKTHT
CPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 20 – human AAT-IgG4 Fc 101S

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTLDEKGTE
AAGAEFLEAIPLSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKGSESKYGPPCP
PCPAPEFEGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ
PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGK

SEQ ID NO: 21 – human AAT-IgG4 Fc (symmetric – no signal peptide)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKGGGGSGGGG
SESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 22 – human AAT-IgG4 Fc T366W (asymmetric – no signal peptide)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE

AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKGGGGSGGGG
SESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 23 – human IgG4 T366S, L368A, Y407V, H435R, Y436F (asymmetric – no signal peptide)

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLVSRLTVDKSRWQEGNVFSCSVMHEALHNRFTQKSLSLSLG
K

SEQ ID NO: 24 – GS linker

GGGGSGGGGS

SEQ ID NO: 25 – IgG1 hinge

EPKSCDKTHTCPPCP

SEQ ID NO: 26 – IgG4 hinge

ESKYGPPCPSCP

SEQ ID NO: 27 – IgG4 hinge

ESKYGPPCPPCP

SEQ ID NO: 28 – human AAT (without signal peptide)

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL

PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

SEQ ID NO: 29 – IgG1 Fc (101C)

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO: 30 – IgG1 Fc (101T)

TRTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 31 – IgG1 Fc (101R)

EPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEV
KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

SEQ ID NO: 32 – IgG4 Fc (symmetric)

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 33 – IgG4 Fc (asymmetric)

ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFN
WYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLWCLVKGFYPSDIAVEWESNGQPEN

NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
K

SEQ ID NO: 34 – human AAT-IgG4 Fc

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAEFLEAIPLSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQKGSESKYGPPCP
PCPAPEFEGGPSVFLFPPKPKDTLYISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ
PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS
DGSFFLYSRLTVDKSRWQEGNVFSCSVLHEALHNHYTQKSLSLSLGK

SEQ ID NO: 35 - AAT variant

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTE
AAGAEFLEAIPLSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

SEQ ID NO: 36 – AAT variant

EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFSLYRQLAHQSNSTNIFFSPVSIAT
AFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGFQELLRTLNQPDSQLQLTTGNG
LFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEAKKQINDYVEKGTQGKIVDLVK
ELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFHVDQVTTVKVPMMKRLGMFNIQ
HCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLENEDRRSASLHL
PKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTLDEKGTE
AAGAEFLEAIPLSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

EXAMPLES

Example 1

Neutrophil elastase and ADAM17 activity upon inhibition with AAT multiple sources

Materials and Methods

SDS PAGE

SDS-PAGE coomassie-stained with AAT from multiple sources. Reduced samples were prepared for analysis by mixing with NuPage 4x LDS sample buffer (Life Technologies) and NuPage 10x sample reducing agent (Life Technologies), and incubated at 70°C, 10 min. For non-reduced samples, the reducing agent and heat incubation were omitted. 5µg of protein was loaded per lane. Samples were electrophoresed on 4-20% Mini- PROTEAN® Precast Gels (BioRad) with TGS buffer. Then gels were fixed for 30 minutes at room temperature in fixation buffer (50% Methanol + 10% acetic acid + 40% H2O). Coomassie blue (Sigma Aldrich) was added at final concentration of 0.25% and incubated for additional 15 minutes. Finally gels were destained overnight in multiple washes of fixation buffer, and imaged on a G:Box imager (Syngene).

Neutrophil elastase assay

Neutrophil Elastase Inhibition Assay. Substrate MeOSuc-AAPV-pNA 100 µM, purified human neutrophil elastase (ELA2) 2.2µU/µL and several concentrations of AAT (1 nM and 10 nM) were incubated together for 45 min. Absorbance was measured every minute in a spectrophotometer reader (A405nm). Vmax value was determined with the slope of the trendline during 20 min. The Experiment was performed in duplicate. Error bars indicate the standard deviation.

ADAM-17 activity

Here we tested the ability of a recombinant AAT-Fc fusion protein to inhibit ADAM-17 activity.

Human recombinant ADAM-17 protein (930-ADB-010, R&D system) and fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH2 (ES003 R&D system) were used for the assay.

Hr-ADAM-17 protein was reconstituted in sterile water at 100µg/ml and the peptide substrate was diluted to 2mM in DMSO.

500µg of AAT-Fc (YbdYbiotech, SEQ ID NO. 9) were resuspended in sterile water at 60µM. Plasma-derived AAT (Sigma A9160) was resuspended in sterile water at 500µM. The reagents were kept on ice.

Just before starting the reaction, we prepared a working solution (10µM, 4x concentrated to achieve a final concentration of 2.5µM of AAT-Fc and plasma-derived AAT). We diluted the HR-ADAM-17 protein 1:250 and the peptide substrate 1:100 in an assay buffer. The reagents were dispensed in triplicate on a black Maxisorp plate in this order:

- 25µl/well of AAT 10µM
- 25µl/well of HR-ADAM-17
- 50µl/well of peptide substrate.

In control wells, AAT was replaced by 25µl of assay buffer.

Control negative (blank) contains 50µl of assay buffer and 50µl of peptide substrate.

Fluorescence was measured immediately using a SpectraMax iD3 instrument using the following parameters: excitation wavelength 320 nm, emission wavelength 405 µm, kinetic mode for five minutes.

The blank values were subtracted from the other values and an analysis was performed using GraphPad-Prism.

Results

Figure 1 shows the protein size of each AAT sources tested. Figure 2 shows that all source of AAT are not able to inhibit neutrophil elastase at 1nM. At 10nM recombinant AAT4 and recombinant AAT-Fc (CHO) are able to inhibit completely neutrophil elastase activity in comparison to plasma-derived AAT (40% inhibition), recombinant AAT-1 (60%), recombinant AAT-2 (30%) and recombinant AAT-his 3 (0% inhibition). As shown in Figure 3, AAT-Fc at 2.5µM reduced ADAM-17 activity to 87%, whether there is no effect at the same concentration of plasma-derived AAT.

These data together suggest that AAT-Fc has the potential to be more effective than plasma-derived AAT for the treatment of CMT1A.

