

Alpha1-antitrypsin for use in the treatment of diseases or disorders of the nervous system such as chronic inflammatory demyelinating polyneuropathy

The invention relates to alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof and/or a corresponding nucleic acid sequence for use in the treatment of diseases or disorders of the nervous system such as chronic inflammatory demyelinating polyneuropathy. The invention further relates to combinations with IgG antibodies, such as immunoglobulin therapy, for use in these diseases or disorders.

Diseases or disorders of the nervous system are diseases or disorders which can dramatically affect the peripheral and/or central nervous system (PNS/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 peripheral (PNS) and central nervous systems (CNS).

Peripheral neuropathies constitute a highly diverse group of disorders with the major component being damages to the myelin sheaths, either after its abnormal development (dysmyelination) in the inherited forms (CMT1A-F and -X) or direct in the acquired ones such as chronic inflammatory demyelinating polyneuropathy.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune disease that targets myelin sheaths, specifically in the peripheral nerves, and causes progressive weakness and sensory loss. Swelling of nerve roots is also a characteristic of the disease. Although it can occur at any age and in both genders, CIDP is more common in young adults, and it is more common in men than women.

Untreated, CIDP is characterized by accumulating disability that requires physical and occupational therapy, orthotic devices and long-term treatment. Early intervention can prevent permanent damage and disability. Current methods of treatment for CIDP include administration of corticosteroids, such as prednisone, which may be prescribed alone or in combination with immunosuppressant drugs. Immunosuppressant drugs may also be given in the absence of a steroid.

Myelin is produced by Schwann cells (SCs) in the PNS and is crucial for proper transmission of the electric impulse in the nerves. In the intricate neuron/glia cross-

communication that is required for proper myelin regulation (Rao and Pearse 2016), several diverse signaling pathways are involved, which include growth factors, integrins and cell adhesion molecules but more importantly, the pivotal neuregulin 1 type III (NRG1-III) that signals through the ERBB2/3 receptors and its proteolytic sheddase modulator, the tumor necrosis factor- α -converting enzyme, TACE (also known as ADAM17). TACE/ADAM17, is a transmembrane protein that includes an extracellular zinc-dependent protease domain. In the context of the PNS, ADAM17 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 alpha-1-Antitrypsin (AAT), specifically, in 2013 AAT was shown not to interact with TACE (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 and inhibits its activity in a dose dependent manner (Bergin, D. A. et al., 2010, J Clin Invest 120(12): 4236-4250.).

The biological common trait of many PNS and CNS neurodegenerative diseases is a sustained and acute inflammatory response due to cytokine release orchestrated in feed-forward loops (also called "cytokine storm"). Therefore, dampening of 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.

Thus, there is a need for improved therapies for diseases or disorders of the nervous system, in particular chronic inflammatory demyelinating polyneuropathy.

The above technical problem is solved by the embodiments disclosed herein and as defined in the claims.

Accordingly, the invention relates to, inter alia, the following embodiments:

1. A pharmaceutical product for use in the treatment of an inflammatory disease or disorder, the pharmaceutical product comprising:
 - a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has ADAM17 inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or

- b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.
2. The pharmaceutical product for use of embodiment 1, wherein the inflammatory disease or disorder is an autoimmune inflammatory disease.
 3. The pharmaceutical product for use of embodiment 1 or 2, wherein the inflammatory disease or disorder is an inflammatory disease or disorder of the nervous system, preferably wherein the inflammatory disease or disorder is neuropathic pain caused by an inflammatory disease or disorder of the nervous system.
 4. The pharmaceutical product for use of any one of embodiments 1 to 3, wherein the inflammatory disease is chronic inflammatory demyelinating polyneuropathy.
 5. The pharmaceutical product for use of embodiments 1 or 2, wherein the inflammatory disease is complex regional pain syndrome.
 6. The pharmaceutical product for use of any one of embodiments 1 to 3, wherein the inflammatory disease or disorder is inflammatory pain.
 7. A kit of parts for use in the treatment of a disease or disorder of the nervous system, the kit comprising:
 - i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and
 - ii) a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.
 8. A pharmaceutical composition for use in the treatment of a disease or disorder of the nervous system, the composition comprising:
 - i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has

- inhibitory activity or a small molecule having ADAM17 inhibitory activity;
and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and
 - ii) a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof; and
 - iii) at least one pharmaceutically acceptable carrier.
9. A method of treatment comprising administering an effective amount of a pharmaceutical composition to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants, the pharmaceutical composition comprising
- a) AAT protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.
10. A method of treatment comprising administering an effective amount of a pharmaceutical compound comprising a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of
- a) AAT protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

11. The kit of parts for use of embodiment 7, the pharmaceutical composition for use of embodiment 8, the method of treatment of embodiment 9 or 10, wherein the disease or disorder of the nervous system is pain caused by a disease or disorder of the nervous system, preferably chronic pain caused by a disease or disorder of the nervous system.
12. The kit of parts for use of embodiment 7 or 11, the pharmaceutical composition for use of embodiment 8 or 11, the method of treatment of embodiment 9 to 11, wherein the disease or disorder of the nervous system is an autoimmune disease or disorder of the nervous system.
13. The kit of parts for use of embodiment 12, the pharmaceutical composition for use of embodiment 12, the method of treatment of embodiment 12, wherein the disease or disorder of the nervous system is chronic inflammatory demyelinating polyneuropathy.
14. The pharmaceutical product for use of embodiment 4 or 6, the kit of parts for use of embodiment 13, the pharmaceutical composition for use of embodiment 13, the method of treatment of embodiment 13, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.
15. The kit of parts for use of any one of the embodiments 7, 11 to 14, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 14, the method of treatment of any one of the embodiments 9 to 14, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.
16. The kit of parts for use of any one of the embodiments 7, 11 to 14, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 14, the method of treatment of any one of the embodiments 9 to 14, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

17. The kit of parts for use of any one of the embodiments 7, 11 to 16, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 16, the method of treatment of any one of the embodiments 9 to 16, wherein the plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for intravenous administration.
18. The kit of parts for use of any one of the embodiments 7, 11 to 16, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 16, the method of treatment of any one of the embodiments 9 to 16, wherein the plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for subcutaneous administration.
19. The pharmaceutical product for use of any one of the embodiments 1 to 6, the kit of parts for use of any one of the embodiments 7, 11 to 18, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 18, the method of treatment of any one of the embodiments 9 to 18, wherein the AAT protein is recombinant AAT.
20. The pharmaceutical product for use of any one of the embodiments 1 to 6, the kit of parts for use of any one of the embodiments 7, 11 to 18, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 18, the method of treatment of any one of the embodiments 9 to 18, wherein the AAT protein is plasma derived AAT.
21. The pharmaceutical product for use of any one of the embodiments 1 to 6, 19 or 20, the kit of parts for use of any one of the embodiments 7, 11 to 20, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 20, the method of treatment of any one of the embodiments 9 to 20, wherein
 - a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.is/are formulated for intravenous administration.

22. The pharmaceutical product for use of any one of the embodiments 1 to 6, 19 or 20, the kit of parts for use of any one of the embodiments 7, 11 to 20, the pharmaceutical composition for use of any one of the embodiments 8, 11 to 20, the method of treatment of any one of the embodiments 9 to 20, wherein

a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or

b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

is/are formulated for subcutaneous administration.

Accordingly, in one embodiment, the invention relates to a pharmaceutical product for use in the treatment of an inflammatory disease or disorder, the pharmaceutical product comprising: a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

The term "inflammatory disease or disorder", as used herein, refers to a disease, a disorder or a condition that is characterized by increased inflammation in a tissue, organ or the system compared to a corresponding tissue, organ or system of a healthy reference subject. Inflammation is characterized by a dysregulation of inflammation markers and/or increased immune cell infiltration, activation, proliferation, and/or differentiation in the blood and/or the tissue. An inflammation marker is a marker that is indicative for inflammation in a subject. In certain aspects the inflammatory marker described herein is a marker selected from the group of CRP, erythrocyte sedimentation rate (ESR), and procalcitonin (PCT), Interleukin (e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-33, IL-32, IL-33, IL-35 or IL-36) Tumor necrosis factor (e.g., TNF-alpha, TNF-beta), Interferon (e.g., interferon gamma) MIP-I, MCP-I, RANTES, other chemokines and/or other cytokines. An inflammatory marker may also be detectable indirectly, e.g., by detection of an inhibitory factor of an inflammatory marker (e.g., binding factor and/or

antagonist). In some aspects, the inflammatory marker is measured in cells involved in inflammation, in cells affected by cells involved in inflammation, in the cerebrospinal fluid, and/or in the blood. In some aspects, the inflammation marker is indicative for immune cell infiltration, activation, proliferation and/or differentiation. Detection of the inflammation marker or the ratio of two or more inflammation markers is detected outside the normal range. The normal range of inflammation markers and whether a marker(ratio) has to be below or above a threshold to be indicative for inflammation is known to the person skilled in the art. In some aspects, the gene expression level, the RNA transcript level, the protein expression level, the protein activity level and/or the enzymatic activity level of at least one inflammation marker is detected. In some aspects at least one inflammation marker is detected quantitatively and/or qualitatively to determine the inflammatory disease or disorder in a subject in need of treatment and/or prevention.

In some aspects, the inflammatory disease or disorder described herein is characterized by acute inflammation, that is the duration of inflammation symptoms typically takes from about a few minutes (e.g., 2, 5, 10, 15, 30, 45 minutes) to a few days (e.g., 2, 3, 5, 7, 10 or 14 days). In some aspects, the inflammatory disease or disorder is characterized by chronic inflammation, that is the duration of symptoms of inflammation typically take at least about a few days (e.g., 2, 3, 5, 7, 10 or 14 days) or the symptoms of inflammation reoccur at least once (e.g., once or more times, twice or more times or three or more times). In some aspects, the inflammatory disease or disorder is characterized by chronic low-grade inflammation. Chronic low-grade inflammation can occur in the absence of clinical symptoms. In certain embodiments, the inflammatory disease described herein is an inflammatory disease characterized by increase inflammation affecting the Schwann Cell (SC) phenotype or health. In certain embodiments, the inflammatory disease described herein is an inflammatory disease characterized by an increase of inflammatory microglia and/or an increase in microglia inflammatory phenotypes.

The inventor found that AAT is able to inhibit TACE/ADAM17 in a dose-dependent manner which, without being bound by theory, rescues myelin production by SCs, and thus subsequently prevents, or slowing, and/or reverses the progression of inflammatory diseases. Furthermore, alpha1-antitrypsin (AAT) protein and variant thereof attenuate the reactive oxygen species (ROS) response of SCs when stimulated

with the pro-inflammatory cytokine TNF α (TNF- α). This indicates that AAT and its derivatives exert a novel therapeutic effect by acting directly on Schwann Cells (SCs) and rescuing these cells from their inflamed state back to their natural wild type state, e.g., rescuing SCs ability to restore myelination around axons. As such, this anti-inflammatory effect on SCs in the PNS or microglia in the CNS is surprisingly effective in the treatment of inflammatory diseases involving these cells.

Accordingly, the invention is at least in part based on the anti-inflammatory effect of AAT and variants, isoforms and/or fragments thereof.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder is an autoimmune inflammatory disease.

The term "autoimmune inflammatory disease", as used herein refers to a group of diseases or disorders in which tissue injury is associated with a humoral and/or cell-mediated immune response to body constituents or, in a broader sense, an immune response to self. The pathological immune response may be systemic or organ specific. In certain embodiments, autoimmune disease described herein is a disease selected from the group consisting of multiple sclerosis, myasthenia gravis, Pernicious anemia, arthritis, Sjögren syndrome, systemic lupus erythematosus, complex regional pain syndrome and type I diabetes.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder is an inflammatory disease or disorder of the nervous system. In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder is neuropathic pain.

In certain embodiments, the invention relates to the kit of parts, the pharmaceutical composition and the method of treatment for use of the invention, wherein the disease or disorder of the nervous system is neuropathic pain.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder is neuropathic pain caused by an inflammatory disease or disorder of the nervous system.

The term “inflammatory disease or disorder of the nervous system”, as used herein, refers to a disease, a disorder or a condition that is characterized by increased inflammation in the nervous system compared to a healthy reference subject.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder, preferably the inflammatory disease or disorder of the nervous system, is selected from the group consisting of inflammatory pain, neuropathic pain, autoimmune inflammatory disease, complex regional pain syndrome and chronic inflammatory demyelinating polyneuropathy. In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder, preferably the inflammatory disease or disorder of the nervous system, is selected from the group consisting of autoimmune inflammatory disease, complex regional pain syndrome and chronic inflammatory demyelinating polyneuropathy. In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder, preferably the inflammatory disease or disorder of the nervous system, is selected from the group consisting of complex regional pain syndrome and chronic inflammatory demyelinating polyneuropathy.

The term “neuropathic pain” as used herein refers to pain caused by damage or disease affecting the somatosensory system, preferably the pain is caused by an inflammatory disease or disorder. The term “neuropathic pain caused by an inflammatory disease or disorder of the nervous system”, as used herein refers to pain induced by damage to the nerves, wherein the damage is caused by an inflammatory disease or disorder. In certain embodiments, the pain described herein is considered to be “caused” by a disease, if the disease and the pain are present, and the disease is known to have the pain as a symptom, preferably wherein the disease is the most likely cause of the pain of a subject.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease is complex regional pain syndrome.

In certain embodiments, the invention relates to the kit of parts, the pharmaceutical composition and the method of treatment for use of the invention, wherein the disease or disorder of the nervous system is complex regional pain syndrome.

The term “complex regional pain syndrome”, as used herein refers to a syndrome that is characterized by excess and prolonged pain and inflammation that follows an injury, typically in injury to an arm or leg.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the inflammatory disease or disorder is inflammatory pain.

In certain embodiments, the invention relates to the kit of parts, the pharmaceutical composition and the method of treatment for use of the invention, wherein the disease or disorder of the nervous system is inflammatory pain.

The term “inflammatory pain”, as used herein is pain that occurs in response to tissue damage and inflammation. As such, inflammatory pain is pain that is neither nociceptive pain nor neuropathic pain. In certain embodiments, the inflammatory pain described herein is caused by an inflammatory disease.

The term “pain”, as described herein, refers to the term as used in the art. In certain embodiments, the pain described herein is chronic pain such that it occurs for longer than 2, longer than 4, longer than 6, longer than 8 or longer than 12 weeks. In certain embodiments, the pain described herein is breakthrough pain in a subject with chronic pain or in a subject under pain reducing therapy (such as pharmacological pain treatment) or a subject with chronic pain under pain reducing therapy. In certain embodiments, the pain described herein occurs without causal history of injury or operation. In certain embodiments, the pain described herein is self-reported pain. The numeric rating scales (NRS) is the most commonly used pain scale for scaling pain on a scale of 0 to 10. In certain embodiments, the pain described herein is pain rated larger than 1, larger than 2, larger than 3, larger than 4, larger than 5, larger than 6, larger than 7, larger than 8 or larger than 9 on NRS.

In certain embodiments, the invention relates to a pharmaceutical product for use in the treatment of pain caused by a disease or disorder of the nervous system, the pharmaceutical product comprising: a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

Oxidative stress is well established to be associated with neuropathic pain. The inventor found that oxidative stress is downregulated by AAT and its peptide derivatives, in particular in SCs which reduces pain.

Accordingly, the invention is at least in part based the effect of AAT and variants, isoforms and/or fragments thereof on oxidative stress.

In certain embodiments, the invention relates to a pharmaceutical product for use in the treatment of chronic inflammatory demyelinating polyneuropathy, the pharmaceutical product comprising: a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

As such the pharmaceutical product described herein may comprise alpha1-antitrypsin (AAT) protein, a variant of AAT having ADAM17 inhibitory activity, an isoform of AAT having ADAM17 inhibitory activity or a fragment of AAT having ADAM17 inhibitory activity or any combination thereof. Additionally or alternatively may comprise a small molecule having ADAM17 inhibitory activity.

The terms "peptide", "protein", "polypeptide", "polypeptidic" and "peptidic" are used herein interchangeably to designate a series of amino acid residues connected to the other by peptide bonds between the alpha-amino and carboxy groups of adjacent residues.

The term "Alpha1-Antitrypsin protein" or "AAT", as used herein refers to a protease inhibitor belonging to the serpin superfamily. Preferably AAT is mammalian AAT, more preferably human AAT. hAAT and human AAT are herein used interchangeably and refer to plasma derived human AAT. In humans AAT is encoded by the SERPINA1 gene. The term AAT includes natural variants, post-translational modified AAT and isoforms of AAT, preferably produced by alternative splicing. Sequences of AAT of different species and origin can be found in protein and nucleic acid databases, such as UniProt, Genbank, DDBJ and EMBL. In a very preferred embodiment, the term "AAT" includes variants as disclosed in protein and nucleic acid databases. In a very preferred embodiment, the term "AAT" refers to a protein with an amino acid sequence as defined by the SEQ ID NO: 1 or a nucleotide sequence encoding a protein with an

amino acid sequence as defined by the SEQ ID NO: 1. In some embodiments, the AAT described herein is a protein, peptide or polypeptide. AAT protein can be obtained by isolation from blood (e.g. human blood) or can be produced recombinantly.

The term "variant" refers to a protein, peptide or polypeptide having an amino acid sequence that differ to some extent from the AAT native sequence peptide, preferably SEQ ID NO: 1, that is an amino acid sequence that vary from the AAT native sequence, preferably SEQ ID NO: 1, by amino acid substitutions, whereby one or more amino acids are substituted by another with same characteristics and conformational roles. Preferably, a variant described herein is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homologous to amino acids of SEQ ID NO: 1. The amino acid sequence variants can have 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 some embodiments, the AAT variant described herein is an AAT variant having protease inhibitory activity, preferably having a disintegrin and metalloprotease 17 (ADAM17) inhibitory activity. The "disintegrin and metalloprotease 17 (ADAM17) inhibitory activity" is preferably measured by the Recombinant Human ADAM-17 kit (Recombinant Human TACE/ADAM17 Protein, CF: 930-ADB-010 and Mca-PLAQAV-Dpa-RSSSR-NH2 Fluorogenic Peptide Substrate: ES003; R&D Systems, preferably under the conditions described in example 22). The "disintegrin and metalloprotease 17 (ADAM17) inhibitory activity" is at least about 30% ADAM17 activity reduction at 200 uM, preferably at least about 30% ADAM17 activity reduction at 100uM and/or at least about 45% ADAM17 activity reduction at 200uM, more preferably at least about

45% ADAM17 activity reduction at 15uM. In these embodiments the concentration in uM may be the concentration of the AAT variant. In certain embodiments, wherein the AAT variant described herein is Ac-VKFNKPFVFLNleIEQNTK-NH2. In certain embodiments, the AAT variant described herein is a sequence related to Ac-VKFNKPFVFLNleIEQNTK-NH2 that is a sequence having not more than 5, not more than 4 not more than 3 or not more than one amino acid substitution(s) compared to Ac-VKFNKPFVFLNleIEQNTK-NH2, preferably wherein the amino acid substitution(s) are conservative amino acid substitutions. The sequence related to Ac-VKFNKPFVFLNleIEQNTK-NH2 may further comprise one or more terminal insertion(s). In certain embodiments, preferably in embodiments wherein the AAT variant described herein is Ac-VKFNKPFVFLNleIEQNTK-NH2 or a sequence related to Ac-VKFNKPFVFLNleIEQNTK-NH2, the AAT variant is not used in the treatment of acute nociceptive, inflammatory, and neuropathic pain. In certain embodiments, the Ac-VKFNKPFVFLNleIEQNTK-NH2 or a sequence related to Ac-VKFNKPFVFLNleIEQNTK-NH2 described herein is used in the treatment of chronic inflammatory demyelinating polyneuropathy or a symptom 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. In some embodiments, the AAT isoform described herein is an AAT isoform having ADAM17 inhibitory activity, preferably having ADAM17 inhibitory activity, more preferably having a disintegrin and metalloprotease 17 (ADAM17) inhibitory activity.

As used herein, a "fragment" of an AAT 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, in particular less amino acids than the sequence of AAT as set forth in SEQ ID NO:1. The fragment is preferably a functional fragment, e.g. a fragment with the same biological activities as the AAT protein as set forth in SEQ ID NO:1. The functional fragment is preferably derived from the AAT protein as set forth in SEQ ID NO:1. Any AAT fragment 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. In some embodiments, the AAT fragment described herein is an AAT fragment having protease inhibitory activity, preferably having ADAM17 inhibitory activity, more preferably having a disintegrin and metalloprotease 17 (ADAM17) inhibitory activity.

Preferably, the (functional) 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, or more of the native human AAT amino acid sequence as set forth in SEQ ID NO:1. In some embodiments, the (functional) fragment described herein, comprises an expression optimized signal protein. In certain embodiments, the fragment described herein is derived from the C-terminus of AAT, as such 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, or more of the native human AAT amino acid sequence as set forth in SEQ ID NO:2. In a very preferred embodiment, the fragment is derived from the C-terminus of AAT, as such the fragment shares about at least about 200 or more of the native human AAT amino acid sequence as set forth in SEQ ID NO:2. In a very preferred embodiment, the fragment has the sequence of SEQ ID NO: 2.

As used herein, a "small molecule" having ADAM17 inhibitory activity refers to a molecule having a molecular weight of less than 1500kD, less than 1400kD, less than 1300kD, less than 1200kD, less than 1100kD, less than 1000kD, less than 900kD, less than 800kD, less than 700kD, less than 600kD, less than 500kD or less than 400kD. In certain embodiments, the small molecule described herein is a peptide having a molecular weight of between 1500kD and 500kD, preferably between 1300kD and 600kD, more preferably between 1100kD and 700kD. Examples of such peptides are given as peptide 9 and peptide 8 in example 22. In certain embodiments, the small molecule is a variant of peptide 9 and peptide 8, wherein one or two amino acids are

replaced, inserted or removed, preferably replaced by a conservative amino acid substitution. In certain embodiments, the small molecule described herein is a molecule having a molecular weight of between 1000kD and 50kD, preferably between 600kD and 100kD, more preferably between 400kD and 200kD. Examples of such a small molecule is provided in example 22 (peptidomimetic 14). The skilled person is aware how to identify further small molecules for use of the invention, e.g., by following the teaching of Lior, Yotam, et al. (European Journal of Medicinal Chemistry 228 (2022): 113969) and screening for ADAM17 inhibitory activity as described in example 22.

In some embodiments, 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 embodiments, 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.

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.

The term "treatment" (and grammatical variations thereof such as "treat" or "treating"), as used herein, refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the

invention are used to delay development of a disease or to slow the progression of a disease.

CIDP is an acquired polyneuropathy within the peripheral nerve system with an assumed autoimmune-mediated pathogenesis. CIDP is characterized by symmetrical weakness in both proximal and distal muscles that worsens progressively. The condition is usually, but not always, associated with impaired sensation, absent or diminished tendon reflexes, an elevated cerebrospinal fluid protein level, and changes in electrophysiology parameters. Nerve biopsy specimens are characterized by signs of demyelination. The clinical course can be relapsing or chronic and progressive (see, e.g., Mathey E K, et al. *J Neurol Neurosurg Psychiatry* 2015; 86:973-985; Köller H, et al. *N Engl J Med.* 2005; 352(13): 1343-1356), the former being much more common in young adults. CIDP is a rare disease with an estimated prevalence of about 1.6 to 8.9 per 100,000 adults and about 0.5 per 100,000 children. CIDP may be diagnosed as described by the Joint Task Force of the EFNS and the PNS (*Journal of the Peripheral Nervous System* 15:1-9 (2010)).

The following conditions are identical or considered essentially identical to CIDP and are thus encompassed by the term "CIDP": "chronic relapsing polyneuropathy", "chronic idiopathic demyelinating polyneuropathy", "chronic inflammatory demyelinating polyradiculoneuropathy", and "chronic acquired demyelinating polyneuropathy" ("CADP").

The inventors found that AAT can reduce neuronal pathology pathways (Fig. 2 – 4, Table 1 - 10). This reduction of neuronal pathology pathways was observed in resting cells (Fig. 4B) and stimulated cells (Fig. 4C) and is therefore useful in preventing and/or treating diseases or disorders of the nervous system and symptoms thereof.

TACE/ADAM17 activity modulation is involved in myelin regulation and as an inflammation hallmark of chronic inflammatory demyelinating polyneuropathy.

The inventors found that AAT is able to inhibit TACE/ADAM17 in a dose-dependent manner which, without being bound by theory, rescues myelin production by SCs, and thus subsequently prevents, or slowing, and/or reverses the progression of chronic inflammatory demyelinating polyneuropathy. As such, the combined anti-inflammatory and neuroprotective/regenerative effect is surprisingly effective in the treatment of chronic inflammatory demyelinating polyneuropathy.

Accordingly, the invention is at least in part based on the finding that AAT is useful in treating disease or disorders of the nervous system as described herein.

According to some embodiments, the treatment described herein comprises single dose administration of the total amount of AAT. According to certain embodiments, the effective amount of AAT is about 10 mg to about 1000 mg per kg body weight of AAT per week, preferably about 10 mg to about 500 mg per kg body weight of AAT per week (or an equivalent amount of AAT variant, fragment, isoform and/or corresponding nucleic acid). According to certain embodiments, the effective amount of AAT is about 30 mg to about 240 mg per kg body weight AAT per week, preferably about 60 mg to about 120 mg per kg body weight AAT per week (or an equivalent amount of AAT variant, fragment, isoform and/or corresponding nucleic acid). According to certain embodiments, the effective amount of AAT (or an equivalent amount of AAT variant, fragment, isoform and/or corresponding nucleic acid) is about 10 mg/kg/week to about 1000 mg/kg/week, preferably about 10 mg/kg/week to about 500 mg/kg/week. According to certain embodiments, the effective amount of AAT (or an equivalent amount of AAT variant, fragment, isoform and/or corresponding nucleic acid) is above about 100 mg/kg/day, preferably between about 100 mg/kg/day to about 1000 mg/kg/day, more preferably between about 100 mg/kg/day to about 500 mg/kg/day, more preferably between about 150 mg/kg/day to about 250 mg/kg/day, more preferably between about 150 mg/kg/day to about 210 mg/kg/day.

According to other embodiments, the treatment described herein comprises multiple administrations of multiple portion doses to reach the total cumulative dose of AAT. According to certain embodiments, each portion dose comprises from about 5 mg to about 500 mg per kg, preferably about 15 mg to about 240 mg per kg. According to other embodiments, each portion dose comprises about 10, about 20, about 40, about 60, about 80, about 120 or about 240 mg AAT/kg (or an equivalent amount of AAT variant, fragment, isoform and/or corresponding nucleic acid or small molecule). Each possibility represents a separate embodiment of the present invention.

