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(54) **Title**: COMPOSITIONS, METHODS AND USES FOR ALPHA-1 ANTITRYPSIN FUSION MOLECULES

(57) **Abstract**: Compositions of and methods for making and using alpha-l antitrypsin (AAT) fusion molecules or peptide derivatives thereof are disclosed. The compositions and methods relate to generating an AAT fusion molecule of use in pharmaceutically acceptable compositions to treat a subject in need of AAT therapy or treatment. Compositions and methods disclosed herein concern linking AAT or derivative thereof to an immune fragment.

COMPOSITIONS, METHODS AND USES FOR ALPHA-1 ANTITRYPSIN FUSION MOLECULES

PRIORITY

[001] This application claims the benefit under 35 USC § 119(e) of provisional U.S. application No. 61/585,182, filed on January 10, 2012, and provisional U.S. application No. 61/586,038, filed on January 12, 2012, and provisional U.S. application No. 61/614,391, filed on March 22, 2012, all of which are incorporated herein by reference in their entirety for all purposes

FIELD

[002] Embodiments herein relate to compositions, methods and uses for recombinant alpha-1 antitrypsin (α-1 antitrypsin, AAT). In certain embodiments, recombinant AAT disclosed herein can be isolated more readily than other forms of AAT. In other embodiments, recombinant AAT has enhanced anti-inflammatory and anti-immune activities compared to naturally-occurring AAT or other commercial formulations of AAT. In yet other embodiments, 10-fold, 100 fold or even 1000 fold less recombinant AAT (rAAT) or AAT fusion molecules may be used in the place of any and all current forms of AAT for prevention or treatment of a condition or disease in a subject. In some embodiments, AAT fusion molecules can be used to treat a subject having a condition such as an infection or other health condition. Yet other embodiments reported herein concern compositions and methods for treating a myocardial indication, diabetes, inflammatory bowel disease, graft rejection or other known AAT-responsive conditions.

BACKGROUND

AAT

[003] Normal plasma concentration of alpha-1 antitrypsin (AAT) ranges from 1.3 to 3.5 mg/ml. Under certain conditions, AAT easily diffuses into tissue spaces and forms a 1:1 complex with target proteases, principally neutrophil elastase. Other enzymes such as trypsin, chymotrypsin, cathepsin G, plasmin, thrombin, tissue kallikrein, and factor Xa can also serve as substrates. The enzyme/inhibitor complex is then removed from circulation by binding to serpin-enzyme complex (SEC) receptor and catabolized by the liver and spleen.

SUMMARY

[004] Embodiments herein report generating and using recombinant constructs of alpha-1 antitrypsin (AAT) having superior properties to commercially available AAT compositions. Other embodiments report methods for purifying and scaling-up recombinant AAT production for therapeutic uses. In accordance with these embodiments, recombinant AAT can be isolated for use for any AAT-related activity, for example, as an anti-inflammatory agent, an immune modulator and/or a serine protease inhibitor.

[005] In certain embodiments, recombinant AAT disclosed herein includes a full length molecule or carboxyterminal peptide derivative thereof generated by any recombinant technology known in the art. Some embodiments concern constructs including AAT or a carboxyterminal derivative thereof having immunological elements associated with AAT, for example, to use for rapid purification and activity conservation of the AAT or to increase activity of AAT or its peptides. Other embodiments concern simultaneous synthesis of more than one constructs having AAT molecules each associated with an immunological element (*e.g.* an Fc fragment) and co-purified as a unit. Other embodiments can concern generating a construct of one or more carboxyterminal derivative(s) or fragment(s) of AAT including, for example, a fragment of the last 80 AAs or subfragments thereof (e.g. about 40, about 30, about 20 or about 10 AAs, or about 5 AAs) of the molecule associated with one or more immune molecule to form a construct for compositions, methods and uses disclosed herein.

[006] An AAT molecule of a construct contemplated herein can concern naturally occurring alpha-1 antitrypsin (e.g. human) or the most abundant form of AAT or other naturally-occurring form thereof, or fragments, or derivatives thereof, or mutant forms of AAT having no significant serine protease inhibitor activity, or alleles thereof (for example, there are approximately 100 naturally occurring AAT varients and any of these varients can be used in constructs disclosed herein), or analogs thereof or fusion protein thereof (e.g. a human IgG or fragment of human IgG). In accordance with these embodiments, a final construct may include 2 AAT constructs each associated with an immunological fragment (e.g. an Fc fragment) wherein the AAT-immune fragment constructs are linked together by disulfide bonds to form dual AAT-immune fragment constructs joined by one or more disulfide bonds (See for example, Fig. 1 and Fig. 2). In certain methods disclosed herein, rapid purification of AAT- or AAT-peptide linked to an immune molecule significantly reduced inactivation of AAT activities and reduced time to purification. Rapid purification eliminates multiple purification steps while preserving critical activities of the constructs. For example, these

rapidly purified fusion molecules are capable of retaining cytokine inhibiting functions, modulate immune and inflammatory molecule production compared to control plasma derived AAT (e.g. typical purification of naturally occurring AAT and purification of commercially available formulas). Significantly reduced concentrations of fusion molecules can be used to achieve the same or improved modulatory functions. Further, fusion molecules disclosed herein where an Fc region of Fc-AAT has a truncated hinge or deleted hinge region has superior activity when compared to plasma-derived AAT or fusion molecules of Fc-AAT with intact Fc.

[007] In accordance with these embodiments, a unit including two or more AAT-Fc (hinge deletion/truncation) constructs (or carboxyterminal AAT peptide fragments) can be purified and used in compositions and methods disclosed herein. Some of these embodiments of Fc-huAAT (hinge deletion) can be used in any method or composition contemplated herein. Other embodiments can include using IgG1, IgG2, IgG3 or IgG4 Fc fragments (hinge truncated or deleted) linked to an AAT molecule purified by rapid purification methods in order to preserve activity of the AAT molecule.

[008] Certain embodiments disclosed herein concern using Protein A for a minimum step (e.g. one-step) purification of Fc-fusion constructs in order to avoid the deleterious effects of other methods and multiple steps as used in plasma AAT purification. Some embodiments herein concern preserving 85%, 90%, 95% or more AAT's anti-inflammatory activity in the fusion molecule compared to standard purifications used for commercially available products (e.g. Aralast^{TM}, Prolastin^{TM}) and/or compared to naturally-occurring AAT found in blood plasma. In some embodiments, fusion molecules of the instant application have demonstrated 100 to 1000 fold more activity to reduce inflammation or treat a condition compared to commercially available formulations. In other embodiments, AAT-Fc having a truncated or deleted hinge region of the Fc portion demonstated superior activity *in vivo* to Fc-AAT where Fc is intact.

[009] Disclosed herein are methods to create and recover constructs having activities similar and in certain embodiments superior plasma-derived AAT. Certain activities known to be of interest regarding AAT include immunomodulatory or inflammatory modulation activities. It is contemplated herein that constructs described are isolated and assessed for activities other than serine protease inhibitor activities. In some embodiments, constructs disclosed herein have increased IL-1 receptor antagonist activity compared to commercially available compositions and reduced IL-1β production as well as other pro-inflammatory cytokines.

[0010] In certain embodiments, compositions (e.g construct compositions) and methods concern modulating adverse effects of radiation on a subject. In some embodiments, compositions and methods concern treating a subject having radiation therapy or radiation for example, when administered to a subject having cancer or suspected of developing a malignancy or for uncontrolled cellular growth. Other embodiments disclosed herein concern treating a subject having been exposed to radiation, for example, by accident or by a purposeful act.

[0011] Some embodiments concern administering AAT generated using recombinant technology to a subject in need of AAT therapy. In accordance to these embodiments, a subject could have an AAT-deficiency, an inflammatory or immune condition or other AAT-related condition known in the art. Certain embodiments herein include administering a composition having at least one construct and a pharmaceutically acceptable carrier to a subject in need of such a treatment. In certain embodiments, doses administered to a subject can include a 10-fold, 100-fold or 1,000 fold reduction in dose (e.g. of an Fc-AAT3 construct) to the subject compared to commercially available formulations. In certain embodiments, a dose can be about 1 mg/kg to about 10 mg/kg to a subject compared to 10 mg/kg to 100 mg/kg (concentrations of commonly used commercially available AAT such as Aralast™ or Prolastin C™).

[0012] Some embodiments of the present invention concern reducing adverse effects of ischemia reperfusion. In accordance with these embodiments, compositions herein can be used to modulate the affects of ischemia reperfusion damage as a consequence of a myocardial infarction or kidney failure or other condition. In other embodiment, fusion constructs reported herein can be used to modulate the onset or progression of cardiac tissue remodeling (*e.g.* enlargement and necrosis of cardiac tissue), for example, left or right ventricular (LV) remodeling. In accordance with these embodiments, intervention for example, by administering a composition disclosed herein, can modulate onset, severity (*e.g* of damage) or progression before, during, or after a cardiac event that can lead to heart muscle damage. In yet other embodiment, compositions disclosed herein can be administered to a subject having a heart condition to reduce early or late infarct size. In accordance with these embodiments, an early infarct can be one measured before (for example, a baseline), during or within 48 hours after surgery or other cardiac event. In other embodiments, a late infarct can be one measured after 48 hours or up to days or weeks after surgery or other cardiac event, for example 7 days after a cardiac event. In yet other embodiments,

compositions disclosed herein can be used to treat a subject having a cardiac event (e.g. myocardial infarction), to modulate cardiac enlargement and dysfunction as a consequence of the cardiac event by about 5%, or about 10%, or about 15%, or about 20% or about 25%, or about 30% or more compared to a subject not treated with these compositions.

[0013] Certain embodiments concern compositions for treating a subject having a cardiac event. In accordance with these embodiments, a composition can include, an AAT-Fc (hinge deletion or truncation) (e.g. human AAT or fragment thereof), or mutants thereof having no significant serine protease inhibitor activity, or alleles thereof (for example, there are approximately 100 naturally occurring AAT varients), or analogs thereof or fusion protein thereof (e.g. a human IgG or fragment of human IgG (Fc)). Some embodiments concern administering naturally-occurring AAT to a subject having or having had a cardiac event in order to modulate LV remodeling. Other embodiments can concern administering a composition of one or more carboxyterminal derivative(s) or fragment(s) of AAT including, for example, a fragment of the last 80 AAs of the 394 AA naturally occurring AAT (SEQ ID NO. 1 and 33). Some embodiments concern treating a subject having a cardiac condition with a recombinantly-produced AAT fusion peptide disclosed herein, in order to ameliorate the cardiac condition.

[0014] Other embodiments include treating a subject having an infection (e.g. bacteria or viral infection) or preventing a subject from getting an infection using compositions disclosed herein. In certain embodiments, a viral infection can be an infection due to HIV or influenza (e.g. H1N1, influenza A or B). In other embodiments, a bacterial infection can include bacterial pneumonia, a mycobacterial infection, exposure to bacillis anthracis or other bacterial infection.

[0015] Some embodiments concern compositions disclosed herein to reduce or prevent graft rejection. In other embodiments, compositions disclosed herein can be used to reduce the incidence or prevent Graft versus Host disease (GVHD). In certain embodiments, a composition disclosed herein can be used to treat a subject before, during or after organ, tissue or cellular transplantation. In other embodiments, an organ, tissue or cell culture can be exposed to a composition having an Fc-AAT (hinge deleted or truncated) fusion molecule in order to preserve the organ, tissue or cell culture prior to and during transplantation.

[0016] In certain embodiments, compositions for administration can be in a range of between about 0.1 ng and about 10 mg per ml or mg of the formulation. A therapeutically effective

amount of AAT peptides or constructs that have similar activities as AAT or peptides may be measured in molar concentrations and may range between about 1 nM and about 10 mM. The formulation is also contemplated in combination with a pharmaceutically or cosmetically acceptable carrier. Precise doses can be established by well known routine clinical trials without undue experimentation. In one embodiment, a subject may be treated for a conditions with a single dose (e.g 0.6 mg/kg to 0.8 mg/kg by IV infusion depending on the potency of the construct composition compared to a control) of an active agent (*e.g.* AAT construct or AAT peptide derivative thereof). In accordance with this embodiment, the subject can be treated with follow-on treatments (*e.g.* 5 to 10 days following a single dose or more) as determined by a health professional. Other embodiments can include using a control population having a placebo (*e.g.* human serum albumin administration or other comparable placebo) and comparing a placebo effect to a population receiving compositions disclosed herein.

[0017] In other embodiments, a composition disclosed herein can be administered to a subject every time a subject undergoes radiation and/or chemotherapy. Some embodiments disclosed herein concern treatment of a subject undergoing cancer therapies. Cancer treatments include, but are not limited to, treatment for bladder, breast, kidney, leukemia, lung, myeloma, liposarcoma, lymphoma, tongue, prostate, stomach, colon, uterine cancers, melanoma, pancreatic, eye and other known cancers.

[0018] Some embodiments disclosed herein concern treating a subject having prostate cancer. In accordance with these embodiments, a male subject having prostate cancer can be treated with compositions disclosed herein before, during or after radiation and/or chemotherapy in order to reduce development of impotence or erectile dysfunction, common side effects of prostate cancer therapies.

[0019] In certain embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In yet other embodiments, the subject is a pregnant female or young child. In other embodiments, the subject is a pet, a domesticated animal or livestock.

[0020] In other embodiments, the subject or mammal can be a non-domesticated mammal such as a captive or free wild animal.

[0021] In certain embodiments, compositions comprising human AAT mutants having no significant serine protease inhibitor activity can be used in constructs disclosed herein for use in methods described (*e.g* AAT fusion peptide derivative or Reactive Center Loop related

mutant fusion polypeptide). In accordance with these embodiments, recombinant molecules or fusion protein constructs disclosed herein have no significant serine protease inhibition activity. These constructs can be generated where they associate with an immune molecule (e.g. Fc). Association with the immune molecule can be used for rapid purification of the construct thereby preserving activities of the AAT or carboxyterminal thereof by reducing purification steps. In certain embodiments, the purification step is a single step using an affinity process (e.g. Protein A). These processes preserve conformation of the constructs disclosed herein by reducing deleterious purification steps used in other commercially available formulations (e.g. Aralast™, Zemaira™, Prolastin C™, and Glassia™). Other embodiments concern AAT-derived fragment constructs adapted to have no significant serine protease inhibitor activity. Constructs herein can include, but are not limited to constructs including a carboxy-terminal peptide or amino-terminal peptides corresponding to AAT, an analog thereof, any derivative of AAT carboxy terminus that binds to serpin-enzyme complex (SEC) receptor or a combination thereof linked to an immune molecule (e.g. IgG molecule).

[0022] Pharmaceutical compositions contemplated herein may further include an agent selected from the group consisting of an anti-inflammatory agent, an immunosuppressive agent, an immunomodulatory agent, an anti-viral agent, an anti-pathogenic agent, an anti-bacterial agent, a protease inhibitor, and a combination thereof. Some of these agents include, but are not limited to, one or more of interferon, interferon derivatives including betaseron, beta-interferon, prostane derivatives including iloprost, cicaprost; glucocorticoids including cortisol, prednisolone, methyl-prednisolone, dexamethasone; immunsuppressives including cyclosporine A, FK-506, methoxsalene, thalidomide, sulfasalazine, azathioprine, methotrexate; lipoxygenase inhibitors comprising zileutone, MK-886, WY-50295, SC-45662, SC-41661A, BI-L-357; leukotriene antagonists; peptide derivatives including ACTH and analogs thereof; soluble TNF-receptors; TNF-antibodies; soluble receptors of interleukins, other cytokines, T-cell-proteins; antibodies against receptors of interleukins, other cytokines, T-cell-proteins; and calcipotriols; Celcept®, mycophenolate mofetil, and analogues thereof taken either alone or in combination.

[0023] Other embodiments concern combination therapies for the treatment of a subject undergoing cancer related therapies, for example a composition disclosed herein can be combined with any other agent known to shrink or eliminate a tumor or reduce metastisis of a tumor in the subject or treat other aspects of cancer in the subject.

[0024] In certain embodiments, treating the subject with a composition encompassed herein to modulate normal cell damage can be by at least 10%, or by at least 20% or by at least 30%, or by at least 40%, or by at least 50%, or by at least 60%, or by at least 70%, or by at least 80%, or by at least 90% compared to a subject not treated with the composition.

[0025] As such, those skilled in the art will appreciate that the conception, upon which this disclosure is based, can readily be used as a basis for designing other methods for carrying out the several features and advantages of embodiments of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The following drawings form part of the present specification and are included to further demonstrate certain embodiments disclosed herein. Embodiments may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0027] Fig. 1 represents a schematic of an AAT construct contemplated of use for some embodiments disclosed herein for production of recombinant AAT is certain embodiments. AAT peptide fragments can also be produced using certain embodiments of the present invention.

[0028] Fig. 2 represents a schematic of human AAT constructs with associated immune molecules contemplated of use for some embodiments disclosed herein.

[0029] Fig. 3 represents a SDS-PAGE gel illustrating migration of some fusion molecules generated by certain embodiments disclosed herein.

[0030] Figs. 4A and 4B illustrate, by histogram plot, cytokine production (e.g. TNFα, tumor necrosis factor-alpha) in an *in vitro* cell model exposed to certain AAT fusion molecules disclosed herein (4A) in comparison to a commercially available AAT formulation (4B).

[0031] Figs. 5A and 5B represent reduction in expression of various pro-inflammatory cytokines in the presence or absence of LPS (5A) with or without an AAT fusion molecule (rAAT or recombinant AAT) and expression of IL-1Ra using decreasing amounts of an AAT fusion molecule (rec AAT-Fc; recombinant Fc-AAT) (5B).

[0032] Figs. 6A-6C represent percent expression of CD11b/CD45 positive cells and percent of TLR4 and TLR2 expression in the presence of plasma-derived AAT versus AAT fusion molecule, Fc-AAT2, and found that about 100 to about 1000 fold less recombinant AAT (Fc-AAT2) had the same inhibitory effect on these deleterious molecules. For example, Toll-like

Receptor 4 at either 500 or 100 ng as effective as 500 μg of plasma-derived AAT (see **Fig. 6A**).

[0033] Figs. 7A-7D represent histogram data plots representing an *in vivo* Gouty arthritis assay in mice related to affects of fusion molecules of some embodiments disclosed herein on joint swelling (7A comparing two Fc AAT fusion molecules, 7C an Fc AAT fusion molecule versus controls) and IL- 6 production in the same model (7B) as well as affects of exposure to an Fc AAT fusion molecule and inhibition of IL-1β over time (7D, from 24 to 72 hours).

[0034] Fig. 8 represents a histogram plot representing infarct size in the presence or absence of Fc-AAT2 (recombinant AAT at 2 concentrations) after a cardiac event using a myocardial infarction-induced mouse model where the mouse undergoes ischemia reperfusion.

[0035] Fig. 9 represents a histogram plot representing infarct size in the presence or absence of two Fc-AAT constructs, each having an intact AAT molecule linked to intact Fc (at the same concentration) after a cardiac event using a myocardial infarction-induced mouse model where the mouse undergoes ischemia reperfusion.

[0036] Fig. 10 illustrates schematics of Fc-AAT fusion molecules of certain embodiments of the present invention.

Description of Illustrative Embodiments

Definitions:

[0037] As used herein, "a" or "an" may mean one or more than one of an item.

[0038] As used herein, "about" can mean plus or minus 10%, for example, about 10 minutes can mean from 9 to 11 minutes.

[0039] As used herein, "Fc-AAT" or "AAT-Fc" can mean an Fc fragment linked to AAT or AAT carboxyterminal fragment either at the carboxy-terminal or amino-terminal end of an AAT polypeptide.

DETAILED DESCRIPTION

[0040] In the following sections, various exemplary compositions and methods are described in order to detail various embodiments of the invention. It will be obvious to one skilled in the art that practicing the various embodiments does not require the employment of all or even some of the specific details outlined herein, but rather that concentrations, times and

other specific details may be modified through routine experimentation. In some cases, well known methods, or components have not been included in the description.

[0041] It has been traditionally thought that AAT (alpha-1 antitrypsin) anti-inflammatory activities were attributed to its ability to inhibit serine proteases, and particularly neutrophil elastase. This is the basis for its use in replacement therapy for humans with AAT deficiencies. AAT that is currently commercially available for human use is standardized by its anti-elastase units not for other AAT-related activities. These commercially available formulations are purified from pooled human plasma, but these are not pure (although some are purer than others) because they contain other human serum proteins. The majority of studies on human AAT in vitro as well as in vivo models depend on the use of these commercially available preparations directed to serine protease inhibition activity, each approved for use in humans. Although infusions of AAT in humans with various AAT deficiency states are considered safe, the role of contaminating proteins remains unknown. Certain embodiments herein report quick production of recombinant forms of AAT of high purity and high activity to overcome issues of contaminating co-purified plasma proteins.

[0042] In certain embodiments disclosed herein a challenge is presented regarding a long-held concept that inhibition of neutrophil elastase is the sole mechanism for the therapeutic benefit of augmentation therapy due to AAT. One contribution clarified herein is creation of novel forms of fusion molecules of AAT such as Fc fusions that not only aide in purification of AAT but are more potent in activity than native, commercially available compositions. For decades scientists have attempted to generate recombinant forms of AAT that retain similar or equal beneficial affects of AAT but have been largely unsuccessful. Fc fusion proteins have a long history of having safety and many are used therapeutically by hundreds of thousands (e.g. Enbrel™ and Alabacept™, for example, of use for rheumatoid arthritis treatment). Fc-AAT fusion molecules presented herein can be readily produced and purified in large quantities and have reduced side effects of other constructs. In addition, fusion constructs presented herein have up to 10, to 20, to 30, to 40 to, 50…to 100 and in certain cases up to 1,000 times more potency when compared to commercially available formulations (e.g Zemaira™, Aralast™, Prolastin™ etc) regarding AAT activities such as anti-inflammatory and anti-immune activities. In certain aspects, it is considered superior to commercially available formulations in part because the commercially purified plasma – derived AAT formulations likely destroy anti-inflammatory domains during the purification. In certain methods, one of the initial steps in purifying plasma-derived AAT is cold-alcohol

precipitation which is highly oxidative. Thus, fusion molecules provided herein provide a superior substitute for AAT for any of clinical indications such as a superior treatment for COPD in AAT deficient patients, for reducing effects of graft rejection, for treatment of inflammatory conditions because preparations disclosed herein focus in-part on maintaining anti-inflammatory domains of AAT rather than on elastase inhibition although AAT activities are preserved in total in other formulations.