Example 2

Treatment with AAT and AAT-derived peptides on Human Schwann cells (HSC)

Materials and Methods

Human Schwann cells (HSC) were purchased from the Innoprot company and cultured according to the manufacturer's instructions. The TNFα was used at a concentration of 10ng/ml for 1 hour to activate the downstream pathway of TNFα. The AAT-derived peptides were purchased from IRIS Biotech and resuspended in DMSO.

Sequence of peptide 8: Ac-LFLYVIH-NH2; sequence of peptide 9: Ac-YRAHQGE-NH2; sequence of peptide 14: 4-Hydroxy-Bzl-His-(R/S)-Phg-NH2. We used 50µM concentrations of AAT (plasma-derived, SEQ ID NO. 1) and peptides for 48 hours. Following treatment, HSC were fixed with 4% PFA and permeabilized with 0.1% Triton. An anti-NF-kB/P65 antibody (Thermofisher) was incubated for two hours at room temperature, followed by an anti-rabbit-488 antibody (Alexa). The nuclei were counterstained with DAPI. The pictures were taken using a Leica fluorescent microscope.

Results

The purpose of this study was to investigate the regulation of TNFα signaling by AAT and peptides in HSC stimulated with TNFα.

The cytosol-nuclear translocation of NF-kB is one of the main downstream pathways regulated by TNFα. In the presence of activated signaling, nuclear NF-kB stained, whereas when the pathway is not activated, NF-kB is found in the cytosol.

Following plating, HSC were treated with AAT and peptides for 24 hours. The following day, we performed TNFα treatment in the presence or absence of freshly added AAT or peptide (Figure 4). After TNFα treatment, NF-kB immunostaining revealed a clear nuclear translocation. Some cells showed cytoplasmic and other nuclear localization in the presence of AAT. There is a similar but less potent effect observed in the presence of peptides 8 and 14. Peptide 9 does not appear to change TNFα stimulation's effect on NF-kB localization (Figure 5).

Example 3

ADAM-17 activity inhibition by AAT-Fc and IC50 values for AAT-FC

Materials and Methods

Human recombinant ADAM-17 protein (930-ADB-010, R&D system) and fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH2 (ES003 R&D system) were used for the assay.

Hr-ADAM-17 protein was reconstituted in sterile water at 100ug/ml.

Peptide substrate was diluted to 2mM in DMSO.

50ug of AAT-Fc (Ybdybiotech, SEQ ID NO. 9) were resuspended in sterile water at 5µM. AAT-Fc was tested at the following final concentrations: 0.012, 0.03, 0.11, 0.33, and 1 µM. See Figure 4 for AAT-Fc aminoacidic sequence.

The concentration of 25 µM of AAT (plasma-derived, SEQ ID NO.1) was used as a positive control.

The reagents were kept on ice. Just before starting the reaction, we prepared a working solution. We diluted the HR-ADAM-17 protein 1:250 and the peptide substrate 1:100 in an assay buffer. The reagents were dispensed in triplicate on a black Maxisorp plate in this order:

- 25µl/well of AAT
- 25µl/well of HR-ADAM-17
- 50µl/well of peptide substrate.

In control wells AAT was replaced by 25µl of assay buffer. Control negative (blank) contains 50µl of assay buffer and 50µl of peptide substrate. Fluorescence was measured immediately using a SpectraMax iD3 instrument using the following parameters: excitation wavelength 320 nm, emission wavelength 405 nm, kinetic mode for five minutes. The blank values were subtracted from the other values and the analysis was performed using GraphPad-Prism.

Results

AAT-Fc reduces ADAM-17 activity in a dose-dependent manner. According to the IC50 analysis, 50% inhibition (Figure 6B) is achieved at 0.25 µM of AAT-Fc, suggesting that AAT-

Fc is approximately 60 times more effective at inhibiting ADAM-17 activity than plasma-derived AAT (plasma derived AAT IC50: 15.3 µM, see Zhukovsky et al., 2022).

Example 4

AAT-Fc decreases IFNγ-induced expression of MHCII in microglia derived from Human Induced Pluripotent Stem Cell (hiPSCs)

Materials and Methods

Human microglia cells derived from hiPSCs and transduced with MHCII-FLuc reporter gene were cultured for 24 hours before treatment. The IFNγ was used at a concentration of 1µg/ml for 24 hours to activate the microglia MHCII-FLuc reporter gene. The AAT-derived peptides were purchased from IRIS Biotech, resuspended in DMSO and used at 50 µM for 48 hours. Similarly, AAT (plasma-derived, SEQ ID NO. 1), resuspended in sterile water, was used at 50 µM for 48 hours.

50ug of AAT-FC (Ybdybiotech, SEQ ID NO. 9) were resuspended in sterile water at 10µM and used at 1 µM for 48 hours.

Following treatment, the microglia cell viability was controlled with the Cell Counting Kit-8 (CCK-8), a sensitive colorimetric assay which determines the number of viable cells. CCK-8 solution was added directly to the cells and after 1 hour the absorbance was measured at 450nm using a SpectraMax iD3 instrument. Then, the quantitation of luciferase expression was done with ONE-Glo™ EX Luciferase Assay System. A volume of ONE-Glo™ EX Reagent that is equal to the volume of culture medium in each well was added, and samples were incubated for at least 3 minutes to lyse cells. The luminescence was then read using a SpectraMax iD3 instrument. The luciferase activity was calculated and normalized to the number of viable cells and results were displayed using GraphPad-Prism.

Results

Using microglia derived from hiPSCs and stable expressing the MHCII-Fluc reporter gene, we demonstrated the anti-(neuro)inflammatory properties of AAT-FC and AAT-derived peptides. We stimulated the cells with IFNγ, to increase MHCII expression, thereby indicating active/inflamed microglia. We treated them with AAT and its derivatives.

AAT-FC can reduce the activity of the MHCII reporter by 90% in the presence of IFNγ, as shown in Figure 7. Peptide 8 slightly reduces the activity of MHCII as compared to IFNγ. We did not observe any effect of peptide 9 or peptide 14.