In one embodiment, the invention relates to a kit of parts for use in the treatment of a disease or disorder of the nervous system, the kit comprising: i) a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory

activity and ii) a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

The term “plurality of IgG antibodies”, as used herein, refers to at least two different, at least three different, at least four different, at least five different, at least six different, at least seven different, at least eight different, at least nine different or at least ten different IgG antibodies. In certain embodiments, the plurality of IgG antibodies identify at least two different epitopes or antigens. In certain embodiments, the plurality of IgG antibodies comprises IgG antibodies of several IgG subclasses. In certain embodiments, the plurality of IgG antibodies are polyclonal antibodies. In certain embodiments, the plurality of IgG antibodies are not monoclonal antibodies. In certain embodiments, the plurality of IgG antibodies described herein comprises IgG antibodies human plasma derived IgG antibodies, preferably pooled IgG antibodies. Isoforms of IgG antibodies, fragments of IgG antibodies and/or IgG variants may further be combined with (e.g. added to or not separated from) the plurality of IgG antibodies. In certain embodiments, these isoforms, fragments and variants are naturally occurring isoforms, fragments and/or variants. In other embodiments, the isoforms, fragments and/or variants are separately produced and added to the IgG antibodies.

In general, the term “antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), fully-human antibodies and antibody fragments so long as they exhibit the desired antigen-binding activity. In some embodiments, the IgG antibodies described herein are from the IgG1, IgG2, IgG3, and/or IgG4 subclass. In some embodiments, the IgG antibodies described herein comprise polyclonal IgG antibodies. In some embodiments, the IgG antibodies described herein are part of an immunoglobulin therapy. As used herein, the term “immunoglobulin therapy” refers generally to a therapeutic method of intravenously, subcutaneously, or intramuscularly administering a composition of IgG immunoglobulins to a patient for treating a number of conditions such as immune deficiencies, inflammatory diseases, and autoimmune diseases. The IgG immunoglobulins are typically pooled and prepared from plasma. Whole antibodies or fragments can be used. IgG immunoglobulins can be formulated in higher concentrations (e.g., greater than 10%) for subcutaneous administration, or formulated for intramuscular administration. This is particularly common for specialty IgG

preparations which are prepared with higher than average titres for specific antigens (e.g., Rho D factor, pertussis toxin, tetanus toxin, botulism toxin, rabies, etc.). Preparation of an immunoglobulin therapy is known in the art (see e.g. US8940877B2). The immunoglobulin therapy may be derived from mammalian, preferably human, plasma. In certain embodiments, the plasma of multiple (generally 1000 or more) healthy donors is pooled and optionally further processed. The term "healthy individual" means an individual who meets the current (at the time of donation) standard eligibility criteria for donating blood, bearing in mind that such eligibility criteria are subject to continuous improvement and change. In some embodiments, the immunoglobulin fraction is enriched from the pooled plasma. Preferably, the immunoglobulin is purified from the pooled plasma. More preferably, the immunoglobulin is purified and concentrated. In various embodiments, purified and concentrated immunoglobulin G (IgG) is used. In certain embodiments, the immunoglobulin therapy may contain traces of immunoglobulins of different Ig classes such as IgA or IgM. In certain embodiments, the IgA concentration is 50 µg or less per 100 mg immunoglobulin. In a preferred embodiment, the IgA concentration is 25 µg or less per 100 mg immunoglobulin. Low IgA is desirable in order to avoid adverse events in patients with IgA deficiency. In one embodiment, the IgM concentration is 10 µg or less per 100 mg immunoglobulin. In a preferred embodiment, the IgM concentration is 5 µg or less per 100 mg immunoglobulin. In various embodiments, the immunoglobulin therapy exhibits a purity of the protein fraction of >90% IgG, more preferably >95% IgG, even more preferably >98% IgG. In various embodiments, the immunoglobulin therapy exhibits an immunoglobulin monomer and dimer content of >90%, more preferably >95%, even more preferably >98%. The immunoglobulin therapy preferably exhibits a natural IgG subclass distribution. In one embodiment, the immunoglobulin subclass distribution in the immunoglobulin therapy is 62-74% IgG1, 22-34% IgG2, 2-5% IgG3 and 1-3% IgG4. The immunoglobulin therapy may contain additional ingredients such as stabilizers, for example amino acids such as proline or glycine, or sucrose, maltose, sorbitol, albumin nicotinamide, PEG, polysorbate 80, or others. Preferred stabilizers are amino acids, in particular proline. In various embodiments, the immunoglobulin therapy contains 10-30% (w/v) immunoglobulin. In certain embodiments, the immunoglobulin therapy is provided as a solution containing at least 10% (w/v) immunoglobulin, more preferably at least 15% (w/v) immunoglobulin, most preferably about 20% (w/v) immunoglobulin. The immunoglobulin therapy may also contain about 30% (w/v) immunoglobulin. The

immunoglobulin therapy is virus-safe for enveloped viruses (e.g., HIV, HBV and HCV) and non-enveloped viruses (e.g., HAV and parovirus B19). The immunoglobulin therapy may be provided as a liquid product or a lyophilized product. In a preferred embodiment, the immunoglobulin therapy is provided as a liquid product. Such liquid products are ready-for-use, i.e., it is not necessary to reconstitute the product prior to administration. Liquid products are convenient to use, as no reconstitution is required. Therefore, liquid products are particularly suitable for self-administration by patients. In some embodiments, the dose of IgG antibodies is individually adjusted or a fixed dose. In some embodiments, the IgG antibodies described herein are administered a dose selected from the range of about 0.1 - about 0.4 g/kg patient weight, from the range of about 0.1 - about 0.3 g/kg patient weight, from the range of about 0.15 - about 0.25 g/kg patient weight, from the range of about 0.18 - about 0.22 g/kg patient weight or a dose of about 0.2 g/kg patient weight per 5-10 days or per 6-8 days per week, preferably in a dose selected from the range of about 0.18 - about 0.22 g/kg patient weight per 6-8 days. In some embodiments, the dose of IgG antibodies is administered in a single dose or distributed 2, 3, 4, 5, 6 or 7 times a week.

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 embodiments, the disease or disorder of the nervous system described herein is a disease or disorder selected from the group consisting of 12q14 microdeletion syndrome, 15q13.3 microdeletion syndrome, 15q24 microdeletion syndrome, 22q11.2 deletion syndrome, 22q13.3 deletion syndrome, 2-methylbutyryl-CoA dehydrogenase deficiency, 2q23.1 microdeletion syndrome, 2q37 deletion syndrome, 3-alpha hydroxyacyl-CoA dehydrogenase deficiency, 3MC syndrome, XXXY syndrome, XYYY syndrome, XXXXY syndrome, 5q14.3 microdeletion syndrome, 6-pyruvoyl-tetrahydropterin synthase deficiency, Aarskog syndrome, Abetalipoproteinemia, ABri amyloidosis, absence of septum pellucidum, Aceruloplasminemia, Acrocallosal syndrome, acrofacial dysostosis Catania type, acrofacial dysostosis Rodriguez type, acute cerebellar ataxia, acute cholinergic dysautonomia, acute CNS demyelinating event, acute disseminated encephalomyelitis, acute inflammatory demyelinating polyneuropathy, acute intermittent porphyria, acute motor and sensory axonal neuropathy syndrome, ADCY5-related dyskinesia, adenosine monophosphate deaminase 1 deficiency, adenylosuccinase deficiency, Adie syndrome, adrenomyeloneuropathy, adult polyglucosan body disease, adult-onset nemaline

myopathy, advanced sleep phase syndrome, agenesis of the corpus callosum, age-related peripheral neuropathy, age-related peripheral neuropathy, Agnosia, Aicardi syndrome, Aicardi-Goutieres syndrome, AIDS Dementia Complex, Al Gazali Aziz Salem syndrome, Alaninuria, Albinism deafness syndrome, alcohol or nutritional deficiencies induced sensorimotor deficiency, alcoholic neuropathy, alcoholic peripheral neuropathy, Alexander disease, ALG11-CDG (CDG-Ip), ALG12-CDG (CDG-Ig), ALG13-CDG, ALG1-CDG (CDG-Ik), ALG2-CDG (CDG-Ii), ALG3-CDG (CDG-Id), ALG6-CDG (CDG-Ic), ALG8-CDG (CDG-Ih), ALG9-CDG (CDG-IL), Allan-Herndon-Dudley syndrome, alopecia epilepsy oligophrenia syndrome of Moynahan, alopecia, epilepsy, pyorrhea, mental subnormality, alopecia-contractures-dwarfism-intellectual disability syndrome, alopecia-intellectual disability syndrome, Alpers syndrome, alpha-ketoglutarate dehydrogenase deficiency, alpha-mannosidosis, alpha-thalassemia x-linked intellectual disability syndrome, alternating hemiplegia of childhood, Alzheimer disease type 4, Alzheimer's disease, Alzheimer's disease without neurofibrillary tangles, aminoacylase 1 deficiency, aminolevulinate dehydratase deficiency porphyria, Amish lethal microcephaly, Amish Nemaline Myopathy, amyloid neuropathy, amyopathic dermatomyositis, amyotrophic lateral sclerosis, amyotrophic lateral sclerosis type 6, amyotrophic lateral sclerosis-parkinsonism/dementia complex 1, amyotrophic lateral sclerosis, anaplastic astrocytoma, anaplastic ganglioglioma, anaplastic oligodendroglioma, Andermann syndrome, Andersen-Tawil syndrome, anemia sideroblastic ataxia, spinocerebellar ataxia, Anencephaly, Angioma hereditary neurocutaneous, Aniridia, Aniridia renal agenesis psychomotor retardation, Antisynthetase syndrome, Aortic arch anomaly, Apraxia, Arachnoid cysts, Arachnoiditis, Aromatic L-amino acid decarboxylase deficiency, Arthrogryposis multiplex congenita, distal, X-linked, Arthrogryposis renal dysfunction cholestasis syndrome, Arts syndrome, Aspartylglycosaminuria, Ataxia, Ataxia telangiectasia, Ataxia with oculomotor apraxia type 1, Ataxia with Oculomotor Apraxia Type 2, Ataxia with oculomotor apraxia type 4, Ataxia with vitamin E deficiency, Ataxia-teleangiectasia, Atelosteogenesis type 2, Atelosteogenesis type 3, Atkin syndrome, Atypical Rett syndrome, aseptic meningitis, Autism with port-wine stain, Autosomal dominant centronuclear myopathy, Autosomal dominant cerebellar ataxia/deafness/narcolepsy, autosomal dominant Charcot-Marie-Tooth disease type 2 with giant axons, autosomal dominant deafness-onychodystrophy syndrome, autosomal dominant intermediate Charcot-Marie-Tooth disease, autosomal dominant

leukodystrophy with autonomic disease, autosomal dominant neuronal ceroid lipofuscinosis 4B, autosomal dominant nocturnal frontal lobe epilepsy, autosomal dominant non-syndromic intellectual disability, autosomal dominant optic atrophy plus syndrome, autosomal dominant partial epilepsy with auditory features, autosomal dominant spinal muscular atrophy, autosomal recessive axonal neuropathy with neuromyotonia, autosomal recessive centronuclear myopathy, autosomal recessive Charcot-Marie-Tooth disease with hoarseness, autosomal recessive intermediate Charcot-Marie-Tooth disease type A, autosomal recessive intermediate Charcot-Marie-Tooth disease type B, autosomal recessive juvenile Parkinson disease, Autosomal recessive neuronal ceroid lipofuscinosis 4A, Adult neuronal ceroid lipofuscinosis, Autosomal recessive primary microcephaly, Autosomal recessive spastic ataxia 4, Autosomal recessive spastic paraplegia type 49, Autosomal recessive spinocerebellar ataxia 9, B4GALT1-CDG (CDG-IIId), Bannayan-Riley-Ruvalcaba syndrome, Barth syndrome, Battaglia-Neri syndrome, Becker muscular dystrophy, Behavioral variant of frontotemporal dementia, Behçet disease, Bell's palsy, Benign essential blepharospasm, Benign familial neonatal epilepsy, Benign familial neonatal-infantile seizures, Benign hereditary chorea, Benign rolandic epilepsy (BRE), Beta-Propeller Protein-Associated Neurodegeneration, Bethlem myopathy, bilateral frontal polymicrogyria, bilateral frontoparietal polymicrogyria, bilateral generalized polymicrogyria, bilateral parasagittal parieto-occipital polymicrogyria, bilateral perisylvian polymicrogyria, Binswanger's disease, Biotinidase deficiency, Biotin-thiamine-responsive basal ganglia disease, Birk-Barel syndrome, Bixler Christian Gorlin syndrome, Blepharonasofacial malformation syndrome, Bobble-head doll syndrome, Bohring-Opitz syndrome, Borjeson-Forssman-Lehmann syndrome, Bowen-Conradi syndrome, Brachioskeletogenital syndrome, Brachydactyly-mesomelia-intellectual disability-heart defects syndrome, Brain dopamine-serotonin vesicular transport disease, Brain-lung-thyroid syndrome, Branchial arch syndrome X-linked, Brody myopathy, Brooks Wisniewski Brown syndrome, Brown-Sequard syndrome, Bullous dystrophy, C syndrome, Cabezas syndrome, CADASIL, Camptocormism, Camptodactyly arthropathy coxa vara pericarditis syndrome, CANOMAD syndrome, Cantu syndrome, Cap myopathy, Cardiofaciocutaneous syndrome, Carey-Fineman-Ziter syndrome, Carney complex, Cataract ataxia deafness, Catel Manzke syndrome, Caudal appendage deafness, Caudal regression sequence, Central core disease, Central nervous system germinoma, Central neurocytoma, Central pain syndrome,

Central pontine myelinolysis, Cerebellar ataxia, Cerebellar degeneration, Cerebellar hypoplasia, Cerebellitis, Cerebelloparenchymal disorder 3, Cerebellum agenesis hydrocephaly, Cerebral autosomal recessive arteriopathy, Cerebral cavernous malformation, Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome, Cerebral folate deficiency, Cerebral gigantism jaw cysts, Cerebral palsy, Cerebral palsy ataxic, Cerebral palsy athetoid, Cerebral palsy spastic hemiplegic, Cerebral palsy spastic monoplegic, Cerebral palsy spastic quadriplegic, Cerebral sclerosis, Cerebro-facio-articular syndrome, Cerebro-oculo-facio-skeletal syndrome, Cerebrooculonasal syndrome, Cerebrospinal fluid leak, Cerebrotendinous xanthomatosis, Ceroid lipofuscinosis neuronal 1, Cervical hypertrichosis peripheral neuropathy, Chanarin-Dorfman syndrome, Charcot-Marie-Tooth disease, Charcot-Marie-Tooth disease type 1A, Charcot-Marie-Tooth disease type 2, Charcot-Marie-Tooth disease type 3, Charcot-Marie-Tooth disease type 4, Chediak-Higashi syndrome, Chiari malformation, Chiari malformation type 1, Chiari malformation type 2, Chiari malformation type 4, Childhood apraxia of speech, Childhood-onset nemaline myopathy, Chorea-acanthocytosis, Choroid plexus carcinoma, Choroid plexus papilloma, Christianson syndrome, Chromosome 17p13.1 deletion syndrome, Chromosome 17q11.2 deletion syndrome, Chromosome 19q13.11 deletion syndrome, Chromosome 1p36 deletion syndrome, Chromosome 3p- syndrome, Chronic hiccups, Chronic lymphocytic inflammation, Chronic progressive external ophthalmoplegia, Chudley Rozdilsky syndrome, Cisplatin induced sensory neuropathy, Cleft palate short stature vertebral anomalies, Cluster headache, COACH syndrome, COASY protein-associated neurodegeneration, coats disease, Cobb syndrome, Cockayne syndrome type I, Cockayne syndrome type II, Cockayne syndrome type III, Coenzyme Q10 deficiency, Coffin-Lowry syndrome, Coffin-Siris syndrome, COG1-CDG (CDG-IIg), COG4-CDG (CDG-IIj), COG5-CDG (CDG-IIi), COG7-CDG (CDG-IIe), COG8-CDG (CDG-IIh), Cohen syndrome, cold-induced sweating syndrome, complex regional pain syndrome, congenital central hypoventilation syndrome, congenital cytomegalovirus, congenital fiber type disproportion, congenital fibrosis of extraocular muscles, congenital generalized lipodystrophy type 4, congenital insensitivity to pain, congenital insensitivity to pain with anhidrosis, congenital intrauterine infection-like syndrome, congenital laryngeal palsy, congenital mirror movement disorder, congenital muscular dystrophy, congenital myasthenic syndrome, congenital rubella, congenital toxoplasmosis, continuous spike-wave during slow sleep syndrome, convulsions,

corneal hypesthesia, Cornelia de Lange syndrome, Corpus callosum agenesis, Cortical blindness, Cortical dysgenesis, Corticobasal degeneration, Costello syndrome, Crane-Heise syndrome, cranial nerve palsy, Craniofrontonasal dysplasia, Craniopharyngioma, Craniorachischisis, Craniotelencephalic dysplasia, Creutzfeldt-Jakob disease, Crome syndrome, Curry Jones syndrome, cylindrical spirals myopathy, cyprus facial neuromusculoskeletal syndrome, cytomegalic inclusion disease, D-2-hydroxyglutaric aciduria, Dandy-Walker cyst, Dandy-Walker like malformation, Dandy-Walker malformation, Danon disease, Dapsone induced neuropathy, DDOST-CDG (CDG-Ir), DEAF1-associated disorders, Dentatorubral-pallidoluysian atrophy, Dermatomyositis, Developmental dysphasia familial, Diabetic neuropathy, Dihydrolipoamide dehydrogenase deficiency, Dihydropteridine reductase deficiency, Diphtheria, Distal myopathy with vocal cord weakness, DOOR syndrome, Dopamine beta hydroxylase deficiency, Dopamine transporter deficiency syndrome, Dopa-responsive dystonia, DPAGT1-CDG (CDG-Ij), DPM1-CDG (CDG-Ie), DPM2-CDG, DPM3-CDG (CDG-Io), Dravet syndrome, Duane syndrome, Dubowitz syndrome, Duchenne muscular dystrophy, Dykes Markes Harper syndrome, Dysautonomia like disorder, Dysequilibrium syndrome, Dyskeratosis congenita, Dyskeratosis congenita autosomal dominant, Dyskeratosis congenita autosomal recessive, Dyskeratosis congenita X-linked, Dyssynergia cerebellaris myoclonica, Dystonia 2, DYT-PRKRA, DYT-THAP1, DYT-TOR1A, DYT-TUBB4A, early infantile epileptic encephalopathy, early infantile epileptic encephalopathy 25, early-onset anterior polar cataract, early-onset autosomal dominant alzheimer disease, early-onset parkinsonism-intellectual disability syndrome, eastern equine encephalitis, empty sella syndrome, encephalitis lethargica, encephalocraniocutaneous lipomatosis, encephalopathy, eosinophilic fasciitis, eosinophilic granulomatosis, ependymoma, epidermolysa bullosa simplex with muscular dystrophy, epilepsy juvenile absence, epilepsy occipital calcifications, epilepsy progressive myoclonic type 3, epilepsy with myoclonic-atonic seizures, epiphyseal dysplasia hearing loss dysmorphism, episodic ataxia, erythromelalgia, essential tremor, Fabry disease, facial onset neuronopathy, facioscapulohumeral muscular dystrophy, Fallot complex, familial amyloidosis, familial bilateral striatal necrosis, familial caudal dysgenesis, familial congenital palsy of trochlear nerve, familial dysautonomia, familial encephalopathy, familial exudative vitreoretinopathy, familial focal epilepsy, familial hemiplegic migraine, familial hemophagocytic lymphohistiocytosis, familial infantile convulsions familial infantile paroxysmal

choreoathetosis, familial porencephaly, familial transthyretin amyloidosis, familial or sporadic hemiplegic migraine, farber disease, fatal familial insomnia, fatal infantile encephalomyopathy, fatty acid hydroxylase-associated neurodegeneration, FBXL4-related encephalomyopathic mitochondrial DNA depletion syndrome, Febrile infection-related epilepsy syndrome, Feigenbaum Bergeron Richardson syndrome, Filippi syndrome, Fine-Lubinsky syndrome, Fingerprint body myopathy, Fitzsimmons Walson Mellor syndrome, Fitzsimmons-Guilbert syndrome, Floating-Harbor syndrome, Flynn Aird syndrome, focal dermal hypoplasia, focal motor weakness, focal segmental glomerulosclerosis, Fountain syndrome, FOXP1 syndrome, Fragile X syndrome, Fragile XE syndrome, Friedreich ataxia, Frontometaphyseal dysplasia, Frontotemporal dementia, Frontotemporal lobar dementia, Fryns syndrome, Fucosidosis, Fukuyama type muscular dystrophy, Fumarase deficiency, Galactosialidosis, Galloway-Mowat syndrome, Gamma aminobutyric acid transaminase deficiency, Gangliocytoma, GAPO syndrome, Gaucher disease type 1, Gaucher disease type 2, Gaucher disease type 3, Gemignani syndrome, Genitopatellar syndrome, Genoa syndrome, Gerstmann syndrome, Gerstmann-Straussler-Scheinker disease, Giant axonal neuropathy, Gillespie syndrome, Gliomatosis cerebri, Glucose transporter type 1 deficiency syndrome, Glutamine deficiency, congenital, Glutaric acidemia type I, Glutaric acidemia type II, Glutaric acidemia type III, Glycogen storage disease type 13, Glycogen storage disease type 2, Glycogen storage disease type 3, Glycogen storage disease type 4, Glycogen storage disease type 5, Glycogen storage disease type 7, GM1 gangliosidosis type 1, GM1 gangliosidosis type 2, GM1 gangliosidosis type 3, GM3 synthase deficiency, GMS syndrome, Goldberg-Shprintzen megacolon syndrome, Gomez Lopez Hernandez syndrome, GOSR2-related progressive myoclonus ataxia, Graham-Cox syndrome, Granulomatosis with polyangiitis, Griscelli syndrome type 1, Grubben de Cock Borghgraef syndrome, GTP cyclohydrolase I deficiency, GTPCH1-deficient DRD, Guanidinoacetate methyltransferase deficiency, Guillain-Barre syndrome, Gurrieri syndrome, Gyrate atrophy of choroid and retina, Hair defect-photosensitivity-intellectual disability syndrome, Hallermann-Streiff syndrome, Hall-Riggs syndrome, Hamanishi Ueba Tsuji syndrome, Hansen's disease, Harding ataxia, Harlequin syndrome, Harrod Doman Keele syndrome, Hartnup disease, Hashimoto encephalopathy, Hemangioblastoma, Hemicrania continua, Hemimegalencephaly, Hennekam syndrome, hereditary angiopathy, hereditary coproporphyrria, hereditary diffuse leukoencephalopathy, hereditary fibrosing

poikiloderma with tendon contractures, myopathy, and pulmonary fibrosis, hereditary geniospasm, hereditary hemorrhagic telangiectasia, hereditary hemorrhagic telangiectasia type 2, hereditary hemorrhagic telangiectasia type 3, hereditary hemorrhagic telangiectasia type 4, hereditary hyperekplexia, hereditary motor and sensory neuropathy type 5, hereditary neuropathy with liability to pressure palsies (HNPP), hereditary predisposition to pressure palsies (focal and symmetrical), hereditary proximal myopathy with early respiratory failure, hereditary sensorimotor neuropathy with hyperelastic skin, hereditary sensory and autonomic neuropathy type 1e, hereditary sensory and autonomic neuropathy type 2, hereditary sensory and autonomic neuropathy type 1-7 (HSAN I-VII), hereditary sensory and autonomic neuropathy type v, hereditary sensory neuropathy type 1, hereditary spastic paraplegia, hereditary vascular retinopathy, Hernández-Aguirre Negrete syndrome, herpes simplex encephalitis, herpes zoster oticus, HIBCH deficiency, Homocystinuria, Horizontal gaze palsy with progressive scoliosis, Hoyerall Hreidarsson syndrome, HSD10 disease, HTLV-1 associated myelopathy/tropical spastic paraparesis, Human HOXA1 Syndromes, Human immunodeficiency virus induced neuropathy, Huntington disease, Huntington's disease, Hurler syndrome, Hurler-Scheie syndrome, hydranencephaly, hydrocephalus (e.g. due to congenital stenosis of aqueduct of sylvius), hydrocephalus-cleft palate-joint contractures syndrome, hydroxykynureninuria, hyperbetaalaninemia, hypercoagulability syndrome due to glycosylphosphatidylinositol deficiency, hyperkalemic periodic paralysis, hypermethioninemia, hyperphenylalaninemia, hyperprolinemia, hyperprolinemia type 2, hypertrophic neuropathy of Dejerine-Sottas, hypocalcemia, autosomal dominant, hypokalemic periodic paralysis, hypomelanosis of Ito, hypomyelination (e.g., with atrophy of basal ganglia and/or cerebellum), hypoparathyroidism-intellectual disability-dysmorphism syndrome, hypospadias-intellectual disability, Goldblatt type syndrome, hypothalamic hamartomas, ichthyosis alopecia eclabion ectropion intellectual disability, idiopathic intracranial hypertension, idiopathic spinal cord herniation, inclusion body myositis, incontinentia pigmenti, infantile axonal neuropathy, infantile cerebellar retinal degeneration, infantile choroidocerebral calcification syndrome, infantile myofibromatosis, infantile neuroaxonal dystrophy, infantile onset spinocerebellar ataxia, infantile spasms broad thumbs, infantile-onset ascending hereditary spastic paralysis, infection-induced acute encephalopathy 3, intellectual deficit Buenos-Aires type, athetosis intellectual disability, hypoplastic corpus callosum

intellectual disability, intellectual disability-developmental delay-contractures syndrome, intellectual disability-dysmorphism-hypogonadism-diabetes mellitus syndrome, Intellectual disability-severe speech delay-mild dysmorphism syndrome, intellectual disability-spasticity-ectrodactyly syndrome, Intermediate congenital nemaline myopathy, Internal carotid agenesis, Intraneural perineurioma, IRVAN syndrome, Isaacs' syndrome, Isodicentric chromosome 15 syndrome, Johanson-Blizzard syndrome, Johnson neuroectodermal syndrome, Joubert syndrome, Juberg Marsidi syndrome, Juvenile amyotrophic lateral sclerosis, Juvenile dermatomyositis, Juvenile Huntington disease, Juvenile polymyositis, Juvenile primary lateral sclerosis, Kabuki syndrome, Kanzaki disease, Kapur Toriello syndrome, Kaufman oculocerebrofacial syndrome, KBG syndrome, KCNQ2-Related Disorders, Kearns-Sayre syndrome, Kennedy disease, Keratosis follicularis dwarfism, cerebral atrophy, Kernicterus, Keutel syndrome, King Denborough syndrome, Kleefstra syndrome, Kleine Levin syndrome, Klumpke paralysis, Kosztolanyi syndrome, Kozłowski-Krajewska syndrome, Krabbe's disease, Kuru, Kuzniecky Andermann syndrome, L-2-hydroxyglutaric aciduria, La Crosse encephalitis, Laband syndrome, Lafora disease, Laing distal myopathy, Lambert Eaton myasthenic syndrome, Landau-Kleffner syndrome, L-arginine:glycine amidinotransferase deficiency, Late-onset distal myopathy, Markesbery-Griggs type, lateral meningocele syndrome, Laurence-Moon syndrome, LCHAD deficiency, Leber hereditary optic neuropathy, Leigh syndrome, Lennox-Gastaut syndrome, Lenz Majewski hyperostotic dwarfism, Lenz microphthalmia syndrome, Lesch Nyhan syndrome, Leukodystrophy, Leukoencephalopathy (e.g. with thalamus and brainstem involvement and high lactate), Levic Stefanovic Nikolic syndrome, Lewis-Sumner syndrome, Lhermitte-Duclos disease, Li-Fraumeni syndrome, limb-girdle muscular dystrophy (e.g. type 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 2A, 2B, 2C, 2D, 2E, 2F, 2H, 2I, 2J, 2K, 2L, 2M, 2N, 2O, 2P, 2Q, 2S, 2T), limbic encephalitis with LGI1 antibodies, Limited cutaneous systemic sclerosis, Lipoic acid synthetase deficiency, Lissencephaly 1, Lissencephaly 2, Lissencephaly X-linked, Localized hypertrophic neuropathy, Locked-in syndrome, Logopenic progressive aphasia, Lowe oculocerebrorenal syndrome, Lowry Maclean syndrome, Lujan syndrome, Lyme disease, Mac Dermot Winter syndrome, macrocephaly-short stature-paraplegia syndrome, macrothrombocytopenia progressive deafness, mal de débarquement syndrome, male pseudohermaphroditism intellectual disability syndrome, malignant hyperthermia, malignant hyperthermia