[0043] Other embodiments disclosed herein concern modified Fc molecules associated with various forms of AAT. In accordance with these embodiments, immunoglobulin molecules fused to AAT or a peptide fragment of AAT can be IgG1, IgG2, IgG3, or IgG4. In certain embodiments, a fusion molecule of AAT can include IgG2 where a 12-amino acid hinge region is mutated, truncated or eliminated prior to fusing it to AAT. For example, one fusion molecule disclosed herein in concerns IgG2 with a hinge deletion (also referred to as clone 3 or Fc3) fused to AAT. Truncation, mutation or elimination of the hinge region of IgG2 reduces *in vivo* side effects of the fusion molecule. Some embodiments include reduced ability to activate complement and other actitivies. Fusion molecules disclosed herein retain superior acitivity to a native AAT, plasma-derived composition (e.g. commercially available compositions such as Aralast^{TM}.

[0044] Excess inflammation or inflammation activation can result in the initiation, progression and destructive nature of several chronic diseases, for example chronic destructive or wasting diseases. These include, but are not limited to, autoimmune diseases, such as rheumatoid arthritis, lupus (systemic lupus erythematosus), diabetes such as Type 1 where insulin-producing beta cells can be destroyed by an immune attack. Other conditions that may be treated by compositions and methods disclosed herein include Type 2 diabetes. In addition to autoimmune diseases, chronic inflammation of coronary arteries can increase the risk of a heart attack or stroke. Chronic inflammation also contributes to inflammation in the intestines (e.g. Crohn's Disease, inflammatory bowel disease (IBD) or ulcerative colitis). Several naturally occurring proteins are produced each day in a subject that control inflammation in the subject. AAT is one of these proteins. One drawback of a therapy with AAT is that commercially available AAT is isolated from the plasma of human blood donors therefore supply is limited to available plasma. Uses of therapeutic AAT are growing because its application is not limited to the current uses such as chronic pulmonary obstructive disease (COPD) and AAT replacement therapies.

[0045] Certain embodiments herein report effective recombinant forms of human alpha 1 antitrypsin functional to treat AAT-deficient conditions or AAT-responsive conditions similar to and in certain aspects more efficiently than plasma-derived AAT. In certain embodiments, compositions and methods disclosed herein concern AAT (e.g. human or other mammal) fused to an immune molecule or fragment thereof (e.g. IgG1, IgG2). In other embodiments, fusion proteins can include truncated versions of AAT. In accordance with these embodiments, certain fusion polypeptide can be linked through the amino-terminus of AAT or fragment thereof. Some embodiments concern constructs of AAT fused to an immunoglobulin molecule such as Fc. In certain embodiments, AAT or a carboxyterminal peptide fragment thereof can be linked to Fc derived from IgG1 or IgG2. Fc derived from IgG2 can be used, for example, because the Fc of human IgG1 binds to the complement receptor on myeloid cells and IgG2 was found to be superior in certain compositions and methods.

[0046] Three distinct types of Fc-gamma (γ) receptors occur: designated FcγRI, FcγRII, and FcγRIII are found on human leukocytes. FcγRI (CD64) is a high-affinity receptor expressed on monocytes, macrophages, neutrophils, myeloid precursors and dendritic cells. FcγRI has a high affinity for monomeric human IgG1 and IgG3, but does not bind IgG2. It has been demonstrated that binding of the Fc part of IgG to an FcγR is instrumental in the induction of the cell's effector function, including the release of inflammatory mediators.

[0047] It has been demonstrated that four IgG subclasses differ from each other with respect to their effector functions, for example, the length and flexibility of the hinge region are different. The flexibility of the hinge region decreases in the order IgG3>IgG1>IgG4>IgG2. The Fc IgG2 has 12 amino acids in the hinge region and is less flexible than Fc IgG1. It is contemplated that any hinge region of an Fc fragment can be manipulated to delete or modify the hinge region in order to reduce additional *in vivo* side effects of a fusion molecule including, but not limited to, complement activation. One or more amino acids can be modified or removed from this region to generate fusion molecules with increase AAT activity compared to an unmodified control. In certain embodiments, a hinge region can be shortened in order to modulate flexibility in the construct, to reduce *in vivo* side reactions to the Fc or alter tertiary structure to enhance AAT activities in an Fc-AAT construct contemplated herein. In some embodiments, Fc-AAT constructs disclosed herein have increased half-life compared to a plasma-derived AAT formulation.

[0048] In addition, the Fc IgG2 is resistant to proteases and, as stated previously, does not bind to the high affinity FcγRI, as well as, weak in its ability to activate complement. In certain embodiments, Fc used in fusion proteins contemplated herein may be from IgG1 or IgG2 or IgG3 or IgG4. In other embodiments, Fc can be a mutant molecule that does not bind to a receptor. In yet other embodiments, Fc can be a wildtype or mutant form from IgG2 linked to AAT or carboxyterminal peptide thereof. In certain embodiments, Fc molecules may be associated with AAT molecules to make dimers of Fc-AAT, for example, linked by disulfide bonds. In other embodiments, monomeric molecules of Fc-AAT can be generated and used in methods disclosed herein. Certain constructs disclosed concern Fc-AAT wherein the Fc of the construct is modified to further reduce flexibility in the hinge region, for example by removing additional amino acids in this region. Any of the molecules described herein can be rapidly purified using, for example, Protein A column or matrix or other quick purification or enrichment method for rapid separation to preserve activity.

[0049] Embodiments herein report generating constructs of alpha-1 antitrypsin (AAT) or carboxyterminal fragment thereof having superior properties to current commercially available AAT compositions. Other embodiments report methods for purifying fusion proteins or peptides and subsequent uses for purified AAT fusion molecules disclosed herein. It is contemplated that commercially available AAT derived from blood plasma is in short supply, is currently purified by methods that destroy important properties of AAT and a need exists for synthetic versions of this molecule or updated purification methods where the synthetically produced AATs are capable of performing as well if not better than native forms of AAT or AAT derived peptides.

[0050] With respect to AAT activities other than serine protease inhibition, AAT exerts anti-inflammatory properties by several mechanisms. Preliminary data using a mutation of the anti-protease site (e.g. to reduce anti-protease activity to insignificant levels) support the concept that some of AAT's acivities do not require the anti-protease properties of AAT. In certain embodiments, different recombinant truncated and mutant forms of naturally occurring human AAT (e.g. 394 AA, M_r about 51,000) are generated in order to assess anti-inflammatory properties of the molecule. This approach allows for producing AAT molecules of various compositions, which is extremely difficult and near impossible using the standard methods of plasma-derived AAT. It was demonstrated that anti-inflammatory properties of AAT can be oxidized by currently used purification procedures of commercially

available compositions. Methods disclosed herein provide superior purification methods for preserving this activity in fusion molecules and constructs described.

[0051] In certain methods previously disclosed, it has been demonstrated that AAT blocks toxic activities of IL-1β on mouse model and human pancreatic islet cells. Some embodiments herein concern recombinant production of AAT fusion molecules capable of mimicking this activity. In certain embodiments, recombinantly-produced fusion peptides of the carboxyl terminal region of human AAT are generated for blocking toxic activities or production of IL-1β and for reducing caspase-1 activity (see Example section). These fusion peptides are useful for blocking or reducing production of or activities of pro-inflammatory molecules and therefore are useful for treatment and prevention of many health conditions.

[0052] Alpha 1-Antitrypsin or α1-antitrypsin (AAT) was first classified as a protease inhibitor belonging to the serpin superfamily. It is generally known as serum trypsin inhibitor. AAT can also be referred to as alpha-1 proteinase inhibitor (A1PI) because it inhibits a wide variety of proteases. AAT protects tissues from enzymes of inflammatory cells, especially neutrophil elastase, and typically has a range in blood of about 1.5 to 3.5 gram/liter but the concentration can rise manyfold upon acute inflammation. Over 100 different variants of α_1-antitrypsin have been described in various populations. The most common variety of AAT is termed M, based on its migration in an IEF gel. Other variants are termed A-L and N-Z, depending on whether they run proximal or distal to the M band. The presence of deviant bands on IEF can signify the presence of AAT deficiency. As indicated above, M type AAT has several subtypes and all of these subtypes are contemplated of use herein.

[0053] The current trend for obtaining therapeutic concentrates of AAT is to prepare AAT from the blood plasma of blood donors. This is a limited resource and requires extensive purification steps to get to a marketable product. So far, the United States Food & Drug Administration has approved the use of several commercial products derived from human plasma: For example, some of these products include Prolastin®, ProlastinC®, (Talecris (now Grifols, Raleigh, N.C.), Zemaira®, and Aralast® (Baxter) and Kamada has both an aerosol and an intravenous product (Kamada, Israel). Most of these formulations are administered intravenously for AAT therapy in AAT deficient patients and can cost up to $100,000 per year per patient. It has been demonstrated that plasma isolated AAT has reduced activity compared to AAT derived from blood. Compositions disclosed herein have increased anti-inflammatory activity similar to that of blood not of plasma-derived AAT; and

greater activity than commercially available formulations which have activities that are based on anti-protease activities.

[0054] One study analyzed and compared three of the FDA-approved products in terms of its primary structure and glycosylation. Several of the products showed differences compared to the normal human plasma AAT that are likely introduced during purifications procedures. In addition, it was previously demonstrated that comparison of the commercial formulations in certain studies had large variability regarding serine protease inhibition acitivity and AAT purity. Recently, one of the standard commercially available formulations, Prolastin®, was evaluated and a new formulation ProlastinC® was purified differently than Prolastin®, in order to increase anti-protease activity (e.g. serine protease inhibition activity) in the final product. All of the activities reported for these products are directed to serine protease inhibition activities not anti-inflammatory or immune modulatory activity or alternative AAT-related activities.

[0055] In certain embodiments, compositions generated herein may be more useful as an aerosol formulation than other forms, in part, due to its reach to the lower respiratory tract than intravenous methods. It is contemplated herein that any of the construct formulations can be introduced to a subject by any method known in the art as a pharmaceutically acceptible formula.

[0056] In spite of efforts to improve plasma-derived AAT formulations, there is a finite supply of plasma available where AAT is derived and it is expensive to produce. Therefore, recombinant AAT molecules have been sought. One of the issues encountered by researchers developing recombinant AAT molecules has been reduced activity of these molecules compared to plasma-derived formulations. Recombinant molecules generated previously were often less active when assayed by serine protease inhibitor assays compared to the commercially available formulations previously indicated. Thus, limited supply of plasma and inferior recombinant AAT molecules of the past have left a void for generating adequate supplies of AAT for past and recently discovered methodologies.

[0057] Some embodiments herein concern generating a highly active, highly functional recombinant AAT construct relative to commercially available formulations for use in any AAT method or treatment known in the art. In certain embodiments, recombinant AAT disclosed herein includes a full length molecule or carboxyterminal peptide derivative thereof. Some embodiments concern simultaneous synthesis of more than one construct

having AAT molecules each associated with an immunological element (*e.g.* an Fc fragment or other fragment) and co-purified. Other embodiments can concern generating a construct of one or more carboxyterminal derivative(s) or fragment(s) of AAT including, for example, a fragment of the last 80, 70, 60, 50, 40, 30 amino acids or other fragment of the carboxyterminus of the molecule associated with one or more immune molecule(s) to form a construct for methods and uses disclosed herein.

[0058] An AAT molecule of a construct contemplated herein can concern naturally occurring alpha-1 antitrypsin (e.g. human or other mammal), or fragments, or derivatives thereof, or mutant forms of AAT, any AAT molecule having no significant serine protease inhibitor activity, or alleles thereof (for example, there are approximately 100 naturally occurring AAT varients), or analogs thereof or fusion protein thereof (e.g. a human IgG or fragment of human IgG). In accordance with these embodiments, a construct can include dimeric AAT constructs each associated with an immunological fragment (e.g. an Fc fragment that links two molecules of AAT) wherein the Fc-AAT constructs are linked together by one or more disulfide bond(s). See for example, Fig. 1 and Fig. 2 disclosed herein. In certain methods, purification of recombinant AAT or AAT-peptide and immune molecule complexes increase activity of the AAT or AAT-peptide by significantly reducing purification steps and significantly increasing potency of AAT or AAT-peptide. In accordance with these embodiments, recombinant AAT molecules contemplated herein can be used as a fusion polypeptide (dimer or monomeric form) or can be cleaved from its immune molecule after purification and used as in reduced concentrations compared to commercially available formulations. Some embodiments concern, using $1/100^{th}$ to $1/1000^{th}$ of a concentration compared to commercially available formulations. In certain examples, these molecules can be used in compositions to inhibit cytokines or modulate the immune and inflammatory functions of the molecules compared to controls (e.g. typical purification of naturally occurring AAT and purification of commercially available formulas). In one embodiment, recombinant molecules of the instant application have demonstrated 100 to 1000 fold more activity than commercially available formulation. Certain activities known to be of interest regarding AAT constructs of the instant invention include immunomodulatory or inflammatory modulation activities. In some embodiments, constructs disclosed herein have increased IL-1β receptor antagonist activity compared to commercially available compositions.

Some Uses for Recombinant AAT in the Treatment of Health Conditions

[0059] Some embodiments reported herein concern using recombinant AAT or fusion protein or carboxyterminal fragment fusion molecule thereof to treat a subject in need of AAT therapy, AAT replacement or AAT supplementation. AAT treatments have been reported of use in a variety of conditions including, but not limited to, apoptosis-related conditions, nitric oxide-related conditions, ischemia-reperfusion dysfunction induced conditions, graft rejection and cellular rejection, diabetes, emphysema, other lung conditions, treatment and prevention of bacterial infection, treatment and prevention of viral infections, radiation induced injury and the like.

[0060] Some embodiments herein concern compositions of fusion molecules disclosed herein of use to treat an inflammatory disorder *(e.g.* IBD, Crohn's disease, arthritis). In some embodiments, fusion molecules disclosed herein have enhanced anti-inflammatory activity compared to commercially available AAT compositions. Some embodiments concern a hinge-deleted; truncated or mutated IgG2 Fc fused to synthetically generated AAT or carboxyterminal truncated version thereof (e.g. the last 36 to 80 amino acids of AAT). In one embodiment, Fc-AAT comprises IgG2 hinge deletion with a 2 amino acid linker attached to an intact synthetically generated AAT molecule to make what is referred to in certain cases as *clone 3*.

[0061] In certain embodiments, compositions and methods disclosed herein can be used to reduce or prevent onset of inflammatory bowel disorder in a subject. In accordance with these embodiments, reduction in conditions associated with IBS in a subject may be on the order of about 10-20%, or about 30-40%, or about 50-60%, or about 75-100% reduction or inhibition. In accordance with these embodiments, a subject having IBS or IBD may be treated with a pharmaceutically acceptable composition of recombinant or a fusion protein of AAT or AAT-carboxyterminal peptide (Fc-AAT with a hinge deletion or hinge truncation) to reduce wasting or to reduce loss of or restore barrier function compared to a control subject not receiving such a composition. In other embodiments, compositions disclosed herein can be used to reduce onset of an inflammatory bowel disorder.

[0062] Some embodiments herein concern restoring bowel or intestinal hyperpermeability in a subject having an acute or chronic condition. In accordance with these embodiments bowel or intestinal hyperpermeability or loss of barrier function can be due to chronic diseases such as systemic inflammatory response syndrome (SIRS), inflammatory bowel disease, type 1 diabetes, allergies, and asthma. In certain embodiments, a subject having bowel or intestinal

hyperpermeability can be treated by a health professional by a predetermined regimen such as daily, twice weekly, weekly or other predetermined regimen.

[0063] In certain embodiments, compositions disclosed herein can be used to treat certain indications including but not limited to diabetes (e.g. Type 1 and Type 2), immune diseases such as autoimmune disease, inflammatory diseases, cardiac disorders infectious disease and others. Some diseases disclosed herein may fall under more than one category such as asthma which can be considered an inflammatory disease, an autoimmune disease or a lung disease or other. In certain embodiments, compositions disclosed herein can be used to treat autoimmune diseases that include, but are not limited to, rheumatic diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE),Type I diabetes, and autoimmune diseases of the thyroid, gut, and central nervous system (e.g., rheumatoid arthritis, lupus erythematosus, Sjogren's syndrome, scleroderma, mixed connective tissue disease, dermatomyositis, polymyositis, Reiter's syndrome, and Behcet's disease); autoimmune diseases of the central nervous system (e.g., multiple sclerosis, myasthenia gravis, or encephalomyelitis); autoimmune disease of the gastrointestinal system: (e.g., Crohn's disease, ulcerative colitis, inflammatory bowel disease, Celiac disease, Sprue); autoimmune disease of the thyroid: (e.g., Hashimoto's thyroiditis, or Graves' Disease); and ocular autoimmune disease, (e.g., uveitis). Autoimmune disorder contemplated herein, can concern Alopecia areata, nkylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, Bullous pemphigoid, cardiomyopathy, Celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, Cicatrical pemphigoid, CREST syndrome, Crohn's disease, Discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Glomerulonephritis, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), irritable bowel disease (IBD), IgA neuropathy, Juvenile arthritis, Lichen planus, Lupus erythematosus, Meniere's disease, mixed connective tissue disease, multiple sclerosis, Type 1 or immune-mediated diabetes mellitus, Myasthenia gravis, Pemphigus vulgaris, Pernicious anemia, Polyarteritis nodosa, Polychrondritis, Polyglandular syndromes, Polymyalgia rheumatic, Polymyositis and dermatomyositis, Primary agammaglobulinemia, Primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, Sarcoidosis, Scleroderma,

Sjogren's syndrome, Stiff-man syndrome, Systemic lupus erythematosus, Lupus erythematosus, Takayasu arteritis, Temporal arteristis/giant cell arteritis, ulcerative colitis, Uveitis, Vasculitides such as dermatitis herpetiformis vasculitis, Vitiligo, Wegener's granulomatosis, T cell mediated autoimmune disease, rheumatic disease, rheumatic arthritis, and lupus erythematosus.

[0064] In other embodiments, compositions disclosed herein can include treating conditions such as inflammatory conditions including, but not limited to, allergic disorders, or for example, arthritis, inflammatory osteolysis, asthma, chronic inflammation (e.g. from chronic viral or bacteria infections), chronic obstructive pulmonary disease (COPD), Encephalitis, inflammatory bowel disease (IBD), psoriasis (e.g., plaque psoriasis, pustular psoriasis, erythrodermic psoriasis, guttate psoriasis or inverse psoriasis), pulmonary fibrosis, undifferentiated arthropathy, undifferentitated spondyloarthropathy. Other conditions can include, but are not limited to respiratory conditions, for example, asthma, COPD, emphysema. Certain embodiments concern treating a subject on a monthly, weekly, biweekly, daily, twice daily or other regimen to reduce deleterious effects of inflammation using 10- to 100-fold less AAT in the form of a recombinantly produced fusion molecule where the fusion molecule comprises Fc-AAT (hinge deleted or hinge truncated form). In certain examples, Fc-AAT3 or Fc-AAT4 can be used in a composition to inhibit deleterious affects of these disorders and ameliorate the symptoms associated thereof.

Radiation Protection and Cancer

[0065] In certain embodiments, compositions (e.g construct compositions) and methods concern modulating adverse effects of radiation on a subject. In some embodiments, compositions and methods concern treating a subject having radiation therapy or radiation for example, when administered to a subject having cancer or suspected of developing a malignancy or for uncontrolled cellular growth. Other embodiments disclosed herein concern treating a subject having been exposed to radiation, for example, by accident or by a purposeful act in part, in order to reduce adverse side effects of radiation treatment.

[0066] Some embodiments disclosed herein concern treatment of a subject undergoing cancer therapies. In accordance with these embodiments, a subject undergoing cancer therapies can be treated with a composition disclosed herein to reduce or prevent detrimental affects of the treatment (e.g. from radiation and/or chemotherapy treatments). Cancer treatments include, but are not limited to, treatment for bladder cancer, breast cancer, kidney cancer, leukemia,

lung cancer, myeloma, liposarcoma, lymphoma, tongue cancer, prostate cancer, stomach cancer, colon cancer, uterine cancer, melanoma, pancreatic cancer, brain cancer, eye cancer, skin cancer and other known cancers.

[0067] In other embodiments, compositions disclosed herein can be used to treat a subject having cancer. Cancers contemplated for these embodiments can include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, Kaposi's sarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, melanoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms'tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, myeloma, lymphoma, leukemia, or other known cancer.

[0068] Other embodiments include regarding radioprotection and compositions disclosed herein can concern treatment for trigeminal neuralgia, treatment for severe thyroid eye disease, treatment for pterygium, treatment for pigmented villonodular synovitis, prevention of keloid scar growth, prevention of heterotopic ossification, cosmetic or reconstructive surgical application surgery (e.g. reducing in scar formation), during chemotherapy,in combination with hormone therapy, and/or as an immunotherapy combination.

[0069] Certain side effects can occur during radiation exposure and even as a side effect of radiation therapy or chemotherapy. Some embodiments herein concern reduction or prevention of these side effects in a subject by treating the subject with compositions disclosed herein. Compositions can include AAT, AAT carboxyterminal peptides (e.g 80 mer, 36 mer etc.), recombinant/fusion forms of AAT and/or recombinant/fusion forms of AAT carboxyterminal peptides. Side effects of radiation therapy can include, but are not limited to, cellular damage, pain, swelling, local irritation, fibrosis, scaring, loss of tissue integrity, increased tissue friability, difficulty in swallowing and other symptoms associated with radiation treatment or exposure. Other side affects that can be reduced or prevented

concern side effects from total body irradiation (TBI), for example during bone marrow transplantation. These side effects can include the above and in addition, acute and chronic immunodeficiency and opportunistic infections.