Example 5

AAT decreases IFNγ-induced expression of major histocompatibility complex II (MHCII) in co-culture of neurospheres and microglia derived from hiPSCs

Materials and Methods

Five-thousand human microglia cells derived from hiPSCs and transduced with MHCII-FLuc reporter gene were added to each control neurosphere or neurosphere mutated with the α-synuclein gene (Parkinson's mutation) aged to 25 days of dopaminergic differentiation. Co-cultures were maintained for one week before starting the AAT (plasma-derived, SEQ ID No. 1) pre-treatment at 50μM.

Then, the IFNγ was used at a concentration of 100ng/ml or 1μg/ml for 24 hours to activate the microglia MHCII-FLuc reporter gene.

Following treatment, the quantitation of luciferase expression was done with ONE-Glo™ EX Luciferase Assay System. A ½ volume of ONE-Glo™ EX Reagent and a ½ volume of lysis buffer that is equal to the volume of culture medium in each well was added, and samples were incubated for at least 3 minutes to lyse cells. The luminescence was then read using a SpectraMax iD3 instrument. The luciferase activity was calculated, and results were displayed using GraphPad-Prism.

Results

Using co-culture of microglia/neurospheres derived from hiPSCs, we demonstrated the anti-inflammatory properties of AAT. We stimulated the co-culture organoid with IFNγ, to increase MHCII expression, thereby indicating active/inflamed microglia within the neurospheres.

Treatment with AAT resulted in a 25% reduction in MHCII reporter activity in the presence of IFNγ in control neurospheres, as shown in Figure 8. Furthermore, AAT significantly reduced MHCII activity by 70% in neurospheres mutated in the α-synuclein gene.

Example 6

Measuring inflammatory response in Human Schwann cells (HSCs) following AAT-Fc treatment

Materials and Methods

Human SCs were plated in a 24-well plate at a density of 70 000 cells per well. Two days after seeding, AAT-Fc or pdAAT pre-treatment was initiated for 1 day at 3µM with or without LPS 1µg/mL or TNFα 10ng/mL. Then, the medium was replaced with fresh medium containing 3µM AAT-Fc or pdAAT for 2 days with or without LPS 10µg/mL or TNFα 10ng/mL. Subsequently, the cells were harvested for RNA extraction. Gene expression was analysed by qPCR, with relative expression normalized to a housekeeping gene and visualized using GraphPad Prism.

Results

HSCs express the FcγR and elicit upregulation in pro-inflammatory cytokine mRNA in response to stimulation. IL-1β shows lowest levels with AGX-101R and AGX-101SY. TNFα levels shows no decrease with all compounds. IL-6 levels is decreased with AGX-101SY but not with the other compounds. IL-4 levels are decreased with AGX-SY and AGX-101T compounds but not with the other compounds. Overall, AGX-101R and AGX-101SY compounds exhibit the least immunogenicity with lower levels of IL-1β and IL-6. These results are shown in Figure 14.

After treatment with AGX-101R, HSCs show 59% lower levels of IL-6 after TNFα stimulation, in comparison with AGX-101S treatment (Figure 16A). Also, after LPS stimulation NFκBIA levels, a pro-inflammatory transcription factor, are 26% lower after AGX-101R than after treatment with AGX-101S (Figure 16C). AGX-101C treatment after HSCs stimulation with LPS shows high level of IL-6, TNFα, NFκBIA in comparison with all the other compounds. Also, after TNFα stimulation AGX-101C shows higher levels of IL-6, TNFα and NFκBIA in comparison with AGX-101R. Raw data of the 2(DCt) from each experiment are detailed in Figure 16D. These results are shown in Figure 16.

Example 7

ADAM-17 activity inhibition by AAT-Fc and IC50 values for AAT-FC

Materials and Methods

Human recombinant ADAM-17 protein (930-ADB-010, R&D system) and fluorogenic peptide substrate Mca-PLAQAV-Dpa-RSSSR-NH2 (ES003 R&D system) were used for the assay.

Hr-ADAM-17 protein was reconstituted in sterile water at 100ug/ml.

Peptide substrate was diluted to 2mM in DMSO.

50ug of AAT-Fc (SEQ ID NOs: 17 to 21 and a dimer of SEQ ID NOs: 22 and 23) were resuspended in sterile water at 5µM. AAT-Fc was tested at the following final concentrations: 0.012, 0.03, 0.11, 0.33, and 1 µM.

The concentration of 25 µM of AAT (plasma-derived, SEQ ID NO.1) was used as a positive control.

The reagents were kept on ice. Just before starting the reaction, we prepared a working solution. We diluted the HR-ADAM-17 protein 1:250 and the peptide substrate 1:100 in an assay buffer. The reagents were dispensed in triplicate on a black Maxisorp plate in this order:

- 25µl/well of AAT
- 25µl/well of HR-ADAM-17
- 50µl/well of peptide substrate.

In control wells AAT was replaced by 25µl of assay buffer. Control negative (blank) contains 50µl of assay buffer and 50ul of peptide substrate. Fluorescence was measured immediately using a SpectraMax iD3 instrument using the following parameters: excitation wavelength 320 nm, emission wavelength 405 nm, kinetic mode for five minutes. The blank values were subtracted from the other values and the analysis was performed using GraphPad-Prism.

Results

ADAM17 activity inhibition was tested using different AAT-Fc derivatives AGX-101R, AGX-101S, AGX101C, AGX-101A and AGX-101SY. IC50 was measured using

fluorescence based assay, the values obtained are the followings : AGX-101R IC50:0.85µM; AGX-101S IC50=0.74µM; AGX-101C IC50=0.73µM; AGX-101A IC50=0.65µM; AGX-101SY IC50=0.95µM. All the compounds show IC50 in the same range with AGX-101A showing the lowest IC50 = 0.65µM and AGX101SY IC50 the highest with 0.95µM. Results are shown in Figure 12.

Example 8

Expression and purification of AAT-Fc constructs

Materials and methods

AAT-Fc constructs were expressed in CHO K1 cells cultured in shake flasks (see Table 2). Cell culture supernatants were collected and purified by Affinity Chromatography (MabSelect SuRe resin) followed by Size Exclusion Chromatography (Superdex 200 pg resin). After chromatography purification, the proteins were dialyzed into water, and sucrose was added to a final concentration of 8% (w/v) before lyophilization. The final product was in the form of the lyophilized powder. The QC testing panel included protein concentration by A280, non-reduced/reduced SDS-PAGE, SEC-HPLC, endotoxin level testing, and LC-MS.