arthrogryposis torticollis, malignant migrating partial seizures of infancy, MAN1B1-CDG, Mandibulofacial dysostosis (e.g. with microcephaly), Mannosidosis, Marchiafava Bignami disease, Marden-Walker syndrome, Marfanoid habitus-autosomal recessive intellectual disability syndrome, Marinesco-Sjogren syndrome, Martsolf syndrome, McDonough syndrome, McLeod neuroacanthocytosis syndrome, Meckel syndrome, MECP2 duplication syndrome, medrano roldan syndrome, medulloblastoma, megalencephalic leukoencephalopathy(e.g. with subcortical cysts), megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome, megaloblastic anemia, megalocornea-intellectual disability syndrome, Mehes syndrome, MEHMO syndrome, Meier-Gorlin syndrome, Meige syndrome, Melnick-Needles syndrome, Meningioma, meningitis, menkes disease, meralgia paresthetica, metaphyseal dysostosis-intellectual disability-conductive deafness syndrome, methionine adenosyltransferase deficiency, methylcobalamin deficiency cbl g type, methylmalonic acidemia with homocystinuria type cblc, mgat2-cdg (cdg-iiia), micro syndrome, microbrachycephaly ptosis cleft lip, microcephalic osteodysplastic primordial dwarfism type 1, microcephalic osteodysplastic primordial dwarfism type 2, microcephalic primordial dwarfism, (e.g., Montreal type, Toriello type), microcephaly, microcephaly autosomal dominant, microcephaly brain defect spasticity hypernatremia, microcephaly cervical spine fusion anomalies, microcephaly deafness syndrome, microcephaly glomerulonephritis marfanoid habitus, microcephaly microcornea syndrome, microcephaly-cardiomyopathy, Microduplication Xp11.22-p11.23 syndrome, microphthalmia syndromic 10, microphthalmia syndromic 4, microphthalmia syndromic 8, microphthalmia with linear skin defects syndrome, microscopic polyangiitis, migraine (e.g. with brainstem aura), mild phenylketonuria, Miller-Dieker syndrome, Miller-Fisher syndrome, minicore myopathy with external ophthalmoplegia, mitochondrial complex I deficiency, mitochondrial complex II deficiency, mitochondrial DNA depletion syndrome, encephalomyopathic form with methylmalonic aciduria, mitochondrial DNA-associated Leigh syndrome, mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes, mitochondrial membrane protein-associated neurodegeneration, mitochondrial myopathy and sideroblastic anemia, mitochondrial myopathy with diabetes, mitochondrial myopathy with lactic acidosis, mitochondrial neurogastrointestinal encephalopathy syndrome, mitochondrial trifunctional protein deficiency, mixed connective tissue disease, Miyoshi myopathy, Moebius syndrome, MOGS-CDG (CDG-IIb), Mohr-Tranebjaerg syndrome, molybdenum cofactor

deficiency, monoamine oxidase A deficiency, monoclonal gammopathy of undetermined significance (MGUS), Morse-Rawnsley-Sargent syndrome, Morvan's fibrillary chorea, Mousa Al din Al Nassar syndrome, Moyamoya disease, MPDU1-CDG (CDG-If), MPI-CDG (CDG-Ib), MPV17-related hepatocerebral mitochondrial DNA depletion syndrome, mucopolipidosis type 4, mucopolysaccharidosis type III, mucopolysaccharidosis type IIIA, mucopolysaccharidosis type IIIB, mucopolysaccharidosis type IIIC, mucopolysaccharidosis type IIID, multifocal motor neuropathy, multiple congenital anomalies-hypotonia-seizures syndrome, multiple congenital anomalies-hypotonia-seizures syndrome type 2, multiple myeloma, multiple sulfatase deficiency, multiple system atrophy, multiple system atrophy, multisystemic smooth muscle dysfunction syndrome, muscle eye brain disease, muscular dystrophy white matter spongiosis, megaconial type muscular dystrophy, Muscular phosphorylase kinase deficiency, Musculocontractural Ehlers-Danlos syndrome, Myasthenia gravis, Myelitis, Myelocerebellar disorder, Myelomeningocele, MYH7-related scapuloperoneal myopathy, Myhre syndrome, Myoclonic epilepsy with ragged red fibers, Myoclonus cerebellar ataxia deafness, Myoclonus-dystonia, Myoglobinuria recurrent, Myopathy with extrapyramidal signs, Myosin storage myopathy, Myotonia congenita, Myotonic dystrophy type 1, Myotonic dystrophy type 2, N syndrome, Nance-Horan syndrome, Narcolepsy, NBIA/DYT/PARK-PLA2G6, Necrotizing autoimmune myopathy, Neonatal adrenoleukodystrophy, Neonatal meningitis, Neonatal progeroid syndrome, Neu Laxova syndrome, Neuroblastoma, Neurocutaneous melanosis, Neurofaciodigitorenal syndrome, Neuroferritinopathy, Neurofibromatosis type 1, Neurofibromatosis type 2, Neuroleptic malignant syndrome, Neuromyelitis optica spectrum disorder, Neuronal ceroid lipofuscinosis, Neuronal ceroid lipofuscinosis 10, Neuronal ceroid lipofuscinosis 2, Neuronal ceroid lipofuscinosis 3, Neuronal ceroid lipofuscinosis 5, Neuronal ceroid lipofuscinosis 6, Neuronal ceroid lipofuscinosis 7, Neuronal ceroid lipofuscinosis 9, Neuronal intranuclear inclusion disease, Neuropathic pain, Neuropathy ataxia retinitis pigmentosa syndrome, Neuropathy, distal hereditary motor, Jerash type, Neuropathy, hereditary motor and sensory, Okinawa type, neurologic bladder, Neuropathy, hereditary motor and sensory, Russe type, Neutral lipid storage disease with myopathy, Nevoid basal cell carcinoma syndrome, New-onset refractory status epilepticus, Nicolaides-Baraitser syndrome, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Non-sleep wake disorder, Nondystrophic myotonia,

Noonan syndrome, Norrie disease, Northern epilepsy, Oculocerebrocutaneous syndrome, Oculofaciocardiodental syndrome, Oculopharyngeal muscular dystrophy, Oculopharyngodistal myopathy, Okamoto syndrome, Olfactory neuroblastoma, Oligoastrocytoma, Oligodendroglioma, Oliver syndrome, Olivopontocerebellar atrophy, Omphalocele cleft palate syndrome lethal, OPHN1 syndrome, Opsoclonus-myoclonus syndrome, Optic atrophy 2, Optic pathway glioma, optic neuritis, Ornithine transcarbamylase deficiency, Orofaciodigital syndrome 1, Orofaciodigital syndrome 10, Orofaciodigital syndrome 2, Orofaciodigital syndrome 3, Orofaciodigital syndrome 4, Orofaciodigital syndrome 5, Orofaciodigital syndrome 6, Orthostatic intolerance due to NET deficiency, Osteopenia and sparse hair, Osteoporosis-pseudoglioma syndrome, Oto-palato-digital syndrome type 1, Oto-palato-digital syndrome type 2, Ouvrier Billson syndrome, Pachygyria-intellectual disability-epilepsy syndrome, PACS1-related syndrome, painful orbital and systemic neurofibromas-marfanoid habitus syndrome, Pallidopyramidal syndrome, Pallister W syndrome, Pallister-Killian mosaic syndrome, Pantothenate kinase-associated neurodegeneration, paralysis agitans, paralysis juvenile, paralysis of Hunt, Paramyotonia congenita, Paraneoplastic/autoimmune (anti-Hu-associated) neuropathy, Parkinson, Parkinson disease type 3, Parkinson disease type 9, Paroxysmal exertion-induced dyskinesia, Paroxysmal extreme pain disorder, Paroxysmal hemicrania, Paroxysmal kinesigenic choreoathetosis, Paroxysmal nonkinesigenic dyskinesia, Parsonage Turner syndrome, Partington syndrome, PCDH19-related female-limited epilepsy, Pediatric autoimmune neuropsychiatric disorders associated with Streptococcus infections, PEHO syndrome, Pelizaeus-Merzbacher disease, Periventricular heterotopia, Periventricular leukomalacia, Perry syndrome, Peters plus syndrome, Pfeiffer Mayer syndrome, Pfeiffer Palm Teller syndrome, Pfeiffer-type cardiocranial syndrome, PGM3-CDG, PHACE syndrome, Phosphoglycerate kinase deficiency, Phosphoglycerate mutase deficiency, Phosphoserine aminotransferase deficiency, Photosensitive epilepsy, Pitt-Hopkins syndrome, Pitt-Hopkins-like syndrome, Plasmacytoma, Pleomorphic xanthoastrocytoma, PMM2-CDG (CDG-Ia), polyneuropathy organomegaly endocrinopathy or edema M-protein and skin abnormalities syndrome (POEMS), Poliomyelitis, POLR3-Related Leukodystrophy, Polyarteritis nodosa, Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, Polyneuropathy-intellectual disability-acromicria-premature menopause syndrome, Pontine tegmental cap dysplasia, Pontocerebellar hypoplasia,

Pontocerebellar hypoplasia type 1, Pontocerebellar hypoplasia type 2, Pontocerebellar hypoplasia type 3, Pontocerebellar hypoplasia type 4, Pontocerebellar hypoplasia type 5, Pontocerebellar hypoplasia type 6, post-Polio syndrome, Porphyria, posterior column ataxia, posterior column ataxia with retinitis pigmentosa, postnatal progressive microcephaly, postherpetic neuralgia, postnatal seizures, and postnatal brain atrophy, Potassium aggravated myotonia, Potocki-Lupski syndrome, PPM-X syndrome, Prader-Willi habitus, Primary amebic meningoencephalitis, Primary angiitis of the central nervous system, Primary basilar impression, Primary carnitine deficiency, Primary central nervous system lymphoma, Primary Familial Brain Calcification, Primary lateral sclerosis, Primary melanoma of the central nervous system, Primary orthostatic tremor, Primary progressive aphasia, Primrose syndrome, Progressive bulbar palsy, Progressive encephalomyelitis with rigidity and myoclonus, Progressive external ophthalmoplegia, autosomal recessive 1, progressive hemifacial atrophy, progressive non-fluent aphasia, Progressive Supranuclear Palsy, prolidase deficiency, Proteus syndrome, Proud syndrome, pseudoaminopterin syndrome, Pseudocholinesterase deficiency, pseudoneonatal adrenoleukodystrophy, pseudoprogeria syndrome, pseudotrisomy 13 syndrome, pseudoxanthoma elasticum, Pudendal Neuralgia, Pure autonomic failure, pyridoxal 5'-phosphate-dependent epilepsy, pyridoxine-dependent epilepsy, pyruvate dehydrogenase phosphatase deficiency, Qazi Markouizos syndrome, Radiation induced brachial plexopathy, Ramos Arroyo Clark syndrome, Ramsay Hunt syndrome I, Ramsay Hunt syndrome II, Rapid-onset dystonia-parkinsonism, Rasmussen encephalitis, Reardon Wilson Cavanagh syndrome, Reducing body myopathy, Refsum disease, Renal dysplasia-limb defects syndrome, Renier Gabreels Jasper syndrome, Restless legs syndrome, Retinal arterial macroaneurysm with supraaortic pulmonic stenosis, Retinal vasculopathy with cerebral leukodystrophy, retrobulbar neuritis, Rett syndrome, Reversible cerebral vasoconstriction syndrome, RFT1-CDG (CDG-In), Rhabdoid tumor, Rhizomelic chondrodysplasia punctata type 1, Riboflavin transporter deficiency, Richards-Rundle syndrome, Richieri Costa Da Silva syndrome, Rigid spine syndrome, Ring chromosome 10, Ring chromosome 14, Ring chromosome 20, Rippling muscle disease, RNase T2-deficient leukoencephalopathy, Roussy Levy syndrome, RRM2B-related mitochondrial DNA depletion syndrome, Ruvalcaba syndrome, Salla disease, Sandhoff disease, Sandifer syndrome, Sarcoidosis induced neuropathy, Say Barber Miller syndrome, Say Meyer syndrome, Scapuloperoneal syndrome, SCARF

syndrome, Schaaf-Yang syndrome, Scheie syndrome, Schimke immunoosseous dysplasia, Schindler disease type 1, Schinzel Giedion syndrome, Schisis association, Schizencephaly, Schwannomatosis, Schwartz Jampel syndrome, Scott Bryant Graham syndrome, Seaver Cassidy syndrome, Seckel syndrome, Semantic dementia, Sensory ataxic neuropathy, Sepiapterin reductase deficiency, Septo-optic dysplasia spectrum, SeSAME syndrome, SETBP1 disorder, severe congenital nemaline myopathy, severe intellectual disability-progressive spastic diplegia syndrome, Gustavson type severe X-linked intellectual disability, Shapiro syndrome, Short-chain acyl-CoA dehydrogenase deficiency, Shprintzen omphalocele syndrome, Shprintzen-Goldberg craniosynostosis syndrome, Sialidosis type I, Sialidosis, type II, Sickle cell anemia, Simpson-Golabi-Behmel syndrome, Single upper central incisor, Sjogren-Larsson syndrome, SLC35A1-CDG (CDG-II_f), SLC35A2-CDG, SLC35C1-CDG (CDG-II_c), Slow-channel congenital myasthenic syndrome, Smith-Fineman-Myers syndrome, Smith-Lemli-Opitz syndrome, Smith-Magenis syndrome, Sneddon syndrome, Snyder-Robinson syndrome, Sonoda syndrome, spasmodic dysphonia, spastic ataxia charlevoix-saguenay type, spastic diplegia cerebral palsy, spastic diplegia infantile type, spastic paraplegia 1, spastic paraplegia 10, spastic paraplegia 11, spastic paraplegia 12, spastic paraplegia 13, spastic paraplegia 14, spastic paraplegia 15, spastic paraplegia 16, spastic paraplegia 17, spastic paraplegia 18, spastic paraplegia 19, spastic paraplegia 2, spastic paraplegia 23, spastic paraplegia 24, spastic paraplegia 25, spastic paraplegia 26, spastic paraplegia 29, spastic paraplegia 3, spastic paraplegia 31, spastic paraplegia 32, spastic paraplegia 39, spastic paraplegia 4, spastic paraplegia 51, spastic paraplegia 5a, spastic paraplegia 6, spastic paraplegia 7, spastic paraplegia 8, spastic paraplegia 9, spastic paraplegia facial cutaneous lesions, Spastic paraplegia-epilepsy-intellectual disability syndrome, Spastic paraplegia-glaucoma-intellectual disability syndrome, Spastic tetraplegia-retinitis pigmentosa-intellectual disability syndrome, spastic tetraplegia-thin corpus callosum-progressive postnatal microcephaly syndrome, Spina bifida occulta, spinal atrophy ophthalmoplegia pyramidal syndrome, spinal meningioma, spinal muscular atrophy 1, spinal muscular atrophy type 2, spinal muscular atrophy type 3, spinal muscular atrophy-progressive myoclonic epilepsy syndrome, spinal shock, spinocerebellar ataxia, spinocerebellar ataxia 1, spinocerebellar ataxia 10, spinocerebellar ataxia 11, spinocerebellar ataxia 12, spinocerebellar ataxia 13, spinocerebellar ataxia 14, spinocerebellar ataxia 15, spinocerebellar ataxia 17,

spinocerebellar ataxia 18, spinocerebellar ataxia 19 and 22, spinocerebellar ataxia 2, spinocerebellar ataxia 20, spinocerebellar ataxia 21, spinocerebellar ataxia 23, spinocerebellar ataxia 25, spinocerebellar ataxia 26, spinocerebellar ataxia 27, spinocerebellar ataxia 28, spinocerebellar ataxia 29, spinocerebellar ataxia 3, spinocerebellar ataxia 30, spinocerebellar ataxia 31, spinocerebellar ataxia 34, spinocerebellar ataxia 4, spinocerebellar ataxia 5, spinocerebellar ataxia 7, spinocerebellar ataxia 8, spinocerebellar ataxia 9, spinocerebellar ataxia autosomal recessive 3, spinocerebellar ataxia autosomal recessive 4, spinocerebellar ataxia autosomal recessive 5, spinocerebellar ataxia autosomal recessive 6, spinocerebellar ataxia autosomal recessive 7, spinocerebellar ataxia autosomal recessive 8, spinocerebellar ataxia type 6, spinocerebellar ataxia with axonal neuropathy type 1, spinocerebellar ataxia with dysmorphism, spinocerebellar ataxia x-linked type 2, spinocerebellar ataxia x-linked type 3, spinocerebellar ataxia x-linked type 4, spinocerebellar degeneration and corneal dystrophy, split hand urinary anomalies spina bifida, split spinal cord malformation, spondyloepiphyseal dysplasia congenita, SRD5A3-CDG (CDG-Iq), SSR4-CDG, STAC3 Disorder, Status epilepticus, Steinfeld syndrome, Stiff person syndrome, Stocco dos Santos syndrome, Striatonigral degeneration infantile, Sturge-Weber syndrome, subacute sclerosing panencephalitis, subcortical band heterotopia, subependymal giant cell astrocytoma, Subependymoma, Succinic semialdehyde dehydrogenase deficiency, Susac syndrome, Symmetrical thalamic calcifications, Syndromic X-linked intellectual disability 7, Tangier disease, TANGO2-Related Metabolic Encephalopathy and Arrhythmias, Tarlov cysts, Tay-Sachs disease, Tel Hashomer camptodactyly syndrome, Telfer Sugar Jaeger syndrome, Temple syndrome, Temple-Baraitser syndrome, Temporal epilepsy, Temtamy syndrome, Tethered cord syndrome, Thoracic dysplasia hydrocephalus syndrome, Thoracic outlet syndromes, Thyrotoxic periodic paralysis, TMEM165-CDG (CDG-IIk), Toriello-Carey syndrome, Tourette syndrome, Toxic neuropathies (e.g. alcoholic neuropathy, chemotherapy-induced neuropathy), Tranebjaerg Svejgaard syndrome, Transverse myelitis, Trichinosis, Trichorhinophalangeal syndrome type 2, Trigeminal neuralgia, Triosephosphate isomerase deficiency, Triple A syndrome, Troyer syndrome, Tuberous sclerosis complex, Tubular aggregate myopathy, Tumefactive multiple sclerosis, Typical congenital nemaline myopathy, Tyrosine hydroxylase deficiency, Tyrosinemia type 1, Ullrich congenital muscular dystrophy, Unverricht-Lundborg disease, Van Benthem-Driessen-Hanveld syndrome, Van Den

Bosch syndrome, Variant Creutzfeldt-Jakob disease, Variegate porphyria, Vasculitis induced neuropathy, Vein of Galen aneurysm, Vici syndrome, Viljoen Kallis Voges syndrome, Vincristine induced neuropathy, Visual snow syndrome, Vitamin B6 induced neuropathy, VLCAD deficiency, Vogt-Koyanagi-Harada disease, Von Hippel-Lindau disease, Walker-Warburg syndrome, Weaver syndrome, Welanders distal myopathy, Wernicke-Korsakoff syndrome, West syndrome, Whipple disease, White matter hypoplasia-corporum callosum agenesis-intellectual disability syndrome, Wiedemann Oldigs Oppermann syndrome, Williams syndrome, Wilson disease, Wilson-Turner syndrome, Wolf-Hirschhorn syndrome, Wolman disease, Woodhouse Sakati syndrome, Worster Drought syndrome, Wrinkly skin syndrome, Wyburn-Mason syndrome, Xeroderma pigmentosum, Xia-Gibbs syndrome, XK aprosencephaly, X-linked cerebral adrenoleukodystrophy, X-linked Charcot-Marie-Tooth disease type 1, X-linked Charcot-Marie-Tooth disease type 1A, X-linked Charcot-Marie-Tooth disease type 2, X-linked Charcot-Marie-Tooth disease type 3, X-linked Charcot-Marie-Tooth disease type 4, X-linked Charcot-Marie-Tooth disease type 5, X-linked Charcot-Marie-Tooth disease type 6, X-linked complicated corpus callosum agenesis, X-linked complicated spastic paraplegia type 1, X-linked creatine deficiency, X-linked dystonia-parkinsonism/Lubag, X-linked hereditary sensory and autonomic neuropathy with deafness, X-linked intellectual disability - corpus callosum agenesis - spastic quadriplegia, X-linked intellectual disability - short stature - obesity, X-linked intellectual disability, Najm type, X-linked intellectual disability, Schimke type, Siderius type X-linked intellectual disability, Turner type X-linked intellectual disability, X-linked intellectual disability-dysmorphism-cerebral atrophy syndrome, X-linked intellectual disability-plagiocephaly syndrome, X-linked lissencephaly with abnormal genitalia, X-linked myopathy with excessive autophagy, X-linked myotubular myopathy, X-linked non-specific intellectual disability, X-linked periventricular heterotopia, X-linked skeletal dysplasia-intellectual disability syndrome, Zechi Ceide syndrome, Zellweger syndrome, and ZTTK syndrome. In some embodiments, the disease or disorder of the nervous system is a psychiatric disorder. In some embodiments, the disease or disorder of the nervous system is a disease or disorder classified according to the DSM-V (American Psychiatric Association, & American Psychiatric Association, 2013, Diagnostic and statistical manual of mental disorders: DSM-5. Arlington, VA.). In some embodiments, the disease or disorder of the nervous system is a disease or disorder of the central nervous system. In some embodiments, the disease or disorder of the

nervous system is an inflammatory disease or disorder of the nervous system. In some embodiments, the disease or disorder of the nervous system described herein is a disease or disorder selected from the group consisting of dementia, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal lobar dementia, ataxia-teleangiectasia, multiple system atrophy, progressive supranuclear palsy, Krabbe's disease, agenesis of the corpus callosum associated with peripheral neuropathy, Duchenne muscular dystrophy, Guillain-Barré syndrome, Charcot-Marie-Tooth disease Type 1A, hereditary neuropathy with liability to pressure palsies, diabetic neuropathy, toxic neuropathies, age-related peripheral neuropathy, epilepsy, sleep disorders, encephalopathy and neuropathic pain. In some embodiments, the disease or disorder of the nervous system is a neurodegenerative disease or disorder.

In some embodiments, the disease or disorder of the nervous system described herein, is a toxin and/or drug-induced neuropathy. In some embodiments, the drug-induced neuropathy described herein is induced by, partially induced by or suspected to be induced by at least one agent selected from the group consisting of chemotherapeutic agents, TNF α inhibitors, antiretroviral agents, cardiac medications, statins and antibiotics.

In some embodiments, the drug-induced neuropathy described herein is induced by, partially induced by or suspected to be induced by at least one agent selected from the group consisting of thalidomide, disulfiram, pyridoxine, colchicine, phenytoin, lithium, chloroquine, hydroxychloroquine, cisplatin, oxaliplatin, taxane, vinca alkaloids, bortezomib, suramin, misonidazole, infliximab, etanercept, zalcitabine, didanosine, stavudine, amiodarone, perhexiline, metronidazole, dapsone, podophyllin, fluoroquinolones, isoniazid and nitrofurantoin.

In certain embodiments, the disease or disorder of the nervous system described herein is a disease or disorder of the peripheral nervous system.

In some embodiments, the toxin-induced neuropathy described herein is induced by, partially induced by or suspected to be induced by at least one agent selected from the group consisting of organic solvents, heavy metals and organophosphates.

In some embodiments, the toxin and/or drug-induced neuropathy described herein is induced by, partially induced by or suspected to be induced by alcohol and/or cigarette smoke.

In some embodiments, the toxin and/or drug-induced neuropathy described herein is characterized by at least one selected from the group of dorsal root ganglion toxicity, microtubular axon transport function abnormalities, voltage gated abnormalities, sodium channel abnormalities and demyelination.

The inventors found that AAT can reduce neuronal pathology pathways (Fig. 2 – 4, Table 1 - 10). This reduction of neuronal pathology pathways was observed in resting cells (Fig. 4B) and stimulated cells (Fig. 4C) and is therefore useful in preventing and/or treating diseases or disorders of the nervous system and symptoms thereof.

As such, the effect of the combination of AAT and IgG antibodies in providing immunomodulatory, anti-inflammatory, and neuroprotective/regenerative effects in diseases and disorders of the nervous system that go beyond the expectation of the person skilled in the art.

Accordingly, the invention is at least in part based on the finding that combining AAT activity with IgG antibody activity is useful in treating diseases or disorders of the nervous system as described herein.

In one embodiment, the invention relates to a pharmaceutical composition for use in treatment of a disease or disorder of the nervous system, the composition comprising: i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof having protease inhibitory activity; and/or b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof having protease inhibitory activity; iii) a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants; and ii) at least one pharmaceutically acceptable carrier.

In some embodiments the pharmaceutical acceptable carrier described herein is a pharmaceutically acceptable diluent or carrier.

“Pharmaceutically acceptable diluent or carrier” means a carrier or diluent that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes carriers or diluents that are acceptable for human pharmaceutical use.

Such pharmaceutically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

Pharmaceutically acceptable diluent or carrier include starch, glucose, lactose, sucrose, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like.

The pharmaceutical compositions may further contain one or more pharmaceutically acceptable salts such as, for example, a mineral acid salt such as a hydrochloride, a hydrobromide, a phosphate, a sulfate, etc.; and the salts of organic acids such as acetates, propionates, malonates, benzoates, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, gels or gelling materials, flavorings, colorants, microspheres, polymers, suspension agents, etc. may also be present herein. In addition, one or more other conventional pharmaceutical ingredients, such as preservatives, humectants, suspending agents, surfactants, antioxidants, anticaking agents, fillers, chelating agents, coating agents, chemical stabilizers, etc. may also be present, especially if the dosage form is a reconstitutable form. Suitable exemplary ingredients include macrocrystalline cellulose, carboxymethyl cellulose sodium, polysorbate 80, phenylethyl alcohol, chlorobutanol, potassium sorbate, sorbic acid, sulfur dioxide, propyl gallate, the parabens, ethyl vanillin, glycerin, phenol, parachlorophenol, gelatin, albumin and a combination thereof. A thorough discussion of pharmaceutically acceptable excipients is available in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Pub. Co., N.J. 1991) which is incorporated by reference herein.