[0070] Some embodiments disclosed herein concern treating a subject having or suspected of developing prostate cancer. In accordance with these embodiments, a male subject having or suspected of developing prostate cancer can be treated with compositions disclosed herein before, during or after radiation and/or chemotherapy treatment(s) in order to reduce side effects attributed to these therapies. For example, side effects can be, but are not limited to, development of impotence or erectile dysfunction.

[0071] Other conditions contemplated herein include systemic lupus erythematosis (SLE, or lupus), rheumatoid arthritis, sepsis, , systemic lupus erythematosis (SLE, or lupus), rheumatoid arthritis, inflammatory bowel disease, sepsis, autoimmune diseases, atherosclerosis, Alzheimer's disease, arthritis, muscular dystrophy, Downs syndrome, multiple sclerosis, stroke, neurodegenerative disorders, other inflammatory diseases or conditions and sero-negative spondyloarthropathies.

[0072] In certain embodiments, compositions disclosed herein can be used to treat a subject in septic shock (see animal models for these confirmation studies: Doi *et al* The Journal of Clinical Investigation Volume 119 Number 10 October 2009; for an animal model of sepsis and sepsis-induced kidney injury). It has been demonstrated that plasma-derived AAT can be used systemically to treat both viral and bacterial infections in mouse models and in human cohort studies therefore, Fc-AAT (hinge deletion or hinge truncation of Fc *e.g.* FcAAT3) having been demonstrated as an improvement compared to plasma-derived AAT can be used to treat sepsis. For example, a subject having sepsis due to one or more infection or other cause can be treated with a composition disclosed herein to ameliorate the condition and potentially prevent death in the subject.

[0073] In certain embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In yet other embodiments, the subject is a male, a female, a pregnant female, an infant or a juvenile.

Graft Rejection and Graft Survival

[0074] In other embodiments, recombinant or fusion polypeptides (e.g. Fc-AAT or Fc-AAT fragment) contemplated herein can be used to treat a subject undergoing a transplant, such as an organ or non-organ (e.g. cellular) transplant. In certain embodiments, cellular

transplantation can include bone marrow, islet cell (e.g. islet allograft), corneal cell, stem cell, skin (e.g. cellular or larger), temporary cadaver transplants of skin (e.g. soft tissue, facial or other) or conditions related to cellular transplant rejection such as graft versus host disease (GVHD). Embodiments of the present invention provide for methods for ameliorating symptoms or signs experienced by a subject having or in need of a transplant. In accordance with these embodiments, symptoms or signs may include conditions associated with graft versus host disease (GVHD), or graft rejection. In one example, methods disclosed herein may be used to treat a subject undergoing bone marrow transplantation. In other embodiments, methods disclosed herein may be used to treat a subject undergoing stem cell or other cellular transplantation. In accordance with these embodiments, a subject may be treated to reduce transplantation rejection, preserve the cells of a transplant and/or prolong transplanted cell (graft) survival. Other embodiments can include treating a subject undergoing an organ transplant such as a heart, lung, intestinal, liver, pancreas, kidney or other organ transplant.

[0075] In one example, methods disclosed herein may be used to treat a subject undergoing bone marrow transplantation. In accordance with these embodiments, a subject can be treated before, during or after bone marrow transplantation to reduce or prevent graft rejection and/or GVHD in the subject.

[0076] In other embodiments, compositions and methods disclosed herein concern prevention or reducing the occurrence of organ transplant rejection. In other embodiments, compositions and methods disclosed herein concern prolonging organ transplantation. Transplants contemplated herein can concern transplantation of kidney, heart, liver, soft tissue, facial component transplant, intestinal transplants, and pancreas transplant. In addition, compositions disclosed herein can concern reduction or prevention of symptoms associated with transplantation of an organ or non-organ. Symptoms that can be reduced or prevented by treating a subject undergoing a transplant with compositions disclosed herein can include, graft rejection, kidney failure, lung failure, heart failure, mucosal ulcerations, reduced islet function (increased glucose, diabetes mellitus), graft versus host disease (GVHD), gastrointestinal (GI), ulceration, pulmonary failure, skin ulceration, coagulopathy, CNS dysfunction, and coma.

[0077] Yet other aspects of the present invention concern organ or cell preservation prior to transplantation. For example, cryoprotection or protection during transport or other preservation method may be enhanced by exposing an organ, tissues or cells to compositions

disclosed herein. Certain embodiments herein concern using a composition disclosed herein for preserving an organ, tissue or cells in preparation for transplantation or for cryoprotection. In accordance with these embodiments, organs, tissue or cells can include any of those disclosed herein, for example, pancreatic islet cells, stem cells, bone marrow cells, kidney, liver, lung and other organ or cellular transplants.

[0078] Embodiments of the present invention provide methods for promoting prolonged graft survival and function in a subject including administering to a subject in need thereof a therapeutically effective amount of a composition including a substance of recombinant AAT or fusion protein thereof and a pharmaceutically acceptible excipient.

[0079] In certain embodiments of the present invention, compositions disclosed herein can further include combination therapy. For example, combination therapies can include one or more of interferon, interferon derivatives including betaseron, beta-interferon, prostane derivatives including iloprost, cicaprost; glucocorticoids including cortisol, prednisolone, methyl-prednisolone, dexamethasone; immunsuppressives including cyclosporine A, FK-506, methoxsalene, thalidomide, sulfasalazine, azathioprine, methotrexate; lipoxygenase inhibitors comprising zileutone, MK-886, WY-50295, SC-45662, SC-41661A, BI-L-357; leukotriene antagonists; peptide derivatives including ACTH and analogs thereof; soluble TNF-receptors; TNF-antibodies; soluble receptors of interleukins, other cytokines, T-cell-proteins; antibodies against receptors of interleukins, other cytokines, T-cell-proteins; and calcipotriols; Celcept®, mycophenolate mofetil, and analogues thereof taken either alone or in combination.

Plastic Surgery and Reduction/Prevention of Scarring

[0080] Other aspects disclosed herein concern reducing side effects and enhancing recovery post-reconstructive surgery, enhancement or cosmetic surgery (e.g. elective, cosmetic, burn victims or due to treatment such as radiation etc.). Reconstructive plastic surgery can performed to correct functional impairments caused by for example, burns; traumatic injuries, such as facial bone fractures and breaks; congenital abnormalities, such as cleft palates or cleft lips; developmental abnormalities; viral or bacterial infection and disease; and cancer or tumors. Reconstructive plastic surgery can be performed to improve function, but it may be done to reform a subject to a normal appearance.

[0081] One of the most common reconstructive procedures is tumor removal, laceration repair, scar repair, hand surgery, and breast reduction. Some other common reconstructive

surgical procedures include breast reconstruction after a mastectomy, cleft lip and palate surgery, contracture surgery for burn survivors, and creating a new outer ear when one is congenitally absent. Medical professionals often use microsurgery to transfer tissue for coverage of a defect when no local tissue is available. Flaps of skin, muscle, bone, fat, or a combination can be excised from a subject's own body and moved to another site on the body, and reconnected to a blood supply etc. Therefore, compositions disclosed herein can be used before, during or after reconstructive or cosmetic surgery to reduce scarring and enhance tissue transfer and retension (*e.g.* reduction of graft rejection and scarring), if applicable. In certain embodiments, therapeutic compositions that include AAT fusion molecules such as Fc-AAT (hinge deletion or intact hinge region) can be used to reduce side effects of cosmetic and reconstructive procedures such as preventing or reducing inflammation, a common side effect of these surgeries that can lead to swelling and tissue damage. Other embodiments can include treating a subject having undergone or undergoing a reconstructive procedure to reduce recovery time and enhance the reconstructive process using compositions disclosed herein to augment or ameliorate inflammatory and immune reactions in a subject undergoing such a process. Compositions disclosed herein may be used to treat the subject systemically or by direct application to an affected area (e.g. applied as a salve or lotion or other mode) depending on need as determined by a health professional.

Diabetes

[0082] Some embodiments concern using compositions disclosed herein to treat a subject having or suspected of developing diabetes. In accordance with these embodiments, a subject can be administered a composition disclosed herein to any subject having diabetes to treat the disease in the subject. A subject having Type 1 or Type 2 diabetes can be treated with a composition disclosed herein. These treatments can be combined with any treatment known in the art for diabetes. In certain embodiments, compositions disclosed herein can be administered to a subject at reduced levels (e.g. concentrations) compared to currently available commercial formulations to treat a subject having diabetes. In accordance with these embodiments, a subject having diabetes can be a subject having early onset diabetes Type 1 such as one diagnosed within 5 years having with for example, detectible c-peptide levels, and/or with detectible insulin production, and/or with residual islet cell function.

[0083] Other embodiments can concern using a composition disclosed herein to protect islet cells *in vivo* (*e.g.* to preserve or rejuvenate islet cell function) or *in vitro* (e.g. during transport for transplantation). It is contemplated that compositions disclosed herein can be used to

treat a subject having diabetes that has some remaining islet cell function and/or treat islet cells prior to transplanting them into a subject, to preserve islet cell integrity and function. Thus, it is contemplated that a subject may be treated before, during or after islet cell transplantation. In other embodiments, diabetes treatments can include treating a subject having insulin resistant diabetes, Type I and Type II. It has been demonstrated that Fc-AAT fusion molecules disclosed herein are capable of modulating production of pro-inflammatory cytokines as observed for plasma-derived AAT, only at significantly reduced concentrations. Compositions including Fc-AAT fusion molecules (hinge deleted or hinge truncation) can be used to preserve islet cell populations in a subject in need thereof.

Cardiac Conditions

[0084] Some embodiments of the present invention comprise treating a subject having a cardiac condition or undergoing cardiac intervention (e.g. surgery, preventative treatment). In accordance with these embodiments, a subject having a cardiac condition may have one or more of the following conditions including, but not limited to, myocardial infarction, myocardial ischemia, chronic systemic arterial and venous hypertension, pulmonary arterial and venous hypertension, congenital heart disease (with and without intracardiac shunting), valvular heart disease, idiopathic dilated cardiomyopathy, infectious and non-infectious myocarditis, stress cardiomyopathy (as seen associated with critical care illnesses, physical and emotional stress, and intracranial hemorrhage and stroke), septic cardiomyopathy, atrial and ventricular arrhythmias, endocarditis, pericarditis, damage to heart muscle, cardioplegia, cardiac arrest, acute myocardial infarction (AMI), myocardial ischemia-reperfusion injury, ventricular remodeling, concentric hypertrophy,eccentric hypertrophy and any other known cardiac condition.

[0085] In certain embodiments, a subject having or suspected of having a myocardial infarction can be administered a composition disclosed herein to ameliorate the conditions such as the symptoms or side effects of the cardiac condition. In certain embodiments, compositions disclosed herein that include an Fc-AAT fusion molecule and a pharmaceutically acceptable carrier can be used to reduce or prevent cardiac ventricular remodeling or reduce the effects of ischemia reperfusion. Methods for treating any cardiac condition disclosed herein can include administering a composition before, during or after a cardiac event. In certain embodiments, compositions can be administered to a subject for a period determined by health professional to have optimum benefit after a cardiac event has occurred in a subject. For example, a subject may be treated with a composition for up to one

week, up to two weeks or more following an event. In certain embodiments, compositions administered to a subject described herein can be 5-fold, 10-fold, 100-fold or 1,000 fold less than using a commercially available AAT formulation (e.g. Aralast™, Zemaira™, Prolastin C™), such as .001 mg/kg to 10 mg/kg recombinant or Fc-AAT fusion molecule per dose.

Gastrointestinal Disorders

[0086] Some embodiments of the present invention include treating a subject having a gastrointestinal order or condition (e.g. intermittent, solitary or chronic condition) or inflammatory bowel disorder. In accordance with these embodiments, a subject having a gastrointestinal condition may have one or more of the following conditions including, but not limited to, inflammatory bowel disease (e.g. IBS or IBD), ulcerative colitis (UC), Crohn's disease (CD), systemic inflammatory response syndrome (SIRS), allergy-linked bowel disease, bowel disease linked to Type 1 diabetes, other colitis types (e.g. collagenous colitis, ischaemic colitis, diversion colitis, indeterminate colitis), Behçet's syndrome associated with inflammation of the bowels and other bowel disorders. In certain embodiments, symptoms or side effects of bowel disorders can be treated by compositions disclosed herein. For example, side effects of bowel disorders include, but are not limited to, skin manifestations, weight loss, colon shortening, intestinal mucosa, bowel or intestinal hyperpermeability can be ameliorated with a composition having an Fc-AAT fusion construct (e.g. hinge deletion or hinge truncation) and a pharmaceutically acceptable carrier. Certain embodiments can include treating a subject having a bowel disorder with compositions disclosed herein to reduce or prevent weight loss in a subject having the disorder. Compositions disclosed herein are supported by previous observations that Fc-AAT (IgG1) has anti-inflammatory activity superior to plasma-derived AAT demonstrated in a gastrointestinal mouse model and Fc-AAT3 (hinge deletion of Fc from IgG1) has comparible anti-inflammatory activities as Fc-AAT (IgG1).

Bacterial Conditions

[0087] Some embodiments of the present invention include treating a subject having a bacterial infection. Other embodiments can include administering a composition disclosed herein to prevent a bacterial infection in a subject. Bacterial infections contemplated herein can include, but are not limited to, Gram negative or Gram positive bacteria or mycobacterial organisms. Gram negative bacteria can include, but are not limited to, *N. gonorrhoeae, N. men ingitidi, M. catarrhalis, H. injiuenzae, E. coli,* all *Klebsiela spp.,* all *Enterobacter spp.,*

all Serratia spp, all *Salmonella spp., Proteus mirabilis, Proteus vulgaris,* all *Providencia spp.,* all *Morganella spp., Pseudomonas aeruginosa,* all *Citrobacter spp.,* all *Pasteurella spp.,* all *Aeromonas spp., Pseudomonas cepacia,* all *Shigella spp, Stenotrophomonas maltophilia,* all *Acinetobacter spp.,* all *Legionella spp., Y. enterocolitica, other Yersinoiiosis, H. ducreyeii,* all *Chlamyidia spp., Mycoplasma pneumonia, Mycoplasma hominis, Bacteroides fragilis, P. melaninogenica,* all *Moraxella spp.,* all *Bortedella spp.,* and *P. multocida.*

[0088] Mycobacteria contemplated herein can include, but are not limited to, *M. bovis, M. tuberculosis, Mycobacterium avium complex (MAC)* organisms, *M. intracellulare, M. avium, M. paratuberculosis, leprosy causing (M. leprae , M. flavascens, M. lepraemurium, M. microti, M. chelonei, M. africanum, M. marinium, M. buruli, M. fortuitum, M. haemophilum, M. kansasii, M. littorale, M.malmoense, M. marianum, M. simiae, M. szulgai, M. ulcerans, M. gordonae, M. gastri, M. phlei, M. nonchromogenicum, M. smegmatis, M. terrae, M. trivial, M. scrofulaceum, M. xenopi, M. gordonae, M. haemophilum, M. genavense, M. simiae, M. vaccae.*

[0089] Gram positive bacteria contemplated herein include, but are not limited to, *C. tetani, C. botulinum, C.difficile, Group A, B C, and G Streptococcus, Streptococcus pneumonia, Streptococcus milleri group, Viridans streptococcus, all Listeria spp., all Staphylococcus spp, S. aureus (MSSA), S. aureus (MRSA), S epidermidis, Enterococcus faecalis, Enterococcus faecium,* all *Clostridium spp., C. diptheriea, C. jeikium,* all *Rhodococcus spp.,* all *Leukonostoc spp. and Bacillus anthracis (e.g. that causes anthrax).*

[0090] In certain embodiments, compositions disclosed herein can be used to treat a subject having a bacterial condition, reducing or preventing onset of a bacterial associated condition.

[0091] Yet other embodiments concern treating or reducing septic shock in a subject. Septic shock can be caused by systemic bacterial infection of a subject, for example, to bacterial endotoxins, such as Gram negative lipopolysaccharides. In certain embodiments, it is thought that nitric oxide overproduction contributes to septic shock. Reduction in NO production has been demonstrated to reduce symptoms of septic shock. In accordance with these embodiments, methods disclosed herein relate to treating septic shock by administering an AAT fusion molecule such as Fc-AAT. Some embodiments include administering an AAT fusion molecule in conjunction with other therapies, e.g., antibodies to proinflammatory cytokines etc. Or agents that reduce lipopolysaccharides, reduce tumor necrosis factor or interleukin-1 expression, or interleukin-1 receptor antagonist expression, or soluble TNF or

IL-1 receptors. In certain embodiments, macrophages and endothelium can be cellular targets for inhibition of nitric oxide activity. To date, septic shock has eluded successful therapies.

Viral Conditions

[0092] Some embodiments of the present invention include treating a subject having a viral infection. Other embodiments herein can include administering a composition disclosed herein to prevent a viral infection from developing in a subject exposed to a virus. Viral infections contemplated herein can include, but are not limited to, Human Immunodeficiency Virus (HIV) AIDS, influenza virus (e.g. type A, B, C, influenza A H1N1, H1N2, H3N2, H9N2, H7N2, H10N7), Herpes zoster, Herpes simplex, human papilloma virus,Variola major virus (small pox), Lassa fever virus, avian flu, AIDS Related Complex, Chickenpox (Varicella), Cytomegalovirus (CMV), Colorado tick fever, Dengue fever, Ebola haemorrhagic fever, Hand, foot and mouth disease, Hepatitis, HPV, infectious mononucleosis, Mumps, Poliomyelitis, Progressive multifocal leukencephalopathy, Rabies, Rubella, SARS, viral encephalitis, viral gastroenteritis, viral meningitis, West Nile disease, Yellow fever, Marburg haemorrhagic fever, Measles and other viral-related disorders.

[0093] Other embodiments disclosed herein concern reducing or preventing developing cancer attributed to infection by a virus by inhibiting viral replication and/or infection in a subject using compositions disclosed herein. Cancers induced by viruses can include, but are not limited to, Rous sarcoma induced cancer, human papilloma virus (HPV) induced cancer (e.g cervical cancer), polyoma induced cancer, Hepatitis B virus induced cancer, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, angiosarcoma, chordoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, rhabdosarcoma, colorectal carcinoma, pancreatic cancer, breast cancer, melanoma, prostate cancer, ovarian cancer, squamous cell carcinoma, basal cell carcinoma, sebaceous gland carcinoma, adenocarcinoma, sweat gland carcinoma, papillary carcinoma, hepatoma, cystadenocarcinoma, papillary adenocarcinomas, bronchogenic carcinoma, medullary carcinoma, renal cell carcinoma, seminoma, bile duct carcinoma, cervical cancer, Wilms' tumor, embryonal carcinoma, lung carcinoma, choriocarcinoma, testicular tumor, bladder carcinoma, epithelial carcinoma, small cell lung carcinoma, craniopharyngioma, medulloblastoma, astrocytoma, glioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma,

myeloma, lymphoma, and leukemia. Yet other embodiments concern viral pneumonia and bronchial pneumonia.

[0094] In certain embodiments, compositions disclosed herein can be used to treat a subject having a viral infection, reducing or preventing onset of a viral associated condition. For example, compositions disclosed herein can be used to treat a subject having a viral infection to reduce transmission of the virus and reduce viral replication in the subject (e.g. influenza or other disease transmitted from subject to subject) thereby reducing subject to subject transmission.

Constructs of Various Peptides

[0095] Embodiments herein provide for rapidly generating and using AAT fusion molecules either full-length AAT or carboxyterminal peptides derived from AAT (e.g. a carboxyternminal peptide of AAT found in the last 80 amino acids of AAT or a carboxyterminal peptide of AAT found in the last 36 amino acids of AAT etc).

[0096] In one embodiment of the present invention, a composition may include constructs for treating a subject in need of AAT therapy (e.g. mammalian derived AAT) for example, a series of peptides including carboxyterminal amino acid peptides corresponding to AAT and derivatives thereof. These peptides can include, pentapetides including, FVFLM (SEQ ID NO:2), FVFAM (SEQ ID NO:3), FVALM (SEQ ID NO:4), FVFLA (SEQ ID NO:5), FLVFI (SEQ ID NO:6), FLMII (SEQ ID NO:7), FLFVL (SEQ ID NO:8), FLFVV (SEQ ID NO:9), FLFLI (SEQ ID NO:10), FLFFI (SEQ ID NO:11), FLMFI (SEQ ID NO:12), FMLLI (SEQ ID NO:13), FIIMI (SEQ ID NO:14), FLFCI (SEQ ID NO:15), FLFAV (SEQ ID NO:16), FVYLI (SEQ ID NO:17), FAFLM (18), AVFLM (SEQ ID NO:19), and any combination thereof.

[0097] In other embodiments, AAT peptides contemplated for use in constructs, pharmaceutical compositions and methods herein are also intended to include any and all of those specific AAT peptides of SEQ ID NO:1 or SEQ ID NO:33 (naturally-occurring AAT of 394 amino acids, the most common form is the M type with subtypes M1, M2, M3 etc. are also contemplated herein) associated with the carboxyterminal amino acids. All AAT polypeptides are contemplated of use in methods disclosed herein, that possess anti-inflammatory activity and/or immune regulatory activity. Any combination of consecutive amino acids simulating AAT or AAT-like activity may be used, such as amino acids ranging from 315-394, amino acids ranging from 325-384, 358-394, 340-380 etc. In addition,

combinations of consecutive amino acid sequences such as 5-mers, 10-mers, 15-mers, 20-mers, 25-mers, 30-mers, 35-mers etc. of the carboxyterminus can also be used. For example, any combinations of consecutive amino acids of 5-mers,10-mers, 15-mers, 20-mers from SEQ ID NO:1 AAs 314-394 can be used in developing or purifying a construct contemplated herein.