Table 2 – AAT-Fc constructs

Protein ID	Host Cell	Production Volume
symmetric_A1AT-Fc (AGX-101SY)	CHO K1	0.1 L
asymmetric_A1AT-Fc (AGX-101A)	CHO K1	0.1 L
AGX-101S	CHO K1	0.05 L
AGX-101C	CHO K1	0.05 L
AGX-101T	CHO K1	0.05 L
AGX-101R	CHO K1	0.1 L

DNA and transfection reagent were added to the host cells for transfection. The transfected culture was incubated by shaking at 150 rpm for 7 days. The temperature was shifted from

36.5 degrees to 33 degrees at 24 hours post-transfection. Feeding was performed on day 0 and day 4, while the cell culture was harvested on day 7. Cell culture titer is provided in Table 3.

Protein ID	Titer (mg/L)
symmetric_A1AT-Fc (AGX-101SY)	2143
asymmetric_A1AT-Fc (AGX-101A)	1171
AGX-101S	3244
AGX-101C	2681
AGX-101T	2270
AGX-101R	2197

The cell culture supernatants were harvested by centrifugation at 10000 x g for 40 minutes, followed by sterile filtration through a 0.22 µm filter. The supernatants were then purified using Affinity Chromatography with MabSelect SuRe resin. The ÄKTA M150 system was employed for column chromatography. The Affinity Chromatography was performed in bind-elute mode, with the bound protein eluted using acid elution.

Superdex 200 pg resin was used for size exclusion chromatography (SEC). The sample was directly loaded onto a 320 mL SEC column. PBS at pH 7.4 was used as the running buffer for SEC.

Slide-A-Lyzer™ Dialysis Cassettes, 3.5K MWCO was used for Dialysis. The dialysis cassette is first hydrated according to the manufacturer's instructions. After hydration, the sample is loaded into the dialysis cassette. The cassette is then placed in 50 times the sample volume of dialysis buffer (sterile water). The dialysis buffer is changed every 3 hours, for a total of 3 times.

The sample was pre-frozen at -80°C for 6 hours before lyophilization. The frozen sample was then placed in the freeze dryer to begin the vacuum freeze-drying process. The entire process includes three stages: pre-freezing (4 hours), primary drying (sublimation, 12 hours), and

secondary drying (desorption, 12 hours), following the manufacturer's built-in programs of the instrument. After lyophilization, the sample was sealed for storage at -80°C.

The final products were subjected to quality control testing, including SDS-PAGE, SEC-HPLC, A280, endotoxin level testing, and LC-MS.

Results

Yields for each construct are shown in Table 4. The 101R construct showed significantly higher yield than the other constructs, despite not having increased expression.

Table 4 – Final Products List

Construct	Yield (mg/L)	Concentration (mg/mL)	Volume (mL)	Amount (mg)
symmetric_A1AT-Fc	125	1.09	6.0	6.5
symmetric_A1AT-Fc		N/A*	N/A*	6.0
asymmetric_A1AT-Fc	191	2.10	4.5	9.5
asymmetric_A1AT-Fc		N/A*	N/A*	9.6
AGX-101S	176	N/A*	N/A*	8.8
AGX-101C	132	N/A*	N/A*	6.6
AGX-101T	104	N/A*	N/A*	5.2
AGX-101R	250	N/A*	N/A*	25.0

*The products are in lyophilized powder form.

Example 9

Neutrophil Elastase activity inhibition by AAT-Fc and IC50 values for AAT-FC

Materials and methods

Neutrophil Elastase Inhibition Assay. Substrate MeOSuc-AAPV-pNA l00 µM, purified human neutrophil elastase (ELA2) 2.2µU/µL and several concentrations of AAT-Fc (100, 30, 10 , 5, 3, 2 nM) were incubated together for 30min. Absorbance was measured every minute in a spectrophotometer reader (A405nm). Vmax value was determined with the slope of the trendline during 10 min. The Experiment was performed in duplicate. Error bars indicate the standard deviation.

Results

Neutrophil elastase activity inhibition was tested using different AAT-Fc derivatives AGX-101R, AGX-101S, AGX101C, AGX-101A and AGX-101SY. IC50 was measured using

fluorescence based assay, the values obtained are the followings: AGX-101R IC50=4.55nM; AGX-101S IC50=8.07nM; AGX-101C IC50=7.50nM; AGX-101A IC50=9.46nM; AGX-101SY IC50=7.17nM; AGX-101T IC50=8.52nM. Almost all the compounds show IC50 in the same range of 8nM, except AGX-101R that shows lower IC50 of 4.5nM. Results are shown in Figure 13.

Example 10

Mouse pharmacokinetics study

Materials and methods

This study evaluated the PK characteristics of AGX-101S, AGX-101T and AGX-101R. For each of these molecules, one group of 8 male C57BL/6 mice received a single sub-cutaneous (SC) dose of 10 mg/kg. Each group was divided in 2 sub-groups of 4 mice (group 1 and group 2) and blood samples were taken as indicated below. Pre-treatment (T=0) blood samples were taken from pooled samples from untreated backup mice.

Sampling time	T=0	30 min	Day 1	Day 2	Day 3	Day 4	Day 6	Day 8	Day 10	Day 14	Day 17	Day 21
Pool from backup	X	-	-	-	-	-	-	-	-	-	-	-
Group 1	-	X	-	X	-	X	-	X	-	X	-	X
Group 2	-	-	X	-	X	-	X	-	X	-	X	-

Plasma was prepared and the average concentration (mean ± SD) from 4 animals was determined for each molecule using a qualified ELISA assay (Figure 15).

Briefly, the ELISA consisted in a goat anti-human IgG coated on a microtiter plate. The samples were added to the plate and after a first incubation, the secondary antibody (goat anti-human IgG-biotin) was added to the wells. Following incubation, streptavidin-horseradish peroxidase (HRP) and tetramethylbenzidine (TMB) were added, and then absorbance was measured at 450 nm.