In some embodiments, the pharmaceutical acceptable carrier described herein is an agent (e.g. a molecule or a cell) that improves drug delivery properties of the agents for use of the invention. In some embodiments the drug delivery property described herein comprises at least one property selected from the group of penetration ability (e.g. cell-membrane and/or blood brain barrier), site specific delivery (e.g. brain specific delivery), controlled release delivery and stability (e.g., reduction of enzymatic degradation). In some embodiments, the pharmaceutical carrier described herein is an

agent selected from the group of delivery cell, liposome, nanoparticle, fusion protein, niosome, nanosphere, micelle, nanocapsule, nanoshell, lipid particle and dendrimer.

In one embodiment, the invention relates to a method of treatment comprising administering an effective amount of a pharmaceutical composition comprising AAT protein and/or a nucleic acid encoding AAT to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

An "effective amount" of an agent, e.g., a therapeutic agent, 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, an effective amount of the compositions/compounds/products described herein 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 compositions/compounds/products described herein can be any amount that reduces the number of diseased cells, autoantibodies, and/or other disease markers (e.g. cytokines) without producing significant toxicity to the subject.

As used herein the terms "subject"/"subject in need thereof", or "patient"/"patient in need thereof" 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. In some embodiments, the subject to be treated is a subject above the age of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 12, preferably above the age of 1. According to certain embodiments, the subject is selected from the group consisting of a pre-pubertal child, a pre-pubertal adolescent, an adolescent and an adult. In some embodiments, the subject to be treated is female.

In one embodiment, the invention relates to a method of treatment comprising administering an effective amount of a pharmaceutical compound comprising a

plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of AAT protein and/or a nucleic acid encoding AAT.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the disease or disorder of the nervous system is chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the disease or disorder of the nervous system is chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the disease or disorder of the nervous system is chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.

The term “symptom of chronic inflammatory demyelinating polyneuropathy”, as used herein, refers to at least one symptom selected from the group consisting of diminished

or absent deep-tendon reflexes, sensory ataxia, weakness, numbness, tingling, pain, difficulty in walking, proximal muscle weakness in the limbs and distal muscle weakness in the limbs.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for intravenous administration.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for intravenous administration.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for intravenous administration.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the AAT protein is recombinant AAT.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the AAT protein is recombinant AAT.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the AAT protein is recombinant AAT.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the AAT protein is recombinant AAT.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein the AAT protein is plasma derived AAT.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein the AAT protein is plasma derived AAT.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein the AAT protein is plasma derived AAT.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein the AAT protein is plasma derived AAT.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for intravenous administration.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for intravenous administration.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for intravenous administration.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for intravenous administration.

In certain embodiments, the invention relates to the pharmaceutical product for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the kit of parts for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment

thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the pharmaceutical composition for use of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for subcutaneous administration.

In certain embodiments, the invention relates to the method of treatment of the invention, wherein a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and/or b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity is/are formulated for subcutaneous administration.

The present invention also contemplates a gene delivery vector encoding AAT (or an isoform, a fragment or variant thereof) and pharmaceutical compositions containing the same. The gene delivery vector may be used for any of the therapeutic uses described herein. Preferably, the gene delivery vector is in the form of a plasmid or a vector that comprises one or more nucleic acid encoding the AAT protein, a variant, an isoform and/or a fragment thereof of the invention. Examples of gene delivery vectors comprise e.g., viral vectors, non-viral vectors, particulate carriers, and liposomes. The gene delivery is preferably performed *in vitro* or *ex vivo*.

The kits, pharmaceutical compositions and/or products of the present invention may be administered to a subject by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, 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 person skilled in the art will readily determine the dosing regimen and route of administration that is most appropriate for a particular patient. The kits, pharmaceutical compositions and/or products 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 composition can also be administered by intravenous injection, intravenous infusion, infusion with a dosator pump, inhalation nasal-spray, eye-drops, skin-patches, slow release formulations, ex vivo gene therapy or ex vivo cell-therapy, preferably by intravenous injection.

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 AAT 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 adenovirus associated virus as described herein.

All definitions and combinations provided herein apply to these embodiments, if applicable and unless indicated otherwise.

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. 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.

The term “about” particularly in reference to a given quantity, is meant to encompass deviations of plus or minus 20 percent, preferably 10 percent, preferably 5 percent, even more preferably 2 percent and most preferably 1 percent.

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.

"or" should be understood to mean either one, both, or any combination thereof of the alternatives.

"and/or" should be understood to mean either one, or both of the alternatives.

Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

The terms "include" and "comprise" are used synonymously. "preferably" means one option out of a series of options not excluding other options. "e.g." means one example without restriction to the mentioned example. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of."

Reference throughout this specification to "one embodiment", "an embodiment", "a particular embodiment", "a related embodiment", "a certain embodiment", "an additional embodiment", "some embodiments", "a specific embodiment" or "a further embodiment" or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. 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. In case of conflict, the present

specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The general methods and techniques described herein may be performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990).

While embodiments of the invention are illustrated and described in detail in the figures and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive. It will be understood that changes and modifications may be made by those of ordinary skill within the scope and spirit of the following claims. In particular, the present invention covers further embodiments with any combination of features from different embodiments described above and below.

Brief description of Figures

Fig. 1: IFN γ -mediated microglial activation

Fig. 2: AAT decreases IFN γ -mediated microglial activation

Fig. 3: Validation of AAT anti-inflammatory effect on the experiment used for RNAseq

Fig. 4: Validation of microglial activation and AAT anti-inflammatory effect by GSEA analysis

Fig. 5: AAT (Sigma Aldrich, batch A6150) inhibits TACE activity in a cell-free assay shown as relative fluorescence unit (A) or as percentage of the control activity (B) with an IC₅₀ of 15.3 μ M (C)

Fig. 6: Results of the sciatic nerve electrophysiology (EMG) (A) Amplitude (B) conduction velocity

Fig. 7: Results of the grip strength test (A) absolute values, (B) % of 6 week timepoint

Fig. 8: Results of the rotarod test (A) absolute values, (B) % of 6 week timepoint

Fig. 9: DNAJB9 and PLA2G4B gene expression in response to AAT treatment

Fig. 10: Number of axons

Fig. 11: Axonal diameter

Fig. 12: g ratio

Fig. 13: Individual histology images of sciatic nerve semithin cross sections. Scale bar 10 μ m A) Group 1: WT control, B) CMT1A + vehicle, C) CMT1A + AAT

Fig. 14: Plasma IL-6 concentration

Fig. 15: Plasma TNF α concentration

Fig. 16: Study scheme for CMT1A mouse model and AAT administration

Fig. 17: Cell morphology and count after treatments: SH-SY5Y morphological analysis and cell count after 6-OHDA administration and AAT treatment. Brightfield pictures (20x) and cell count (D0 vs D4 of culture) showing the effect of treatments on SH-SY5Y cell phenotype and proliferation and AAT positive action. A) Example images B) Quantification

Fig. 18: Cell viability: Graph represents cell viability measured through absorbance (450 nm) for control and samples

Fig. 19: IL-6 quantification in cell supernatant

Fig. 20 : Study scheme

Fig. 21 : Neuromuscular tests : A Rotarod latency; B Grip strength ; C Von Frey test

Fig. 22 : Sciatic nerve electrophysiology: A : Compound muscle action potential; B : Nerve conduction velocity

Fig. 23 : A) Graphical representation of plasma TMRSS5 B) Graphical representation of plasma NfL conc

Fig. 24 : Graphical representation of plasma TNF α concentration

Fig. 25 : Rotarod latency to fall of CMT mice treated with AAT (180 mg/kg sc)

Fig. 26 : Grip strength (Newton) of CMT mice treated with AAT (180 mg/kg sc)

Fig. 27 : CMAP (mV) of CMT mice treated with AAT (180 mg/kg sc)

Fig. 28 : NCV (m/s) of CMT mice treated with AAT (180 mg/kg sc)

Fig. 29 : Plasma IL-6 concentration

Fig. 30 : Plasma TNF α concentration

Fig. 31: Effect of peptide 8 on ADAM17 activity. (***, ****: $P < 0.001$, $P < 0.0001$, one-way ANOVA (multiple comparison); ns=not significant vs DMSO

Fig. 32: Effect of AAT on the gene expression of the NF- κ B pathway following TNF α -dependent activation of Schwann Cells

Fig. 33: Effect of AAT on the gene expression related to the oxidative stress response following TNF α -dependent activation of Schwann Cells

Fig. 34: Effect of AAT on the gene expression related to the NRF2-related oxidative stress response following TNF α -dependent activation of Schwann Cells

Fig. 35: Effect of AAT and its derivative peptides on TNF induced Schwann cells.

Fig. 36: Number of axons/100 μm^2

Fig. 37: axonal diameter

Fig. 38: g-ratio

Fig. 39: A) Number of axons/100 μm^2 B) Axonal diameter and C) g-ratio

Fig. 40: Plasma NfL concentration

Fig. 41: Effect of AAT and its derivative peptides in IFN γ -induced microglia

Fig. 42: Synthesis of peptide #8 and #9 (Synthesis: Syro-1 automatic peptide synthesizer; Double coupling conditions: DIC (4 eq)/Oxyma (4 eq), 40 min; HATU (4 eq) /DIPEA (8 eq), 30 min; Deprotection: 40% piperidine in DMF, 3 min and 20% piperidine in DMF, 12 min)

Fig. 43: Synthesis peptidomimetic #14 (Synthesis: Manual Synthesis; Coupling condition: DIC (3 eq)/Oxyma (3 eq), 2 h; Deprotection: 20% piperidine in DMF, 5 min; 20% piperidine in DMF, 20 min)

Examples

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 embodiments 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.

Example 1

A) Human microglial cells *HMC3-MHCII^{Luc}* cells were plated at day 0, activated with IFN γ at day1 until day 2 and measurement of the luciferase activity and cell viability were done at day 4.

B) Activation was measured by the activity of MHCII-driven luciferase and normalized to cell viability. Luciferase activity for all conditions is represented as fold of the untreated control. A potential effect of the highest concentration of the buffer used for drug presentation (IFN γ , AATs) was precluded. All conditions were performed in triplicates, error bars represent standard deviation (Figure 1).

Example 2

A) Human microglial *HMC3-MHCII^{Luc}* and *HMC3-MHCII^{Luc};Ubi^{AAT}* cells were plated at day 0, presented IFN γ at day 1 until day 2 and measurement of the luciferase activity and cell viability were done at day 4.

B) AATs were applied from day 0 to day 4 on *HMC3-MHCII^{Luc}* cells. Activation was measured by the activity of MHCII-driven luciferase and normalized to cell viability. Luciferase activity for all conditions is represented as percentage of IFN γ control. All conditions were performed in triplicates, error bars represent standard deviation. (Figure 2)

Example 3

A) Human microglial *HMC3-MHCII^{Luc}* cells were plated at day 0, presented IFN γ at day 1 until day 2 and measurement of the luciferase activity and cell viability were done at day 4 .

B) AATs were applied from day 0 to day 4 on *HMC3-MHCII^{Luc}* cells. Activation was measured by the activity of MHCII-driven luciferase and represented as percentage of IFN γ control for all conditions. All conditions were performed in triplicates, error bars represent standard deviation.

C) Bulk RNA extraction was performed on the same *HMC3-MHCII^{Luc}* cultures. Quality control (QC) were applied to RNA before sequencing. QC of the sequencing was done prior to mapping on the human genome. Mapped reads were counted, and differential gene expression was measured between the conditions (see Table 1 - 10). (Figure 3)

Example 4

RNAseq data from plasma-derived and recombinant AATs were pooled, normalized and analyzed through Gene Set Enrichment Analysis (GSEA) (<https://www.gsea-msigdb.org/gsea/index.jsp>; Subramanian, A., et al. Proc. Natl. Acad. Sci. USA, 102 (43): 15545–15550, 2005.). Upregulated gene families (grey bars) and downregulated ones (black bars) are shown according to normalized enrichment score.

A) The inflammatory profile induced by IFN γ was confirmed by upregulation of several processes related to inflammation (bold italic).

B-C) AAT treatment, in absence (B) or presence (C) of IFN γ was able to significantly downregulate some of these pathways (bold italic). Importantly, both the IFN γ and inflammatory responses were dampened by AAT. However, the downregulation of the inflammatory response genes in (C) was just under significance.

Regarding other gene families, KRAS signaling is of importance since it has been involved in oncogenic process and immunomodulation (Dias Carvalho, P., et al., 2018, Cancer Res, 78: 7-14.). As well, p53 pathway is of note as a mediator of response to stress. (Figure 4)

Example 5

AAT treatment counterbalances the expression of inflammatory genes

Tables of genes related to antigen presentation (Table 1), cytokine signaling (Table 2), interferon signaling (Table 3) and complement activation (Table 4). Inflammatory top gene (FC>2; p-value<0.05; left column), were defined by differential expression between untreated and IFN γ -treated cells (inflammation; middle column). Top genes that were significantly and oppositely regulated by AAT treatment are highlighted (right column; bold underline).

A substantial number (~35%) of inflammatory top genes were found affected by AAT treatment (6 of 23 genes related to antigen presentation; 14 of 33 genes related to cytokine signaling; 1 of 7 genes related to complement activation; 5/13 genes related to interferon signaling) showing its anti-inflammatory potential. For example, the promoter of HLA-DRA gene which was used as a driver for luciferase expression in the *HMC3-MHCII^{Luc}* line was consistently up- and down-regulated in inflammation and with AAT treatment, respectively (bold italic). Of note, FCs shown in AAT-treated inflammation condition should not be directly compared to those found in inflammation since for the former, a FC=2 represents already a 50% counterbalance on inflammation induced/repressed genes.

Second order inflammatory genes (p-value<0.05; FC<2) significantly and oppositely regulated by AAT treatment in inflammatory condition or resting condition (regulated in AAT) are presented at the bottom of the table. (Figure 5)

Table 1

Antigen presentation				
Top Gene	p-value	FC	p-value	FC
	UP in inflammation		DOWN in inflammation+AAT	
CD74	4,15E-14	75,81		
LAMP3	9,39E-12	7,94		
TAP1	5,99E-11	6,12		
<u>PSMB9</u>	<u>5,73E-11</u>	<u>5,96</u>	<u>3,11E-02</u>	<u>-1,23</u>
<u>HLA-DOB</u>	<u>5,11E-04</u>	<u>4,48</u>	<u>6,82E-03</u>	<u>-4,49</u>
PSMB8	5,18E-11	4,22		
<u>HLA-DRA</u>	<u>4,99E-10</u>	<u>4,76</u>	<u>4,68E-02</u>	<u>-2,22</u>

SOCS1	1,21E-05	3,99		
TAP2	8,97E-13	3,54		
NCF2	2,57E-05	2,88		
CTSS	2,29E-05	3,71		
HLA-DPA1	8,31E-09	3,22		
BATF3	1,45E-06	2,55		
NFKB1	6,21E-10	2,41		
<u>IL1B</u>	<u>5,54E-04</u>	<u>2,08</u>	<u>1,11E-03</u>	<u>-2,07</u>
FYN	2,18E-07	1,80		
BCL10	3,21E-05	1,65		
MALT1	1,75E-04	1,62		
	DOWN in inflammation		UP in inflammation+AAT	
TUBA1A	2,29E-12	-5,00		
KIF20A	7,29E-12	-3,75		
<u>CALR</u>	<u>6,15E-11</u>	<u>-3,53</u>	<u>4,74E-04</u>	<u>1,35</u>
<u>PYCARD</u>	<u>6,92E-03</u>	<u>-2,86</u>	<u>1,53E-02</u>	<u>2,20</u>
CDC20	1,05E-08	-2,80		
Other Gene	p-value	FC	p-value	FC
	DOWN in inflammation		UP in inflammation+AAT	
NFATC2	<0.05	-1,37	<0.05	1,63
MRC2	<0.05	-1,61	<0.05	1,47
CTSD	<0.05	-1,34	<0.05	1,47
HLA-DPB1	<0.05	-1,23	<0.05	1,31
TAPBP	<0.05	-1,90	<0.05	1,23
SOCS3	<0.05	-1,99	<0.05	1,20
CTSA	<0.05	-1,31	<0.05	1,17
	Regulated in inflammation		Regulated in AAT	
NFATC1	<0.05	-1,29	<0.05	1,44
TRAF6	<0.05	1,08	<0.05	-1,26

Table 2

Cytokine signalling				
Top Gene	p-value	FC	p-value	FC
UP in inflammation			DOWN in inflammation+AAT	
IL6	3,53E-10	17,139		
TNFSF10	2,53E-07	10,595		
TRAF1	4,39E-07	7,789		
CCL5	5,17E-09	7,540		
ATF3	6,86E-13	7,218	1,85E-03	-1,26
PSMB9	6,09E-10	5,963	3,11E-02	-1,23
CEBPB	2,57E-10	5,808		
CSF1	3,52E-09	5,455		
PSMB8	1,63E-10	4,219		
IL23A	6,49E-03	4,218		
STAT1	2,04E-09	4,110		
IL15RA	4,63E-07	3,660		
FGF2	1,66E-06	3,608	9,13E-03	-1,57
CXCL2	6,42E-05	3,467		
IL21R	1,16E-05	3,235		
CEBPG	3,19E-09	2,909		
CXCL1	1,36E-04	2,82	7,22E-03	-2,09
CASP1	1,07E-05	2,740		
TNFRSF9	2,39E-04	2,434	1,23E-03	-2,35
CXCL5	5,07E-05	2,36	5,98E-03	-2,15
IL1B	2,14E-03	2,08	1,11E-03	-2,07
DOWN in inflammation			UP in inflammation+AAT	
FSCN1	9,42E-08	-6,312	8,09E-04	1,48
CCL2	2,95E-03	-6,086		
IL4I1	3,22E-03	-4,19	3,22E-03	1,95
IL27RA	2,22E-07	-3,848	2,67E-02	1,36
STAT5A	7,66E-04	-3,53	9,78E-03	1,45
CEBPD	5,07E-05	-3,102		
EGF	6,13E-03	-2,973		
FGF18	2,11E-05	-2,754		
IL6R	6,00E-08	-2,630		
IL1R1	1,27E-07	-2,566	3,94E-02	1,18
JAK3	3,03E-08	-2,43	3,20E-03	1,43
RASAL1	1,36E-06	-2,34	4,65E-03	1,52
Other Gene	p-value	FC	p-value	FC
UP in inflammation			DOWN in inflammation+AAT	
IL18	<0.05	1,83	<0.05	-1,75
NFKBIZ	<0.05	1,94	<0.05	-1,37
ANXA1	<0.05	1,17	<0.05	-1,33
HBEGF	<0.05	1,36	<0.05	-1,30
DUSP6	<0.05	1,96	<0.05	-1,26

	DOWN in inflammation		UP in inflammation+AAT	
CX3CL1	<0.05	-1,38	<0.05	1,61
MAP2K2	<0.05	-1,28	<0.05	1,51
MAPK11	<0.05	-1,22	<0.05	1,44
TRAF2	<0.05	-1,66	<0.05	1,42
MAP2K3	<0.05	-1,56	<0.05	1,37
MAPK12	<0.05	-1,14	<0.05	1,33
MAPK13	<0.05	-1,27	<0.05	1,23
SOCS3	<0.05	-1,99	<0.05	1,20
	Regulated in inflammation		Regulated in AAT	
BIRC2	<0.05	1,53		-1,53
STAT1	<0.05	4,11		-1,47
RASGRF2	<0.05	1,32		-1,45
MYD88	<0.05	1,09		-1,35
CEBPG	<0.05	2,91		-1,31
RIPK2	<0.05	2,03		-1,30
TRAF6	<0.05	1,14		-1,26
SPTBN1	<0.05	1,13		-1,21
TM7SF3	<0.05	1,15		-1,18
TNFSF9	<0.05	-1,92		1,26

Table 3

Interferon signaling				
Top Gene	p-value	FC	p-value	FC
	UP in inflammation		DOWN in inflammation+AAT	
<u>MX2</u>	<u>7,26E-09</u>	<u>21,98</u>	<u>3,69E-03</u>	<u>-2,14</u>
<u>MX1</u>	<u>1,09E-09</u>	<u>19,39</u>	<u>3,90E-03</u>	<u>-2,00</u>
IRF1	8,56E-12	8,63		
<u>IFIT1</u>	<u>9,65E-11</u>	<u>6,73</u>	<u>3,11E-03</u>	<u>-1,78</u>
ISG15	4,97E-06	5,27		
<u>HLA-DRA</u>	<u>5,04E-07</u>	<u>4,76</u>	<u>4,68E-02</u>	<u>-2,22</u>
<u>USP18</u>	<u>5,44E-08</u>	<u>4,68</u>	<u>3,59E-03</u>	<u>-1,76</u>
PSMB8	1,63E-10	4,22		
STAT1	2,04E-09	4,11		
SOCS1	8,94E-06	3,99		
HLA-DPA1	7,92E-07	3,22		
IRF7	1,84E-04	2,83		
IRF5	2,36E-04	2,11		
Other Gene	p-value	FC	p-value	FC
	UP in inflammation		DOWN in inflammation+AAT	
IFNAR1	<0.05	1,47	<0.05	-1,63
FLNB	<0.05	1,25	<0.05	-1,20

	DOWN in inflammation		UP in inflammation+AAT	
IRF3	<0.05	-1,45	<0.05	1,56
HLA-DPB1	<0.05	-1,23	<0.05	1,31
SOCS3	<0.05	-1,99	<0.05	1,20
CD44	<0.05	-1,14	<0.05	1,14
	Regulated in inflammation		Regulated in AAT	
STAT1	<0.05	4,11	<0.05	-1,47
IRF2	<0.05	1,24	<0.05	-1,17

Table 4

Complement activation			
Top Gene	p-value	FC	p-value FC
	UP in inflammation		DOWN in inflammation+AAT
SERPINB2	1,47E-05	25,49	6,63E-06 -4,63
C5AR1	1,78E-10	22,55	
C5	2,05E-07	4,22	
THBD	1,89E-04	2,81	
C3	1,97E-06	2,34	
PLAU	5,48E-09	2,17	
	DOWN in inflammation		UP in inflammation+AAT
PROS1	2,84E-08	-3,43	
Other Gene	p-value	FC	p-value FC
	UP in inflammation		DOWN in inflammation+AAT
C3AR1	<0.05	2,44	<0.05 -1,50
	DOWN in inflammation		UP in inflammation+AAT
PLAUR	<0.05	-1,84	<0.05 1,32
ITGAX	<0.05	-1,60	<0.05 1,41
	Regulated in inflammation		Regulated in AAT
PLAU	<0.05	2,17	<0.05 -1,27

Example 6 - AAT treatment enhances the expression of hallmark genes related to M2 anti-inflammatory microglia

M2 microglia gene expression is promoted following a M2-type induction (FC study; Satoh 2017). AAT treatment in resting microglia (FC AAT) and in activated microglia (FC inflammation+AAT) was able to similarly enhance expression of M2 genes, while at lower magnitude. ~60% of the modified genes were common among AAT treatment conditions (Table 5 in bold).

Table 5

M2 Gene	FC study	FC AAT	M2 Gene	FC study	FC inflammation + AAT
MMP1	7,36	2,51	HLA-DRB5	53,99	2,09
ITGA11	4,78	1,65	ANPEP	8,90	1,25
COL1A1	4,38	1,58	THBS1	8,16	1,34
TAGLN	2,79	1,37	MMP1	7,36	2,39
GUK1	2,76	1,57	BGN	6,68	1,27
PAPPA	2,74	1,24	ITGA11	4,78	1,54
PLTP	2,52	1,28	OLFML2B	4,78	1,44
NTN4	2,38	1,25	GUK1	2,76	1,31
FOXC1	2,37	1,26	FAT1	2,75	1,34
PTPRS	2,34	1,27	PAPPA	2,74	1,31
COL11A1	2,30	1,14	KDM2B	2,69	1,17
STC2	2,29	1,42	CLMP	2,46	1,22
MXRA8	2,28	1,40	PTPRS	2,34	1,23
TEKT4P2	2,17	2,46	COL11A1	2,30	1,16
SART1	2,11	1,44	STC2	2,29	1,32
FZD2	2,09	1,39	MXRA8	2,28	1,49
AP2A2	2,04	1,26	TGFB1	2,19	1,18
FEZ1	1,99	1,45	LOXL2	2,10	1,25
ACAN	1,98	1,37	FZD2	2,09	1,37
TGFB1I1	1,94	1,31	FEZ1	1,99	1,34
NID1	1,90	1,16	AHNAK2	1,96	1,40
RASA4B	1,87	1,46	TGFB1I1	1,94	1,26
GREM1	1,82	1,69	CLIP3	1,89	1,25
FGD3	1,82	1,76	SH3PXD2B	1,88	1,19
GADD45GIP1	1,70	1,55	IL1R1	1,84	1,18
MOSPD3	1,69	1,51	GREM1	1,82	1,56
APRT	1,69	1,16	FGD3	1,82	2,31
RAB3IL1	1,68	1,55	ARHGDIB	1,79	1,28
TNFRSF1B	1,67	1,46	GATA6	1,78	1,47
SLC22A5	1,66	1,22	PRSS23	1,78	1,16
ENG	1,66	1,29	KANK2	1,74	1,25
SEMA3B	1,65	1,50	GADD45GIP1	1,70	1,51
DBN1	1,65	1,42	LDHA	1,67	1,21
IGFBP2	1,63	1,63	TNFRSF1B	1,67	1,23
TBCD	1,63	1,26	ENG	1,66	1,35
METRNL	1,63	2,23	SEMA3B	1,65	1,39
SERPINH1	1,62	1,21	DBN1	1,65	1,31
MBOAT7	1,61	1,44	IGFBP2	1,63	1,34
PREX1	1,61	1,25	KIAA1549L	1,63	1,44

TTC9	1,61	1,65	TBCD	1,63	1,26
RASAL1	1,60	1,52	METRN	1,63	1,64
ZIC2	1,60	2,42	PLLP	1,62	1,46
CHPF	1,58	1,80	SERPINH1	1,62	1,20
PLEC	1,58	1,69	MBOAT7	1,61	1,37
APLP1	1,58	1,41	PREX1	1,61	1,28
HSPG2	1,57	1,58	RASAL1	1,60	1,45
BAIAP2	1,55	1,45	ZIC2	1,60	2,77
MIF	1,55	1,83	ZNF319	1,59	1,25
FZD7	1,55	1,24	CHPF	1,58	1,58
MARCH4	1,54	1,28	PLEC	1,58	1,54
PRSS36	1,52	1,58	HSPG2	1,57	1,41
C16orf45	1,51	1,31	TCEAL2	1,56	1,30
EXTL1	1,50	2,19	BAIAP2	1,55	1,24
			MIF	1,55	1,72
			FZD7	1,55	1,36
			FADS2	1,54	1,20
			HCK	1,51	1,44
			EXTL1	1,50	1,78
			SLC16A3	1,50	1,65

Example 7 - AAT treatment affect the expression of neurodegenerative diseases risk genes

Risk genes related to PD (21 genes), AD (15 genes), MS (53 genes), MCT (50 genes) PN (88 genes) and GBS (30 genes; FC) were extracted from public libraries (Timmerman, V., A. V. Strickland, and S. Zuchner. 2014., Genes (Basel), 5: 13-32; Parnell, G. P., and D. R. Booth. 2017. Front Immunol, 8: 425.; Nikolac Perkovic, M., and N. Pivac. 2019. Adv Exp Med Biol, 1192: 27-52.; Blauwendraat, C., M. A. Nalls, and A. B. Singleton. 2020. Lancet Neurol, 19: 170-78.) and their expression in AAT-treated resting microglia (FC AAT) and activated microglia (FC inflammation+AAT) was assessed. Common modified genes among AAT treatment conditions are highlighted (bold underline). Of note, the expression of several risk genes in all abovementioned diseases were modified by AAT.