[0098] Certain embodiments concern generating a recombinant fusion protein including linking an entire AAT molecule (e.g. SEQ ID NO: 1 or 33) or a peptide molecule derived from the carboxyterminal amino acid region of AAT, to an IgG (e.g. Fc or mutant Fc for example, to reduce the hinge region) or fragment thereof. One common form of AAT is denoted by SEQ ID NO:33. One construct contemplated herein is referenced as SEQ ID NO:32 (*e.g.* full-length AAT, a leader sequence and an Fc portion/fragment of an immunoglobulin molecule). These constructs can be used in dimer form or as a monomeric form in compositions disclosed herein. In accordance with these embodiments, a pharmaceutically acceptable composition can include a dimer of Fc-AAT or a monomer of Fc-AAT or AAT cleaved from the Fc or combinations thereof, and a pharmaceutically acceptable excipient. In addition, point mutations can be made in the Fc region to reduce the flexibility of the hinge region and generate novel Fc-AAT molecules. In other embodiments, the hinge region of Fc derived from IgG1, IgG2, IgG3 or IgG4 can be deleted or truncated prior to linking an Fc to AAT or AAT peptide. Fc can be further manipulated to modify the region to reduce receptor interactions and enhance Fc-AAT construct activity. For example, point mutations can be made in the Fc region to reduce the flexibility of the hinge region or deletions or additions to this region can be made to affect secondary interactions regarding this region or that alter tertiary structure of the fusion molecule to generate novel Fc-AAT molecules.

[0099] SEQ ID NO:33: EDPQGDAAQKTDTSHHDQDHPTFNKITPNLAEFAFS
LYRQLAHQSNSTNIFFSPVSIATAFAMLSLGTKADTHDEILEGLNFNLTEIPEAQIHEGF
QELLRTLNQPDSQLQLTTGNGLFLSEGLKLVDKFLEDVKKLYHSEAFTVNFGDTEEA
KKQINDYVEKGTQGKIVDLVKELDRDTVFALVNYIFFKGKWERPFEVKDTEEEDFH
VDQATTVKVPMMKRLGMFNIQHCKKLSSWVLLMKYLGNATAIFFLPDEGKLQHLE
NELTHDIITKFLENEDRRSASLHLPKLSITGTYDLKSVLGQLGITKVFSNGADLSGVTE
EAPLKLSKAVHKAVLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKS
PLFMG KVVNPTQK

[00100] In other embodiments, AAT protease binding domain can be mutated in order to reduce or eliminate the protease function of the molecule and not inhibit elastase activity; these molecules can be used in any construct contemplated herein such as a Fc-AAT mutant. In certain embodiments, a mutated AAT can be used to generate an AAT construct by methods disclosed herein. In other embodiments, a mutated molecule (e.g. having reduced or essentially no protease activity) retains its anti-inflammatory effects and/or immunomodulatory effects and can be used as an anti-inflammatory molecule in a subject having a need for AAT therapy. One skilled in the art would understand a non-protease binding domain of AAT as well as what is termed the carboxyterminal last 80 amino acids of naturally-occurring AAT.

[00101] In each of the above-recited methods, αl-antitrypsin or carboxyterminal peptide derivatives thereof are contemplated for use in a composition herein. These peptide derivatives may include but are not limited to amino acid peptides containing the last 80 carboxyterminal derived amino acids of AAT, GITKVFSNGA (SEQ ID NO:20), DLSGVTEEAP (SEQ ID NO:21), LKLSKAVHKA (SEQ ID NO:22), VLTIDEKGTE (SEQ ID NO:23), AAGAMFLEAI (SEQ ID NO:24), PMSIPPEVKF (SEQ ID NO:25), NKPFVFLMIE (SEQ ID NO:26), QNTKSPLFMG (SEQ ID NO:27), KVVNPTQK (SEQ ID NO:28), LEAIPMSIPPEVKFNKPFVFLM (SEQ ID NO:29); and LEAIPMSIPPEVKFNKPFVF (SEQ ID NO:30), GADLSGVTEEAPLKLSKAVHKA VLTIDEKGTEAAGAMFLEAIPMSIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK (SEQ ID NO:31), SEQ ID NO:34 or any combination thereof. In certain embodiments, the carboxyterminal peptides of AAT are 80%, or 85%, or 90%, or 95%, or 99% identical to the naturally occurring M type amino acid sequence identified by SEQ ID NO. 33. In certain embodiments, about 3, or about 4, or about 5 amino acids can vary (e.g. point mutations) from an 80-mer from the carboxy terminal of M type sequence.

[00102] Certain embodiments include compositions of the fusion molecule SEQ ID NO: 32 or other Fc–AAT fusion molecule with or without an Fc hinge region where an Fc region originates from IgG1, IgG2, IgG3 or IgG4 or even IgD. In accordance with these embodiments, the compositions can be a pharmaceutical composition.

[00103] In certain embodiments, compositions of recombinant AAT or AAT-derived carboxyterminal peptides capable of binding to SEC receptors or compositions with AAT-like activities may be administered to a subject in need thereof. As disclosed herein the carboxy terminal region of AAT includes the last 80 amino acids (SEQ ID NO:31) or other

human AAT molecule or other naturally occurring AAT molecule. In other embodiments, peptides derived from AAT can include 5-mers, 10-mers, 20-mers, 25-mers, 30-mers, 35-mers, 40-mers, 50-mers, and up to an 80 mer of an AAT molecule wherein any of the contemplated peptides have no significant serine protease inhibitor activity, are derived from the carboxyterminus of AAT and are capable of being used for treating subjects undergoing radiation or subjects exposed to large doses of radiation by accident or other cause.

[00104] In one embodiment of the present invention, a construct may include compounds that engage or associate with the SEC receptor. In some of the recited methods, an AAT-mutant or AAT derived peptide (*e.g.* mammalian derived) having no significant serine protease inhibitor activity contemplated for use within the methods of the present invention can include a series of peptides including carboxyterminal amino acid peptides corresponding to AAT. In addition, combinations of amino acid 5-mers or 10-mers or 20-mers or 30-mers or more can also be used. For example, one or more 5-mers or 10-mers or 20-mers etc can include consecutive amino acids starting from AA 315 and ending with AA 394 of naturally occurring AAT represented as SEQ ID NO:1. As contemplated herein, the later half of a sequence toward the carboxy end is referred to as the carboxyterminus. In certain embodiments, the carboxyl domain of AAT going backwards from the carboxyl terminus is defined as those amino acids most conserved among the difference species and do not participate in the protease binding domain of AAT. In addition, in other embodiments, AAT protease binding domain can be mutated in order to reduce or eliminate the protease function of the molecule and this molecule can be used in any composition contemplated herein. In other embodiments, a mutated molecule can retain its anti-inflammatory and/or immunomodulatory effects. Also contemplated herein is that the carboxyl domain is the non-protease binding domain. One skilled in the art would understand a non-protease binding domain of AAT.

[00105] In each of the above-recited methods, compositions herein may include peptides derived from the carboxyterminus of AAT. In certain embodiments, AAT-associated molecules used in the methods and compositions herein can include, but are not limited to, compositions of SEQ ID NO:1, naturally occurring AAT (394 AA length molecule making up approximately 90% of AAT isolated from serum), other AAT M-types or other AAT molecules.

Grid:

Underline= restriction site

No marking= human AAT molecule

Fc= shaded

Hinge region= ***italic and bold*** (Lucida console)

SEQ ID NO:47

AAT-Fc2 (pCAG.neo-hAAT-hIgG1 Fc) (nucleic acid sequence to SEQ ID NO:32) Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG1)

< DNA sequence > dsDNA 1977 bp

GAATTCGCCA CCATGCCGTC TTCTGTCTCG TGGGGCATCC TCCTGCTGGC AGGCCTGTGC 60

TGCCTGGTCC CTGTCTCCCT GGCTGAGGAT CCCCAGGGAG ATGCTGCCCA GAAGACAGAT 120

ACATCCCACC ACGATCAGGA TCACCCAACC TTCAACAAGA TCACCCCCAA CCTGGCTGAG 180

TTCGCCTTCA GCCTATACCG CCAGCTGGCA CACCAGTCCA ACAGCACCAA TATCTTCTTC 240

TCCCCAGTGA GCATCGCTAC AGCCTTTGCA ATGCTCTCCC TGGGGACCAA GGCTGACACT 300

CACGATGAAA TCCTGGAGGG CCTGAATTTC AACCTCACGG AGATTCCGGA GGCTCAGATC 360

CATGAAGGCT TCCAGGAACT CCTCCGTACC CTCAACCAGC CAGACAGCCA GCTCCAGCTG 420

ACCACCGGCA ATGGCCTGTT CCTCAGCGAG GGCCTGAAGC TAGTGGATAA GTTTTTGGAG 480

GATGTTAAAA AGTTGTACCA CTCAGAAGCC TTCACTGTCA ACTTCGGGGA CACCGAAGAG 540

GCCAAGAAAC AGATCAACGA TTACGTGGAG AAGGGTACTC AAGGGAAAAT TGTGGATTTG 600

GTCAAGGAGC TTGACAGAGA CACAGTTTTT GCTCTGGTGA ATTACATCTT CTTTAAAGGC 660

AAATGGGAGA GACCCTTTGA AGTCAAGGAC ACCGAGGAAG AGGACTTCCA CGTGGACCAG 720

GCGACCACCG TGAAGGTGCC TATGATGAAG CGTTTAGGCA TGTTTAACAT CCAGCACTGT 780

AAGAAGCTGT CCAGCTGGGT GCTGCTGATG AAATACCTGG GCAATGCCAC CGCCATCTTC 840

TTCCTGCCTG ATGAGGGGAA ACTACAGCAC CTGGAAAATG AACTCACCCA CGATATCATC 900

ACCAAGTTCC TGGAAAATGA AGACAGAAGG TCTGCCAGCT TACATTTACC CAAACTGTCC 960

ATTACTGGAA CCTATGATCT GAAGAGCGTC CTGGGTCAAC TGGGCATCAC TAAGGTCTTC 1020

AGCAATGGGG CTGACCTCTC CGGGGTCACA GAGGAGGCAC CCCTGAAGCT CTCCAAGGCC 1080

GTGCATAAGG CTGTGCTGAC CATCGACGAG AAAGGGACTG AAGCTGCTGG GGCCATGTTT 1140

TTAGAGGCCA TACCCATGTC TATCCCCCCC GAGGTCAAGT TCAACAAACC CTTTGTCTTC 1200

TTAATGATTG AACAAAATAC CAAGTCTCCC CTCTTCATGG GAAAAGTGGT GAATCCCACC 1260

CAAAAA**ACGC GT**GAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCCACC GTGCCCAGCA 1320

CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA GGACACCCTC 1380

ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT 1440

GAGGTCAAGT TCAACTGGTA CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG 1500

CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG 1560

GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC 1620

ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT GTACACCCTG 1680

CCCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC 1740

TTCTATCCCA GCGACATCGC CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC 1800

AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC 1860

GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT 1920

CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG AGGATCT 1977

SEQ ID NO:32

AAT-Fc2 < Amino acid sequence >

652 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60

LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120

QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180

INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240

KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300

ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360

VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQKTR 420

EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF 480

NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT 540

ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 600

PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 652

SEQ ID NO:48

AAT-Fc3 (pCAG.neo-hAAT-hIgG1 Fc; hinge deletion)

(Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG1 hinge deletion)

< DNA sequence > dsDNA 1950 bp

GAATTCGCCA CCATGCCGTC TTCTGTCTCG TGGGGCATCC TCCTGCTGGC AGGCCTGTGC 60

TGCCTGGTCC CTGTCTCCCT GGCTGAGGAT CCCCAGGGAG ATGCTGCCCA GAAGACAGAT 120

ACATCCCACC ACGATCAGGA TCACCCAACC TTCAACAAGA TCACCCCCAA CCTGGCTGAG 180

TTCGCCTTCA GCCTATACCG CCAGCTGGCA CACCAGTCCA ACAGCACCAA TATCTTCTTC 240

TCCCCAGTGA GCATCGCTAC AGCCTTTGCA ATGCTCTCCC TGGGGACCAA GGCTGACACT 300

CACGATGAAA TCCTGGAGGG CCTGAATTTC AACCTCACGG AGATTCCGGA GGCTCAGATC 360

CATGAAGGCT TCCAGGAACT CCTCCGTACC CTCAACCAGC CAGACAGCCA GCTCCAGCTG 420

ACCACCGGCA ATGGCCTGTT CCTCAGCGAG GGCCTGAAGC TAGTGGATAA GTTTTTGGAG 480

GATGTTAAAA AGTTGTACCA CTCAGAAGCC TTCACTGTCA ACTTCGGGGA CACCGAAGAG 540

GCCAAGAAAC AGATCAACGA TTACGTGGAG AAGGGTACTC AAGGGAAAAT TGTGGATTTG 600

GTCAAGGAGC TTGACAGAGA CACAGTTTTT GCTCTGGTGA ATTACATCTT CTTTAAAGGC 660

AAATGGGAGA GACCCTTTGA AGTCAAGGAC ACCGAGGAAG AGGACTTCCA CGTGGACCAG 720

GCGACCACCG TGAAGGTGCC TATGATGAAG CGTTTAGGCA TGTTTAACAT CCAGCACTGT 780

AAGAAGCTGT CCAGCTGGGT GCTGCTGATG AAATACCTGG GCAATGCCAC CGCCATCTTC 840

TTCCTGCCTG ATGAGGGGAA ACTACAGCAC CTGGAAAATG AACTCACCCA CGATATCATC 900

ACCAAGTTCC TGGAAAATGA AGACAGAAGG TCTGCCAGCT TACATTTACC CAAACTGTCC 960

ATTACTGGAA CCTATGATCT GAAGAGCGTC CTGGGTCAAC TGGGCATCAC TAAGGTCTTC 1020

AGCAATGGGG CTGACCTCTC CGGGGTCACA GAGGAGGCAC CCCTGAAGCT CTCCAAGGCC 1080

GTGCATAAGG CTGTGCTGAC CATCGACGAG AAAGGGACTG AAGCTGCTGG GGCCATGTTT 1140

TTAGAGGCCA TACCCATGTC TATCCCCCCC GAGGTCAAGT TCAACAAACC CTTTGTCTTC 1200

TTAATGATTG AACAAAATAC CAAGTCTCCC CTCTTCATGG GAAAAGTGGT GAATCCCACC 1260

CAAAAA**ACGC GT**ACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 1320

TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 1380

TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 1440

GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 1500

CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1560

TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1620

GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1680

AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1740

TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1800

GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1860

AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1920

CTCTCCCTGT CTCCGGGTAA ATGA**GGATCT** 1950

SEQ ID NO:49

AAT-Fc3 < Amino acid sequence > new sequence 49 (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG1)

643 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60

LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120

QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180

INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240

KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300

ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360

VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQK**TR** 420

TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV 480

HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR 540

EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 600

FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 643

SEQ ID NO:50

AAT-Fc4 (pCAG.neo-hAAT-hIgG2 Fc, intact) >

(Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG2)

< DNA sequence > dsDNA 1962 bp

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TGCCTGGTCC CTGTCTCCCT GGCTGAGGAT CCCCAGGGAG ATGCTGCCCA GAAGACAGAT 120

ACATCCCACC ACGATCAGGA TCACCCAACC TTCAACAAGA TCACCCCCAA CCTGGCTGAG 180

TTCGCCTTCA GCCTATACCG CCAGCTGGCA CACCAGTCCA ACAGCACCAA TATCTTCTTC 240
TCCCCAGTGA GCATCGCTAC AGCCTTTGCA ATGCTCTCCC TGGGGACCAA GGCTGACACT 300
CACGATGAAA TCCTGGAGGG CCTGAATTTC AACCTCACGG AGATTCCGGA GGCTCAGATC 360
CATGAAGGCT TCCAGGAACT CCTCCGTACC CTCAACCAGC CAGACAGCCA GCTCCAGCTG 420
ACCACCGGCA ATGGCCTGTT CCTCAGCGAG GGCCTGAAGC TAGTGGATAA GTTTTTGGAG 480
GATGTTAAAA AGTTGTACCA CTCAGAAGCC TTCACTGTCA ACTTCGGGGA CACCGAAGAG 540
GCCAAGAAAC AGATCAACGA TTACGTGGAG AAGGGTACTC AAGGGAAAAT TGTGGATTTG 600
GTCAAGGAGC TTGACAGAGA CACAGTTTTT GCTCTGGTGA ATTACATCTT CTTTAAAGGC 660
AAATGGGAGA GACCCTTTGA AGTCAAGGAC ACCGAGGAAG AGGACTTCCA CGTGGACCAG 720
GCGACCACCG TGAAGGTGCC TATGATGAAG CGTTTAGGCA TGTTTAACAT CCAGCACTGT 780
AAGAAGCTGT CCAGCTGGGT GCTGCTGATG AAATACCTGG GCAATGCCAC CGCCATCTTC 840
TTCCTGCCTG ATGAGGGGAA ACTACAGCAC CTGGAAAATG AACTCACCCA CGATATCATC 900
ACCAAGTTCC TGGAAAATGA AGACAGAAGG TCTGCCAGCT TACATTTACC CAAACTGTCC 960
ATTACTGGAA CCTATGATCT GAAGAGCGTC CTGGGTCAAC TGGGCATCAC TAAGGTCTTC 1020
AGCAATGGGG CTGACCTCTC CGGGGTCACA GAGGAGGCAC CCCTGAAGCT CTCCAAGGCC 1080
GTGCATAAGG CTGTGCTGAC CATCGACGAG AAAGGGACTG AAGCTGCTGG GGCCATGTTT 1140
TTAGAGGCCA TACCCATGTC TATCCCCCCC GAGGTCAAGT TCAACAAACC CTTTGTCTTC 1200
TTAATGATTG AACAAAATAC CAAGTCTCCC CTCTTCATGG GAAAAGTGGT GAATCCCACC 1260
CAAAAA**ACGC GT**CGCAAATG TTGTGTCGAG TGCCCACCGT GCCCAGCACC ACCTGTGGCA 1320
GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC 1380
CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC 1440
TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC 1500
AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC 1560
AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC 1620
TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT 1680
GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC 1740
ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC 1800
GTGCTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG 1860
TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC 1920
ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA**GGAT CT** 1962

SEQ ID NO:51

AAT-Fc4 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG2)

647 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300

ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360

VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQK**TR** 420

RKCCVECPPC PAPPVAGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD 480

GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK 540

GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS 600

DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 647

SEQ ID NO:52

AAT-Fc5 (pCAG.neo-hAAT-hIgG3 Fc, intact) (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG3)

< DNA sequence > dsDNA 1995 bp

GAATTCGCCA CCATGCCGTC TTCTGTCTCG TGGGGCATCC TCCTGCTGGC AGGCCTGTGC 60

TGCCTGGTCC CTGTCTCCCT GGCTGAGGAT CCCCAGGGAG ATGCTGCCCA GAAGACAGAT 120

ACATCCCACC ACGATCAGGA TCACCCAACC TTCAACAAGA TCACCCCCAA CCTGGCTGAG 180

TTCGCCTTCA GCCTATACCG CCAGCTGGCA CACCAGTCCA ACAGCACCAA TATCTTCTTC 240

TCCCCAGTGA GCATCGCTAC AGCCTTTGCA ATGCTCTCCC TGGGGACCAA GGCTGACACT 300

CACGATGAAA TCCTGGAGGG CCTGAATTTC AACCTCACGG AGATTCCGGA GGCTCAGATC 360

CATGAAGGCT TCCAGGAACT CCTCCGTACC CTCAACCAGC CAGACAGCCA GCTCCAGCTG 420

ACCACCGGCA ATGGCCTGTT CCTCAGCGAG GGCCTGAAGC TAGTGGATAA GTTTTTGGAG 480

GATGTTAAAA AGTTGTACCA CTCAGAAGCC TTCACTGTCA ACTTCGGGGA CACCGAAGAG 540

GCCAAGAAAC AGATCAACGA TTACGTGGAG AAGGGTACTC AAGGGAAAAT TGTGGATTTG 600

GTCAAGGAGC TTGACAGAGA CACAGTTTTT GCTCTGGTGA ATTACATCTT CTTTAAAGGC 660

AAATGGGAGA GACCCTTTGA AGTCAAGGAC ACCGAGGAAG AGGACTTCCA CGTGGACCAG 720

GCGACCACCG TGAAGGTGCC TATGATGAAG CGTTTAGGCA TGTTTAACAT CCAGCACTGT 780

AAGAAGCTGT CCAGCTGGGT GCTGCTGATG AAATACCTGG GCAATGCCAC CGCCATCTTC 840

TTCCTGCCTG ATGAGGGGAA ACTACAGCAC CTGGAAAATG AACTCACCCA CGATATCATC 900

ACCAAGTTCC TGGAAAATGA AGACAGAAGG TCTGCCAGCT TACATTTACC CAAACTGTCC 960

ATTACTGGAA CCTATGATCT GAAGAGCGTC CTGGGTCAAC TGGGCATCAC TAAGGTCTTC 1020

AGCAATGGGG CTGACCTCTC CGGGGTCACA GAGGAGGCAC CCCTGAAGCT CTCCAAGGCC 1080

GTGCATAAGG CTGTGCTGAC CATCGACGAG AAAGGGACTG AAGCTGCTGG GGCCATGTTT 1140

TTAGAGGCCA TACCCATGTC TATCCCCCCC GAGGTCAAGT TCAACAAACC CTTTGTCTTC 1200

TTAATGATTG AACAAAATAC CAAGTCTCCC CTCTTCATGG GAAAAGTGGT GAATCCCACC 1260

CAAAAA**ACGC GT**CCATGCCC ACGGTGCCCA GAGCCCAAAT CTTGTGACAC ACCTCCCCCG 1320

TGCCCAAGGT GCCCAGCACC TGAACTCCTG GGGGGACCGT CAGTCTTCCT CTTCCCCCCA 1380

AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCCTGAGG TCACATGCGT GGTGGTGGAC 1440

GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG TGGACGGCGT GGAGGTGCAT 1500

AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TACAACAGCA CGTACCGTGT GGTCAGCGTC 1560

CTCACCGTCC TGCACCAGGA CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC 1620

AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA 1680

CCACAGGTGT ACACCCTGCC CCCATCCCGG GATGAGCTGA CCAAGAACCA GGTCAGCCTG 1740

ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG 1800
CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG CTCCTTCTTC 1860
CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC 1920
TCCGTGATGC ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG 1980
GGTAAATGA**G GATCT** 1995

SEQ ID NO:53

AAT-Fc5 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG3)

658 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQK**TR** 420
PCPRCPEPKS CDTPPPCPRC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE 480
DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP 540
APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN 600
NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 658

SEQ ID NO:54

AAT-Fc6 (pCAG.neo-hAAT-hIgG4 Fc, intact) Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG4)

< DNA sequence > dsDNA 1965 bp

GAATTCGCCA CCATGCCGTC TTCTGTCTCG TGGGGCATCC TCCTGCTGGC AGGCCTGTGC 60
TGCCTGGTCC CTGTCTCCCT GGCTGAGGAT CCCCAGGGAG ATGCTGCCCA GAAGACAGAT 120
ACATCCCACC ACGATCAGGA TCACCCAACC TTCAACAAGA TCACCCCCAA CCTGGCTGAG 180
TTCGCCTTCA GCCTATACCG CCAGCTGGCA CACCAGTCCA ACAGCACCAA TATCTTCTTC 240
TCCCCAGTGA GCATCGCTAC AGCCTTTGCA ATGCTCTCCC TGGGGACCAA GGCTGACACT 300
CACGATGAAA TCCTGGAGGG CCTGAATTTC AACCTCACGG AGATTCCGGA GGCTCAGATC 360
CATGAAGGCT TCCAGGAACT CCTCCGTACC CTCAACCAGC CAGACAGCCA GCTCCAGCTG 420
ACCACCGGCA ATGGCCTGTT CCTCAGCGAG GGCCTGAAGC TAGTGGATAA GTTTTTGGAG 480
GATGTTAAAA AGTTGTACCA CTCAGAAGCC TTCACTGTCA ACTTCGGGGA CACCGAAGAG 540
GCCAAGAAAC AGATCAACGA TTACGTGGAG AAGGGTACTC AAGGGAAAAT TGTGGATTTG 600
GTCAAGGAGC TTGACAGAGA CACAGTTTTT GCTCTGGTGA ATTACATCTT CTTTAAAGGC 660
AAATGGGAGA GACCCTTTGA AGTCAAGGAC ACCGAGGAAG AGGACTTCCA CGTGGACCAG 720
GCGACCACCG TGAAGGTGCC TATGATGAAG CGTTTAGGCA TGTTTAACAT CCAGCACTGT 780
AAGAAGCTGT CCAGCTGGGT GCTGCTGATG AAATACCTGG GCAATGCCAC CGCCATCTTC 840
TTCCTGCCTG ATGAGGGGAA ACTACAGCAC CTGGAAAATG AACTCACCCA CGATATCATC 900

ACCAAGTTCC TGGAAAATGA AGACAGAAGG TCTGCCAGCT TACATTTACC CAAACTGTCC 960
ATTACTGGAA CCTATGATCT GAAGAGCGTC CTGGGTCAAC TGGGCATCAC TAAGGTCTTC 1020
AGCAATGGGG CTGACCTCTC CGGGGTCACA GAGGAGGCAC CCCTGAAGCT CTCCAAGGCC 1080
GTGCATAAGG CTGTGCTGAC CATCGACGAG AAAGGGACTG AAGCTGCTGG GGCCATGTTT 1140
TTAGAGGCCA TACCCATGTC TATCCCCCCC GAGGTCAAGT TCAACAAACC CTTTGTCTTC 1200
TTAATGATTG AACAAAATAC CAAGTCTCCC CTCTTCATGG GAAAAGTGGT GAATCCCACC 1260
CAAAAA**ACGC GT**TCCAAATA TGGTCCCCCA TGCCCATCAT GCCCAGCACC TGAGTTCCTG 1320
GGGGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ACACCCTCAT GATCTCCCGG 1380
ACCCCTGAGG TCACATGCGT GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC 1440
AACTGGTACG TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG 1500
TACAACAGCA CGTACCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAT 1560
GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC
GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC 1620
ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA CCACAGGTGT ACACCCTGCC CCCATCCCGG 1680
GATGAGCTGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC 1740
GACATCGCCG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT 1800
CCCGTGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC 1860
AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC 1920
TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGA**G GATCT** 1965

SEQ ID NO:55

AAT-Fc6 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG4)

648 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQK<u>TR</u> 420
SKYGPPCPSC PAPEFLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV 480
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA 540
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD 600
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK 648

SEQ ID NO:56

AAT-Fc7 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG2 with hinge deletion)

634 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60

LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQKTR 420
PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE 480
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP 540
SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD 600
KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK 634

SEQ ID NO:57

AAT-Fc7 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG2 with hinge deletion)

634 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQKTR 420
GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ 480
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR 540
DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS 600
RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 632

SEQ ID NO:58

AAT-Fc9 < Amino acid sequence > (Artificial: derived from human alpha-1 antitrypsin and human Fc fragment of IgG4 with hinge deletion)

632 a.a.

MPSSVSWGIL LLAGLCCLVP VSLAEDPQGD AAQKTDTSHH DQDHPTFNKI TPNLAEFAFS 60
LYRQLAHQSN STNIFFSPVS IATAFAMLSL GTKADTHDEI LEGLNFNLTE IPEAQIHEGF 120
QELLRTLNQP DSQLQLTTGN GLFLSEGLKL VDKFLEDVKK LYHSEAFTVN FGDTEEAKKQ 180
INDYVEKGTQ GKIVDLVKEL DRDTVFALVN YIFFKGKWER PFEVKDTEEE DFHVDQATTV 240
KVPMMKRLGM FNIQHCKKLS SWVLLMKYLG NATAIFFLPD EGKLQHLENE LTHDIITKFL 300
ENEDRRSASL HLPKLSITGT YDLKSVLGQL GITKVFSNGA DLSGVTEEAP LKLSKAVHKA 360
VLTIDEKGTE AAGAMFLEAI PMSIPPEVKF NKPFVFLMIE QNTKSPLFMG KVVNPTQKTR 420
GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ 480
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR 540
DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS 600

RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK 632

[00106] Commercially available formulations for comparisons and/or controls with recombinant or fusion molecules disclosed herein can include plasma-derived AAT in commercially available formulations of Aralast™ (Baxter), Zemaira™ (Aventis Behring), Prolastin™ or ProlastinC™ (Talecris), Aprotonin™ or Trasylol™ (Bayer Pharmaceutical Corporation), Ulinistatin™ (Ono Pharmaceuticals, Inc.), and inhalation and/or injectible AAT, Glassia™ (Kamada, Ltd., Israel), or any other commercially available AAT compositions or any combination thereof.

[00107] Other embodiments concern mutants of human AAT where the mutant is generated to have no significant serine protease inhibitor activity. Any method known in the art for generating mutants is contemplated. Some embodiments include using site-directed mutageneis to generate a hATT having no significant serine protease inhibitor activity (see Examples section and pEF-hAAT). In some embodiments, compositions can be a pharmaceutical composition having a mutated human alpha-1 antitrypsin (hAAT) wherein the AAT includes AAT with one or more point mutations at AAT's protease-binding site within AAT's reactive center loop (RCL). These one or more point mutations can significantly reduces or eliminate serine protease inhibition activity of the AAT compared to a control human AAT. Other methods include disrupting the serine protease inhibiting region of hAAT by other disruption methods such as heating hAAT, or generating a mutant such as an RCL mutant with a modified proline to cysteine residue at position 357 within the RCL to eliminate or dramatically reduce serine protease inhibitor activity, or chemically modifying AAT (e. g. human AAT). In certain embodiments, a fusion molecule can include linking manipulated Fc (e.g. IgG1, 2, 3 or 4) or FAB to an AAT mutant having one or more point mutations at one or more of amino acids within the RCL, (e.g. amino acids 355-363 of native AAT), wherein the AAT mutant has no significant serine protease inhibition activity and the RCL remains intact.

Pharmaceutical Compositions

[00108] Embodiments herein provide for administration of compositions to subjects in a biologically compatible form suitable for pharmaceutical administration in vivo. By "biologically compatible form suitable for administration in vivo" is meant a form of the active agent (e.g. pharmaceutical chemical, protein, gene, antibody etc of the embodiments) to be administered in which any toxic effects are outweighed by the therapeutic effects of the

active agent. Administration of a therapeutically active amount of the therapeutic compositions is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of antibody to elicit a desired response in the individual. Dosage regima may be adjusted to provide the optimum therapeutic response.

[00109] Pharmaceutical compositions containing AAT or peptide fragment thereof, or analog thereof, or mutant thereof, or a functional derivative thereof (*e.g.* pharmaceutical chemical, protein, peptide of some of the embodiments) may be administered to a subject, for example by subcutaneous, intravenous, intracardiac, intracoronary, intramuscular, by oral administration, by inhalation, transdermal application, intravaginal application, topical application, intranasal or rectal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the degradation by enzymes, acids and other natural conditions that may inactivate the compound. In a preferred embodiment, the compound may be orally administered. In another preferred embodiment, the compound may be administered intravenously. In one particular embodiment, the composition may be administered intranasally, such as inhalation.

[00110] Some embodiments disclosed herein concern using a stent or a catheter to deliver one or more chemotherapeutic agents (*e.g.* along with compositions disclosed herein) to a subject having or suspected being treated for cancer. Any stent or other delivery method known in the art that can deliver one or more agents directly to tumor site is contemplated. These delivery techniques can be used alone or in combination with other delivery methods.

[00111] A compound (*e.g.* a peptide, protein or mixture thereof) may be administered to a subject in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. It may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. The active agent may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

[00112] Pharmaceutical compositions suitable for injectable use may be administered by means known in the art. For example, sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion may be used.

[00113] Sterile injectable solutions can be prepared by incorporating active compound (*e.g.* a compound that reduces serine protease activity) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[00114] Aqueous compositions can include an effective amount of a therapeutic compound, peptide, epitopic core region, stimulator, inhibitor, and the like, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Compounds and biological materials disclosed herein can be purified by means known in the art. Solutions of the active compounds as free-base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose.

[00115] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above. It is contemplated that slow release capsules, timed-release microparticles, and the like can also be employed. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

[00116] The active therapeutic agents may be formulated within a mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 1 to 10 gram per dose. Single dose or multiple doses can also be administered on an appropriate schedule for a predetermined condition such as daily, bi-weekly, weekly, bi-monthly etc. Pharmaceutical compositions are administered in an amount, and with a frequency, that is effective to modulate side effects. The precise dosage and duration of treatment may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Dosages may also vary with the severity of the condition. In certain embodiments, the composition range can be between 1.0 and 75 mg/kg introduced daily or weekly to a subject. A therapeutically effective amount of α1-antitrypsin, peptides, or drugs that have similar activities as α1-

antitrypsin or peptides can be also measured in molar concentrations and can range between about 1 nM to about 2 mM.

[00117] In another embodiment, nasal solutions or sprays, aerosols or inhalants may be used to deliver the compound of interest. Additional formulations that are suitable for other modes of administration may include suppositories and pessaries. A rectal pessary or suppository may also be used. In general, for suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

[00118] Liposomes or microparticles can be used as a therapeutic delivery system and can be prepared in accordance with known laboratory techniques. In addition, dried lipids or lyophilized liposomes prepared as previously described may be reconstituted in a solution of active agent (*e.g.* nucleic acid, peptide, protein or chemical agent), and the solution diluted to an appropriate concentration with a suitable solvent known to those skilled in the art. The amount of active agent encapsulated can be determined in accordance with standard methods.

[00119] In some embodiments, pharmaceutical construct compositions concerns a construct derived from an AAT molecule having no significant serine protease inhibitor activity but having other α1-antitrypsin activity or analog thereof may be used in a single therapeutic dose, acute manner or a chronic manner to treat a subject. For example, the fusion polypeptides contemplated herein can be a fusion polypeptide having no significant protease inhibition activity.

[00120] In certain embodiments, compositions herein can be administered orally, systemically, via an implant, time released or slow-release compositions (*e.g.* gel, microparticles etc.), intravenously, topically, intrathecally, subcutaneously, by inhalation, nasally, or by other means known in the art or a combination thereof.

Expression Proteins and Constructs

[00121] Once the target gene or portion of a gene has been determined, the gene can be inserted into an appropriate expression system. The gene can be expressed in any number of different recombinant DNA expression systems to generate large amounts of the polypeptide product, which can then be purified and used in compositions and methods disclosed herein.

[00122] Examples of expression systems known to the skilled practitioner in the art include bacteria such as *E. coli*, yeast such as *Pichia pastoris*, baculovirus, and mammalian

expression systems such as in Cos or CHO cells. A complete gene can be expressed or, alternatively, fragments of the gene encoding portions of polypeptide can be produced.

[00123] The AAT gene or gene fragment encoding a polypeptide may be inserted into an expression vector by standard subcloning techniques. An E. coli expression vector may be used which produces the recombinant polypeptide as a fusion protein, allowing rapid affinity purification of the protein. Examples of such fusion protein expression systems are the glutathione S-transferase system (Pharmacia, Piscataway, NJ), the maltose binding protein system (NEB, Beverley, MA), the FLAG system (IBI, New Haven, CT), and the 6xHis system (Qiagen, Chatsworth, CA).

[00124] Amino acid sequence variants of the polypeptide may also be prepared. These may, for instance, be minor sequence variants of the polypeptide which arise due to natural variation within the population or they may be homologues found in other species. They also may be sequences which do not occur naturally but which are sufficiently similar that they function similarly and/or elicit an immune response that cross-reacts with natural forms of the polypeptide. Sequence variants may be prepared by standard methods of site-directed mutagenesis such as those described herein for removing the transmembrane sequence.

[00125] Amino acid sequence variants of the polypeptide may be substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein which are not essential for function or immunogenic activity, and are exemplified by the variants lacking a transmembrane sequence.

[00126] The engineering of DNA segment(s) for expression in a prokaryotic or eukaryotic system may be performed by techniques generally known to those of skill in recombinant expression. It is believed that virtually any expression system may be employed in the expression of the claimed nucleic acid sequences.

[00127] As used herein, the terms "engineered" and "recombinant" cells are intended to refer to a cell into which an exogenous DNA segment or gene, such as an AAT full-length cDNA or gene has been introduced through the hand of man. Therefore, engineered cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced exogenous DNA segment or gene. Recombinant cells include those having an introduced cDNA or genomic gene, and also include genes positioned adjacent to a heterologous promoter not naturally associated with the particular introduced gene.

[00128] To express a recombinant encoded protein or peptide, whether full-length AAT mutant or wild-type or carboxyterminal peptide thereof, in accordance with embodiments herein, one could prepare an expression vector that includes an isolated nucleic acid under the control of, or operatively linked to, one or more promoters as known in the art. Many standard techniques are available to construct expression vectors containing the appropriate nucleic acids and transcriptional/translational control sequences in order to achieve protein or peptide expression in a variety of host-expression systems. Cell types available for expression include, but are not limited to, bacteria, such as *E. coli* and *B. subtilis* transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors.

[00129] Certain examples of prokaryotic hosts are *E. coli* strain RR1, *E. coli* LE392, *E. coli* B, *E. coli* X 1776 (ATCC No. 31537) as well as *E. coli* W3110 (F-, lambda-, prototrophic, ATCC No. 273325); bacilli such as *Bacillus subtilis*; and other enterobacteriaceae such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species.

[00130] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells.

[00131] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism may be used as transforming vectors in connection with these hosts. For example, the phage lambda GEM^{TM}-11 may be utilized in making a recombinant phage vector which may be used to transform host cells, such as *E. coli* LE392.

[00132] Further useful vectors include pIN vectors (Inouye *et al.*, 1985); and pGEX vectors, for use in generating glutathione S-transferase (GST) soluble fusion proteins for later purification and separation or cleavage. Other suitable fusion proteins are those with ß-galactosidase, ubiquitin, or the like.

[00133] Promoters that are most commonly used in recombinant DNA construction include the β-lactamase (penicillinase), lactose and tryptophan (trp) promoter systems. While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published, enabling those of skill in the art to ligate them functionally with plasmid vectors.

[00134] For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used. This plasmid already contains the *trp*l gene which provides a selection marker for a

mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, 1977). The presence of the *trp*l lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Suitable promoting sequences in yeast vectors are known in the art. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other suitable promoters, which have the additional advantage of transcription controlled by growth conditions, are also contemplated of use herein.

[00135] In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. In addition to mammalian cells, these include insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus); and plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV or other plants) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing one or more coding sequences. Insect systems are also contemplated.

[00136] Examples of useful mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, W138, BHK, COS-7, 293, HepG2, 3T3, RIN and MDCK cell lines. In addition, a host cell strain may be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the encoded protein.

[00137] Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cells lines or host systems may be chosen to ensure the correct modification and processing of the foreign protein expressed. Expression vectors for use in mammalian cells ordinarily include an origin of replication (as necessary), a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences. The origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (*e.g.*, Polyoma, Adeno, VSV, BPV) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is

often sufficient. The promoters may be derived from the genome of mammalian cells. Further, it is also possible, and may be desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

[00138] In cases where an adenovirus is used as an expression vector, the coding sequences may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing proteins in infected hosts.

[00139] Specific initiation signals may also be required for efficient translation of the claimed isolated nucleic acid coding sequences. These signals include the ATG initiation codon and adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may additionally need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be in-frame (or in-phase) with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons may be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements or transcription terminators (Bittner *et al.*, 1987).

[00140] In eukaryotic expression, one will also typically desire to incorporate into the transcriptional unit an appropriate polyadenylation site (e.g., 5'-AATAAA-3') if one was not contained within the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides "downstream" of the termination site of the protein at a position prior to transcription termination.

[00141] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express constructs encoding proteins may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells may be transformed with vectors controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The

selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn may be cloned and expanded into cell lines.

[00142] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance may be used as the basis of selection for dhfr, that confers resistance to methotrexate; gpt, that confers resistance to mycophenolic acid; neo, that confers resistance to the aminoglycoside G-418 and hygro, that confers resistance to hygromycin or any other method known the art.

[00143] It is contemplated that the isolated nucleic acids of the invention may be "overexpressed", *i.e.*, expressed in increased levels relative to its natural expression in human prostate, bladder or breast cells, or even relative to the expression of other proteins in the recombinant host cell. Such overexpression may be assessed by a variety of methods, including radio-labeling and/or protein purification. However, simple and direct methods are preferred, for example, those involving SDS/PAGE and protein staining or Western blotting, followed by quantitative analyses, such as densitometric scanning of the resultant gel or blot. A specific increase in the level of the recombinant protein or peptide in comparison to the level in natural human prostate, bladder or breast cells is indicative of overexpression, as is a relative abundance of the specific protein in relation to the other proteins produced by the host cell and, e.g., visible on a gel.

[00144] It is contemplated herein that constructs generated utilizing an immune molecule (e.g. Fc portion) can be isolated using various affinity columns. In addition, Fc fragments can also be further manipulated such as removing the hinge region. These Fc fragments can include any of of IgG1, IgG2, IgG3, IgG4 or IgD. The hinge region can be eliminated or truncated or mutated prior to linking the immune fragment to an AAT target molecule.

Isolated Proteins

[00145] One embodiment pertains to isolated proteins, and biologically active peptides thereof. In one embodiment, the native polypeptide can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In certain embodiments, the native polypeptide may be heated or otherwise treated to reduce or eliminate serine protease inhibitor activity. In certain particular embodiments, serine

protease inhibitor activity is reduced where no significant activity remains. In another embodiment, polypeptides contemplated herein are produced by recombinant DNA techniques. Alternative to recombinant expression, a polypeptide can be synthesized chemically using standard peptide synthesis techniques. Any of the peptide or protein molecules contemplated of use in compositions disclosed herein can be compositions having no significant serine protease inhibitor activity. For example, AAT compositions may be mutated or truncated in order to reduce or eliminate serine protease inhibitor activity or an AAT polypeptide may be isolated wherein the polypeptide has reduced or no significant serine protease inhibitor activity.

[00146] An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. Thus, protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium. When the protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals. For example, such preparations of the protein have less than about 30%, 20%, 10%,5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest.

[00147] In certain embodiments, nucleotides that encode polypeptides can be inserted to any construct known in the art for generating a peptide or protein. These peptides can include a polypeptide having a consecutive amino acid sequence corresponding to a portion or all of the last 80 amino acids of carboxyterminus of AAT or AAT allele. Other useful proteins are substantially identical to any portion of the carboxyterminus, and retain the functional activity of the peptide of the corresponding naturally-occurring protein other than serine protease inhibitor activity yet differ in amino acid sequence due to natural allelic variation or mutagenesis.

[00148] In certain embodiments, purification of Fc-AAT constructs disclosed herein can include using a Protein A column or protein A matrix or the like (Pierce or other IgG purification kit). In certain embodiments, purification of constructs disclosed herein can be by using minimal steps to preserve anti-inflammatory or immune modulatory activity of a target AAT protein or peptide. In accordance with these embodiments, purification of constructs

contemplated herein may be by a single step (e.g. protein A column purification of Fc-AAT molecules) (See for example Kin-Ming et al. Protein Engineering vol.11 no.6 pp.495–500, 1998; expression/Fc/Fc-X/fusion protein; and diabody technologies).