Results

The PK characteristics of each molecule were calculated using Phoenix WinNonlin 8.0 and are summarized in the table below.

Compound	AGX-101S	AGX-101T	AGX-101R
Dose	10 mg/kg SC	10 mg/kg SC	10 mg/kg SC
T½ (h)	201	169	238
C_{max} (µg/mL)	53.7	25.8	64.2
AUC_{0-t} (h* µg/mL)	9318	4393	9793
Cl obs (mL/day/kg)	20.6	44.3	18.1
MRT_{INF} obs (g)	255	251	307

The calculated data suggest that AGX-101R shows the highest level of exposure when compared to AGX-101S and to AGX-101T. Especially, the half-life of AGX-101R was higher by 16% and 29% relative to AGX-101S and AGX-101T, respectively.

EMBODIMENTS

1. A fusion protein comprising:

(i) an immunoglobulin IgG Fc domain, and

(ii) an alpha1-antitrypsin (AAT) polypeptide,

wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, and wherein:

(a) the Fc domain comprises mutations corresponding to L234A or F234A, L235A, and P329G;

(b) the Fc domain comprises mutations corresponding to M252Y, S254T, and T256E; and/or

(c) the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

2. The fusion protein of embodiment 1, wherein:

(a) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to L234A, L235A, and P329G;

(b) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to M252Y, S254T, and T256E;

(c) the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(d) the Fc domain is an IgG4 Fc domain and the fusion protein comprises a linker between the Fc domain and the AAT polypeptide, wherein the linker comprises at least 5 amino acids, optionally wherein the linker is a glycine-serine linker and/or comprises the amino acid sequence of SEQ ID NO: 24.

3. The fusion protein of embodiment 1 or embodiment 2, wherein:

(a) the Fc domain is an IgG1 Fc domain and the IgG1 Fc domain comprises a hinge region, optionally wherein the hinge region comprises or consists of the amino acid sequence of SEQ ID NO: 25; or

(b) the Fc domain is an IgG4 Fc domain and the IgG4 Fc domain comprises a hinge region, optionally wherein the hinge region comprises or consists of the amino acid sequence of SEQ ID NO: 26 or 27.

4. The fusion protein of any one of the preceding embodiments, wherein the linker is between 5 and 15 amino acids in length, optionally wherein the linker is 10 amino acids in length.

5. The fusion protein of any one of the preceding embodiments, wherein the AAT polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36.

6. The fusion protein of any one of the preceding embodiments, wherein the AAT polypeptide comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36.

7. The fusion protein of any one of the preceding embodiments, wherein the Fc domain comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NOs: 29 to 33.

8. The fusion protein of any one of the preceding embodiments, wherein the Fc domain comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NOs: 29 to 33.

9. The fusion protein of any one of the preceding embodiments, wherein the fusion protein comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NOs: 17 to 19, 21, or 22.

10. The fusion protein of any one of the preceding embodiments, wherein the fusion protein comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NOs: 17 to 19, 21, or 22.

11. A dimer comprising the fusion protein of any one of the preceding embodiments and a second protein.

12. The dimer of embodiment 11, wherein the second protein comprises a fusion protein of any one of the preceding embodiments, optionally wherein the fusion protein and the second protein comprise or consist of the same amino acid sequence.

13. The dimer of embodiment 12, wherein the second protein comprises or consists of an IgG4 Fc domain.

14. The dimer of embodiment 11 or embodiment 13, wherein the second protein does not comprise an AAT polypeptide.

15. The dimer of embodiment 14, wherein the second protein comprises or consists of an amino acid sequence of SEQ ID NO: 23.

16. The fusion protein or the dimer of any one of the preceding embodiments, wherein the fusion protein or the dimer has a lower immunogenicity than:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34;

(b) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, and P329G;

(c) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E;

(d) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; and/or

(e) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

17. The fusion protein or the dimer of embodiment 16, wherein the immunogenicity is measured by contacting human Schwann cells with the fusion protein or the dimer, harvesting the cells, extracting RNA, and analysing the expression of at least one cytokine.

18. The fusion protein or the dimer of embodiment 17, wherein the at least one cytokine is selected from IL-1β, TNFα, IL-6, and IL-4.

19. The fusion protein or the dimer of embodiment 17 or embodiment 18, wherein the fusion protein promotes less than 100%, less than 90%, less than 80%, less than 70%, less than 60%,

less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine expression promoted by:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34;

(b) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, and P329G;

(c) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E;

(d) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; and/or

(e) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

20. The fusion protein or the dimer of any one of the preceding embodiments, wherein the fusion protein or the dimer can inhibit and/or impair the activity of ADAM metallopeptidase domain 17 (ADAM-17).

21. The fusion protein or the dimer of embodiment 20, wherein inhibition of ADAM-17 is measured by incubating ADAM-17 with increasing concentrations of the fusion protein or the dimer and a fluorogenic ADAM-17 substrate, measuring the fluorescence, and calculating an IC50 value for the fusion protein or the dimer.

22. The fusion protein or the dimer of embodiment 21, wherein the IC50 of the fusion protein or the dimer is less than 10µM, less than 9µM, less than 8µM, less than 7µM, less than 6µM, less than 5µM, less than 4µM, less than 3µM, less than 2µM, or less than 1µM.

23. The fusion protein or the dimer of any one of the preceding embodiments, wherein the fusion protein or the dimer can inhibit and/or impair the activity of neutrophil elastase (NE).

24. The fusion protein or the dimer of embodiment 23, wherein inhibition of NE is measured by incubating NE with the fusion protein or the dimer and a fluorogenic NE substrate, measuring the fluorescence, and calculating an IC50 value for the fusion protein or the dimer.

25. The fusion protein or the dimer of embodiment 23 or embodiment 24, wherein the IC50 of the fusion protein or the dimer is less than 100nM, less than 90nM, less than 80nM, less than 70nM, less than 60nM, less than 50nM, less than 40nM, less than 30nM, less than 20nM, less than 10nM, or less than 5nM.