Table 6

Risk gene PD	AAT		Risk gene PD	Inflammation+AAT	
	p-value	FC		p-value	FC
<u>PLA2G6</u>	<u>9,48E-03</u>	<u>1,55</u>	<u>ATP13A2</u>	<u>6,94E-03</u>	<u>1,49</u>
<u>ATP13A2</u>	<u>1,92E-02</u>	<u>1,42</u>	<u>PLA2G6</u>	<u>4,87E-02</u>	<u>1,37</u>
<u>LRP10</u>	<u>4,49E-02</u>	<u>1,29</u>	<u>LRP10</u>	<u>3,15E-02</u>	<u>1,23</u>
<u>DNAJC6</u>	<u>1,23E-02</u>	<u>-1,14</u>	PINK1	4,12E-02	1,20
GIGYF2	1,29E-02	-1,16	GBA	4,81E-02	1,15
<u>SYNJ1</u>	<u>1,08E-02</u>	<u>-1,33</u>	UCHL1	3,66E-02	-1,10
DNAJC13	3,07E-03	-1,37	<u>DNAJC6</u>	<u>3,43E-02</u>	<u>-1,12</u>
VPS13C	1,84E-02	-1,41	<u>SYNJ1</u>	<u>4,94E-02</u>	<u>-1,23</u>
TMEM230	3,43E-03	-1,42			
VPS35	2,46E-03	-1,42			
LRRK2	1,98E-02	-3,98			

Table 7

Risk gene AD	AAT		Risk gene AD	Inflammation+AAT	
	p-value	FC		p-value	FC
<u>TREM2</u>	<u>2,50E-03</u>	<u>2,22</u>	<u>APOE</u>	<u>3,13E-02</u>	<u>1,55</u>
<u>ABCA7</u>	<u>3,77E-04</u>	<u>1,75</u>	<u>TREM2</u>	<u>4,38E-02</u>	<u>1,47</u>
<u>APOE</u>	<u>1,05E-02</u>	<u>1,74</u>	<u>BIN1</u>	<u>4,26E-03</u>	<u>1,43</u>
<u>PSEN2</u>	<u>3,51E-03</u>	<u>1,45</u>	<u>ABCA7</u>	<u>2,26E-02</u>	<u>1,36</u>
<u>BIN1</u>	<u>3,90E-02</u>	<u>1,33</u>	<u>PSEN2</u>	<u>1,00E-02</u>	<u>1,28</u>
CLU	5,24E-03	-1,30	<u>PICALM</u>	<u>3,38E-02</u>	<u>-1,29</u>
CD2AP	1,25E-02	-1,39			
<u>PICALM</u>	<u>4,42E-03</u>	<u>-1,48</u>			

Table 8

Risk gene MS	AAT		Risk gene MS	Inflammation+AAT	
	p-value	FC		p-value	FC
<u>CYP24A1</u>	<u>8,25E-06</u>	<u>2,55</u>	DKKL1	4,05E-02	2,12
<u>HLA-A</u>	<u>1,88E-03</u>	<u>1,48</u>	<u>HLA-A</u>	<u>2,42E-05</u>	<u>1,57</u>
MPV17L2	3,93E-02	1,31	HLA-DRB1	1,41E-02	1,51
<u>AGAP2</u>	<u>3,00E-02</u>	<u>1,30</u>	ZMIZ1	8,06E-04	1,47
CDC37	4,54E-02	1,26	<u>AGAP2</u>	<u>1,92E-02</u>	<u>1,45</u>
METTL1	3,26E-02	1,22	<u>CYP24A1</u>	<u>1,66E-02</u>	<u>1,34</u>
CD40	2,67E-02	1,17	MPHOSPH9	2,92E-02	-1,19
RPS6KB1	4,07E-02	-1,23	NFKBIZ	4,75E-02	-1,37
CBLB	8,31E-03	-1,24	<u>SLC30A7</u>	<u>3,20E-02</u>	<u>-1,56</u>
PTGER4	3,38E-02	-1,29			
PTPRK	1,93E-04	-1,38			
AHI1	6,58E-03	-1,46			
HLA-DRB1	3,40E-02	-1,47			
CD58	4,85E-02	-1,61			
EXTL2	2,52E-03	-1,76			
<u>SLC30A7</u>	<u>9,06E-03</u>	<u>-1,77</u>			

Table 9

Risk gene MCT* / PN	AAT		Risk gene MCT* / PN	Inflammation+AAT	
	p-value	FC		p-value	FC
SCN11A	2,65E-02	4,49	<u>TRPV4*</u>	<u>2,02E-02</u>	<u>1,72</u>
KIF1A	1,99E-02	2,76	<u>HSPB1*</u>	<u>1,06E-02</u>	<u>1,56</u>
<u>TRPV4*</u>	<u>1,14E-03</u>	<u>1,92</u>	TUBB3	2,33E-02	1,44
<u>HSPB1*</u>	<u>2,22E-02</u>	<u>1,67</u>	TRPV4*	4,70E-02	1,40
PLEKHG5*	4,11E-03	1,49	LMNA*	9,07E-03	1,39
<u>IGHMBP2*</u>	<u>9,58E-03</u>	<u>1,39</u>	MED25*	2,22E-02	1,38
<u>DNAJB2*</u>	<u>9,15E-03</u>	<u>1,39</u>	INF2	4,80E-02	1,35
LRSAM1*	9,19E-03	1,36	NDRG1*	3,75E-03	1,30
IFRD1	2,59E-02	1,23	DST	4,71E-02	1,30
ARHGEF10	3,57E-03	1,19	SEPT9	1,36E-02	1,29
WNK1	4,34E-02	-1,14	<u>IGHMBP2*</u>	<u>3,80E-02</u>	<u>1,21</u>
<u>CCT5</u>	<u>1,18E-02</u>	<u>-1,17</u>	<u>DNAJB2*</u>	<u>2,76E-02</u>	<u>1,20</u>
SBF2	3,60E-03	-1,20	<u>CCT5</u>	<u>1,91E-02</u>	<u>-1,13</u>
KIF1B*	5,00E-03	-1,21	KARS*	2,35E-02	-1,13
<u>GARS*</u>	<u>1,91E-03</u>	<u>-1,21</u>	<u>TFG*</u>	<u>1,91E-02</u>	<u>-1,19</u>
GAN*	2,14E-02	-1,21	<u>GARS*</u>	<u>1,12E-03</u>	<u>-1,24</u>
SETX	2,68E-02	-1,23	<u>GDAP1*</u>	<u>1,32E-02</u>	<u>-1,24</u>
SPTLC1	3,73E-02	-1,23	<u>SCN9A</u>	<u>1,99E-03</u>	<u>-1,66</u>
<u>TFG*</u>	<u>2,73E-03</u>	<u>-1,28</u>			
DST	2,99E-02	-1,35			

MTMR2*	3,72E-03	-1,36		
TRIM2*	2,09E-03	-1,39		
DYNC1H1*	1,10E-02	-1,40		
FIG4*	1,84E-03	-1,42		
<u>SCN9A</u>	<u>1,08E-02</u>	<u>-1,50</u>		
<u>GDAP1*</u>	<u>5,03E-05</u>	<u>-1,54</u>		
GNB4*	6,11E-03	-1,56		

Table 10

Risk gene	FC	AAT		Risk gene	FC	AAT	
		p-value	FC AAT			p-value	FC AAT
<u>GUK1</u>	<u>-2,13</u>	<u>1,38E-02</u>	<u>1,57</u>	<u>GUK1</u>	<u>-2,13</u>	<u>3,31E-02</u>	<u>1,31</u>
HAGH	-2,14	1,07E-02	1,40	NFIL3	2,83	6,38E-03	-1,22
GLRX5	-2,48	4,32E-02	1,12	<u>ZNF12</u>	2,79	<u>4,52E-02</u>	<u>-1,23</u>
HMGB2	3,63	7,91E-03	-1,20	<u>FOS</u>	4,02	<u>1,19E-02</u>	<u>-1,54</u>
<u>ZNF12</u>	2,79	<u>3,05E-02</u>	<u>-1,26</u>	<u>MARCKS</u>	2,81	<u>2,06E-03</u>	<u>-1,84</u>
CDC42	2,88	8,15E-03	-1,35	LY96	3,27	1,72E-02	-2,31
SMCHD1	2,81	3,33E-02	-1,39				
PKN2	2,77	1,66E-02	-1,47				
SENP6	2,76	1,91E-02	-1,47				
<u>MARCKS</u>	<u>2,81</u>	<u>5,40E-04</u>	<u>-2,06</u>	-		-	-
<u>FOS</u>	4,02	<u>6,60E-04</u>	<u>-2,31</u>				

Example 8

Further experiments include CSF-1 treatment for (M ϕ) to M1 macrophage transition optimization, Dose dependance of IFN γ for M1 macrophage activation, AAT treatment on resting and IFN γ -activated macrophage and RNA extraction and RNAseq and analysis. Whereby the cells are human primary resting (M ϕ) or M1-differentiated macrophages. The treatment includes pre-treatment for 24h with AATs, followed by cell activation (or not) with CSF-1 or IFN in presence of AAT for 24h. Cells are further treated 48h in AAT and finally tested for pro-inflammatory cytokine release (IL-6, TNF α , IL-1 β , IL-8; multiplexing readout) using the culture supernatant or alternatively test for Luciferase activity (*MHCII^{Luc}* line) and concomitantly extract RNA for microarray analysis. Cultures with consistent readouts for the cytokine release or the luciferase assay will be used as samples for RNA extraction and microarray analysis.

Experimental conditions: every condition is performed in triplicate,

- Untreated (no AAT, no CSF-1) Resting macrophage control
- IFN γ : activated macrophage control
- AAT (Plasma-derived; Sigma or recombinant; Lonza): AAT effect on resting macrophage
- IFN γ and AAT (Plasma-derived; Sigma or Lonza): AAT anti-inflammatory effect on activated macrophage

Output:

- Resting state macrophage gene expression
- Pro-inflammatory differentially regulated genes (fold of untreated; significant p-value; fold up/down-regulation threshold)
- AAT-driven gene expression change on resting and activated macrophage
- Difference between recombinant and plasma-derived AAT gene regulation

METHODS

Human microglial cell line culture

HMC3-*MHCII^{Luc}* cell line coding for *Renilla* luciferase under major histocompatibility complex II promoter (HLA-DRA) has been described as a valuable tool to study human microglial activation by and was obtained from Prof. Karl-Heinz Krause, University of Geneva. It was transduced with a lentiviral vector to obtain the HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* cell line (See Fig. 3). Both HMC3-*MHCII^{Luc}* and HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* cell lines were cultured on TC treated cell culture dishes (CELLSTAR®, Greiner, 7.664160) in DMEM high glucose + glutamine (Gibco, 41965039) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, 10270106) and 100 μ g/ml penicillin/streptomycin (Pen/Strep, ThermoFisher, 15070063). Cultures were maintained at 37°C in a 5% CO₂ atmosphere. Passage was done by quickly rinsing the cells in PBS 1X, 3min trypsinization at RT (Tryple Express, ThermoFisher, 12604021) followed by centrifugation (5min, 1000RPM) and resuspension in the abovementioned supplemented DMEM. Cells were counted and plated at desired concentration.

Human microglial cell line transduction

The lentivirus coding for human AAT under ubiquitin promoter and GFP under human PGK promoter was obtained according to the protocol described in Marc Giry-Laterrière, Els Verhoeyen, and Patrick Salmon, 2011, Methods in molecular biology. In brief, 4.5×10^6 HEK cells were plated in a Ø100 mm dish and transfected 16h later with 15 µg of pCWXPB-UBI-SP::AAT, 10 µg of packaging plasmid (psPAX2, gift from Didier Trono [Addgene plasmid 12260]), and 5 µg of envelope (pMD2G, gift from Didier Trono [Addgene plasmid 12259]). The medium was changed 8h post-transfection. After 48h, viral supernatant was collected and filtered using 45 µm PVDF filters and stored at -80°C. Titer of the virus was done and HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* cell lines with approximately 100% and 50% of cells expressing the AAT were selected for experimental conditions.

IFN γ -mediated human microglial activation

HMC3-*MHCII^{Luc}* cell line was seeded into 96-well plates at a density of approximately 2500 cells/well. 24h after, their activation was induced with a 24h-long IFN γ (Sigma, SRP3058) presentation at ranged concentrations (0.1; 1; 10 or 100 ng/ml). IFN γ was then removed and cells cultured for 48h before being assessed for cell viability and activation (see Fig. 2).

Exogenous/endogenous AATs treatment on IFN γ -activated human microglia

HMC3-*MHCII^{Luc}* and HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* (endogenous AAT) cell lines were seeded into 96-well plates at a density of approximately 2500 cells/well. HMC3-*MHCII^{Luc}* cell line was plated and was added plasma-derived AAT and recombinant AATs (produced in CHO cells, AAT 1 and AAT 2) 3h later and at ranged concentration (1; 10 or 25 µM). 24h after, still in the presence of exogenous or endogenous AAT, microglial activation was induced with a 24h-long IFN γ presentation (10ng/ml). IFN γ was then removed and both HMC3-*MHCII^{Luc}* and HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* cells were cultured for 48h in exogenous or endogenous presence of AAT before cell cultures were being assessed for cell viability and activation (See Fig. 3 and 4).

Human microglia cell viability and activation measurement

Viability (Cell Counting Kit-8, Sigma, 96992) and activation (Renilla-Glo® Luciferase Assay System, Promega, E2710) of HMC3-*MHCII^{Luc}* and HMC3-*MHCII^{Luc}*; *Ubi^{AAT}* cell cultures were measured according to the manufacturers' protocols.

RNA collection, sequencing and differential expression analysis

RNA extraction was achieved with RNeasy Mini kit (Qiagen) according to manufacturer's protocol. RNA samples from plasma-derived AAT and recombinant AAT Nr. 2 were checked for quality (2100 Bioanalyzer, Agilent) and libraries prepared with Truseq RNA Library Kit (Illumina, RS-122-2001). Libraries were sequenced (HiSeq 4000, Illumina) controlled for the quality of sequencing (FastQC), mapped on the human genome (STAR v.2.7.0f; UCSC hg38), reads were counted (HTSeq v0.9.1) and the differential expression analysis was performed with the R/Bioconductor package (edgeR 1.30.1.).

RNA collection, sequencing and differential expression analysis

Human monocyte-derived M1 macrophage (GM-CSF, PromoCell, C-12916) were cultured on fibronectin-coated cell culture dishes in M1-Macrophage Generation Medium XF and activated with CSF-1 (50ng/ml, Sigma, SRP3058) according to the manufacturer's protocol. Cultures were maintained at 37°C in a 5% CO₂ atmosphere.

Cytokine multiplex assay

Cell culture supernatant was collected and measure for IL-6, TNF α , IL-1 β and IL-8 with bead based Luminex assay according to the manufacturer's protocol.

Cell free TACE/ADAM17 activity

TACE activity and its inhibition by human AAT (AAT) was performed with Recombinant Human TACE/ADAM17 kit (930-ADB and ES003, R&D Systems) in black 96-well immuno plates (437111, ThermoFisher Scientific). The enzymatic activity of TACE/ADAM17 was measured by mixing 0.005 μ g of rhTACE with 10 μ M of Mca-PLAQAV-Dpa-RSSSR-NH₂ fluorogenic peptide substrate III in assay buffer (25 mM Tris, 2.5 μ M ZnCl₂, 0.005% Brij-35 (w/v), pH 9.0) to a final volume of 100 μ l. AAT (Sigma Aldrich, batch A6150) was resuspended in water (vehicle), control TACE/ADAM17 activity was assessed in presence of the vehicle (amount used for AAT

100 μ M). AAT was added at different concentration (0, 6.25, 12.5, 25, 50 and 100 μ M) to assess its dose-dependent inhibition of TACE/ADAM17. All conditions were performed in triplicates. Activity was measured as relative fluorescent unit (RFU) in a kinetic mode (9 time points over 5 min) with a SpectraMax iD3 Microplate Reader (low PMT gain, 1s exposition, top read at 1mm, wavelength: excitation 320nm, emission 405nm). Bar graph as percentage of control activity was obtained by averaging the values obtained over the 5 min for each of the condition.

Animals

As a murine model of CMT1A we used C3-PMP22 transgenic mice (B6.Cg-Tg(PMP22)C3Fbas/J, The Jackson Laboratory) which express three copies of a wild-type human peripheral myelin protein 22 (PMP22) gene (Verhamme, Camiel, et al. *Journal of Neuropathology & Experimental Neurology* 70.5 (2011): 386-398). Mice were housed in macrolon cages with filter hoods, in a continuously air-filtered room, thereby avoiding contamination. During experiments, paired animals were caged at a constant temperature with a day/night cycle of 12/12 hours. Animals were fed (control tap water and nutrition) ad libitum. Animal protocol is approved by the Animal Studies Committee of Languedoc Roussillon. This protocol and the laboratory procedures comply with French legislation, which implements the European Directives (Reference Number: D3417223, APAFIS#23920-2020020320279696 v3). Animal health was followed on a daily basis to ensure that only animals in good health enter the testing procedures and follow up the study.

In vivo study paradigm

Animals were split in 3 groups (wild-type control (subcutaneous 0.9% NaCl), CMT1A-vehicle (subcutaneous 0.9% NaCl), CMT1A-human alpha-1 antitrypsin (subcutaneous, twice daily, 50 mg/kg per injection)) of 3 mice each (3 weeks old males of 18 ± 2.5 g at the beginning of study) and all went through the following protocol after 7 days of acclimation on site.

Starting from the age of 4 weeks, animals were subjected to blood samplings for the determination of interleukin-6 (IL-6) and tumor necrosing factor alpha (TNF α) levels, as described in Figure 16.

Plasma levels of AAT were evaluated every 5 days from the first day of treatment to the last treatment day.

On the first and last day of treatment, the neuromuscular performance of animals was tested with a rotarod test, grip test and sciatic nerve electrophysiology test. After the last treatment at 8 weeks, these tests were repeated, and animals were sacrificed and the left sciatic nerve was sampled for histological evaluation of the number and size of neurons.

Example 9

TACE activity is assessed according to the manufacturer's instruction (Recombinant Human TACE/ADAM17 kit, 930-ADB, R&D Systems) in kinetic mode, without or with different AAT concentration. All conditions were done in triplicates and are shown as mean $\pm$ SD (Figure 5).

Example 10

The most common type of CMT is CMT1A, characterized by a duplication of the PMP22 gene leading to an accumulation of the pmp22 protein in the Schwann cell and progressive demyelination. PMP22 is a tetraspan glycoprotein contained in compact myelin of the peripheral nervous system. Duplication of PMP22 has been associated with the onset of Charcot-Marie-Tooth disease type 1A (CMT1A). The C3-PMP22 transgenic mice (B6.Cg-Tg(PMP22)C3Fbas/J) express three copies of a wildtype human peripheral myelin protein 22 (PMP22) gene. The cause and effect between the additional PMP22 gene and CMT1A are still not well understood and remain elusive to this day. Several plausible hypotheses are, nevertheless, available to link the genetic abnormality, that is the duplication of the PMP22 gene, to the pathology's manifestation. Without being bound to theory, PMP22 overexpression may exert a negative effect on the formation of myelin sheaths in the peripheral nervous system (PNS). These mice present an age-dependent demyelinating neuropathy characterized by predominantly distal loss of strength and sensation. C3-PMP mice show no overt clinical signs at 3 weeks and develop progressive and observable neuromuscular impairment after 4 weeks. The mice have stable, low nerve conduction velocities the same way as in adults with human CMT1A. Myelination is delayed in these mice, and they contain reduced numbers of myelinated axons at 3 weeks of age. This mouse model was used to study the effect of AAT in different paradigms.

Positive efficacy of AAT administration was observed after two weeks in the CMT1A mice by increasing rotarod latency, grip strength and nerve conduction performances

compared to an untreated control group. Moreover, there is no observable body weight loss in the AAT treated group compared to the vehicle group suggesting the absence of systemic toxicology of the compound at these experimental conditions (Table 11).

Table 11 Body weight

WT control					
	4 weeks old	5 weeks old	6 weeks old	7 weeks old	8 weeks old
Mouse 1,1	18,6	19,5	19,8	20,2	21,1
Mouse 1,2	19,1	19,7	19,8	20,3	20,9
Mouse 1,3	18,4	18,9	19,4	19,8	20,6
MEAN	18,70	19,37	19,66	20,12	20,90
SD	0,36	0,40	0,20	0,25	0,25
SEM	0,21	0,23	0,12	0,14	0,14
CMT1A+ vehicle					
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old
Mouse 2,1	19,5	20,4	20,8	21,3	21,4
Mouse 2,2	19,2	19,8	20,4	20,6	21,2
Mouse 2,3	18,1	18,4	19,0	19,7	20,5
MEAN	18,93	19,57	20,08	20,51	21,05
SD	0,74	1,03	0,98	0,79	0,49
SEM	0,43	0,60	0,57	0,46	0,29
CMT1A + hAAT					
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old
Mouse 3,1	18,7	19,2	19,6	20,1	20,8
Mouse 3,2	19,2	20,1	20,5	21,1	21,8
Mouse 3,3	18,6	18,6	19,1	19,9	20,6
MEAN	18,83	19,31	19,73	20,33	21,06
SD	0,32	0,78	0,74	0,63	0,62
SEM	0,19	0,45	0,43	0,36	0,36

Sciatic nerve electrophysiology (EMG) provides sensitive and quantitative approach to measure compound muscle action potential and nerve conduction velocity amplitude in the animals and was done by stimulation of the sciatic nerve. Similar compound muscle action potential (CMAP) amplitudes were observed between groups at the baseline (6 weeks old). As expected, a strong and significant decrease of CMAP amplitude was observed in the CMT1A+vehicle group compared to the wild type control group at 8 weeks old. Results show improvement of the EMG parameters for CMT1A mice treated with AAT compared to the control (Figure 6 and Table 12, 13, 14) suggesting a positive efficacy of AAT on axonal degeneration induced by CMT1A disorder.

Lower nerve conduction velocities (NCV) were observed in both CMT1A groups compared to the wild type control group at the baseline (6 weeks old). At the baseline, the differences of NCVs between groups were not statistically significant. As expected, a strong and significant decrease of NCV was observed in the CMT1A+vehicle group

compared to the wild type control group at 8 weeks old. The CMT1A+AAT treated group presented an increase of the NCV compared to the vehicle treated group. Because the nerve conduction velocity depends on the myelin sheath integrity, these data also suggest a positive efficacy of AAT on the Schwann cell demyelination induced by CMT disorder.

Table 12 - Sciatic nerve electrophysiology

	WT control					
	Amplitude (mV)		T1 distal (s)		T2 proximal (s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 1,1	8.881	7.462	0.00251	0.00456	0.00298	0.00508
Mouse 1,2	6.982	6.204	0.00331	0.00426	0.00376	0.00468
Mouse 1,3	7.140	8.721	0.00271	0.00739	0.00319	0.00795
MEAN	7.668	7.462				32.032
SD	1.054	1.259				1.309
SEM	0.608	0.727				0.756
						2.727
	CMT1A+vehicle					
	Amplitude (mV)		T1 distal (s)		T2 proximal (s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 2,1	6.942	1.785	0.00228	0.00215	0.00292	0.00354
Mouse 2,2	6.452	3.162	0.00293	0.00235	0.00357	0.00379
Mouse 2,3	5.835	3.735	0.00137	0.00213	0.00191	0.00302
MEAN	6.410	2.894				27.863
SD	0.555	1.002				25.034
SEM	0.320	0.579				2.451
						1.415
						2.124
	CMT1A + haAT					
	Amplitude (mV)		T1 distal (s)		T2 proximal (s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 3,1	7.030	3.799	0.00202	0.00251	0.00250	0.00313
Mouse 3,2	6.025	5.260	0.00207	0.00236	0.00295	0.00318
Mouse 3,3	6.359	7.822	0.00160	0.00228	0.00217	0.00302
MEAN	6.471	5.627				26.268
SD	0.511	2.036				24.909
SEM	0.295	1.176				7.145
						4.125
						20.892
						2.821
						1.629

Table 13 – Mean compound muscle action potential

Compound muscle action potential, mV (Mean $\pm$ SEM)		
	6 weeks old	8 weeks old
WT control	7.67 $\pm$ 0.61	7.46 $\pm$ 0.73
CMT1A + vehicle	6.41 $\pm$ 0.32	2.89 $\pm$ 0.58***
CMT1A + AAT	6.47 $\pm$ 0.30	5.63 $\pm$ 1.18†

2-way ANOVA with repeated measures and Bonferroni t-test

***: $p < 0.001$ vs WT control; †: $p < 0.05$ vs CMT1A+vehicle

Table 14: Mean nerve conduction velocity

Nerve conduction velocity, m/s (Mean $\pm$ SEM)		
	6 weeks old	8 weeks old
WT control	32.03 $\pm$ 0.76	30.59 $\pm$ 2.73
CMT1A + vehicle	25.03 $\pm$ 1.42	12.68 $\pm$ 2.12***
CMT1A + AAT	24.91 $\pm$ 4.13	20.89 $\pm$ 1.63*

2-way ANOVA with repeated measures and Bonferroni t-test

*, ***: $p < 0.01$; $p < 0.001$ vs WT control

Grip strength test measures neuromuscular strength by assessing the animal's grasp of a metal grid. Lower grip strength was observed in the CMT groups compared to the wild type control group at the baseline (6 weeks old). At the baseline, the differences of grip strengths between groups were not statistically significant. As expected, a strong and significant decrease of grip strength was observed in the CMT1A+vehicle group compared to the wild type control group at 8 weeks old. Results show improvement of the grip strength for CMT1A mice treated with AAT compared to the control group (Figure 7 and Table 15, 16).