[00149] It is contemplated herein that a nucleic acid encoding any protein or peptide capable of reversibly binding to itself (e.g. through disulfide or other binding) can be used to generate AAT constructs disclosed herein. These constructs can be used as doublets of AAT for increased purification with reduced loss of function and can also be used as a dimeric molecule for use in therapeutic applications or for research purposes. In accordance with these embodiments, the portion linked to AAT or the carboxyterminal fragment can be inert or essentially non-immunogenic unless increased immugenicity is desired. Further, Fc is manipulated in constructs disclosed herein to reduce or eliminate complement interaction or activation (e.g. hinge is deleted). Positioning Fc at the carboxyterminal region of AAT has been demonstrated to not interfere with certain AAT activities such as anti-inflammatory and elastase inhibition.

Other Uses

[00150] Some compositions disclosed herein may be used as therapeutic agents in the treatment of a physiological condition caused in whole or part, by excessive serine protease activity. In addition, a physiological condition can be inhibited in whole or part. Peptides contemplated herein may be administered in a composition as free peptides or pharmaceutically acceptable salts thereof. Peptides may be administered to a subject as a pharmaceutical composition, which, in most cases, will include the fusion molecule and a pharmaceutically acceptable excipient, or pharmaceutically acceptable carrier or a pharmaceutically acceptible salt formulation thereof.

[00151] Biologically active portions of AAT or a peptide derivative thereof can include amino acid sequences sufficiently identical to or derived from the amino acid sequence of the protein (*e.g.*, the amino acid sequence captured by any of SEQ ID NOs:2 to 32, 34, 49 or 51 which exhibit at least one activity of the corresponding full-length protein). A biologically active portion of a protein of the invention can be a polypeptide, which is, for example, 5, 10, 20, 30, 40 or more amino acids in length. Moreover, other biologically active portions having no significant serine protease inhibitor activity, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of the native form of a polypeptide disclosed herein.

[00152] In certain embodiments, polypeptides may have the amino acid sequence of SEQ ID NOs:2 to 32, 34, 49 or 51. Other useful proteins are substantially identical (*e.g.*, at least about, 85%, 90%, 95%, or 99%) to any of SEQ ID NOs1: to 34, 49 and 51, and AAT linked to Fc represented by SEQ ID No. 49, 56, 57, 58 or other construct with or without an Fc hinge region manipulation.

[00153] Variants of AAT molecules having no significant serine protease activity can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation. For example, a point mutation may be generated in AAT or peptide derivative thereof that still leaves the reactive center loop intact (RCL) while interfering with or preventing serine protease binding capabilities with the AAT or peptide but retaining its ability to modulate radiation adverse effects. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein except no significant serine protease activity remains. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

Fusion Polypeptides

[00154] In other embodiments, agents such as AAT and/or analog thereof, or peptide derivative or fragment thereof may be part of a fusion polypeptide. In one example, a fusion polypeptide may include AAT (*e.g.* naturally occurring mammalian α1-antitrypsin, such as human) or an analog thereof or fragment thereof and a different amino acid sequence that may be an immunofragment such as an IgG fragment (e.g. Fc hinge deletion or hinge truncation or mutant thereof). In addition, a fusion polypeptide disclosed herein can include a pharmaceutically acceptable carrier, excipient or diluent. Any known methods for generating a fusion protein or fusion peptide are contemplated herein.

[00155] In yet another embodiment, AAT polypeptide or peptide fusion protein can be a GST fusion protein in which is fused to the C-terminus of GST sequences. Fusion expression vectors and purification and detection means are known in the art. Expression vectors can routinely be designed for expression of a fusion polypeptide of the invention in prokaryotic (*e.g.*, *E. coli*) or eukaryotic cells (*e.g.*, insect cells (using baculovirus expression

vectors), yeast cells or mammalian cells) by means known in the art.In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector as described in the art.

[00156] When examining effects of plasma-derived AAT formulations on a system compared to fusion molecules disclosed herein, protein concentration is taken into consideration because Fc-AAT disclosed herein occur as a doublet of 2 AAT molecules unless they are cleaved or reduced which generates two Fc-AAT single molecules. Fc-AAT fusion molecules of use in compositions disclosed herein can include a pharmaceutically acceptable composition of one or more of SEQ ID NO: 32, 49, 51, 53, or 55-58 to treat a subject having an inflammatory condition or other condition responsive to plasma-derived AAT treatment as provided herein or known in the art. In certain embodiments, Fc linked to AAT, a mutant AAT form or AAT peptide fragment may increase the halflife of AAT *in vivo* or facilitate cellular uptake and transport of the construct *in vivo*. Thus, novel molecules have been made where multiple improvements have been observed regarding generating a recombinant form of AAT compared to plasma-derived AAT and other recombinants, as well as improvements *in vivo* compared to Fc-AAT (IgG1, Fc-AAT2).

Combination Therapies

[00157] Any of the embodiments detailed herein may further include one or more other therapeutically effective agent in combination with compositions disclosed herein. In certain embodiments, these alternative agents can include cancer-related medications in the treatment of cancer. For example, these therapies can include, but are not limited to, aspirin and other antiplatelet therapy including for example, clopidogrel, prasugrel, ticagrelor, abciximab, eptifibatide, tirofiban; heparin and derivatives; direct thrombin inhibitors or Xa inhibitors; warfarin; angiotensin converting enzyme inhibitors or angiotensin receptor blockers; beta- and alpha-adrenergic receptor blockers; calcium channel blockers; HMGCoA reductase inhibitors (e.g. statins); niacin and derivatives; fenofibrate; fish oil; aldosterone blockers; hydralazine and nitroderivates; phosphodiesterase inhibitors; direct guanylil cyclase activators, anti-microbial drugs, anti-inflammatory agent, immunomodulatory agent, or immunosuppressive agent or combination thereof.

[00158] Examples of anti-bacterial agents include, but are not limited to, penicillins, quinolonses, aminoglycosides, vancomycin, monobactams, cephalosporins, carbacephems,

cephamycins, carbapenems, and monobactams and their various salts, acids, bases, and other derivatives.

[00159] Anti-fungal agents contemplated of use herein can include, but are not limited to, caspofungin, terbinafine hydrochloride, nystatin, amphotericin B, griseofulvin, ketoconazole, miconazole nitrate, flucytosine, fluconazole, itraconazole, clotrimazole, benzoic acid, salicylic acid, and selenium sulfide.

[00160] Anti-viral agents contemplated of use herein can include, but are not limited to, valgancyclovir, amantadine hydrochloride, rimantadin, acyclovir, famciclovir, foscamet, ganciclovir sodium, idoxuridine, ribavirin, sorivudine, trifluridine, valacyclovir, vidarabin, didanosine, stavudine, zalcitabine, zidovudine, interferon alpha, and edoxudine.

[00161] Anti-parasitic agents contemplated of use herein can include, but are not limited to, pirethrins/piperonyl butoxide, permethrin, iodoquinol, metronidazole, diethylcarbamazine citrate, piperazine, pyrantel pamoate, mebendazole, thiabendazole, praziquantel, albendazole, proguanil, quinidine gluconate injection, quinine sulfate, chloroquine phosphate, mefloquine hydrochloride, primaquine phosphate, atovaquone, co-trimoxazole, (sulfamethoxazole/trimethoprim), and pentamidine isethionate.

[00162] Immunomodulatory agents can include for example, agents which act on the immune system, directly or indirectly, by stimulating or suppressing a cellular activity of a cell in the immune system, (*e.g.*, T-cells, B-cells, macrophages, or antigen presenting cells (APC)), or by acting upon components outside the immune system which, in turn, stimulate, suppress, or modulate the immune system (*e.g.*, hormones, receptor agonists or antagonists, and neurotransmitters); other immunomodulatory agents can include immunosuppressants or immunostimulants. Anti-inflammatory agents can include, for example, agents which treat inflammatory responses, tissue reaction to injury, agents that treat the immune, vascular, or lymphatic systems or any combination thereof.

[00163] Anti-inflammatory or immunomodulatory drugs or agents contemplated of use herein can include, but are not limited to, interferon derivatives, e.g., betaseron, β–interferon; prostane derivatives, iloprost, cicaprost; glucocorticoids such as cortisol, prednisolone, methylprednisolone, dexamethasone; immunsuppressive agents such as cyclosporine A, FK-506, methoxsalene, thalidomide, sulfasalazine, azathioprine, methotrexate; lipoxygenase inhibitors, e.g., zileutone, MK-886, WY-50295, SC-45662, SC-41661A, BI-L-357; leukotriene antagonists; peptide derivatives for example ACTH and analogs; soluble TNF

(tumor necrosis factor)-receptors; TNF-antibodies; soluble receptors of interleukines, other cytokines, T-cell-proteins; antibodies against receptors of interleukins, other cytokines, and T-cell-proteins.

[00164] Other agents of use in combination with compositions herein can be molecules having serine protease inhibitor activity. For example other serine protease inhibitors contemplated of use herein can include, but are not limited to, leukocyte elastase, thrombin, cathepsin G, chymotrypsin, plasminogen activators, and plasmin.

[00165] In addition, other combination compositions of methods disclosed herein can include certain antibody-based therapies. Non-limiting examples include, polyclonal anti-lymphocyte antibodies, monoclonal antibodies directed at the T-cell antigen receptor complex (OKT3, TIOB9), monoclonal antibodies directed at additional cell surface antigens, including interleukin-2 receptor alpha. In certain embodiments, antibody-based therapies may be used as induction therapy in combination with the compositions and methods disclosed herein.

[00166] Subjects contemplated herein can include human subjects, male or female, adult or infant, or fetus, or other subjects such as non-human subjects, including but not limited to, primates, dogs, cats, horses, cows, pigs, guinea pigs, birds and rodents.

AAT

[00167] Human AAT is a single polypeptide chain with no internal disulfide bonds and only a single cysteine residue normally intermolecularly disulfide-linked to either cysteine or glutathione. One reactive site of AAT contains a methionine residue, which is labile to oxidation upon exposure to tobacco smoke or other oxidizing pollutants. Such oxidation reduces the elastase-inhibiting activity of AAT; therefore substitution of another amino acid at that position, e.g., alanine, valine, glycine, phenylalanine, arginine or lysine, produces a form of AAT which is more stable. Native AAT can be represented by the formula of SEQ ID NO:1 or 33 or other known naturally-occurring AAT molecule.

[00168] Any means known for producing and purifying fusion molecules disclosed herein is contemplated (*e.g.* in mammalian cells, by bacteria, by fungi or other organisms or produced in plants).

Kits

[00169] In still further embodiments, kits for use with compositions, constructs (e.g. recombinant and/or fusion molecules) and methods described above are contemplated. Kits may include AAT fusion or recombinant constructs (e.g. Fc-AAT; Fc-mutant AAT, IgG2 mutant linked to AAT or carboxyterminal derivative of AAT or Fc, hinge deleted constructs, SEQ ID NO. 32, 49-58 etc.), constructs of one or more peptides derived from AAT, a mutant AAT construct composition, a mutant AAT molecule associated with a gene therapy delivery system or other combinations. Small molecules, proteins or peptides may be employed for use in any of the disclosed methods. In addition, other agents such as anti-bacterial agents, immunosuppressive agents, anti-inflammatory agents may be provided in the kit. The kits can include, suitable container means, a protein or a peptide or analog agent, and optionally one or more additional agents.

[00170] The kits may further include a suitably aliquoted construct composition of the encoded protein or polypeptide antigen, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay or for therapeutic applications described.

[00171] Containers of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means or other delivery device (e.g. a stent or catheter).A kit will also generally contain a second, third or other additional container into which other combination agents may be placed. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[00172] In certain embodiments, a kit can include a composition including, but not limited to, constructs of AAT, AAT fragment, or an AAT analog or polypeptide, having no significant serine protease inhibitor activity. In accordance with these embodiments, a kit can contain AAT or an analog thereof having no significant serine protease inhibitor activity.

EXAMPLES

[00173] The following examples are included to illustrate various embodiments. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered to function well in the practice of the claimed methods, compositions and apparatus. However, those of skill in the art should, in light of the present disclosure, appreciate that changes may be made in the some embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Generation of Expression Plasmid for Production of Recombinant human AAT

[00174] In one exemplary method, Fc-AAT constructs can be generated. Recombinant AAT can be generated for fusion molecules as indicated in **Fig. 1**. Insertion of human AAT (or AAT peptides such as carboxyterminal peptides) sequences into an expression vector, pCAGGS. Human full-length AAT cDNA of 1260 base pairs was isolated from a human liver library and inserted into pCAGGS as illustrated in **Fig. 1**. Chinese Hamster Ovay (CHO) cells were transfected with the plasmid for expression. Using limiting dilution, AAT-positive clones were selected and grown in serum free media. The supernatants were collected and pooled. Using an antibody to human AAT, a band of about 55 kDa was observed on Western blots (data not shown) verifying AAT. A fusion protein with the human IgG1, IgG2, IgG3 or IgG4 (with or without varying hinge region deletions, mutations and up to a total hinge region deletion) Fc receptor was used to generate recombinant AAT or fusion molecules thereof. These constructs were purified. In certain exemplary methods, these constructs were purified using Protein A (as a column or matrix etc.) to bind Fc and rapidly isolate a target fusion molecule from a solution. See **Fig. 3** for a representative SDS-PAGE gel separation of fusion molecules produced herein (e.g. Fc-AAT2/AAT-Fc2 and Fc-AAT-6/AAT-Fc6)

Example 2

[00175] In another exemplary method, fusion constructs disclosed herein can be purified and used for methods or therapeutic treatment for any condition known to be treated by commercially available AAT compositions or other inflammatory condition.

[00176] Human Fc IgG plasmids can be purchased from Qiagen (e.g. IgG1, IgG2, IgG3 and IgG4 etc.). The human cDNA was excised and inserted into the human Fc vector via PCR cloning. The in-frame sequence was performed for validation. The plasmid was transfected into CHO cells and after limiting dilutions to obtain single clones, several stable clones were isolated. The stable clones were expanded and further selected using serum-free medium. Large scale cell culture was performed and the supernatants collected and pooled.

[00177] Supernatant containing Fc-AAT fusion molecules can be purified using Protein A as a matrix, in a gel or in a column. In certain methods, human Fc-AAT generated herein was eluted from the protein A using glycine (about pH 2.4) and then rapidly neutralized to physiological pH, about pH 7.4. These methods produced a single band on an

SDS-PAGE gel under reducing conditions. Purified Fc-AAT fusion constructs could then be readily compared to commercially available formulations such as Aralast™, Glassia ™, ProlastinC™ for AAT-related activities such as elastase inhibition assay, anti-inflammatory assays (*e.g.* affects on cytokine levels etc).

[00178] Purification of human AAT Fc: A Western blot demonstrated bands (about 170 kDa) that represent intact dimer of two Fc-AAT full length molecules without manipulation to the Fc from IgG1. Other lanes on the Western blot represented when all disulfide bonds were broken to form 2 singular molecules of FC-AAT. Both non-reducing gels as well as reducing gels demonstrated level of purity of the AAT constructs. Fc-AAT can be purified in a single step from a mammalian cell culture supernatant using protein A chromatography thus dramatically reducing side-effects of purification deleterious to AAT activities. The following clones were generated Clone 2: Fc-AAT using IgG1 and a linker to the carboxyterminus of AAT: Clone 3: Fc-AAT using IgG1 where the hinge region of Fc is removed and a linker that is again linked to the carboxyterminus of AAT. Other clones have been generated that include Fc from IgG2, IgG3 and IgG4 with and without hinge region deletions. It is noted that Fc-AAT2 and Fc-AAT3 retain elastase inhibition activity but behave differently under certain conditions when compared both *in vivo* and *in vitro* implicating another active region of AAT other than the serine protease inhibition activity region is involved and proposed to be anti-inflammatory and anti-immune active regions AAT.

Example 3

[00179] It was hypothesized that affects of Fc-AAT (or mouse AAT Fc) on cytokine-induced TNFα from mouse RAW macrophages would be more potent to reduce TNFα than that of native AAT (*e.g.* commercially available formulations) due in part to rapid purification and conserved AAT activity of the clones. It is also hypothesized that clone 3, having a complete hinge deletion may be more potent *in vitro* in certain activities tested but also an improved formulation for *in vivo* use due to reduced secondary activity issues (e.g. reduced complement activation etc.): In one exemplary method, ATT-Fc2/Fc-AAT2 (clone 2 intact IgG1 hinge) and AAT-Fc3/Fc-AAT (clone 3, deleted IgG1 hinge) were examined for effects on spontaneous production of immune stimulatory activities, mouse TNFα production, in order to examine an unwanted immune activities.

[00180] Cytokine Assays for AAT fusion molecules: assays on cell cultures for cytokine production *in vitro*. RAW macrophages were used for the following experiments.

Raw 264.7 cells in 96 well plate (3 x 10^5 cells per well) were used. Increased concentrations of AAT-Fc2 and AAT-Fc3 were applied to stimulate the mouse RAW cells as indicated in the figures. Mean ± SEM of mouse TNFα production by the AAT-Fcs were measured by a standard ELISA kit according to manufactures' instruction (R&D Systems, Minneapolis MN). Here, the difference in spontaneous induction of mouse TNFα by two different AAT-Fc molecules was examined. These results support that AAT-Fc3 (hinge deleted) is more effective in reducing TNFα production, a pro-inflammatory cytokine marker, even in this *in vitro* model. **Fig. 4A** represents a comparison of two fusion molecules, Fc-AAT2 and FcAAT3 and affects of TNFα production in an in vitro system. **Fig. 4B** represents a comparison of two fusion molecules, Fc-AAT2 and Fc-AAT3, with a commercially available plasma-derived AAT formulation (Aralast™). Fc-AAT3 demonstrates superior results comparible to plasma-derived AAT (Aralast™) Of note, tumor necrosis factor (TNF), cachexin, or cachectin, and formerly known as tumor necrosis factor-alpha or TNF-α) is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages (M1), although it can be produced by many other cell types as CD4+ lymphocytes, NK cells and neurons. This in vitro study supports that Fc-AAT where the hinge region is deleted or modified has certain superior qualities to Fc-AAT with an intact Fc hinge region and is as active as plasma-derived formulations to inhibit TNF production.

[00181] These experiments were performed three times in order ensure that the observed results of AAT-Fc2 (IgG1, clone 2) and AAT-Fc3 (hinge deleted, IgG2, clone 3) were comparible and an accurate reflection of their potency compared to a commercially available formulations (see for example, **Figs. 4A and 4B**). Here, it was observed that there was a significant difference in spontaneous production of mouse TNFα by AAT-Fc2 (IgG1) and AAT-Fc3 (hinge deleted) (Fig. 4A) where AAT-Fc3 induction of TNFα is dramatically reduced compared to AAT-Fc2. Further, there was a dramatic difference when comparing commercially available formulations (e.g. Aralast) with AAT-Fc2 (clone 2) and AAT-Fc3 (clone 3).

[00182] Similar data were observed using human IL-33 as a stimulant. Recombinant mouse IL-33 was also tested and demonstrated consistent suppression of TNFα by 100 and 500 ng/mL levels of Fc-AAT (IgG1 Fc intact with AAT full length (data not shown)).

Example 4

IL-1 Receptor Antagonist Induction and IL-8 induction

[00183] In this exemplary method, production of IL-8 is assessed. IL-8 is an inflammatory molecule and its production is an indication of an induced inflammatory response. In this example, human blood neutrophils (3 x 106 cells/ml) were incubated for 6 hours alone or in the presence of LPS (10ng/ml), recombinant AAT (clone 2) (10 μg/ml) or a combination of the two. Production of IL-8 was measured in the cell culture supernantants (N=3). It was demonstrated that recombinant AAT dramatically reduced IL-8 expression in the presence of the stimulant LPS. It is proposed that clone 3 (hinge deletion of IgG1) will have a similar activity as reflected in this experiment (**see Fig. 5A**) because the AAT portion of this clone is intact while the Fc is manipulated. This data is supported by previous data using plasma-derived AAT (data not shown). Thus, these molecules are capable of inhibiting inflammation.

[00184] In another exemplary method, IL-1 receptor antagonist (IL-1Ra) was analyzed in order to assess recombinant molecule formulations affects on another inflammation marker. In this example, productions of IL-1 receptor antagonists from human neutrophils cells were measured in various concentrations of recombinant AAT (Fc-AAT2, clone 2: See Fig. Y2, con equals a negative control having no induction of the molecule). These experiments revealed that recombinant AAT at very low levels was able to dramatically inhibit IL-1Ra production. Because the region of AAT found in this clone is identical to Fc-AAT3 (without hinge), this data supports similar activity in Fc-AAT3 would be obtained as compared to Fc-AAT2 demonstrated here (**see Fig. 5B**).

Example 5

[00185] Other cytokine expression has been examined where affects of FcAAT (clone 2) were analyzed (e.g. IL-1beta, IFNgamma, IL-17 etc), parts of these results are illustrated in Table 1 below. It was demonstrated that recombinant AAT having an Fc fusion was capable of blocking deleterious cytokine production.