26. The fusion protein or the dimer of any one of embodiments 1 to 25, wherein the fusion protein or the dimer has increased serum half-life compared to:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34, or a dimer consisting of two fusion proteins consisting of the amino acid sequence of SEQ ID NO: 20 or 34;

(b) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, and P329G;

(c) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E;

(d) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; and/or

(e) an equivalent fusion protein not comprising mutations corresponding to M252Y, S254T, and T256E and not comprising a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

27. The fusion protein or the dimer of embodiment 26, wherein the serum half-life is measured by administering the fusion protein to a mouse, taking blood samples from the mouse at various time points, and measuring the concentration of the fusion protein in the samples using an ELISA.

28. The fusion protein or the dimer of embodiment 27 or embodiment 27, wherein the fusion protein or the dimer has a serum half-life of at least 100 hours, at least 110 hours, at least 120 hours, at least 130 hours, at least 140 hours, at least 150 hours, at least 160 hours, at least 170 hours, at least 180 hours, at least 190 hours, or at least 200 hours.

29. A vector comprising a nucleic acid sequence encoding the fusion protein or the dimer of any one of the preceding embodiments.

30. A host cell comprising, or expressing, the fusion protein or the dimer of any one of embodiments 1 to 28 or the vector of embodiment 29.

31. A pharmaceutical composition comprising the fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, or the host cell of embodiment 30 and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

32. The pharmaceutical composition of embodiment 31, wherein the carrier is a blood-brain barrier permeability enhancer.

33. The fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, the host cell of embodiment 30, or the pharmaceutical composition of embodiment 31 or embodiment 32 for use in a method of treating or preventing a disease or disorder.

34. A method of treating or preventing a disease or disorder comprising administering the fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, the host cell of embodiment 30, or the pharmaceutical composition of embodiment 31 or embodiment 32 to a subject.

35. Use of the fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, the host cell of embodiment 30, or the pharmaceutical composition of embodiment 31 or embodiment 32 in the manufacture of a medicament for use in a method of treating or preventing a disease or disorder.

36. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use according to embodiment 33, the method of treatment of embodiment 34, or the use of embodiment 35, wherein the disease or disorder is a disease or disorder of the nervous system, optionally wherein the disease of disorder is a disease or disorder of the peripheral nervous system (PNS), further optionally a demyelinating and/or dysmyelinating PNS disease or associated disorder.

37. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use, method, or use of embodiment 36, wherein the disease or disorder of the PNS is selected from the group

comprising Charcot–Marie–Tooth (CMT), Guillain-Barré Syndrome (GBS), neuropathic pain and chronic inflammatory demyelinating polyneuropathy (CIDP).

38. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use, method, or use of embodiment 37, wherein CMT is selected from the group comprising CMT1A, CMT1B, CMT1X, CMT2A, CMT3, and CMT4.

39. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use, method, or use of any one of embodiments 36 to 38, wherein the treating comprises promoting remyelination and/ or arresting demyelination of the axons of the PNS.

40. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use according to embodiment 33, the method of treatment of embodiment 34, or the use of embodiment 35, wherein the disease or disorder is AAT deficiency (AATD) or Chronic Obstructive Pulmonary Disease (COPD).

41. The fusion protein, dimer, vector, host cell or pharmaceutical composition for use, method, or use of any one of embodiments 36 to 40, wherein the fusion protein, dimer, vector, host cell or pharmaceutical composition is administered by a route of administration selected from the group comprising inhalation, intra-arterial, intradermal, intramuscular, intraperitoneal, intravenous, intranasal, intracerebral, parenteral, pulmonary, instillation, subcutaneous, ex vivo gene therapy or ex vivo cell-therapy.

42. A method of inhibiting ADAM-17 in a cell, the method comprising contacting the cell with the fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, the host cell of embodiment 30, or the pharmaceutical composition of embodiment 31.

43. A method of promoting remyelination and/ or arresting demyelination of the axons of the PNS in a subject, the method comprising administering to the subject the fusion protein or the dimer of any one of embodiments 1 to 28, the vector of embodiment 29, the host cell of embodiment 30, or the pharmaceutical composition of embodiment 31.

44. A method of producing a fusion protein or a dimer according to any one of embodiments 1 to 28.

45. The method of embodiment 44, wherein the method comprises expressing the fusion protein or the dimer in a cell, optionally wherein the cell is a CHO cell, optionally a CHO HK1 cell.

46. The method of embodiment 45, wherein expressing the fusion protein or the dimer comprises transfecting the cell with a nucleic acid or vector encoding the fusion protein or the dimer.

47. A method of purifying a fusion protein or a dimer according to any one of embodiments 1 to 28.

48. The method of embodiment 47, wherein the method comprises a step of affinity chromatography.

49. The method of embodiment 48, wherein the step of affinity chromatography uses a column comprising protein A, optionally wherein the protein A comprises a Z domain.

50. The method of any one of embodiments 47 to 49, wherein the fusion protein or the dimer comprises an IgG Fc domain comprising:

(i) mutations corresponding to M252Y, S254T, and T256E;

(ii) mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G; or

(iii) mutations corresponding to M252Y, S254T, and T256E and a wild-type hinge region, e.g. a hinge region comprising or consisting of the amino acid sequence of SEQ ID NO: 25.

51. The method of any one of embodiments 47 to 50, wherein the method comprises a step of size exclusion chromatography.

52. A fusion protein for use in the prevention and/or treatment of a disease or disorder of the nervous system, wherein said fusion protein comprises

(i) an immunoglobulin IgG Fc domain, a variant or a fragment thereof, and

(ii) an alpha1-antitrypsin (AAT) polypeptide, a variant, an isoform and/or a fragment thereof,

wherein the AAT polypeptide, variant, isoform and/or fragment thereof, is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, variant or fragment thereof.

53. The fusion protein for use of embodiment 52, wherein the disease or disorder of the nervous system is a disease or disorder of the peripheral nervous system (PNS), preferably a demyelinating and/or dysmyelinating PNS disease or associated disorder.

54. The fusion protein for use of embodiment 52 or 53, wherein the PNS disease or disorder is selected from the group comprising Charcot–Marie–Tooth (CMT), Guillain-Barré Syndrome (GBS), neuropathic pain and chronic inflammatory demyelinating polyneuropathy (CIDP).

55. The fusion protein for use of embodiment 54, wherein CMT is selected from the group comprising CMT1A, CMT1B, CMT2A, CMT3 and CMTX1.