Table 15 Grip strength

	Grip strenght at 6 weeks old (baseline) (Newtons)			
	WT control			
Animal number	set 1	set 2	set 3	Mean / animal
1.1	5.45	6.96	8.64	7.02
1.2	3.51	6.73	9.20	6.48
1.3	8.84	6.86	8.61	8.10
	CMT1A + vehicle			
	set 1	set 2	set 3	Mean / animal
2.1	1.71	6.57	7.76	5.35
2.2	4.74	5.76	5.29	5.26
2.3	6.90	5.09	7.59	6.53
	CMT1A + AAT			
	set 1	set 2	set 3	Mean / animal
3.1	5.84	7.62	1.82	5.10
3.2	7.10	7.19	6.62	6.97
3.3	5.94	3.08	7.52	5.51

	Grip strenght at 8 weeks old (Newtons)			
	WT control			
Animal number	set 1	set 2	set 3	Mean / animal
1.1	6.31	4.90	8.83	6.68
1.2	5.46	7.51	7.41	6.79
1.3	6.46	9.38	7.29	7.71
	CMT1A + vehicle			
	set 1	set 2	set 3	Mean / animal
2.1	4.29	4.76	2.90	3.98
2.2	5.58	1.27	2.05	2.96
2.3	0.61	3.43	3.16	2.40
	CMT1A + AAT			
	set 1	set 2	set 3	Mean / animal
3.1	6.55	4.38	5.43	5.45
3.2	4.01	6.93	3.63	4.86
3.3	2.76	3.67	5.21	3.88

Table 16 mean grip strength

Grip strength, newtons (Mean $\pm$ SEM)		
	6 weeks old	8 weeks old
WT control	7.20 $\pm$ 0.48	7.06 $\pm$ 0.33
CMT1A + vehicle	5.71 $\pm$ 0.41	3.12 $\pm$ 0.46***
CMT1A + AAT	5.86 $\pm$ 0.57	4.73 $\pm$ 0.46**

2-way ANOVA with repeated measures and Bonferroni t-test

, *: $p < 0.01$; $p < 0.001$ vs WT control

The rotarod test measures neuromuscular coordination by assessing the capacity of the animals to stay in balance on a rotating cylinder. Similar rotarod latency was observed between groups at the baseline (6 weeks old). As expected, a strong and significant decrease of rotarod latency was observed in the CMT1A+vehicle group compared to the wild type control group at 8 weeks old. Results show improvement of the rotarod latency for CMT1A mice treated with AAT compared to the control group (Figure 8 and Table 17,18).

Table 17 Rotarod latency

Rotarod latency at 6 weeks old (baseline) (seconds)				
WT control				
Animal number	set 1	set 2	set 3	Mean / animal
1.1	64.7	94.3	88.4	82.46
1.2	105.8	98.8	84.9	96.49
1.3	77.2	115.0	46.3	79.50
CMT1A + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
2.1	69.5	82.5	65.8	72.62
2.2	56.6	77.8	101.8	78.77
2.3	61.2	72.3	84.3	72.62
CMT1A + AAT				
Animal number	set 1	set 2	set 3	Mean / animal
3.1	85.0	102.6	65.5	84.36
3.2	51.4	93.4	68.8	71.19
3.3	59.6	92.1	67.1	72.91

Rotarod latency at 8 weeks old (seconds)				
WT control				
Animal number	set 1	set 2	set 3	Mean / animal
1.1	61.9	106.2	84.0	84.03
1.2	60.0	72.9	67.4	66.77
1.3	110.8	70.2	106.2	95.69
CMT1A + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
2.1	30.6	78.2	51.0	53.26
2.2	40.8	51.0	26.3	39.36
2.3	35.7	38.3	24.9	32.95
CMT1A + AAT				
Animal number	set 1	set 2	set 3	Mean / animal
3.1	62.1	35.7	51.5	49.76
3.2	83.2	39.5	71.3	64.65
3.3	46.0	46.0	76.4	56.12

Table 18 mean rotarod latency

Rotarod latency, seconds (Mean $\pm$ SEM)		
	6 weeks old	8 weeks old
WT control	86.15 $\pm$ 5.24	82.16 $\pm$ 8.40
CMT1A + vehicle	74.67 $\pm$ 2.05	41.86 $\pm$ 5.99***
CMT1A + AAT	76.15 $\pm$ 4.13	56.84 $\pm$ 4.31*

2-way ANOVA with repeated measures and Bonferroni t-test

*, ***: $p < 0.05$; $p < 0.001$ vs WT control

Example 11

It appears that PMP22 protein is particularly important in protecting nerves from physical pressure, helping them restore their structure after being pinched or squeezed (compressed). Compression can interrupt nerve signaling, leading to the sensation commonly referred to as a limb "falling asleep." The ability of nerves to recover from normal, day-to-day compression, for example when sitting for long periods, keeps the limbs from constantly losing sensation. In CMT1A patients the myelination process is not properly complete, and the pathological symptoms associated with the disease become apparent most often after the second decade of life.

The PMP22 gene also plays a role in the growth of Schwann cells and the process by which cells mature to carry out specific functions (differentiation). Before they become part of myelin, newly produced PMP22 proteins are processed and packaged in specialized cell structures called the endoplasmic reticulum and the Golgi apparatus. Completion of these processing and packaging steps is critical for proper myelin function. CMT1A's pathomechanics is characterized by the absence of myelin sheaths due to an extra PMP22 gene, which is responsible for the abnormally high concentration of the peripheral myelin protein 22 (PMP22) in Schwann cells. GSEA analysis done on human microglial cells, AAT treatment has shown upregulation of genes related to the unfolded protein response (UPR) pathway and cell-survival (anti-apoptotic (Fig. 4C, Fig. 9).

Example 12

ADAM17, also known as TACE, is a transmembrane protein that includes an extracellular zinc-dependent protease domain. In the context of CMT1A, ADAM17 is known for its inhibitory effect on SCs mediated myelination through neuregulin 1 type III (NRG1-III). It is postulated that AAT was able to cross the blood nerve barrier (BNB) and interact with ADAM17 to successfully inhibit its activity and by doing so allowing SCs to “manually” overcome the distress signal that an overloaded ER with PMP22 generates and facilitating the formation of myelin sheaths around axons.

Example 13 Plasma AAT levels

AAT was not detected in plasma of the wild type control and CMT1A mice treated with vehicle at the analyzed time points (day 14, day 19, day 24 and day 29). The AAT was detected in plasma of CMT1A +AAT group at a mean of 6.07 µg/mL, 6.99 µg /mL, 8.14 µg/mL and 5.22 µg /mL at day 14, day 19, day 24 and day 29 respectively.

Example 14A Sciatic nerve histology

As expected a decrease of the total number of axons per surface, the axonal diameter and a significant increase of the g-ratio was observed in the CMT1A+vehicle group compared to the wild type control group at 8 weeks old (Table 19, Figure 10 to Figure 13).

Slight increase of the total number of axons per surface was observed in the CMT1A+AAT treated group compared to the vehicle group. Moreover, significant increase of the axonal diameter and decrease of the g-ratio (equal to the ratio of the inner-to-outer diameter of a myelinated axon) were observed in the CMT1A animals treated with AAT compared to the vehicle treated group. Even if, the CMT1A+AAT animals also presented a number of axons, axonal diameter and g-ratio statistically different than the wild type control group (Table 19, Figures 10 to 13). Taken together these data suggest a positive but partial efficacy of AAT on the histopathology induced by the CMT1A disorder when administrated at 50 mg/kg twice daily by subcutaneous route.

Table 19 Sciatic nerve histology

Sciatic nerve histology (Mean $\pm$ SEM)			
	Axons/100 μm^2	Axonal diameter (μm)	g-ratio
WT control	57.67 $\pm$ 0.88	3.52 $\pm$ 0.13	0.54 $\pm$ 0.005
CMT1A + vehicle	28.00 $\pm$ 7.02*	1.65 $\pm$ 0.09***	0.73 $\pm$ 0.010***
CMT1A + AAT	39.33 $\pm$ 4.81	2.53 $\pm$ 0.15*** †††	0.63 $\pm$ 0.009*** †††

Example 14B - IL-6

Similar plasma IL-6 concentrations were observed between groups at the baseline (day1) and day 8.

As expected, significant increase of plasma IL-6 concentration was observed in the CMT+vehicle group at day 14 and day 29. The terms CMT and CMT1A are used interchangeably in the example section.

The CMT+AAT treated group also presented a significant increase of plasma IL-6 concentration compared to the baseline concentration. However, the plasma IL-6 concentration of animals treated with AAT was lower than animals treated with vehicle at day 29 (Table 20 and Figure 14) suggesting a direct or indirect effect of AAT on this inflammatory cytokine.

Table 20: Mean plasma IL-6 concentration of CMT mice treated with and without AAT

Plasma IL-6 concentration, pg/mL (Mean $\pm$ SD)				
	Day 1	Day 8	Day 14	Day 29
WT control	28.50 $\pm$ 3.04	29.74 $\pm$ 2.67	35.90 $\pm$ 4.516	37.20 $\pm$ 3.391
CMT1A + vehicle	26.90 $\pm$ 3.86	38.32 $\pm$ 4.78	85.04 $\pm$ 2.296***	127.84 $\pm$ 7.810***
CMT1A + AAT	34.19 $\pm$ 1.67	39.10 $\pm$ 1.91*	84.21 $\pm$ 4.727**	90.20 $\pm$ 3.085*** †

Student t-test *, **, ***: $p < 0.05$; $p < 0.01$; $p < 0.001$ vs WT control at this timepoint; †: $p < 0.05$ vs CMT1A+vehicle at this timepoint

Example 15 - TNF α

A significant increase of plasma TNF α concentration was observed in the CMT1A+vehicle group at day 14 and day 29.

The CMT1A+AAT treated group also presented a significant increase of plasma TNF α concentration at day 14 and day 29 compared to the baseline concentration (Table 21

and Figure 15) suggesting that AAT has no effect on the levels of this inflammatory cytokine in this model of CMT1A.

Table 21: Plasma TNF α levels

Plasma TNF α concentration, pg/mL (Mean $\pm$ SEM)				
	Day 1	Day 8	Day 14	Day 29
WT control	2.60 $\pm$ 0.05	2.89 $\pm$ 0.14	2.80 $\pm$ 0.187	2.80 $\pm$ 0.187
CMT1A + vehicle	2.76 $\pm$ 0.17	3.99 $\pm$ 0.11**	5.80 $\pm$ 0.175***	11.81 $\pm$ 0.897***
CMT1A + AAT	2.94 $\pm$ 0.17	3.70 $\pm$ 0.41	5.15 $\pm$ 0.393**	10.67 $\pm$ 0.833***

Student t-test **: p<0.01; ***: p<0.001 vs WT control at this timepoint.

Example 16

AAT's effect on SH-SY5Y treated with 6-OHDA was evaluated by cell morphology and cell proliferation followed by cell viability quantification.

Cells and treatments

SH-SY5Y cells, which are commonly used to model neurodegenerative disorders, were used to produce an in vitro Parkinson's model (Que R, et al., 2021, Front. Immunol. 12). Cells were cultured in DMEMF12/Glutamax supplemented with 10% FBS.

Cells were treated for 24 hours with the neurotoxin 6-hydroxydopamine (6-OHDA, Sigma Aldrich 162957) at 50, 100 μ M concentrations alone or in combination with AAT 25 μ M (AAT plasma derived, Sigma Aldrich batch A9024). Cells treated with AAT alone or in combination with 6-OHDA for 24 hours, were then incubated with fresh AAT for additional 24 hours. Control cells were treated with PBS.

Cell growth and viability assay

At day 0 cells were plated in equally number in 24-well plate, the day after they were treated as described above, and final total cell count was performed at day 4 of culture (cell growth). Cell viability was assessed with Cell Counting Kit-8 (CCK-8; Sigma Aldrich 96992). After treatments, cells were incubated with 10 μ l of CCK-8 solution for 2 hours in the incubator. Absorbance was measured at 450 nm using SpectraMax iD3 Microplate Reader. Experiments were performed in triplicate.

Human IL-6 Immunoassay

Human IL-6 immunoassay (R&D D6050) was performed on cells supernatant. Briefly, 40000 cells were plated in 24-well plate and treated the day after as described in paragraph "Cells and treatment". At the end of the treatment, cells were washed with PBS and incubated with 2% FBS medium for 24 hours, then cell culture supernatants were collected and centrifuged to remove particulates. Assay procedure was performed as described by manufactured instructions, on standard and samples duplicates. Absorbance was measured at 450 nm using SpectraMax iD3 Microplate Reader. A standard curve was prepared from seven IL-6 standard dilutions and IL-6 sample concentrations determined.

6-OHDA at 50-100 μ M for 24 hours induced cells proliferation impairment by inducing cell death and reducing the number of cells after 4 days of culture (Figure 17). In contrast combined treatment with AAT significantly increased the number of cells counted compared to 6-OHDA alone (Figure 17), indicating a positive effect of AAT on cell survival/proliferation. T-test p-values are significative for: ctrl vs 6ohda $p=0.001$; 6ohda vs AAT+6ohda $p=0.003$. Ctrl vs AAT is not significant. Error bars are S.E.M.

Treated cells were then challenged in a cell viability assay. SH-SY5Y treated with 6-OHDA alone showed strong reduction of cell viability compared to control cells and cells treated with AAT only (Figure 18).

Cell count as well as the viability of the cells were significantly enhanced when 6-OHDA treatment was combined with AAT, compared to 6-OHDA treatment alone (Figure 18). P-values are calculated with t-test, non-significant differences were observed between control and AAT alone. All conditions were performed in triplicates. Based on these results we can conclude that AAT administration in a PD cell model (SH-SY5Y induced by 6-OHDA) has favorable effect on cell growth and viability, possibly protecting cells from 6-OHDA induced death.

In addition, to explore the role of AAT on pro-inflammatory cytokines we performed IL-6 quantification in cells supernatant. Medium from cells induced by 6-OHDA had increased level of IL-6 compared to control medium, on the contrary the medium collected from cells treated with AAT displayed less concentration of IL-6 (Figure 19). T-test p-value are significant for ctrl vs 6-OHDA $p=0.05$ and not significant for the other comparison.

Example 17

C57BL/6 mice were received at 8 weeks of age and housed under controlled conditions for the duration of the study. Three groups of 4 animals each were assigned to the following subcutaneous treatments twice daily at 7:00AM and 7:00PM for 2 weeks, from 20 to 35 days after the start of disease induction:

- Wild type (WT) mice receiving the vehicle, 0.9% NaCl (positive control)
- CIDP mice receiving the vehicle, 0.9% NaCl (negative control)
- CIDP mice receiving human AAT (AAT), 50 mg/kg per injection

Disease induction consisted in subcutaneous injection of mouse sciatic nerve homogenate in phosphate buffer saline (PBS) + Freund's adjuvant mixture at 10 mL/kg. For each treatment, 3 different body sites injections were performed. Control mice also received three subcutaneous injections of PBS + Freund's adjuvant at the same 3 different body sites.

On the day preceding disease induction (Day 1), on the first (Day 20) and on the last (Day 35) treatment day, animals were subjected to blood samplings for the determination of neurofilament light chain (NfL), tumour necrosing factor alpha (TNF α) and TMRSS5 levels, as described in Figure 20. In addition, plasma levels of AAT were evaluated on the last treatment day.

On the first (Day 20) and last (Day 35) day of treatment, the neuromuscular and performance of animals was tested with a rotarod test, grip test and a sensory test using the Von Frey filament test, as well as sciatic nerve electrophysiology test. After the last treatment at Day 35, these tests were repeated, animals were sacrificed and the left sciatic nerve was sampled for histological evaluation of the number and size of neurons.

Rotarod test

A strong decrease of rotarod latency was observed in the CIDP groups at Day 20, compared to the control group. On Day 35, the CIDP+AAT treated group presented an increase of rotarod latency compared to the vehicle treated group (Table 22, Figure 21 A).

Table 22: Mean rotarod latency

Rotarod latency, seconds (Mean $\pm$ SEM)		
	Day 20	Day 35
WT control	84.65 $\pm$ 5.55	81.50 $\pm$ 7.44
CIDP + vehicle	24.21 $\pm$ 5.16	25.17 $\pm$ 4.39
CIDP + AAT	25.96 $\pm$ 6.16	34.96 $\pm$ 9.47

Grip strength test

Lower grip strength was observed in the CIDP groups compared to the wild type control group at the baseline (Day 20). On Day 35, the CIDP+AAT treated group presented an increase of grip strength compared to the vehicle treated group (Table 23 and 21 B).

Table 23: Mean grip strength

Grip strength, newtons (Mean $\pm$ SD)		
	Day 20	Day 35
WT control	5.77 $\pm$ 0.99	5.88 $\pm$ 0.43
CIDP + vehicle	2.88 $\pm$ 0.40	2.72 $\pm$ 0.20
CIDP + AAT	2.89 $\pm$ 0.32	3.59 $\pm$ 0.35

Von Frey test

Lower paw withdrawal threshold was observed in the CIDP groups compared to the wild type control group at the baseline (Day 20). On Day 35, the CIDP+AAT treated group presented an increased threshold of paw withdrawal compared to the vehicle treated group (Table 24 and Figure 21 C). The results from this test suggest that AAT presents a statistically significant positive effect on the pain threshold (alleviating pain) in these CIDP mice when administrated with AAT twice a day at 50 mg/kg by the subcutaneous route.

Table 24: Mean paw withdrawal threshold

Von Frey, paw withdrawal threshold, grams (Mean $\pm$ SD)		
	Day 20	Day 35
WT control	7.25 $\pm$ 0.92	6.89 $\pm$ 0.75
CIDP + vehicle	3.86 $\pm$ 0.50	4.09 $\pm$ 0.47
CIDP + AAT	3.16 $\pm$ 0.95	5.71 $\pm$ 0.41

Compound muscle action potential

A strong and significant decrease of CMAP amplitude was observed in both CIDP groups compared to the wild type control group on Day 20.

At Day 35, the CIDP+AAT treated group presented an increase of CMAP amplitude compared to the CIDP+vehicle treated group (Table 25 and Figure 22A).

Table 25: Mean compound muscle action potential

Compound muscle action potential, mV (Mean $\pm$ SD)		
	Day 20	Day 35
WT control	8.15 $\pm$ 0.85	8.54 $\pm$ 1.17
CIDP + vehicle	3.77 $\pm$ 0.34	2.78 $\pm$ 0.28
CIDP + AAT	3.73 $\pm$ 0.42	4.56 $\pm$ 0.76

Nerve conduction velocity

A strong decrease of NCV was observed in both CIDP groups compared to the wild type control group at Day 20. The CIDP+AAT treated group presented an increase of the NCV compared to the vehicle treated group (Table 26 and Figure 22B).

Table 26: Mean nerve conduction velocity

Nerve conduction velocity, m/s (Mean $\pm$ SD)		
	Day 20	Day 35
WT control	26.11 $\pm$ 7.45	26.16 $\pm$ 6.99
CIDP + vehicle	12.24 $\pm$ 1.70	9.20 $\pm$ 1.66
CIDP + AAT	13.10 $\pm$ 4.49	16.85 $\pm$ 2.43

Neuromuscular impairment and decrease of nerve conduction amplitude and velocity were observed in the preclinical CIDP mouse model treated with vehicle, compared to the wild type control group, 20 days after the start of disease induction.

An increase of rotarod latency, grip strength, paw withdrawal threshold and nerve conduction velocity and amplitude was observed in AAT treated CIDP mice compared to the vehicle treated group.

These data suggest that AAT presents a trend to a positive effect on CIDP when administrated twice a day at 50 mg/kg by subcutaneous route in CIDP mice.

Raw data

Table 27 A: Rotarod latency

Baseline (Day 20) _ Rotarod latency (seconds)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	102.55	68.92	96.99	89.48
1.2	67.95	90.98	71.03	76.65
1.3	82.89	99.21	76.55	86.22
1.4	88.28	68.13	102.28	86.23
MEAN				84.65
SD				5.55
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	36.9	21.6	20.6	26.34
2.2	28.7	20.9	40.7	30.12
2.3	25.6	14.3	26.6	22.18
2.4	26.4	15.8	12.4	18.20
MEAN				24.21
SD				5.16
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	19.1	17.7	16.2	17.68
3.2	28.0	26.7	32.9	29.19
3.3	28.7	33.0	33.8	31.82
3.4	24.1	24.0	27.3	25.13
MEAN				25.96
SD				6.16

Table 27 B: Rotarod latency

Day 35 _ Rotarod latency (seconds)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	90.87	53.01	71.22	71.70
1.2	71.77	72.47	95.36	79.87
1.3	109.55	69.15	85.85	88.18
1.4	98.93	74.19	85.68	86.27
MEAN				81.50
SD				7.44
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	27.4	21.5	33.1	27.30
2.2	27.9	34.4	18.1	26.78
2.3	27.5	12.1	16.3	18.63
2.4	7.3	42.3	34.4	27.97
MEAN				25.17
SD				4.39
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	32.02	36.34	48.35	38.90
3.2	44.79	31.48	32.56	36.28
3.3	52.44	34.61	42.63	43.23
3.4	37.74	19.21	7.29	21.41
MEAN				34.96
SD				9.47

Table 28 A: Grip strength

Baseline (Day 20) _ Grip strength (Newton)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	5.59	8.13	5.90	6.54
1.2	3.89	3.90	5.21	4.33
1.3	6.30	6.11	6.51	6.31
1.4	5.17	6.88	5.66	5.90
			MEAN	5.77
			SD	0.99
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	3.29	2.17	3.31	2.93
2.2	3.02	3.92	2.31	3.08
2.3	3.10	1.83	2.01	2.31
2.4	1.54	4.44	3.65	3.21
			MEAN	2.88
			SD	0.40
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	2.92	2.43	2.04	2.46
3.2	3.06	2.09	4.42	3.19
3.3	2.89	2.45	3.14	2.83
3.4	3.50	2.62	3.13	3.08
			MEAN	2.89
			SD	0.32

Table 28 B: Grip strength

Day 35 _ Grip strength (Newton)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	5.57	6.28	4.19	5.34
1.2	5.84	5.67	7.70	6.40
1.3	6.46	5.98	5.37	5.94
1.4	4.64	6.00	6.86	5.84
			MEAN	5.88
			SD	0.43
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	1.76	3.16	3.63	2.85
2.2	3.24	1.67	2.60	2.50
2.3	3.47	2.36	1.99	2.61
2.4	2.58	2.51	3.72	2.94
			MEAN	2.72
			SD	0.20
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	4.92	3.53	2.58	3.68
3.2	4.55	2.00	4.52	3.69
3.3	5.15	3.04	3.53	3.91
3.4	3.47	2.61	3.23	3.10
			MEAN	3.59
			SD	0.35

Table 29 A: Von Frey test

Baseline (Day 20) _ von Frey test (Paw withdrawal threshold in grams)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	6.6	9.3	8.0	7.97
1.2	7.3	8.3	6.8	7.47
1.3	6.1	4.0	7.6	5.90
1.4	7.0	8.6	7.4	7.66
MEAN				7.25
SD				0.92
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	3.0	3.9	4.3	3.74
2.2	2.9	3.3	3.6	3.28
2.3	3.0	4.3	4.4	3.92
2.4	3.7	2.8	6.9	4.49
MEAN				3.86
SD				0.50
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	4.6	2.4	6.0	4.30
3.2	2.1	1.8	2.4	2.10
3.3	3.3	2.1	2.8	2.74
3.4	4.5	2.2	3.8	3.48
MEAN				3.16
SD				0.95

Table 29 B: Von Frey test

Day 35 _ von Frey test (Paw withdrawal threshold in grams)				
Sham control group				
Animal	set 1	set 2	set 3	Mean / animal
1.1	7.0	9.5	4.5	7.02
1.2	6.1	6.3	6.6	6.34
1.3	5.3	8.0	6.8	6.70
1.4	6.0	9.2	8.2	7.83
MEAN				6.97
SD				0.63
CIDP + vehicle				
Animal	set 1	set 2	set 3	Mean / animal
2.1	4.4	4.2	2.9	3.86
2.2	4.9	2.9	3.8	3.84
2.3	2.8	3.9	4.9	3.88
2.4	4.6	3.4	2.3	3.46
MEAN				3.76
SD				0.20
CIDP + hAAT				
Animal	set 1	set 2	set 3	Mean / animal
3.1	5.3	5.1	6.4	5.60
3.2	5.9	3.7	6.1	5.24
3.3	7.7	5.3	5.7	6.21
3.4	5.1	7.0	6.3	6.14
MEAN				5.80
SD				0.46

Table 30: Electrophysiology

Sham control group									
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)		
	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 35
Mouse 1,1	7.256	7.815	3.298	7.273	3.713	7.964	36.155		21.697
Mouse 1,2	8.113	7.759	4.337	6.809	5.034	7.549	21.538		20.261
Mouse 1,3	7.932	8.331	3.538	5.834	4.307	6.391	19.510		26.949
Mouse 1,4	9.292	10.256	3.254	6.937	3.805	7.357	27.223		35.716
MEAN	8.148	8.540					26.106		26.156
SD	0.847	1.172					7.452		6.992
SEM	0.268	0.371					2.357		2.211

CIDP + vehicle									
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)		
	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 35
Mouse 2,1	3.862	3.050	2.489	5.848	3.976	7.946	10.086		7.149
Mouse 2,2	3.941	2.482	3.332	2.859	4.592	4.197	11.898		11.211
Mouse 2,3	3.267	2.604	1.869	2.222	2.932	3.863	14.113		9.142
Mouse 2,4	4.024	2.978	3.733	2.462	4.899	4.074	12.866		9.308
MEAN	3.773	2.778					12.241		9.203
SD	0.344	0.278					1.699		1.660
SEM	0.109	0.088					0.537		0.525

CIDP + hAAT									
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)		
	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 20	Day 35	Day 35
Mouse 3,1	3.734	3.490	2.673	4.089	3.605	5.026	16.096		16.007
Mouse 3,2	3.562	5.146	3.177	3.902	4.533	4.833	11.057		16.112
Mouse 3,3	3.318	5.068	2.864	4.177	4.793	5.185	7.775		14.881
Mouse 3,4	4.308	4.527	3.114	3.970	3.973	4.705	17.463		20.402
MEAN	3.731	4.558					13.098		16.851
SD	0.421	0.763					4.492		2.432
SEM	0.133	0.241					1.421		0.769

Table 31A: Bodyweight

Animal number / days	Sham negative control												
	1	5	9	13	17	20	23	26	29	32	35		
1.1	19.40	19.91	19.98	20.27	20.66	20.86	21.14	21.24	21.97	22.27	22.96		
1.2	21.26	21.36	21.90	22.36	23.26	23.46	23.61	23.78	24.34	25.16	25.79		
1.3	20.69	20.88	21.08	21.50	21.97	22.21	22.70	23.28	23.85	24.10	25.02		
1.4	19.48	19.98	20.48	20.49	20.93	21.10	21.46	22.09	23.04	23.98	24.35		
CIDP + vehicle													
Animal number	1	5	9	13	17	20	23	26	29	32	35		
2.1	19.73	20.52	21.02	21.32	21.41	21.42	21.56	21.69	21.70	21.86	21.97		
2.2	20.77	20.81	21.64	21.87	21.94	21.97	22.04	22.21	22.39	22.43	22.58		
2.3	20.28	21.05	21.77	21.28	22.31	22.47	22.66	22.83	22.83	22.89	22.97		
2.4	19.45	19.74	19.80	20.06	20.62	20.77	20.85	20.96	21.14	21.15	21.19		
CIDP + hAAT													
Animal number	1	5	9	13	17	20	23	26	29	32	35		
3.1	20.74	20.92	21.26	21.76	21.92	22.06	22.21	22.31	22.49	22.68	22.79		
3.2	20.81	21.07	21.26	21.37	21.54	21.58	21.71	21.78	21.87	22.00	22.14		
3.3	19.50	20.12	20.54	20.24	21.35	21.41	21.43	21.47	21.53	21.64	21.65		
3.4	20.65	20.71	21.00	21.18	21.90	22.04	22.13	22.20	22.30	22.45	22.62		

Histology

A significant decrease of the total number of axons and the axonal diameter and significant increase of the g-ratio was observed in the CIDP+vehicle group compared to the sham control group at day 35 (Table 31B and Figure 37 to Figure 39). Slight but non-significant increase of the total number of axons per surface was observed in the CIDP+AAT treated group compared to the vehicle treated group. A significant increase of the axonal diameter and decrease of the g-ratio was also observed in the CIDP+AAT treated group compared to the CIDP+vehicle treated group at day 35.