Table 1

Percent Inhibition

TNF-a

	Donor 1	Donor 2	Donor 3
10 ug/mL FcAAT + Anti CD3/CD28	54%	54%	47%
1 ug/mL FcAAT + Anti CD3/CD28	17%	50%	29%
0.1 ug/mL FcAAT + Anti CD3/CD28	28%	56%	100%
0.01 ug/mL FcAAT + Anti CD3/CD28	-15%	63%	100%
0.001 ug/mL FcAAT + Anti CD3/CD29		0%	

IL-6

	Donor 1	Donor 2	Donor 3
10 ug/mL FcAAT + Anti CD3/CD28	-35%	-250%	100%
1 ug/mL FcAAT + Anti CD3/CD28	52%	-344%	47%
0.1 ug/mL FcAAT + Anti CD3/CD28	30%	77%	100%
0.01 ug/mL FcAAT + Anti CD3/CD28	-35%	69%	100%
0.001 ug/mL FcAAT + Anti CD3/CD29		15%	

IL-1beta

	Donor 1	Donor 2	Donor 3
10 ug/mL FcAAT + Anti CD3/CD28	-55%	-305%	100%
1 ug/mL FcAAT + Anti CD3/CD28	30%	-532%	72%
0.1 ug/mL FcAAT + Anti CD3/CD28	7%	8%	100%
0.01 ug/mL FcAAT + Anti CD3/CD28	-45%	17%	97%
0.001 ug/mL FcAAT + Anti CD3/CD29		-100%	

IFN-g

	Donor 1	Donor 2	Donor 3
10 ug/mL FcAAT + Anti CD3/CD28	-262%	30%	
1 ug/mL FcAAT + Anti CD3/CD28	-9%	20%	
0.1 ug/mL FcAAT + Anti CD3/CD28	17%	100%	
0.01 ug/mL FcAAT + Anti CD3/CD28	65%	100%	
0.001 ug/mL FcAAT + Anti CD3/CD29		14%	

IL-17

Suzhao Trial 1		Donor 1	Donor 2	Donor 3
26%	10 ug/mL FcAAT + Anti CD3/CD28			100%
19%	1 ug/mL FcAAT + Anti CD3/CD28			100%
51%	0.1 ug/mL FcAAT + Anti CD3/CD28			100%
	0.01 ug/mL FcAAT + Anti CD3/CD28			92%
	0.001 ug/mL FcAAT + Anti CD3/CD29			

				AAT-Fc 2	AAT-Fc 2	AAT-Fc 3	AAT-Fc 3				
IL-1b pg/ml	Donor 1	*Alone*	Control	0	0	0	0	*Alone*	Control	0	0
			30 ug/ml	12	25	5	7		E. LPS 100 ng/ml	5	8
			15 ug/ml	5	13	2	2		E. LPS 10 ng/ml	4	3
		Plus Bartonella	Control	0	2		2	*Plus Bartonella*	Contol	0	0
			30 ug/ml	11	42	5	5		E. LPS 100 ng/ml	3	3
			15 ug/ml	7	13	2	3		E. LPS 10 ng/ml	2	2
				AAT-Fc 2	AAT-Fc 2	AAT-Fc 3	AAT-Fc 3				
	Donor 2	*Alone*	Control	3	1	0	2	*Alone*	Control	0	0
			30 ug/ml	373	119	2	2		E. LPS 100 ng/ml	73	
			15 ug/ml	138	30	0	0		E. LPS 10 ng/ml	34	55
		Plus Bartonella	Control	3	1	0	2	*Plus Bartonella*	Contol	0	0
			30 ug/ml	227	75	2	1		E. LPS 100 ng/ml	14	13
			15 ug/ml	53	36	2	1		E. LPS 10 ng/ml	1	1
				AAT-Fc 2	AAT-Fc 2	AAT-Fc 3	AAT-Fc 3				
IL-6 pg/ml	Donor 1	*Alone*	Control	0	0	0	0	*Alone*	Control	0	0
			30 ug/ml	865	923	0	0		E. LPS 100 ng/ml	2488	2141
			15 ug/ml	777	787	0	0		E. LPS 10 ng/ml	1718	1444
		Plus Bartonella	Control	0	0	0	0	*Plus Bartonella*	Contol	0	0
			30 ug/ml	873	930	0	0		E. LPS 100 ng/ml	2398	2132
			15 ug/ml	888	805	0	0		E. LPS 10 ng/ml	1606	1623
				AAT-Fc 2	AAT-Fc 2	AAT-Fc 3	AAT-Fc 3				
	Donor 2	*Alone*	Control	35	0	0	0	*Alone*	Control	0	0
			30 ug/ml	1939	1829	0	0		E. LPS 100 ng/ml	5205	
			15 ug/ml	1926	1319	0	0		E. LPS 10 ng/ml	4447	4828
		Plus Bartonella	Control	35	0	0	0	*Plus Bartonella*	Contol	0	0
			30 ug/ml	1128	1054	0	0		E. LPS 100 ng/ml	2812	2908
			15 ug/ml	183	112	0	0		E. LPS 10 ng/ml	772	858
				AAT-Fc 2	AAT-Fc 2	AAT-Fc 3	AAT-Fc 3				
TNF-a pg/ml	Donor 1	*Alone*	Control	7	0	7	0	*Alone*	Control	7	0
			30 ug/ml	595	629	174	18		E. LPS 100 ng/ml	901	
			15 ug/ml	520	386	88	101		E. LPS 10 ng/ml	776	724
		Plus Bartonella	Control	0	6	0	6	*Plus Bartonella*	Contol	0	6
			30 ug/ml	645	583	123	92		E. LPS 100 ng/ml	442	516
			15 ug/ml	537	403	28	33		E. LPS 10 ng/ml	274	266

[00186] **Figs. 6A-6C** represent percent expression of CD11b/CD45 positive cells and percent TLR4 and TLR2 expression in the presence of plasma-derived AAT versus Fc-AAT2 and found that about 100 to about 1000 fold less recombinant AAT (Fc-AAT2) had the same inhibitory effect on these deleterious molecules. For example, Toll-like Receptor 4 at either 500 or 100 ng as effective as 500 μg of plasma-derived AAT (see **Fig. 6A**).

Example 6

Gout Model

[00187] Effect of recombinant Fc-AAT on IL-1β production in PBMC stimulated with monosodium urate crystals, a model for gouty arthritis. Effects of Fc-AAT induced IL-1β production in PBMC stimulated with monosodium urate crystals (MSU) together with C-18 (C18) fatty acids were analyzed using a previously described gout model.

IL-1β production in In Vivo inflammation study

[00188] Experiments were performed using the mouse gout (Ggouty arthritis) model to compare *in vivo*, affects of Fc-AAT2 (IgG1) versus Fc-AAT3 (hinge deletion) (see **Fig. 7A**). It was hypothesized that Fc-AAT3 (and other Fc having a deleted hinge) would have superior results in vivo to Fc-AAT2 (intact hinge of IgG1). First, protein concentrations of AAT-Fc-2 and AAT-Fc-3 were determined. Mice were weighed and dosing adjusted to 2.0mg/kg. After 2 hours, MSU C16.0 was injected intra-articularly. After 4 hours, mice were euthanized and joints scored. Synovial tissues were homogenized for cytokine levels (e.g. 144,000 g/L = 1 mole; 144,000 mg/mL = 1M:144 mg/mL= 1 mM:144 μg/mL = 1 μM: 14 μg/mL = 100 nM) Molecular weight of plasma-derived AAT = 42,000 (less glycosylations) 42 micrograms/mL of plasma-derived AAT is 240 nM and the molecular weight of AAT-Fc =144,000 (less glycosylations)14 micrograms/mL AAT-Fc is 7 nM. Additional studies concerned assessment of IL-6 in the presence or absence of AAT where a commercial formula (Zemaira™) was compared to Fc-AAT2 and Fc-AAT3 (**Fig. 7B**). It was noted that using an *in vitro* model of human blood monocyte cells induced by Candida albicans that IL-6 expression was dramatically reduced. Both recombinant formulations outperformed native AAT formulations (Zemaira™) (See **Fig. 7B**). The commercial formulation failed to significantly inhibit IL-6 expression compared to the Fc-AAT2 and Fc-AAT3.

[00189] Another experiment was performed using a gout mouse model to observe total IL-1 receptor blockade (see **Fig, 7C**). In yet another exemplary method, a time course analysis of Fc-AAT2 (clone 2) affect on levels of IL-1β was assessed. The time course was between 0 to 72 hours after exposure to various amounts of recombinant AAT.A time-course study of Fc-AAT2 was performed where the fusion molecule was introduced as a pretreatment intraperitoneally before instillation of monosodium urate (NSU) crystals into the knee joint. About 4 hours after instillation, the mice were sacrificed and the knee joint excised and cultured. After about 2 hours in culture, IL-1β was measured in supernatants of the cultures (N=10 per group). The data is illustrated in **Fig. 7D** where IL-1β was inhibited with the pretreatment of FcAAT2 for greater than 48 hours thus supporting a role for novel recombinants in the inhibition of IL-1β adverse effects and as a potential treatment in Gout patients. See for example experimental procedures of Joosten *et al*, Arthritis Rheum. 2010 November; 62(11): 3237–3248.

[00190] Methods in brief: joint inflammation can be induced by intraarticular injection (i.a.) of a dose-range highly pure MSU (30–300μg), 200μM C18.0, MSU/C18.0 (300μg/200μM) or 25μg SCW (rhamnose content) in 10 μl of PBS into the right knee joint of naïve mice. 4 hour after i.a. injection, joint swelling was determined, synovial tissue was isolated and knee joints were removed for histology. Joint swelling measurement can be measured by either macroscopic scoring or by the 99mTc uptake method. Macroscopic joint swelling is scored on a scale ranging from 0–3. After the skin is removed the knee joint was scored, 0 = no swelling and 3 = severe swelling. 99mTcuptake method was performed as previously described (21,22). Joint swelling is expressed as the ratio of the 99mTc uptake in the inflamed over the control joint (left knee joint). All values exceeding 1.10 are assigned as joint swelling.

Example 7

Myocardial Infarction Model

[00191] In another exemplary method, a myocardial infarction mouse model was used to assess the ability of Fc-AAT fusion molecules to inhibit cardiac remodeling, reduce infarct size as previously demonstrated for plasma-derived AAT models (data not shown). The experimental model of AMI (acute myocardial infarction) due to transient myocardial ischemia (30 min) simulates the clinical setting of patients with reperfused AMI. The mice experience an ischemic damage followed by a reperfusion injury. The average infarct size is 15-20% of the left ventricle. The mice develop a mild form of dilated cardiomyopathy with dilatation and dysfunction of the left ventricle. In this model of AMI, an increase in the LVEDD and LVESD, and a fall in LVFS ($p<0.05$ vs Sham for all comparisons) at 7 days was observed. It is demonstrated that at increasing concentrations of 10 or 50 microgram of Fc-AAT fusion molecules that infarct size measures as percent of left ventricle affected by infarct was significantly reduced at both concentrations (**Fig. 8**). This concentration is dramatically reduced compared to plasma-derived AAT formulations (approximately 100 times more were used in a comparible study, 2 milligrams intraperitoneally) (data not shown).

[00192] Another experimental model of AMI due to permanent coronary artery occlusion simulates a clinical setting of patients with non-reperfused large AMI. The mice experience a severe ischemic damage. The average infarct size is 25-35% of the left ventricle. The mice develop a severe form of dilated cardiomyopathy with dilatation and dysfunction of

the left ventricle, and high mortality rate. This model also demonstrated that plasma derived AAT is effective at reducing the affects of an acute myocardial infarction.

[00193] Further, as illustrated in **Fig. 9** using another mouse model simulating a heart attack, Fc-AAT1 (clone 1) which was demonstated to have no elastase activity and Fc-AAT2 (clone 2) demonstrated to have elastase inhibition were equally effective at reducing cardiac remodeling measured as infarct size. Both recombinant molecules were active as a *single* dose (50 micrograms/mouse) compared to 5 days of 2 mg/mouse of other agents (IVIG). It is proposed that Fc-AAT3 (clone3, without hinge of IGg1) will be even more effective in this in vivo model to reduce cardiac remodeling and other effects of ischemia reperfusion. Thus, these experiments support that fusion molecules disclosed here are effective at reducing the deleteruious effects of ischemia-reperfusion injury and adverse cardiac conditions.

[00194] Fc-AAT fusion molecules limit ischemia-reperfusion damage and reduce infarct size.

[00195] Fc-AAT fusion molecules limit the cytokine release after ischemia-reperfusion.

[00196] plasma-derived AAT does not reduce infarct size in the non-reperfused AMI which suggests that AAT affects the inflammatorycomponent related to reperfusion injury (data not shown) thus supporting a role for Fc-AAT fusion moleculesfor treatment of adverse cardiac conditions.

4) Fc-AAT fusion molecules limit adverse cardiac remodeling in both models of AMI

Example 8

[00197] **Fig. 10** illustrates some Fc-AAT fusion molecules contemplated herein where the hinge region is deleted, truncated or mutated.

Example 9

Colitis/IBD Model

[00198] As presented above, Fc-AAT3 both *in vivo* and *in vitro* inhibits the production of cytokines thus modulating deleterious affects of pro-inflammatory cytokines. It is thought that this data and previous studies of plasma-derived AAT in colitis/IBD models both support a role for Fc-AAT (hinge deleted) in the treatment of or prophylactic for inflammatory bowel diseases.

[00199] It has been demonstrated that plasma-derived AAT treatment attenuates loss of weight in DSS colitis model of mice, which is one of the most dependable indicators of inflammatory bowel disease activity in this model. In addition, there is significant reduction in cytokines secreted into the supernatant of colonic explants.

[00200] Mice can be with Fc-AAT (2 micrograms to 2 mg/day i.p) in the DSS model compared to vehicle treated mice and control plasma-derived AAT formulations (2 mg/day). Mice will be weighed at various times and then sacrificed to assess body weight and assess decrease in colon shortening as observed for native AAT. It is hypothesized that the supportive evidence presented herein will be further substantiated by observations of decreased weight loss and colon shortening in the fusion molecule treated DSS colitis mice. Further, it expected that the concentration to see the same or similar results as plasma-derived AAT (positive control) will be about 10- and up to 1000- fold less for the fusion molecule. In addition cytokine production (e.g. IL-1 , IL-6, MCP-1 and KC) by colonic explants will be assessed and compared to the control mice.

Example 10

[00201] A mouse model for assessing glucose regulation in islet cell toxicity induced scenario will be used to assess Fc-AAT fusion molecules ability to reduce cellular transplant rejection and reduce for example, islet cell degradation as further supported by the previous observations that plasma-derived AAT is capable of protecting islet cells from degradation. In certain methods, a standard toxin can be used on a mouse model islet beta cell toxicity assay the toxin streptozotozin (STZ). As previously illustrated, a commercially available source of AAT (Aralast^{TM}) demonstrated protection in STZ-induced diabetes to protect islet cells. The previous study used a single dose of STZ to induce beta cell death. After two injections of STZ, mice become diabetic (blood sugar rises to over 400 mg/dL). This double dose is used as an acceptible model of immune destruction of the beta cells. Fc-AAT fusion molecules will be compared to plasma-derived AAT to assess protective effects on the islets. Either control (PBS), plasma-derived AAT (commercially available) or Fc-AAT (hinge intact and deleted at about 1microgram or 10 times less per mouse) will be injected each day. It was previously observed that commercially available formulations of AAT reverse the adverse effects and preserve islet cell function. It is predicted that Fc-AAT (hinge deletion) will have superior effects compared to plasma-derived AAT and demonstrate less side effects than Fc-AAT without hinge deletion thus supporting the use of compositions disclosed herein to aid in cellular and organ transplantation to reduce organ rejection and preserve transplants.

[00202] Use of fusion molecules disclosed herein before, during and/or after transplantation are supported. In certain embodiments, Fc-AAT fusion molecules disclosed herein can be used to maintain a graft and/or reduce adverse affects of graft rejection such as GVHD.

[00203] Preliminary data also support that fusion molecules of Fc-AAT (hinge deletion) can be used to reduce or prevent the onset of diabetes by for example, protecting islet cells in a subject from adverse effects of inflammation and immune responses.

Example 11

[00204] Construction of truncated variants of Fc-AAT. In certain exemplary embodiments, protease cleavage of AAT can be a simple insertion of a protease site within the sequence of AAT, for example, tobacco mosaic virus protease. Insertion of the protease recognition site generates a truncated carboxyl end of AAT. This site is upstream from a Carboxy-36-terminal peptide of naturally-occurring AAT: SIPPEVKFNKPFVFLMIEQNTKSPLFMGKVVNPTQK (SEQ.ID NO. 34)

[00205] These truncated AAT molecules are capable of inhibiting LPS-induced IL-1β, IL-6 and TNFα. A bi-valent truncated fusion molecule will be superior to the peptide itself in terms of increased plasma half-life. Given the likelihood that natural AAT is found in the lipid rafts of the cell membrane, it would be unlikely that the insertion would be at the N-terminus but rather the C-terminus. Therefore, having the C-terminal 36 amino acids linked to Fc for a bi-valent structure will likely be more effective in the lipid rafts.

[00206] Cleavage of the Fc domain. The other cleavage site is that of the Fc itself, in order to remove the Fc fragment. This site generates monomeric AAT or truncated AAT. However, the enzyme for Fc-IgG1 differs from that of Fc-IgG2.

[00207] A fusion protein of the N-terminus. The construct of N-terminal AAT is a novel concept that is based on data showing the anti-inflammatory properties of AAT are independent of the elastase inhibition property. Thus using the N-terminal for an inframe construction facilitates the formation of a molecule with a bi-valent C-terminal. For each construct, the expression in CHO is essential as glycosylation is an important component of the molecule. Therefore, CHO cells will be used for the expression of wild-type as well as truncated AAT-Fc. Other examples include constructs linked at the carboxy terminus (e.g. Fc-AAT2 and Fc-AAT3)

[00208] Purification and assays of truncated AAT-Fc. In the case of the protease insertion site, the protease to cleave the molecule can be introdued first and then use Protein A to isolate only fragments. That would yield a near pure form of product. In certain methods, such as in the case of the Fc cleavage site, the molecule would be best purified on Protein A, add the Fc cleavage protease and then remove the Fc fragment on protein A leaving the remaining protein nearly pure.

Example 12

[00209] Effect of Fc-AAT on IL-1β-induced IL-17 in Type 2 Diabetes (T2D). Evidence demonstrates that immune system cells, especially monocytes of the PBMC fraction, play pro-inflammatory roles in type 2 diabetes (T2D). Monocytes from T2D patients hyper-produce key pro-inflammatory cytokines, including IL-1β. IL-1β is implicated in skewing of human T cells to the pro-inflammatory IL-17 production by T cells from T2D patients (compared to non-diabetic donors) is elevated constitutively and in response to stimuli. Effects of Fc-AAT (rec-AAT, rAAT) will be tested on production of IL-1β-induced IL-17 in PBMC as a model.

[00210] Generation of AAT Tg^{wt} mice. The unique aspect of these mice that differs from the AAT Tg strain is the promoter. The new strain will have the chicken beta-actin global promoter and blood levels will be higher than those of the AAT Tg mice expressing AAT in the type 2 lung epithelial. Once generated, heterozygous mice (expressing one copy of wild-type AAT) will be subjected to *in vivo* challenge assays. a. Characterization of AAT Tg^{wt} mice. Once there are heterozygous mice by DNA analysis, western blot assessment will be carried out using various tissues to examine steady state expression. These include histological examinations of the tissues.b. assays on primary cells for cytokine production in vitro. Similar to humans, it is possible to study cytokines from PBMC of mice. The stimulants include all Toll Like Receptors (TLR) agonists, and the combination of IL-18 plus IL-12. c. cytokine responses in vivo following various challenges. LPS or heat-killed *Staphylococcus epidermidis* will be injected intraperitoneally and circulating cytokines will be measured at specific time intervals. Models of IL-18 plus IL-2 will also be tested. In this model, mice develop a wasting syndrome with hypothermia, colitis and hypoglycemia. In preliminary data, the AAT Tg mouse is resistant to this model.d. effect on islet allograft rejection. Once demonstrated that the AAT Tg^{wt} mice are resistant to TLR challenges, the mice will assessd for islet allograft rejection and related studies on mouse islets.

[00211] Mutation and cloning of AAT without serine protease inhibitory activity (AAT Tg^{mu}) . As previously demonstrated, mutation of a cyteine in human AAT results in a molecule without the ability to inhibit protease (listed as non-functional, in the table below). The AAT plasmid shown above will be mutated by standard PCR methods and the Cys will be replaced with Ala. The sequence will be confirmed. This has already been mutated where Cys in human AAT was inserted into an EBV-gene expression vector. When the wild-type AAT EBV-gene expression vector is injected using high pressure bolus into the tail vein of a wild-type mouse, the DNA enter hepatocytes and AAT is expressed in the liver and serum levels of AAT rise.

Table 3. Reactive centre: engineered and natural variants

Serpin	P_4	P_3	P_2	P_1	P_1'	P_2'	P_3'	P_4'	P_5'	Inhibits	Oxidation
α_1-Antitrypsin	Ala	Ile	Pro	Met	Ser	Ile	Pro	Pro	Glu	elastase	+
Pittsburgh variant	Ala	Ile	Pro	Arg	Ser	Ile	Pro	Pro	Glu	thrombin	–
Val-recombinant	Ala	Ile	Pro	Val	Ser	Ile	Pro	Pro	Glu	elastase	–
Leu-recombinant	Ala	Ile	Pro	Leu	Ser	Ile	Pro	Pro	Glu	Cat G. elastase	–
P_2Cys-recombinant	Ala	Ile	Cys	Met	Ser	Ile	Pro	Pro	Glu	non-functional	
Ala-recombinant	Ala	Ile	Pro	Ala	Ser	Ile	Pro	Pro	Glu	elastase	–
Christchurch variant	Ala	Ile	Pro	Met	Ser	Ile	Pro	Pro	Lys	elastase	+
P_2P_1'-recombinant	Ala	Ala	Gly	Arg	Ser	Leu	Asn	Pro	Glu	non-functional	
Antithrombin	Ile	Ala	Gly	Arg	Ser	Leu	Asn	Pro	Asn	thrombin	–
Denver variant*	Ile	Ala	Gly	Arg	Leu	Leu	Asn	Pro	Asn	non-functional	–

Stephens, Thalley and Hirs (1985).

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All of the COMPOSITIONS and METHODS disclosed and claimed herein may be made and executed without undue experimentation in light of the present disclosure. While the COMPOSITIONS and METHODS have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variation may be applied to the COMPOSITIONS and METHODS and in the steps or in the sequence of steps of the METHODS described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein

while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED:

1. A construct comprising:

a nucleic acid encoding alpha-1 antitrypsin (AAT) or fragment or peptide cleavage molecule thereof; and

a nucleic acid encoding an immune fragment or a fragment capable of being joined to itself, the nucleic acid further manipulated to truncate or eliminate the immune fragment hinge region.