56. The fusion protein for use of any one of embodiments 52-55, wherein the Fc domain of an IgG is selected from the group comprising a human IgG 1, a human IgG2, a human IgG3 and a human IgG4, a fragment or a variant thereof.

57. A vector comprising a nucleic acid sequence encoding a fusion protein for use of anyone of embodiments 52 to 56.

58. A host cell comprising, or expressing, a fusion protein for use of anyone of embodiments 52 to 56 or a vector of embodiment 57.

59. A pharmaceutical composition comprising a therapeutically effective amount of i) a fusion protein for use of any one of embodiments 52-56, ii) a vector of embodiment57 or iii) a host cell of embodiment 58, and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

60. The pharmaceutical composition of embodiment 59, wherein the carrier is a blood-brain barrier permeability enhancer.

61. The pharmaceutical composition of any one of the embodiments 59-60, wherein the pharmaceutical composition is administered by a route of administration selected from the group comprising inhalation, intra-arterial, intradermal, intramuscular, intraperitoneal, intravenous, intranasal, intracerebral, parenteral, pulmonary, instillation, subcutaneous, ex vivo gene therapy or ex vivo cell-therapy.

62. The fusion protein of embodiments 52 to 56 or the pharmaceutical composition of embodiments 59 to 61, wherein the fusion AAT-Fc inhibits and/or impairs the activity of ADAM metallopeptidase domain 17 (ADAM-17).

63. The fusion protein of embodiments 52 to 55 or the pharmaceutical composition of embodiments 58 to 60, wherein the fusion AAT-Fc promotes remyelination and/ or arrests demyelination of the axons of the PNS.

64. A method of treatment and/or prevention of a disease or disorder of the nervous system, comprising administering a fusion protein of embodiments 52 to 56 or a pharmaceutical composition of embodiments 59 to 61, to a subject in need thereof.

65. The method of treatment and/or prevention of embodiment 64, wherein the disease or disorder of the nervous system is a disease or disorder of the peripheral nervous system (PNS), preferably a demyelinating and/or dysmyelinating PNS disease or associated disorder.

CLAIMS

1. A fusion protein comprising:

(i) an immunoglobulin IgG Fc domain, and

(ii) an alpha1-antitrypsin (AAT) polypeptide,

wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain, and wherein the Fc domain comprises mutations corresponding to L234A or F234A, L235A, M252Y, S254T, T256E, and P329G.

2. A fusion protein comprising:

(i) an immunoglobulin IgG Fc domain,

(ii) an alpha1-antitrypsin (AAT) polypeptide, and

(iii) a linker,

wherein the AAT polypeptide is covalently fused to the N-terminus or the C-terminus of the IgG Fc domain via the linker, and wherein the Fc domain comprises mutations corresponding to L234A or F234A, L235A, M252Y, S254T, T256E, and P329G.

3. The fusion protein of claim 1 or claim 2, wherein the Fc domain is an IgG1 Fc domain and comprises mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G.

4. The fusion protein of any one of the preceding claims, wherein the Fc domain is an IgG1 Fc domain and the IgG1 Fc domain comprises a hinge region, optionally wherein the hinge region comprises or consists of the amino acid sequence of SEQ ID NO: 25.

5. The fusion protein of any one of the preceding claims, wherein the AAT polypeptide comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36.

6. The fusion protein of any one of the preceding claims, wherein the AAT polypeptide comprises or consists of an amino acid sequence that is identical to the amino acid sequence of any one of SEQ ID NO: 28, 35, or 36.

7. The fusion protein of any one of the preceding claims, wherein the Fc domain comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of SEQ ID NO: 31.

8. The fusion protein of any one of the preceding claims, wherein the Fc domain comprises or consists of an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 31.

9. The fusion protein of any one of the preceding claims, wherein the fusion protein comprises or consists of an amino acid sequence having at least 90%, at least 95%, or at least 98% identity to the amino acid sequence of SEQ ID NO: 19.

10. The fusion protein of any one of the preceding claims, wherein the fusion protein comprises or consists of an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO: 19.

11. The fusion protein of any one of the preceding claims, wherein the fusion protein has a lower immunogenicity than:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34; and/or

(b) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G.

12. The fusion protein of claim 11, wherein the immunogenicity is measured by contacting human Schwann cells with the fusion protein, harvesting the cells, extracting RNA, and analysing the expression of at least one cytokine.

13. The fusion protein of claim 12, wherein the at least one cytokine is selected from IL-1β, TNFα, IL-6, and IL-4.

14. The fusion protein of claim 12 or claim 13, wherein the fusion protein promotes less than 100%, less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10% of the cytokine expression promoted by:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34; or
(b) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G.

15. The fusion protein of any one of the preceding claims, wherein the fusion protein can inhibit and/or impair the activity of ADAM metallopeptidase domain 17 (ADAM-17).

16. The fusion protein of claim 15, wherein inhibition of ADAM-17 is measured by incubating ADAM-17 with increasing concentrations of the fusion protein and a fluorogenic ADAM-17 substrate, measuring the fluorescence, and calculating an IC50 value for the fusion protein.

17. The fusion protein of claim 16, wherein the IC50 of the fusion protein is less than 10μM, less than 9μM, less than 8μM, less than 7μM, less than 6μM, less than 5μM, less than 4μM, less than 3μM, less than 2μM, or less than 1μM.

18. The fusion protein of any one of the preceding claims, wherein the fusion protein can inhibit and/or impair the activity of neutrophil elastase (NE).

19. The fusion protein of claim 18, wherein inhibition of NE is measured by incubating NE with the fusion protein and a fluorogenic NE substrate, measuring the fluorescence, and calculating an IC50 value for the fusion protein.

20. The fusion protein of claim 18 or claim 19, wherein the IC50 of the fusion protein is less than 100nM, less than 90nM, less than 80nM, less than 70nM, less than 60nM, less than 50nM, less than 40nM, less than 30nM, less than 20nM, less than 10nM, or less than 5nM.

21. The fusion protein of any one of claims 1 to 20, wherein the fusion protein has increased serum half-life compared to:

(a) a fusion protein consisting of the amino acid sequence of SEQ ID NO: 20 or 34; or
(d) an equivalent fusion protein not comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G.