Table 31B: Sciatic nerve histology

Sciatic nerve histology (Mean $\pm$ SEM)			
	Axons/100 μm^2	Axonal diameter (μm)	g-ratio
WT control	51.40 $\pm$ 3.90	5.30 $\pm$ 0.08	0.585 $\pm$ 0.060
CIDP + vehicle	19.20 $\pm$ 1.00	3.31 $\pm$ 0.10	0.749 $\pm$ 0.012
CIDP + AAT	26.00 $\pm$ 2.10	3.82 $\pm$ 0.09	0.715 $\pm$ 0.008

Example 18: Plasma NfL quantification

Similar plasma NfL concentrations were observed between the three groups at the baseline (day1, before model induction).

A significant increase of plasma NfL concentration was observed in the CIDP+vehicle and CIDP+AAT groups compared to the sham control group before compound treatment at day 20 (Table 32, Table 33 and Figure 23B).

Importantly, significant decrease of plasma NfL concentration was observed in the CIDP+AAT treated group compared to the CIDP+vehicle treated group at day 35 (Table 32, Table 33 and Figure 23B) confirming the positive efficacy of the compound on the peripheral axonopathy induced by this inflammatory neuropathy at these experimental conditions.

Example 19: Plasma TNF α quantification

Similar plasma TNF α concentration was observed between the three groups at the baseline (day1, before model induction). A significant increase of plasma TNF α concentration was observed in the CIDP+vehicle and CIDP+AAT groups compared to the sham control group before compound treatment at day 20 (Table 34, Table 35 and Figure 24).

Importantly, significant decrease of plasma TNF α concentration was observed in the CIDP+AAT treated group compared to the CIDP+vehicle treated group at day 35 (Table 34, Table 35 and Figure 24) confirming the positive efficacy of the compound on the cytokines activation induced by the autoimmune chronic inflammatory demyelinating polyneuropathy at these experimental conditions.

In conclusion neuromuscular impairment and decrease of nerve conduction amplitude and velocity were observed in the preclinical CIDP mouse model treated with vehicle, compared to the wild-type control group, 20 days after the start of disease induction.

An increase of rotarod latency, grip strength and nerve conduction velocity were observed in CIDP+AAT treated group compared to the CIDP+vehicle treated group. No statistically significant differences were observed in the plasma TMPRSS5 concentration of CIDP+AAT treated group compared to the vehicle group. However, significant increase of compound muscle action potential amplitude, decrease of neuropathic pain and decrease of plasma TNF α and NfL concentration was observed in CIDP+AAT treated group compared to the CIDP+vehicle treated group at day 35. Finally, the histopathology analysis of sciatic nerve showed a significant increase of the axonal diameter and myelin sheath diameter (decrease of g-ratio) in the CIDP+AAT compared the vehicle treated group confirming the positive efficacy of the compound from a histological point of view.

Taken together these data suggest that AAT presents positive efficacy on the autoimmune chronic inflammatory demyelinating polyneuropathy targeting peripheral nervous system, increasing neuromuscular and electrophysiological performances and decreasing neuropathic pain and plasma neuropathy biomarkers when administrated twice a day at 50 mg/kg by subcutaneous route in a preclinical CIDP mouse model.

Day 1 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
1.1	0.097	0.098	0.097	0.534
1.2	0.075	0.075	0.075	0.410
1.3	0.044	0.045	0.045	0.244
1.4	0.011	0.011	0.011	0.060
	MEAN		MEAN	0.31
	SD		SD	0.21

Day 20 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
1.1	0.0490	0.0492	0.0491	0.2695
1.2	0.0821	0.0827	0.0824	0.4520
1.3	0.0075	0.0077	0.0076	0.0417
1.4	0.0561	0.0568	0.0565	0.3097
	MEAN		MEAN	0.27
	SD		SD	0.17

Day 35 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
1.1	0.0989	0.0995	0.0992	0.5442
1.2	0.0694	0.0697	0.0695	0.3814
1.3	0.0626	0.0630	0.0628	0.3446
1.4	0.0777	0.0784	0.0780	0.4281
	MEAN		MEAN	0.42
	SD		SD	0.09

Day 20 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
2.1	0.4814	0.4847	0.4831	2.6499
2.2	0.5704	0.5701	0.5703	3.1282
2.3	0.7971	0.8034	0.8003	4.3898
2.4	0.5717	0.5805	0.5761	3.1601
	MEAN		MEAN	3.33
	SD		SD	0.74

Day 35 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
2.1	0.4774	0.4687	0.4730	2.5947
2.2	0.8700	0.8780	0.8740	4.7942
2.3	0.5035	0.5082	0.5059	2.7749
2.4	0.5544	0.5616	0.5580	3.0609
	MEAN		MEAN	3.31
	SD		SD	1.01

Day 20 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
3.1	0.4437	0.4476	0.4457	2.4447
3.2	0.3484	0.3449	0.3467	1.9017
3.3	0.4268	0.4299	0.4284	2.3497
3.4	0.4520	0.4615	0.4568	2.5056
	MEAN		MEAN	2.30
	SD		SD	0.27

Day 35 Nfl concentration (ng/ml)				
Animal number	Sham control group		Nfl conc (ng/ml)	
	Abs 1	Abs 2		
3.1	0.4437	0.4476	0.4457	2.4447
3.2	0.3484	0.3449	0.3467	1.9017
3.3	0.4268	0.4299	0.4284	2.3497
3.4	0.4520	0.4615	0.4568	2.5056
	MEAN		MEAN	2.30
	SD		SD	0.27

Table 32B:

Plasma NfL concentration, ng/mL (Mean $\pm$ SD)			
	Day 1	Day 20	Day 35
WT control	0.31 $\pm$ 0.21	0.27 $\pm$ 0.17	0.42 $\pm$ 0.09
CIDP + vehicle	0.34 $\pm$ 0.08	3.33 $\pm$ 0.74	3.31 $\pm$ 1.01
CIDP + AAT	0.29 $\pm$ 0.11	3.12 $\pm$ 0.86	2.30 $\pm$ 0.27

Table 33:

Two-way ANOVA					
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	18.94	< 0.0001	****	Yes	
Row Factor	35.61	< 0.0001	****	Yes	
Column Factor	35.48	< 0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	14.11	4	3.529	F (4, 27) = 12.82	P < 0.0001
Row Factor	26.53	2	13.27	F (2, 27) = 48.21	P < 0.0001
Column Factor	26.44	2	13.22	F (2, 27) = 48.05	P < 0.0001
Residual	7.429	27	0.2752		
Bonferroni's multiple comparisons test					
Groups	Mean Diff.	95% CI of diff.	Significant?	Summary	
Day 1					
Sham negative control vs. CIDP + vehicle	-0.0295	-0.9763 to 0.9173	No	ns	
Sham negative control vs. CIDP + hAAT	0.01878	-0.9280 to 0.9655	No	ns	
CIDP + vehicle vs. CIDP + hAAT	0.04828	-0.8985 to 0.9950	No	ns	
Day 20					
Sham negative control vs. CIDP + vehicle	-3064	-4.011 to -2.117	Yes	****	
Sham negative control vs. CIDP + hAAT	-2855	-3.802 to -1.908	Yes	****	
CIDP + vehicle vs. CIDP + hAAT	0.2088	-0.7379 to 1.156	No	ns	
Day 35					
Sham negative control vs. CIDP + vehicle	-2882	-3.828 to -1.935	Yes	****	
Sham negative control vs. CIDP + hAAT	-1876	-2.823 to -0.9291	Yes	****	
CIDP + vehicle vs. CIDP + hAAT	1006	0.05900 to 1.953	Yes	*	

Table 34 A:

Day 1 TNF-α concentration (pg/mL)				
Sham control group				
Animal number	Abs 1	Abs 2	Mean abs. dilution corr	TNFα conc (pg/mL)
1.1	0.0496	0.0497	0.0496	165.4920
1.2	0.0518	0.0513	0.0516	171.8472
1.3	0.0422	0.0423	0.0422	140.7485
1.4	0.0429	0.0430	0.0430	143.2007
	MEAN			155.32
	SD			15.66

Day 20 TNF-α concentration (pg/mL)				
Sham control group				
Animal number	Abs 1	Abs 2	Mean abs. dilution corr	TNFα conc (pg/mL)
1.1	0.0516	0.0517	0.0516	172.0910
1.2	0.0638	0.0641	0.0640	213.2430
1.3	0.0495	0.0485	0.0490	163.3830
1.4	0.0370	0.0377	0.0373	124.4260
	MEAN			168.29
	SD			36.44

Day 35 TNF-α concentration (pg/mL)				
Sham control group				
Animal number	Abs 1	Abs 2	Mean abs. dilution corr	TNFα conc (pg/mL)
1.1	0.0462	0.0465	0.0464	154.5570
1.2	0.0379	0.0380	0.0380	126.5980
1.3	0.0482	0.0486	0.0484	161.3470
1.4	0.0315	0.0314	0.0315	104.8520
	MEAN			136.84
	SD			26.09

CIDP + vehicle				
Animal number	Abs 1	Abs 2	Mean abs. dilution corr	TNFα conc (pg/mL)
2.1	0.4738	0.4792	0.4765	1588.406
2.2	0.5038	0.5012	0.5025	1674.969
2.3	0.2987	0.3065	0.3026	1008.676
2.4	0.4325	0.4408	0.4366	1455.462
	MEAN			1431.88
	SD			296.23
CIDP + hAAT				
Animal number	Abs 1	Abs 2	Mean abs. dilution corr	TNFα conc (pg/mL)
3.1	0.2735	0.2731	0.2733	910.997
3.2	0.3402	0.3566	0.3484	1161.216
3.3	0.2785	0.2814	0.2799	933.130
3.4	0.3569	0.3520	0.3545	1181.571
	MEAN			1046.73
	SD			144.47

Table 34 B:

Plasma TNF α concentration, pg/mL (Mean $\pm$ SD)			
	Day 1	Day 20	Day 35
WT control	155.32 $\pm$ 15.66	168.29 $\pm$ 36.44	136.84 $\pm$ 26.09
CIDP + vehicle	141.43 $\pm$ 11.58	1209.71 $\pm$ 310.56	1431.88 $\pm$ 296.23
CIDP + AAT	138.46 $\pm$ 18.58	1397.62 $\pm$ 330.96	1046.73 $\pm$ 144.47

Table 35:

Two-way ANOVA					
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	20.94	<0.0001	****	Yes	
Row Factor	36.3	<0.0001	****	Yes	
Column Factor	35.18	<0.0001	****	Yes	
ANOVA table		SS	DF	MS	F (DFn, Dfd)
Interaction		2629000	4	657345	F (4, 27) = 18.64
Row Factor		4558000	2	2279000	F (2, 27) = 64.64
Column Factor		4417000	2	2209000	F (2, 27) = 62.64
Residual		952014	27	35260	

Bonferroni's multiple comparisons test				
Groups	Mean Diff.	95% CI of diff.	Significant?	Summary
Day 1				
Sham negative control vs. CIDP	13.89	-325.0 to 352.8	No	ns
Sham negative control vs. CIDP	16.86	-322.1 to 355.8	No	ns
CIDP + vehicle vs. CIDP + hAAT	2 967	-335.9 to 341.9	No	ns
Day 20				
Sham negative control vs. CIDP	-1041	-1380 to -702.5	Yes	****
Sham negative control vs. CIDP	-1229	-1568 to -890.4	Yes	****
CIDP + vehicle vs. CIDP + hAAT	-187.9	-526.8 to 151.0	No	ns
Day 35				
Sham negative control vs. CIDP	-1295	-1634 to -956.1	Yes	****
Sham negative control vs. CIDP	-909.9	-1249 to -571.0	Yes	****
CIDP + vehicle vs. CIDP + hAAT	385.1	46.24 to 724.1	Yes	*

Example 20

Compound Muscle Action Potential (CMAP) significantly increases after two weeks of AAT therapy.

CMT MOUSE MODEL: Non-humanized CMT1A mice B6.Cg-Tg(PMP22)C3Fbas/J (three copies of the PMP22 gene), three groups of 8 animals each.

DOSAGE: 180 mg/kg/day non-clinical grade AAT (Sigma)

ADMINISTRATION: Subcutaneous (SC) (bi-daily injection of 90mg/kg)

Key disease progression parameters significantly improve after two weeks of AAT treatment (180mg/kg/day) see Fig. 25-30.

Table 36: Rotarod latency

Rotarod latency at baseline (6 weeks old) (in SECONDS)				
WT + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
1.1	110.71	102.76	80.81	98.09
1.2	79.52	64.63	78.92	74.35
1.3	105.19	100.84	101.23	102.42
1.4	99.55	66.29	85.74	83.86
1.5	89.53	73.16	108.00	90.23
1.6	74.92	91.57	68.16	78.21
1.7	86.69	61.17	74.64	74.16
1.8	98.01	67.17	98.26	87.81
Mean				86.14
SD				10.54

CMT + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
2.1	48.42	85.90	68.19	67.50
2.2	29.20	32.19	45.39	35.59
2.3	72.34	53.17	70.72	65.41
2.4	50.97	34.95	101.40	62.44
2.5	118.89	50.77	112.19	93.95
2.6	110.30	108.33	99.29	105.98
2.7	51.52	73.49	93.01	72.67
2.8	66.48	71.66	92.09	76.74
Mean				72.54
SD				21.17

CMT + hAAT				
Animal number	set 1	set 2	set 3	Mean / animal
3.1	53.47	57.11	49.72	53.43
3.2	57.35	51.61	49.96	52.98
3.3	69.94	67.40	62.87	66.74
3.4	62.11	54.79	53.98	56.96
3.5	65.03	72.92	62.30	66.75
3.6	66.86	68.48	61.35	65.56
3.7	110.28	109.55	100.14	106.66
3.8	93.16	105.36	109.32	102.61
Mean				71.46
SD				21.27

Rotarod latency at 8 weeks old (in SECONDS)				
WT + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
1.1	67.78	72.14	62.65	67.52
1.2	69.78	62.80	62.61	65.06
1.3	84.19	84.84	76.98	82.00
1.4	74.26	71.83	68.83	71.64
1.5	82.04	89.50	75.59	82.38
1.6	83.77	82.87	77.37	81.34
1.7	119.07	115.57	116.66	117.10
1.8	113.70	116.61	118.35	116.22
Mean				85.41
SD				20.40

CMT + vehicle				
Animal number	set 1	set 2	set 3	Mean / animal
2.1	29.63	47.21	34.04	36.96
2.2	27.67	46.34	14.19	29.40
2.3	25.63	63.48	30.51	39.87
2.4	45.78	48.26	29.22	41.09
2.5	23.74	61.79	24.54	36.69
2.6	40.17	59.89	29.58	43.22
2.7	26.20	50.80	23.70	33.57
2.8	33.95	55.89	36.66	42.17
Mean				37.87
SD				4.69

CMT + hAAT				
Animal number	set 1	set 2	set 3	Mean / animal
3.1	55.48	61.08	72.89	63.15
3.2	48.88	61.14	45.41	51.81
3.3	60.00	48.80	53.05	53.95
3.4	47.76	53.19	55.82	52.25
3.5	30.45	62.45	22.62	38.51
3.6	96.29	79.58	100.40	92.09
3.7	74.06	65.45	82.56	74.02
3.8	68.88	74.87	81.93	75.23
Mean				62.63
SD				17.07

Table 37: Grip strength

Grip strength at baseline (6 weeks old) (in NEWTONS)				
Animal number	WT + vehide			Mean / animal
	set 1	set 2	set 3	
1.1	4.75	6.48	3.56	4.93
1.2	4.33	5.13	5.26	4.91
1.3	6.14	7.87	5.03	6.35
1.4	6.71	4.20	5.45	5.45
1.5	7.71	7.94	5.48	7.04
1.6	7.44	5.69	5.57	6.24
1.7	9.76	9.84	10.84	10.15
1.8	9.78	9.79	10.62	10.07
			Mean	6.89
			SD	2.11

Animal number	CMT + vehide			Mean / animal
	set 1	set 2	set 3	
2.1	3.48	8.97	4.77	5.74
2.2	2.45	2.14	2.32	2.30
2.3	6.77	4.30	6.43	5.83
2.4	3.55	3.48	8.31	5.12
2.5	5.16	4.98	4.49	4.88
2.6	8.64	5.85	7.83	7.44
2.7	3.48	6.61	9.39	6.50
2.8	4.43	5.46	8.72	6.20
			Mean	5.50
			SD	1.52

Animal number	CMT + hAAT			Mean / animal
	set 1	set 2	set 3	
3.1	6.56	3.79	4.52	4.96
3.2	5.62	4.00	4.69	4.77
3.3	7.24	7.19	5.93	6.79
3.4	5.90	8.88	5.37	6.72
3.5	4.97	7.60	6.66	6.41
3.6	4.35	6.25	3.63	4.74
3.7	6.08	2.61	4.13	4.27
3.8	6.51	3.97	6.57	5.68
			Mean	5.54
			SD	0.99

Grip strength at 8 weeks old (in NEWTONS)				
Animal number	WT + vehide			Mean / animal
	set 1	set 2	set 3	
1.1	5.85	8.59	6.52	6.99
1.2	7.13	5.42	6.25	6.27
1.3	8.48	8.29	7.59	8.12
1.4	6.62	7.19	6.44	6.75
1.5	7.67	8.69	6.17	7.51
1.6	5.47	4.30	4.17	4.65
1.7	7.65	8.82	7.58	8.01
1.8	7.78	8.81	8.00	8.19
			Mean	7.06
			SD	1.20

Animal number	CMT + vehide			Mean / animal
	set 1	set 2	set 3	
2.1	2.47	2.38	2.33	2.39
2.2	2.65	2.65	1.04	2.12
2.3	0.97	7.40	2.83	3.73
2.4	5.29	3.46	3.36	4.03
2.5	0.96	4.18	3.01	2.72
2.6	2.76	4.51	3.10	3.46
2.7	1.93	3.44	1.67	2.35
2.8	3.34	5.06	1.20	3.20
			Mean	3.00
			SD	0.71

Animal number	CMT + hAAT			Mean / animal
	set 1	set 2	set 3	
3.1	3.97	5.37	5.14	4.83
3.2	2.15	4.34	1.69	2.73
3.3	3.47	2.22	3.21	2.97
3.4	4.13	4.58	3.55	4.09
3.5	4.85	3.84	6.47	5.05
3.6	8.17	5.69	8.92	7.59
3.7	6.20	4.40	5.99	5.53
3.8	4.87	5.39	6.77	5.68
			Mean	4.81
			SD	1.57

Table 38: Electrophysiology

WT + vehicle								
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 1,1	7.404	7.700	3.959	4.628	4.296	4.886	44.476	58.132
Mouse 1,2	9.509	5.513	5.755	6.457	6.339	6.964	25.702	29.554
Mouse 1,3	5.676	5.971	4.793	4.463	5.468	5.057	22.227	25.237
Mouse 1,4	3.969	7.500	4.595	4.519	5.056	5.001	32.545	31.101
Mouse 1,5	4.658	6.293	1.995	3.578	2.526	4.131	28.220	27.093
Mouse 1,6	10.121	9.150	3.629	4.959	4.241	5.639	24.508	22.070
Mouse 1,7	6.668	7.655	5.115	5.394	5.406	5.740	51.553	43.468
Mouse 1,8	9.740	8.402	3.940	4.494	4.650	5.116	21.125	24.117
MEAN	7.218	7.273					31.294	32.597
SD	2.386	1.250					11.072	12.241

CMT + vehicle								
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 2,1	4.758	2.137	6.881	1.955	7.521	3.407	23.447	10.327
Mouse 2,2	6.698	1.927	3.939	4.220	4.616	5.557	22.165	11.224
Mouse 2,3	7.307	2.943	2.992	3.423	3.399	4.807	36.829	10.845
Mouse 2,4	5.180	3.395	3.879	3.066	4.645	4.691	19.581	9.231
Mouse 2,5	8.049	4.452	3.120	3.607	3.764	4.828	23.288	12.278
Mouse 2,6	7.274	1.850	3.677	3.999	4.195	5.367	28.930	10.965
Mouse 2,7	7.054	3.429	3.001	5.501	3.874	7.161	17.179	9.039
Mouse 2,8	5.498	1.858	4.094	2.417	4.567	3.911	31.671	10.040
MEAN	6.477	2.749					25.386	10.494
SD	1.182	0.961					6.575	1.069

CMT + hAAT								
	Amplitude (mV)		T1 distal (ms)		T2 proximal (ms)		Conduction velocity (m/s)	
	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old	6 weeks old	8 weeks old
Mouse 3,1	7.828	4.874	7.416	3.916	7.885	4.718	31.949	18.707
Mouse 3,2	3.009	5.376	5.701	3.259	6.130	4.170	34.989	16.462
Mouse 3,3	5.770	5.322	12.428	3.449	12.922	4.945	30.328	10.029
Mouse 3,4	5.919	9.084	5.913	4.291	6.694	5.072	19.190	19.200
Mouse 3,5	7.925	6.115	6.041	3.773	6.869	4.624	18.114	17.615
Mouse 3,6	8.742	3.753	3.664	8.247	4.395	9.802	20.530	9.648
Mouse 3,7	6.803	5.277	4.204	7.467	4.775	8.057	26.247	25.446
Mouse 3,8	7.028	4.139	3.385	11.449	3.906	12.262	28.783	18.460
MEAN	6.628	5.492					26.266	16.946
SD	1.778	1.631					6.332	5.134

Table 39: TNF α

Plasma TNF- α concentration at 8 weeks old (in pg/mL)				
WT + vehicle				
Animal number	Abs 1	Abs 2	Mean Abs	TNF- α concentration (pg/mL)
1.1	0.54375	0.55496	0.54935	3.92
1.2	0.40014	0.39642	0.39828	2.73
1.3	0.39928	0.43564	0.41746	2.88
1.4	0.38018	0.39604	0.38811	2.66
1.5	0.40955	0.44471	0.42713	2.96
1.6	0.49326	0.51042	0.50184	3.54
1.7	0.54415	0.54906	0.54661	3.90
1.8	0.40389	0.41290	0.40840	2.81
Mean				3.18
SD				0.53

CMT + vehicle				
Animal number	Abs 1	Abs 2	Mean Abs	TNF- α concentration (pg/mL)
2.1	1.35282	1.35734	1.35508	10.22
2.2	1.93280	1.92613	1.92946	14.72
2.3	1.44294	1.48421	1.46357	11.07
2.4	1.57975	1.65685	1.61830	12.28
2.5	1.73361	1.68169	1.70765	12.98
2.6	1.21567	1.21985	1.21776	9.15
2.7	1.51298	1.51888	1.51593	11.48
2.8	1.80332	1.78964	1.79648	13.68
Mean				11.95
SD				1.84

CMT + hAAT				
Animal number	Abs 1	Abs 2	Mean Abs	TNF- α concentration (pg/mL)
3.1	0.92930	0.96779	0.94855	7.04
3.2	1.07430	1.04042	1.05736	7.89
3.3	1.07575	1.04322	1.05949	7.91
3.4	0.72589	0.76331	0.74460	5.44
3.5	1.07604	1.04189	1.05897	7.90
3.6	0.71104	0.75262	0.73183	5.34
3.7	1.09751	1.05099	1.07425	8.02
3.8	0.80920	0.80948	0.80934	5.95
Mean				6.94
SD				1.18

Example 21 - Efficacy of AAT in a mouse model of Charcot-Marie-Tooth disease

Charcot-Marie-Tooth disease (CMT) is a hereditary motor and sensory neuropathy of the peripheral nervous system characterized by a progressive loss of muscle tissue and a dysfunction of the tactile sensation in different parts of the body. Currently incurable, this disease is the most prevalent hereditary neurological disorder and affects approximately one in 2,500 people. The most common type of CMT is CMT1A, characterized by a duplication of the *pmp22* gene leading to an accumulation of the *pmp22* protein in the Schwann cell and progressive demyelination. PMP22 is a tetraspan glycoprotein contained in compact myelin of the peripheral nervous system. Duplication of PMP22 has been associated with the onset of Charcot-Marie-Tooth disease type 1A (CMT1A).

The C3-PMP22 transgenic mice (B6.Cg-Tg(PMP22)C3Fbas/J) express three copies of a wild-type human peripheral myelin protein 22 (PMP22) gene. These mice present an age-dependent demyelinating neuropathy characterized by predominantly distal loss of strength and sensation. C3-PMP mice show no overt clinical signs at 3 weeks and develop mild neuromuscular impairment in an age-dependent manner. They have stable, low nerve conduction velocities similar to adults with human CMT1A. Myelination is delayed in these mice, and they contain reduced numbers of myelinated axons at 3 weeks of age.

In CMT1A, the major component of the disease is a developmental abnormality of myelin formation (dysmyelination). Myelin is produced by SC in the peripheral nervous system and is crucial for proper transmission of the electric impulse in the nerves. The primary dysmyelination seen in CMT1A patients leads to an abnormal SC organization around axons and a uniformly slowed nerve conduction velocity.

It has been shown that the transmembrane protein ADAM17 (also known as TACE for Tumor necrosis factor- α -converting enzyme) blocks the myelination of axons in SC by inhibiting neuregulin 1 type III signal (NRG1-III) (La Marca et al., 2011; Pisciotta et al., 2021). AAT can be a promising treatment of CMT by the modulation of NRG1-III signaling via its pharmacological inhibition of ADAM17. The inhibition of ADAM17 by AAT promotes SC myelination in the peripheral nervous system and can therefore reduce the progression of CMT disease.

In addition to its inhibitory function on ADAM17, another mechanism of AAT in the treatment of CMT is its anti-inflammatory activity. AAT exhibits broad anti-inflammatory and immunomodulatory activity. Increasing AAT serum concentration by the administration of exogenous AAT to above-normal levels is expected to be therapeutic in CMT.

B6.Cg-Tg(PMP22)C3Fbas/J transgenic mice express three copies of a wild-type human peripheral myelin protein 22 (PMP22) gene. They present an age-dependent demyelinating neuropathy characterized by predominantly distal loss of strength and sensation. These mice show no overt clinical signs at 3 weeks and develop mild neuromuscular impairment in an age-dependent manner, which are fully established at 8 weeks of age. They have stable, low nerve conduction velocities similar to adults with human CMT1A. Myelination is delayed in these mice, and they contain reduced numbers of myelinated axons.

Materials and methods

Animals were received at 6 weeks of age and housed under controlled conditions for the duration of the study. The study comprised two groups of 8 transgenic mice (CMT mice) each and one group of 8 age matched wild type (WT) C57Bl6 mice, serving as positive control. Animals were assigned to the following subcutaneous treatments twice daily at 7:00AM and 7:00PM for 2 weeks, starting from 8 weeks and up to 10 weeks of age:

- Wild-type (WT, no disease) mice receiving vehicle, 0.9% NaCl (positive control)
- CMT1A mice receiving vehicle, 0.9% NaCl (negative control)
- CMT1A mice receiving AAT at 90mg/kg per injection (daily dose 180 mg/kg)

The efficacy parameters included:

- neuromuscular performance (rotarod latency to fall and grip strength test),
- sciatic nerve electrophysiology (CMAP amplitude and NCV)
- cytokine (IL-6 and TNF α), as well as NfL analysis
- sciatic nerve histology (number of axons and axonal diameter),

On the first day of treatment (Day 1), the neuromuscular and performance of animals was tested with a rotarod test and a grip strength test, as well as sciatic nerve electrophysiology test, which measured the compound muscle action potential (CMAP) and the nerve conduction velocity (NCV). After the last treatment on Day 15, these tests were repeated. Animals were then left untreated for 2 additional weeks to evaluate the duration of the treatment effect. Following the 2-week treatment-free period until 12 weeks of age (Day 30), the behavioural and electrophysiological tests were repeated.

As supportive information for the mode of action of AAT in CMT, plasma concentration of interleukin 6 (IL-6) and $\text{TNF}\alpha$ were measured at Day 1 (8 weeks), Day 15 (10 weeks) and Day 30 (12 weeks). At the same timepoints, plasma levels of the biomarker of nerve damage, NfL were determined.

Results

At 8 weeks of age (baseline), CMT mice show evident clinical signs/symptoms of the disease compared to the normal wild-type mice. The neuromuscular impairment (decreased latency to fall) can be seen in Figure 25 and in Table 40A

Table 40A: Mean rotarod latency in CMT mice treated with and without AAT

Rotarod latency, seconds (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	85.31 $\pm$ 12.71	87.13 $\pm$ 11.91	88.20 $\pm$ 27.50
CMT1A + vehicle	39.02 $\pm$ 4.05	32.31 $\pm$ 5.81	23.56 $\pm$ 3.52
CMT1A + AAT	38.76 $\pm$ 4.90	57.77 $\pm$ 19.19	36.92 $\pm$ 1.66

Decreased grip strength in CMT mice compared to wild-type mice can be seen in Figure 26 and in Table 40B.

Table 40B Mean grip strength of CMT mice treated with and without AAT

Grip strength, newtons (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	7.14 $\pm$ 0.66	7.58 $\pm$ 1.40	7.69 $\pm$ 1.17
CMT1A + vehicle	3.34 $\pm$ 0.39	3.27 $\pm$ 0.32	2.74 $\pm$ 0.57
CMT1A + AAT	3.35 $\pm$ 0.27	5.47 $\pm$ 1.11	3.87 $\pm$ 0.36

The decrease of nerve CMAP amplitude is shown in Figure 27 and in Table 40C

Table 40C: Mean CMAP of CMT mice treated with and without AAT

Compound muscle action potential, mV (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	7.55 $\pm$ 1.26	7.66 $\pm$ 1.15	7.97 $\pm$ 1.33
CMT1A + vehicle	3.72 $\pm$ 0.48	2.63 $\pm$ 0.56	1.81 $\pm$ 0.56
CMT1A + AAT	3.76 $\pm$ 0.50	5.41 $\pm$ 0.71	2.94 $\pm$ 0.65

and the decrease of sciatic nerve velocity is shown in Figure 28 and in Table 40D.

Table 40D: Mean NCV of CMT mice treated with and without AAT

Nerve conduction velocity, m/s (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	32.78 $\pm$ 8.29	34.10 $\pm$ 5.94	33.81 $\pm$ 10.49
CMT1A + vehicle	16.01 $\pm$ 3.05	10.68 $\pm$ 4.12	6.71 $\pm$ 0.75
CMT1A + AAT	15.96 $\pm$ 1.54	21.47 $\pm$ 6.07	10.50 $\pm$ 1.73

The plasma cytokine levels of IL-6 and TNF α are increased in CMT mice (Table 40E, Table 40F and Figure 29 and Figure 30), as well as the biomarker of nerve damage NfL (Table 40G and Figure 40).

Table 40E: Mean plasma IL-6 concentration of CMT mice treated with and without AAT

Plasma IL-6, pg/mL (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	36.23 $\pm$ 5.99	36.50 $\pm$ 5.88	36.42 $\pm$ 3.28
CMT1A + vehicle	132.90 $\pm$ 21.48	150.91 $\pm$ 31.61	165.13 $\pm$ 8.40
CMT1A + AAT	133.48 $\pm$ 31.45	69.78 $\pm$ 11.76	120.90 $\pm$ 6.47

Table 40F: Mean plasma TNF α concentration of CMT mice treated with and without AAT

Plasma TNF α , pg/mL (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	3.46 $\pm$ 0.40	3.01 $\pm$ 0.54	3.13 $\pm$ 0.33
CMT1A + vehicle	9.73 $\pm$ 1.73	12.69 $\pm$ 1.24	14.09 $\pm$ 0.55
CMT1A + AAT	9.80 $\pm$ 2.25	6.55 $\pm$ 2.11	11.61 $\pm$ 1.00

Table 40G: Mean plasma NfL concentration of CMT mice treated with and without AAT

Plasma NfL, ng/mL (Mean $\pm$ SD)			
	8 weeks old	10 weeks old	12 weeks old
WT control	0.31 $\pm$ 0.19	0.32 $\pm$ 0.19	0.36 $\pm$ 0.13
CMT1A + vehicle	3.88 $\pm$ 0.53	4.48 $\pm$ 0.54	6.02 $\pm$ 0.94
CMT1A + AAT	3.83 $\pm$ 0.61	1.96 $\pm$ 0.46	4.25 $\pm$ 0.36

Table 40H: Mean histological data of CMT mice treated with and without AAT

Sciatic nerve histology (Mean $\pm$ SD)			
	Axons/100 μ m ²	Axonal diameter (μ m)	g-ratio
WT control	40.80 $\pm$ 1.90	5.25 $\pm$ 3.56	0.56 $\pm$ 0.12
CMT1A + vehicle	16.90 $\pm$ 1.20	2.38 $\pm$ 0.73	0.75 $\pm$ 0.09
CMT1A + AAT	26.30 $\pm$ 2.00	3.84 $\pm$ 0.25	0.62 $\pm$ 0.16

All these clinical signs/symptoms present in CMT mice were fully established as expected at 8 weeks of age. Furthermore, the signs evolved with time and became more severe at 10 weeks of age and their severity still increased at 12 weeks of age in the control CMT mice.

The treatment with AAT started at 8 weeks of age and lasted 2 weeks, until 10 weeks of age. The results show that AAT increased neuromuscular and electrophysiological performances when administered twice a day at 90 mg/kg by subcutaneous route in CMT mice. CMT mice treated with AAT showed a significant increase in rotarod latency to fall (****: $P < 0.0001$ vs WT + vehicle; ###: $P < 0.001$ vs CMT + vehicle, ANOVA two-way and Bonferroni test; ns: non-significant, $P > 0.05$ Figure 25), significant increase in grip strength (****: $P < 0.0001$ vs WT + vehicle; #, #####: $P < 0.05$, $P < 0.0001$ vs CMT + vehicle, ANOVA two-way and Bonferroni test; ns: non-significant, $P > 0.05$ Figure 26), significant increase in compound muscle action potential amplitude (****: $P < 0.0001$ vs WT + vehicle; #, ###: $P < 0.05$, $P < 0.001$ vs CMT + vehicle, ANOVA two-way and Bonferroni test; ns: non-significant, $P > 0.05$ Figure 27) and significant increase in nerve conduction velocity (****: $P < 0.0001$ vs WT + vehicle; ###: $P < 0.001$ vs CMT + vehicle, ANOVA two-way and Bonferroni test; ns: non-significant, $P > 0.05$ Figure 28) when compared to CMT mice not treated with AAT. AAT reduced the plasma levels of the inflammatory cytokines IL-6 ($P < 0.01$) and TNF α ($P < 0.0001$); (Table 40E, Table 40F, Figure 29 and Figure 30), as well as the level of NfL (Table 40G and Figure 40).

At 12 weeks of age following the 2-week treatment-free period, the effect of AAT was decreased, suggesting that repeated administrations of AAT are necessary to obtain a

sustained effect. At this time, the grip strength and CMAP of the AAT treated mice had decreased relative to the end of the treatment period, while remaining significantly higher than for the untreated CMT animals ($P < 0.05$ for both parameters). The positive effect remained significant for the IL-6, TNF α and NfL plasma levels, and even after 2 weeks treatment free period, the number of axons and the size of the myelin sheath were significantly larger in the treated animals. The only parameters becoming non-significantly different from the untreated group were the rotarod latency to fall and NCV.

Conclusion

AAT therapy was evaluated in a relevant mouse model of CMT disease. At baseline, at 8 weeks of age, the disease was fully established, and the animals showed clear clinical signs and symptoms of CMT1A. AAT treatment was associated with improvement of neuropathy, as shown by a significant increase in neuromuscular and electrophysiological performances as well as an increase of the number of axons and axonal diameter and decrease of g-ratio relative to untreated CMT animals. A decrease of plasma cytokines (TNF α and IL-6) and NfL biomarker was also observed in the treated animals. It has been demonstrated that AAT was able to reduce the progression of the disease. The effects of treatment with AAT were limited in time, and 2 weeks after treatment cessation, the symptoms had worsened, although not reaching the level of the untreated CMT animals.

Taken together, these data demonstrate the potential for AAT to improve CMT1A patient outcomes, by not only reducing the progression of the disease but reversing its symptoms.

Example 22 Inhibitor effect of AAT-related peptides and peptidomimetic on the ADAM17 activity

Biologically active immunoregulatory sites (not associated with canonical anti-protease activity) on the surface of AAT were identified by in silico methods and several peptides were derived from those immunoregulatory sites (Lior, Yotam, et al. European Journal of Medicinal Chemistry 228 (2022): 113969). The potential of two peptides (peptide 8 and peptide 9) and one peptidomimetic (peptide 14) to modulate the ADAM17 activity was investigated.

Peptide 8 (sequence: Ac-YRAHQGE-NH₂; MW: 945.4) and peptide 9 (sequence: Ac-LFLYVIH-NH₂; MW: 901.3) were synthesized using a Syro-1 automatic peptide synthesizer and the Fmoc/tBu strategy (Fig. 42). Peptide 14 (4-Hydroxy-Bzl-His-Phg-NH₂[TFA salt]; MW: 394.2) was synthesized manually using the Fmoc/tBu strategy (Fig. 43).

ADAM-17 activity and its inhibition by human peptide 8 was measured with Recombinant Human ADAM-17 kit (Recombinant Human TACE/ADAM17 Protein, CF: 930-ADB-010 and Mca-PLAQAV-Dpa-RSSSR-NH₂ Fluorogenic Peptide Substrate:ES003; R&D Systems) in black 96-well immunoplates (437111; Thermo Fisher Scientific). The enzymatic activity of ADAM-17 was measured by mixing 0.005 µg of rhADAM-17 with 10 µM of Mca-PLAQAV-Dpa-RSSSR-NH₂ fluorogenic peptide substrate III in assay buffer (25 mM Tris, 2.5 µM ZnCl₂, 0.005% Brij-35 (w/v), pH 9.0) to a final volume of 100 µL. Peptide 8 (Syngene) was dissolved in DMSO and diluted to the desired concentration in assay buffer. Control ADAM-17 activity was assessed in the presence of the assay buffer. Peptide 8 was added at different concentrations in 2 separate experiments (0, 10 and 50 µM in experiment 1; 0, 100, 200 and 250 µM in experiment 2) to assess its dose dependent inhibition of ADAM-17. All conditions were performed in triplicate. Activity was measured as relative fluorescent units (RFU) in endpoint mode (5 min) with a SpectraMax iD3 Microplate Reader (automatic PMT gain, wavelengths: excitation 320 nm, emission 405 nm).

No significant inhibition of ADAM17 activity was noted at concentrations up to 50 µM (Figure 31 left graph), while a significant concentration dependent inhibition was observed at concentrations of 100 (36% inhibition) and 200 (65% inhibition) (Figure 31, right graph).

Example 23 Modulatory effect of AAT on TNFα-induced Schwann cell activation

Human Schwann cells (P10351, Innoprot) were seeded in 12 well plates at 120000 cells per well in DMEM. Cells were pre-treated with either buffer (controls) or AAT (50 µM) for 24 hours before stimulation by TNFα (10 ng/mL). The plates were then incubated at 37°C for 24 hours. The RNA was extracted and selected target gene expression was measured by qPCR.

The rationale for gene selection is explained below.

By binding to TNFR1, TNF can activate nuclear factor κ B (NF- κ B), which drives cell survival signalling as well as cell death. NF- κ B activation induces transcriptional upregulation of inflammasome regulators such as IL-1 β , IL-18, TNF α and IFN- γ , mediating the inflammatory response. Moreover, TNFR1 can directly induce oxidative stress by the activation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) producing enzymes.

The genes evaluated for their regulation included the NF- κ B early gene response NFKBIA, as well as the TNF α and TNFR1 genes. Expression of the gene coding for the IL-6 pro-inflammatory cytokine was also evaluated.

The nuclear factor E2-related factor 2 (Nrf2) plays a crucial role in regulating cellular redox state in various physiological and pathological processes. Under normal physiological conditions, Nrf2 is not biologically active, nor does it activate downstream genes. Through interaction with antioxidant response elements (ARE) of cytoprotective genes, Nrf2 activates antioxidant enzymes, such as SOD, CAT, HO-1, NAD(P)H oxidase (G6PD) and others.

The TXN system plays an important role in maintaining a reduced environment in the cell. Thioredoxin (TXN) is a thiol-oxidoreductase that is a major regulator of cellular redox signaling which protects cells from oxidative stress. TXNIP interacts directly with TXN, inhibiting its ability to scavenge reactive oxygen species (ROS). It has been demonstrated that TXNIP is upregulated in diseases such as type 2 diabetes mellitus as well as neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

As a potential early response gene, TXNIP is tightly regulated and strongly correlated with changes in mRNA levels and protein levels, and it has been shown to play an important role in the dysfunction of Schwann Cells in diabetic peripheral neuropathy.

As shown in Figure 32 to Figure 34, the NF- κ B pathway transcripts were downregulated by AAT treatment after TNF induction. AAT inhibited the expression of genes modulated by TNF-induced oxidative stress response. Taken together, these results show the modulating effects of AAT on the TNF α -induced gene expression in human Schwann cells.

Example 24

Microglial (HMC3) cells were seeded into 96-well plates at a density of approximately 2500 cells/well. Plasma derived AAT (Sigma, 25 μ M) or peptide 8, peptide 9 or peptide 14 (Syngene, 50 μ M) were added, and microglial activation was induced with interferon gamma (IFN γ) (10 ng/mL) for 24 h with no preincubation. Then, the RNA was extracted (RNAeasy mini kit, Qiagen) and the expression of the Interleukin-6 (IL6) and Interleukin-1beta (IL1B) genes were analysed. qPCR was performed with Syber green POWER UP master mix using 10 ng/well of cDNA in all gene expression results.

The results show that IL6 expression was reduced by 30-40% and IL1B expression was reduced by 60-70% by AAT and its derivate peptides (Figure 41), suggesting that all three peptides mimic AAT function in an inflammatory response.

Example 25 Effect of AAT and its derivative peptides on TNF induced Schwann cells.

Schwann cells were seeded at a density of 75000 per well. At the time of seeding, plasma derived AAT (50uM) or peptide 8,9,14 (Syngene, 50uM) was added. The following day, cells were treated with 10ng/ml of TNF alpha with or without AAT and its derivate peptides for 24 hours.

The NAD(P)H oxidase (G6PD) expression is induced by TNF treatment in Schwann cells and reduced by 40-30% in stimulated cells treated with AAT and its derivative peptides (Figure 35). G6PD plays a significant role in the generation of ROS.

The invention further relate to the following items:

1. A pharmaceutical product for use in the treatment of chronic inflammatory demyelinating polyneuropathy, the pharmaceutical product comprising:
 - a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity.
2. A kit of parts for use in the treatment of a disease or disorder of the nervous system, the kit comprising:

- i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity; and
 - ii) a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof.
- 3. A pharmaceutical composition for use in treatment of a disease or disorder of the nervous system, the composition comprising:
 - i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof having ADAM17 inhibitory activity; and
 - ii) a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof; and
 - iii) at least one pharmaceutically acceptable carrier.
- 4. A method of treatment comprising administering an effective amount of a pharmaceutical composition comprising AAT protein and/or a nucleic acid encoding AAT to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof.
- 5. A method of treatment comprising administering an effective amount of a pharmaceutical compound comprising a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof to a subject, wherein the subject is suffering from a disease or disorder of the nervous system and wherein the subject is undergoing a therapy comprising administration of AAT protein and/or a nucleic acid encoding AAT.
- 6. The kit of parts for use of item 2, the pharmaceutical composition for use of item 3, the method of treatment of item 4 or 5, wherein the disease or disorder of the nervous system is chronic inflammatory demyelinating polyneuropathy.

7. The pharmaceutical product for use of item 1, the kit of parts for use of item 6, the pharmaceutical composition for use of item 6, the method of treatment of item 6, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.
8. The kit of parts for use of any one of the items 2, 6 or 7, the pharmaceutical composition for use of any one of the items 3, 6 or 7, the method of treatment of any one of the items 4 to 7, wherein the plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof are plasma derived IgG antibodies, IgG variants, isoforms and/or fragments thereof.
9. The kit of parts for use of any one of the items 2, 6 or 7, the pharmaceutical composition for use of any one of the items 3, 6 or 7, the method of treatment of any one of the items 4 to 7, wherein the plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof are recombinant IgG antibodies, IgG variants, isoforms and/or fragments thereof.
10. The kit of parts for use of any one of the items 2, 6 to 9, the pharmaceutical composition for use of any one of the items 3, 6 to 9, the method of treatment of any one of the items 4 to 9, wherein the plasma derived IgG antibodies, IgG variants, isoforms and/or fragments thereof are formulated for intravenous administration.
11. The kit of parts for use of any one of the items 2, 6 to 9, the pharmaceutical composition for use of any one of the items 3, 6 to 9, the method of treatment of any one of the items 4 to 9, wherein the plasma derived IgG antibodies, IgG variants, isoforms and/or fragments thereof are formulated for subcutaneous administration.
12. The pharmaceutical product for use of any one of the items 1 or 7, the kit of parts for use of any one of the items 2, 6 to 11, the pharmaceutical composition for use of any one of the items 3, 6 to 11, the method of treatment of any one of the items 4 to 11, wherein the AAT protein is recombinant AAT.
13. The pharmaceutical product for use of any one of the items 1 or 7, the kit of parts for use of any one of the items 2, 6 to 11, the pharmaceutical composition for use

of any one of the items 3, 6 to 11, the method of treatment of any one of the items 4 to 11, wherein the AAT protein is plasma derived AAT.

14. The pharmaceutical product for use of any one of the items 1, 7, 12 or 13, the kit of parts for use of any one of the items 2, 6 to 13, the pharmaceutical composition for use of any one of the items 3, 6 to 13, the method of treatment of any one of the items 4 to 13, wherein

a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof having ADAM17 inhibitory activity; and/or

b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity

is/are formulated for intravenous administration.

15. The pharmaceutical product for use of any one of the items 1, 7, 12 or 13, the kit of parts for use of any one of the items 2, 6 to 13, the pharmaceutical composition for use of any one of the items 3, 6 to 13, the method of treatment of any one of the items 4 to 13, wherein

a) the alpha1-antitrypsin (AAT) protein, variant, isoform and/or a fragment thereof having ADAM17 inhibitory activity; and/or

b) the nucleic acid encoding AAT, variant, isoform and/or fragment thereof having ADAM17 inhibitory activity

is/are formulated for subcutaneous administration.

SEQ ID NO: 1:

MPSSVSWGILLLAGLCCLVPVSLAEDPQGDAQAQKTDTSHTDQDHPTFNKITPNLAE
FAFSLYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQ
IHEGFQELLRTLNPDSQLQLTTGNGLFLSEGLKLVDFLEDVKKLYHSEFTVNFG
DTEEAQKQINDYVEKGTQGGKIVDLVKELDRDVFALVNYIFFKGKWERPFVVKDTEE
EDFHVDQVTTVKVPMKRLGMFNIQHCKKLSSWLLMKYLGNAIAIFFLPDEGKLQ
HLENELTHDIITKFLNEDRRSASLHLPKLSITGTDLKSVLGQLGITKVFSNGADLSG
VTTEAPLKLKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQN
TKSPLFMGKVVNPTQK

SEQ ID NO: 2:

VFALVNYIFFKGKWERPFEVKDTEEEEDFHVDQVTTVKVPMMKRLGMFNIQHCKKLS
SWVLLMKYLGNATAIFFLPDEGKLQHLENELTHDIITKFLNEDRRSASLHLPKLSITG
TYDLKSVLGQLGITKVFSNGADLSGVTEEAPLKLSKAVHKAVLTIDEKGTEAAGAMF
LEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK

Claims

1. A pharmaceutical product for use in the treatment of an inflammatory disease or disorder, the pharmaceutical product comprising:
 - a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has ADAM17 inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.
2. The pharmaceutical product for use of claim 1, wherein the inflammatory disease or disorder is an autoimmune inflammatory disease.
3. The pharmaceutical product for use of claim 1 or 2, wherein the inflammatory disease or disorder is an inflammatory disease or disorder of the nervous system, preferably wherein the inflammatory disease or disorder is neuropathic pain.
4. The pharmaceutical product for use of any one of claims 1 to 3, wherein the inflammatory disease or disorder is chronic inflammatory demyelinating polyneuropathy.
5. The pharmaceutical product for use of claims 1 to 3, wherein the inflammatory disease or disorder is complex regional pain syndrome.
6. The pharmaceutical product for use of any one of claims 1 to 3, wherein the inflammatory disease or disorder is inflammatory pain.
7. A kit of parts for use in the treatment of a disease or disorder of the nervous system, preferably an inflammatory disease or disorder of the nervous system, the kit comprising:
 - i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or

- b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and
 - ii) a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.
- 8. A pharmaceutical composition for use in the treatment of a disease or disorder of the nervous system, preferably an inflammatory disease or disorder of the nervous system, the composition comprising:
 - i) a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity; and
 - ii) a plurality of IgG antibodies, IgG variants, isoforms and/or fragments thereof; and
 - iii) at least one pharmaceutically acceptable carrier.
- 9. A method of treatment comprising administering an effective amount of a pharmaceutical composition to a subject, wherein the subject is suffering from a disease or disorder of the nervous system, preferably an inflammatory disease or disorder of the nervous system, and wherein the subject is undergoing a therapy comprising administration of a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants, the pharmaceutical composition comprising
 - a) AAT protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

10. A method of treatment comprising administering an effective amount of a pharmaceutical compound comprising a plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants to a subject, wherein the subject is suffering from a disease or disorder of the nervous system, preferably an inflammatory disease or disorder of the nervous system, and wherein the subject is undergoing a therapy comprising administration of
 - a) AAT protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or
 - b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.
11. The kit of parts for use of claim 7, the pharmaceutical composition for use of claim 8, the method of treatment of claim 9 or 10, wherein the disease or disorder of the nervous system is pain caused by a disease or disorder of the nervous system, preferably chronic pain caused by a disease or disorder of the nervous system.
12. The kit of parts for use of claim 7 or 11, the pharmaceutical composition for use of claim 8 or 11, the method of treatment of claim 9 to 11, wherein the disease or disorder of the nervous system is an autoimmune disease or disorder of the nervous system.
13. The kit of parts for use of claim 12, the pharmaceutical composition for use of claim 12, the method of treatment of claim 12, wherein the disease or disorder of the nervous system, preferably the inflammatory disease or disorder of the nervous system, is chronic inflammatory demyelinating polyneuropathy.
14. The pharmaceutical product for use of claim 4 or 6, the kit of parts for use of claim 13, the pharmaceutical composition for use of claim 13, the method of treatment of claim 13, wherein a subject to be treated has at least one symptom of chronic inflammatory demyelinating polyneuropathy or a history of at least one symptom of chronic inflammatory demyelinating polyneuropathy.
15. The kit of parts for use of any one of the claims 7, 11 to 14, the pharmaceutical composition for use of any one of the claims 8, 11 to 14, the method of treatment

of any one of the claims 9 to 14, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.

16. The kit of parts for use of any one of the claims 7, 11 to 14, the pharmaceutical composition for use of any one of the claims 8, 11 to 14, the method of treatment of any one of the claims 9 to 14, wherein the plurality of IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are recombinant IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants.
17. The kit of parts for use of any one of the claims 7, 11 to 16, the pharmaceutical composition for use of any one of the claims 8, 11 to 16, the method of treatment of any one of the claims 9 to 16, wherein the plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for intravenous administration.
18. The kit of parts for use of any one of the claims 7, 11 to 16, the pharmaceutical composition for use of any one of the claims 8, 11 to 16, the method of treatment of any one of the claims 9 to 16, wherein the plasma derived IgG antibodies, isoforms thereof, fragments thereof, and/or IgG variants are formulated for subcutaneous administration.
19. The pharmaceutical product for use of any one of the claims 1 to 6, the kit of parts for use of any one of the claims 7, 11 to 18, the pharmaceutical composition for use of any one of the claims 8, 11 to 18, the method of treatment of any one of the claims 9 to 18, wherein the AAT protein is recombinant AAT.
20. The pharmaceutical product for use of any one of the claims 1 to 6, the kit of parts for use of any one of the claims 7, 11 to 18, the pharmaceutical composition for use of any one of the claims 8, 11 to 18, the method of treatment of any one of the claims 9 to 18, wherein the AAT protein is plasma derived AAT.
21. The pharmaceutical product for use of any one of the claims 1 to 6, 19 or 20, the kit of parts for use of any one of the claims 7, 11 to 20, the pharmaceutical composition for use of any one of the claims 8, 11 to 20, the method of treatment of any one of the claims 9 to 20, wherein

a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or

b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

is/are formulated for intravenous administration.

22. The pharmaceutical product for use of any one of the claims 1 to 6, 19 or 20, the kit of parts for use of any one of the claims 7, 11 to 20, the pharmaceutical composition for use of any one of the claims 8, 11 to 20, the method of treatment of any one of the claims 9 to 20, wherein

a) alpha1-antitrypsin (AAT) protein, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity or a small molecule having ADAM17 inhibitory activity; and/or

b) a nucleic acid encoding AAT, a variant, an isoform and/or a fragment thereof, wherein said variant, isoform and/or fragment has inhibitory activity.

is/are formulated for subcutaneous administration.