22. The fusion protein of claim 21, wherein the serum half-life is measured by administering the fusion protein to a mouse, taking blood samples from the mouse at various time points, and measuring the concentration of the fusion protein in the samples using an ELISA.

23. The fusion protein of claim 21 or claim 22, wherein the fusion protein has a serum half-life of at least 100 hours, at least 110 hours, at least 120 hours, at least 130 hours, at least 140 hours, at least 150 hours, at least 160 hours, at least 170 hours, at least 180 hours, at least 190 hours, or at least 200 hours.

24. A vector comprising a nucleic acid sequence encoding the fusion protein of any one of the preceding claims.

25. A host cell comprising, or expressing, the fusion protein of any one of claims 1 to 23 or the vector of claim 24.

26. A pharmaceutical composition comprising the fusion protein of any one of claims 1 to 23, the vector of claim 24, or the host cell of claim 25 and at least one pharmaceutically acceptable excipient, diluent, carrier, salt and/or additive.

27. The pharmaceutical composition of claim 26, wherein the carrier is a blood-brain barrier permeability enhancer.

28. The fusion protein of any one of claims 1 to 23, the vector of claim 24, the host cell of claim 25, or the pharmaceutical composition of claim 26 or claim 27 for use in a method of treating or preventing a disease or disorder.

29. A method of treating or preventing a disease or disorder comprising administering the fusion protein of any one of claims 1 to 23, the vector of claim 24, the host cell of claim 25, or the pharmaceutical composition of claim 26 or claim 27 to a subject.

30. Use of the fusion protein of any one of claims 1 to 23, the vector of claim 24, the host cell of claim 25, or the pharmaceutical composition of claim 26 or claim 27 in the manufacture of a medicament for use in a method of treating or preventing a disease or disorder.

31. The fusion protein, vector, host cell or pharmaceutical composition for use according to claim 28, the method of treatment of claim 29, or the use of claim 30, wherein the disease or disorder is selected from the group consisting of alpha-1 antitrypsin deficiency (AATD), chronic obstructive pulmonary disease (COPD), Charcot-Marie-Tooth disease (CMT), Guillain-Barré Syndrome (GBS), Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), neuropathic pain, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), amyloid polyneuropathy (Transthyretin-Related Familial Amyloid Polyneuropathy), panniculitis, vasculitis, anti-proteinase-3 (PR3)-related vasculitis, inflammatory bowel disease (IBD), ulcerative colitis, hypothyroidism, Graft-versus-Host disease (GVHD), Type 1 diabetes mellitus, Type 2 diabetes mellitus, diabetic neuropathies, cardiovascular disease, arterial stiffness, hypertension, osteoporosis, sarcopenia, Alzheimer's disease, neurodegenerative conditions, viral infection such as HIV-1 infection, coronavirus infection, and microbial infection such as Pseudomonas aeruginosa infection.

32. The fusion protein, vector, host cell or pharmaceutical composition for use according to claim 28, the method of treatment of claim 29, or the use of claim 30, wherein the disease or disorder is a disease or disorder of the nervous system, optionally wherein the disease of disorder is a disease or disorder of the peripheral nervous system (PNS), further optionally a demyelinating and/or dysmyelinating PNS disease or associated disorder.

33. The fusion protein, vector, host cell or pharmaceutical composition for use, method, or use of claim 32, wherein the disease or disorder of the PNS is selected from the group comprising Charcot–Marie–Tooth (CMT), Guillain-Barré Syndrome (GBS), neuropathic pain and chronic inflammatory demyelinating polyneuropathy (CIDP).

34. The fusion protein, vector, host cell or pharmaceutical composition for use, method, or use of claim 33, wherein CMT is selected from the group comprising CMT1A, CMT1B, CMT1X, CMT2A, CMT3, and CMT4.

35. The fusion protein, vector, host cell or pharmaceutical composition for use, method, or use of any one of claims 31 to 34, wherein the treating comprises promoting remyelination and/ or arresting demyelination of the axons of the PNS.

36. The fusion protein, vector, host cell or pharmaceutical composition for use according to claim 28, the method of treatment of claim 29, or the use of claim 30, wherein the disease or disorder is AAT deficiency (AATD) or Chronic Obstructive Pulmonary Disease (COPD).

37. The fusion protein, vector, host cell or pharmaceutical composition for use, method, or use of any one of claims 28 to 36, wherein the fusion protein, vector, host cell or pharmaceutical composition is administered by a route of administration selected from the group comprising inhalation, intra-arterial, intradermal, intramuscular, intraperitoneal, intravenous, intranasal, intracerebral, parenteral, pulmonary, instillation, subcutaneous, ex vivo gene therapy or ex vivo cell-therapy.

38. A method of inhibiting ADAM-17 in a cell, the method comprising contacting the cell with the fusion protein of any one of claims 1 to 23, the vector of claim 24, the host cell of claim 25, or the pharmaceutical composition of claim 26.

39. A method of promoting remyelination and/ or arresting demyelination of the axons of the PNS in a subject, the method comprising administering to the subject the fusion protein of any one of claims 1 to 23, the vector of claim 24, the host cell of claim 25, or the pharmaceutical composition of claim 26.

40. A method of producing a fusion protein according to any one of claims 1 to 23.

41. The method of claim 40, wherein the method comprises expressing the fusion protein in a cell, optionally wherein the cell is a CHO cell, optionally a CHO HK1 cell.

42. The method of claim 41, wherein expressing the fusion protein comprises transfecting the cell with a nucleic acid or vector encoding the fusion protein.

43. A method of purifying a fusion protein according to any one of claims 1 to 23.

44. The method of claim 43, wherein the method comprises a step of affinity chromatography.

45. The method of claim 44, wherein the step of affinity chromatography uses a column comprising protein A, optionally wherein the protein A comprises a Z domain.

46. The method of any one of claims 43 to 45, wherein the fusion protein comprises an IgG Fc domain comprising mutations corresponding to L234A, L235A, M252Y, S254T, T256E, and P329G.

47. The method of any one of claims 43 to 46, wherein the method comprises a step of size exclusion chromatography.