2. The constructof claim 1, wherein the nucleic acid encoding AAT comprises a nucleic acid encoding naturally occurring AAT (SEQ ID NO:1).

3. The construct of claim 1 wherein the nucleic acid encoding the AAT peptide cleavage molecule comprises a nucleic acid encoding one or more carboxyterminal fragments of naturally occurring AAT.

4. The construct of claim 3, wherein the carboxyterminal fragment comprises the last 80 amino acids of SEQ ID NO:1 or 10 or more consecutive amino acids thereof.

5. The construct of claim 3, wherein the carboxyterminal fragment comprises the last 36 amino acids of SEQ ID NO:1 (SEQ ID NO. 34).

6. The construct of claim 1, wherein the construct comprises an M-type AAT.

7. The construct of claim 1, wherein the immune fragment comprises Fc.

8. The construct of claim 1, wherein the immune fragment comprises a nucleic acid encoding an Fc fragment from IgG1, IgG2, IgG3 or IgG4.

9. The construct of claim 8, wherein the nucleic acid encoding the Fc fragment hinge region is truncated or deleted.

10. The construct of claim 9, wherein the nucleic acids encoding the Fc fragment encodes the amino acid sequence of SEQ ID NO: 49, SEQ ID NO: 56, SEQ ID NO: 57, or SEQ ID NO: 58.

11. The construct of claim 1, wherein the construct is formed by linking the immune fragment to the N-terminal end of AAT.

12. The construct of claim 1, wherein the construct is formed by linking the immune fragment to the C-terminal end of AAT.

13. The construct of claim 1, wherein the nucleic acid encoding the AAT comprises a nucleic acid encoding a mutant AAT having a mutation at the reactive center loop (RCL).

14. A fusion protein comprising consecutive amino acids which, beginning at the amino terminus of the protein, correspond to consecutive amino acids present in (i) alpha-1 antitrypsin or carboxyterminal fragment thereof, (ii) a peptide linker, and (iii) an Fc immune fragment having a deleted or truncated hinge region, wherein the consecutive amino acids (i) remain bound to (iii) when purified.

15. The fusion protein of claim 14, wherein the Fc immune fragment comprises an IgG1 fragment.

16. The fusion protein of claim 14, wherein the Fc immune fragment comprises an IgG2 fragment.

17. The fusion protein of claim 14, wherein the Fc immune fragment comprises an IgG3 fragment.

18. The fusion protein of claim 14, wherein the Fc immune fragment comprises an IgG4 fragment.

19. A process for purifying a construct of claim 1 or a fusion molecule of claim 14 from a mixture of proteins or peptides comprising:

a. exposing the mixture of proteins or peptides to protein A associated with a resin or protein A bound to a column;

b. allowing immune fragments of the construct or fusion molecules to associate with protein A associated with a resin or protein A bound to a column;

c. removing unbound proteins or peptides of the mixture; and

d. eluting the proteins or peptides from protein A.

20. The process of claim 19, wherein the immune fragment is cleaved from the construct or fusion molecule.

21. The process of claim 19, wherein the construct or fusion molecule recovered retains at least about 90% anti-inflammatory activity compared to naturally-occurring AAT preparation.

22. The process of claim 17, wherein the construct or fusion molecule is used in a pharmaceutical composition.

23. A composition comprising a fusion protein of claim 14 and a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein the fusion protein is present in the composition in a therapeutically effective amount.

25. A vector comprising the nucleic acid construct of claim 1.

26. A transformed cell comprising the vector of claim 25.

27. An isolated preparation of expressed inclusion bodies comprising the fusion protein of claim 14.

28. A method for treating a subject in need of AAT therapy, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to initiate an anti-inflammatory response, wherein the administration reduces the inflammatory response in the subject.

29. A method for treating a subject in need of AAT therapy, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to achieve an immune modulatory response.

30. A method for treating diabetes in a subject, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat diabetes, wherein the administration treats diabetes in the subject.

31. A method for treating ischemic reperfusion injury in a subject, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration treats the ischemic reperfusion injury in the subject.

32. A method for treating a subject undergoing an organ or non-organ transplant, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration modulates transplant rejection in the subject.

33. A method for treating a subject having an infection, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration modulates the infection in the subject.

34. The method of claim 33, wherein the infection comprises a bacterial, viral, fungal or parasitic infection.

35. A method for treating an AAT deficient subject in need of AAT therapy comprising: administering a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration reduces AAT deficiency in the subject.

36. A method for treating a subject having gout, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration modulates gout in the subject.

37. A method for treating a subject having a cardiac condition, the method comprising administering to the subject a therapeutically effective amount of the composition comprising claim 23 in an amount effective to treat the subject, wherein the administration modulates the cardiac condition in the subject.

38. A method for preserving an organ, tissue or cells for transplantation in a subject, the method comprising exposing the organ, tissue or cells to the composition comprising claim 23, in an amount effective to preserve the organ, tissue or cells for transplantation or storage.

39. A construct comprising:

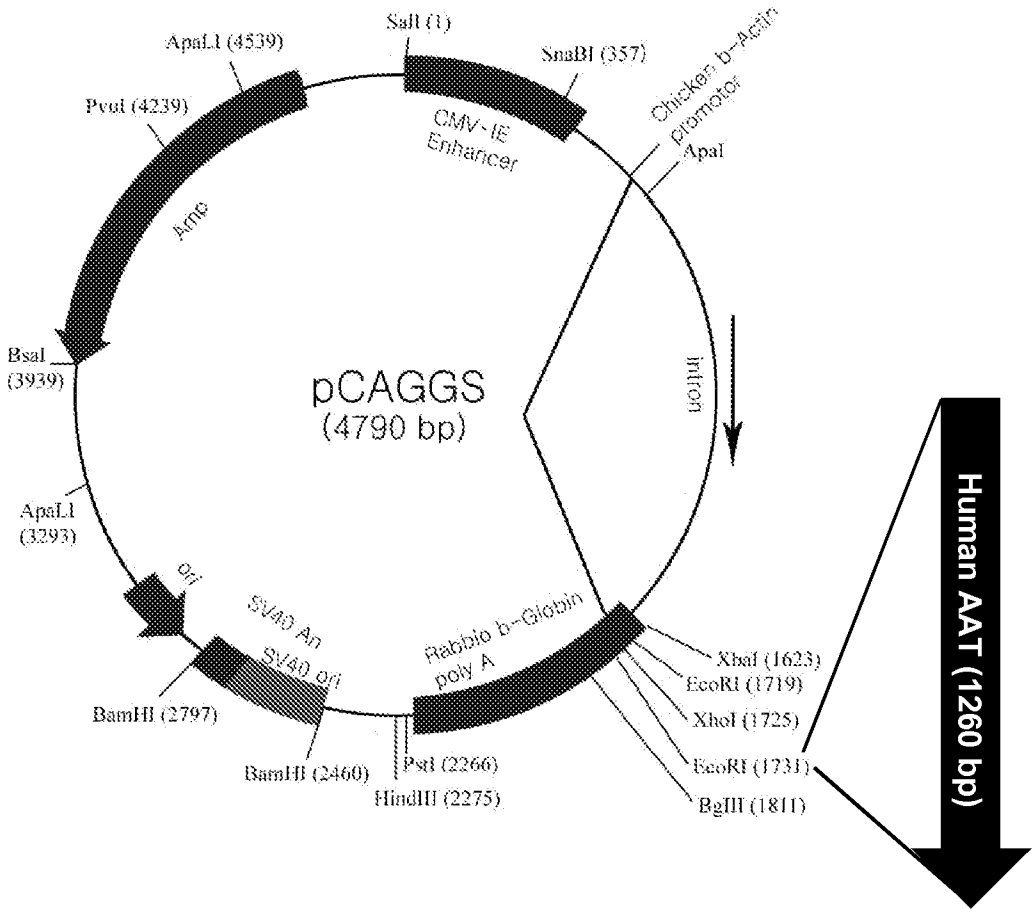
a nucleic acid encoding alpha-1 antitrypsin (AAT) or fragment or peptide cleavage molecule thereof; and

a nucleic acid encoding the amino acid sequence of an Fc fragment without the hinge region.

40. A pharmaceutical composition comprising, the amino acid sequence encoded by the construct of claim 39 and a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier thereof.

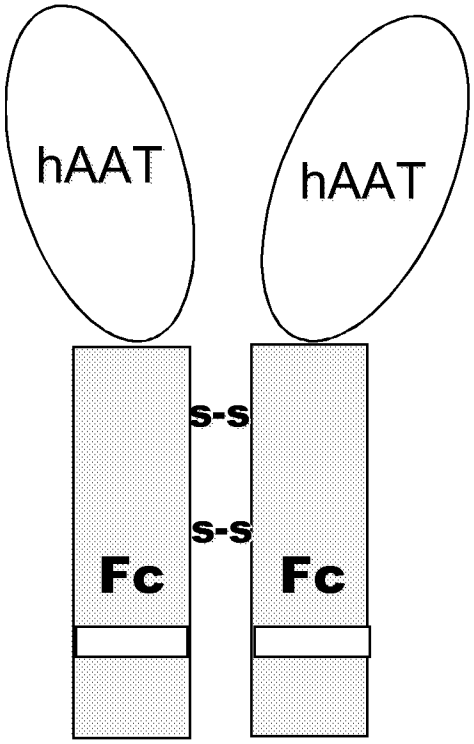
Fig. 1

pCAGGS-human AAT



Human AAT (insert) can also be a carboxyterminal fragment of AAT of the last 80 amino acids or less

Fig. 2



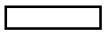
Note: Hinge deletion or mutation: 

Fig. 3

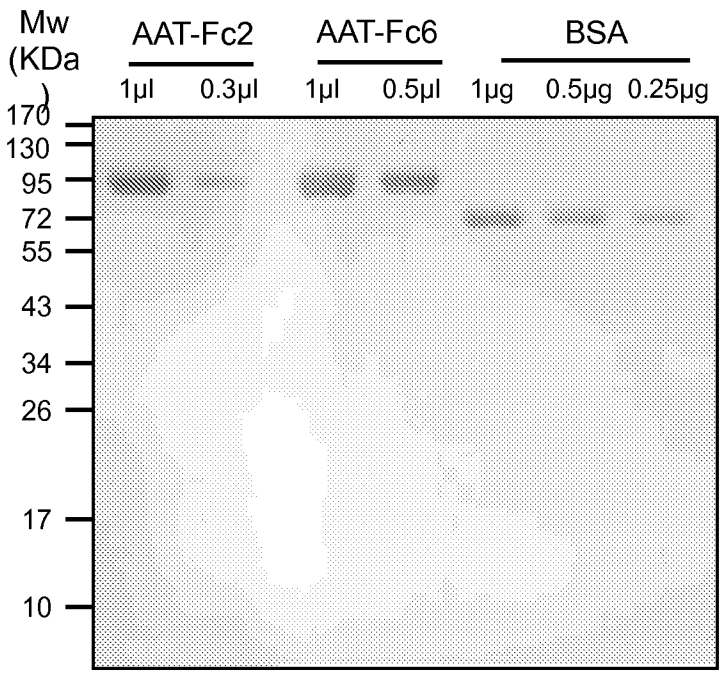

Mw
(KDa
)
AAT-Fc2
AAT-Fc6
BSA
1µl
0.3µl
1µl
0.5µl
1µg
0.5µg
0.25µg
170
130
95
72
55
43
34
26
17
10

Fig. 4A

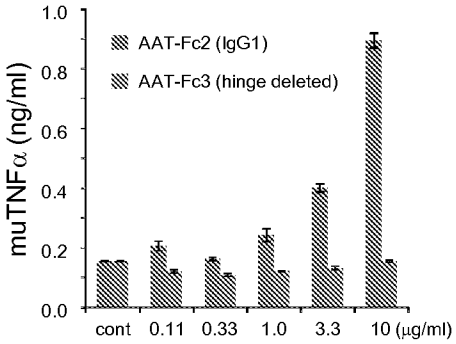

1.0
0.8
0.6
0.4
0.2
0.0
muTNFα (ng/ml)
AAT-Fc2 (IgG1)
AAT-Fc3 (hinge deleted)
cont
0.11
0.33
1.0
3.3
10 (μg/ml)

Fig. 4 B

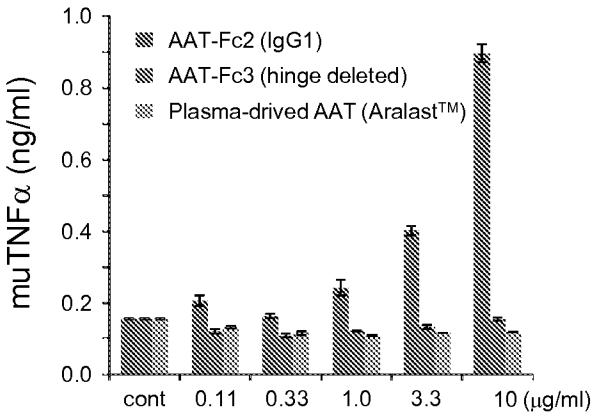

1.0
0.8
0.6
0.4
0.2
0.0
muTNFα (ng/ml)
AAT-Fc2 (lgG1)
AAT-Fc3 (hinge deleted)
Plasma-drived AAT (Aralast™)
cont
0.11
0.33
1.0
3.3
10 (μg/ml)

Fig. 5A

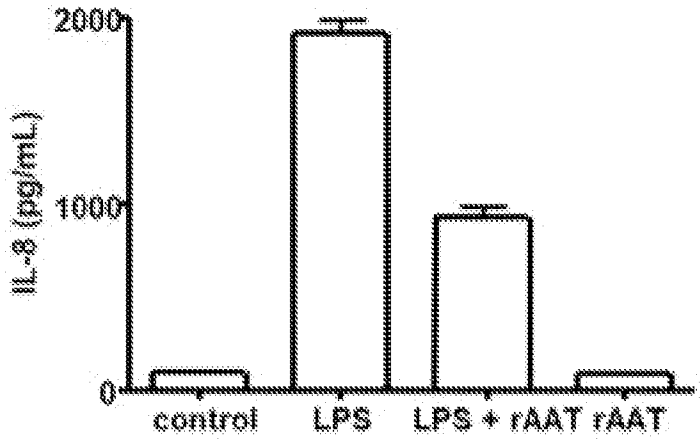
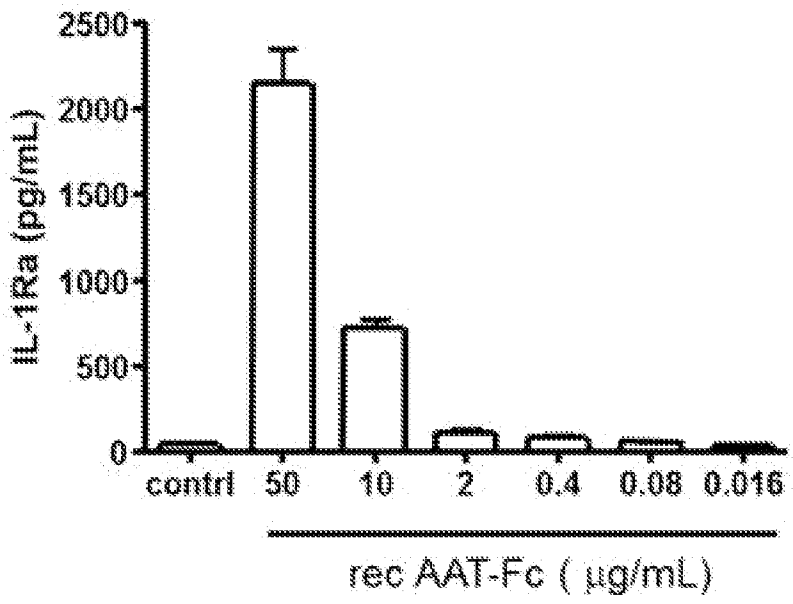
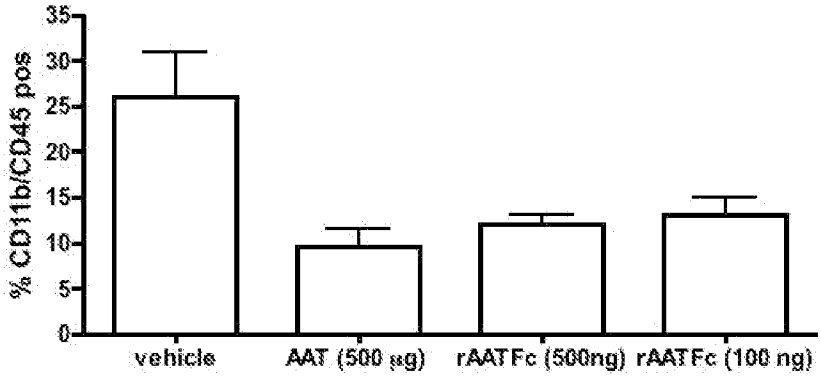

2000
1000
0
IL-8 (pg/mL)
control
LPS
LPS + rAAT
rAAT

Fig. 5B

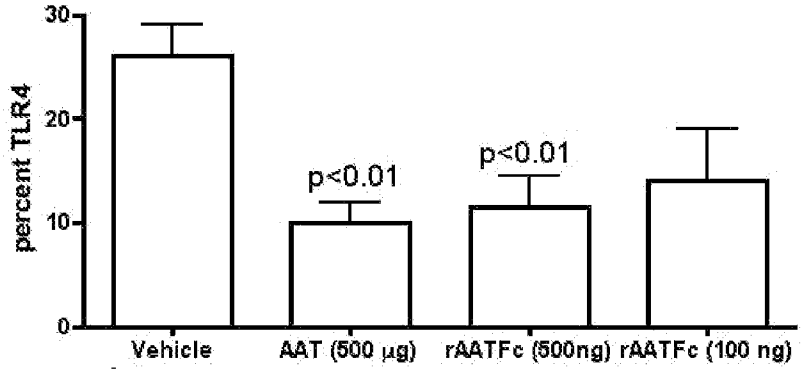

2500
2000
1500
1000
500
0
IL-1Ra (pg/mL)
contrl
50
10
2
0.4
0.08
0.016
rec AAT-Fc (µg/mL)

Figs. 6A-6C

A.



B.



C.

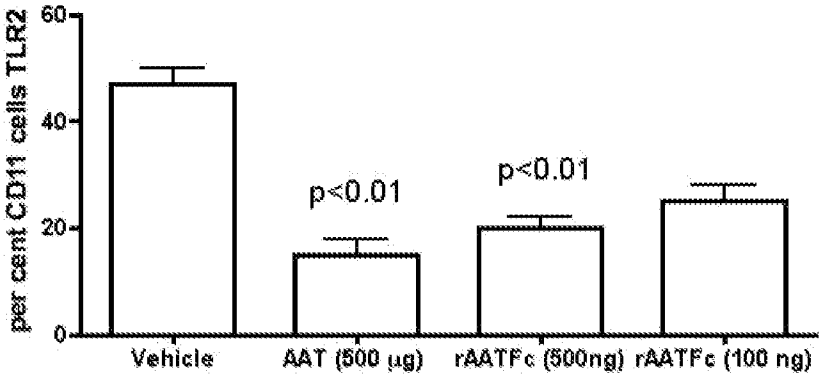


Fig. 7A

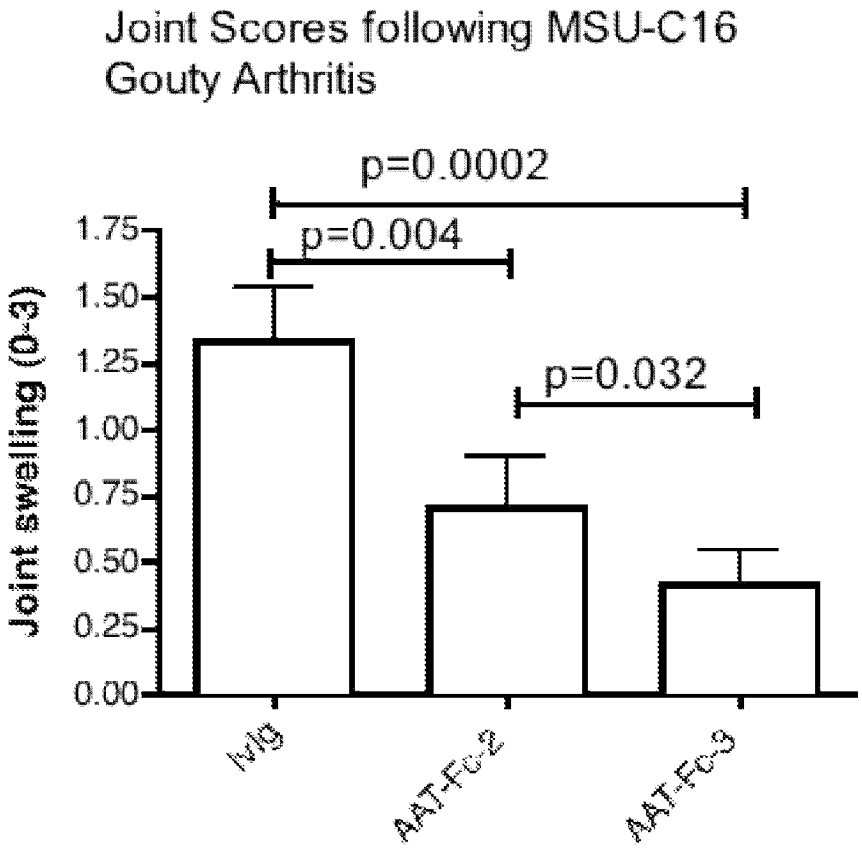

Joint Scores following MSU-C16
Gouty Arthritis
p=0.0002
p=0.004
p=0.032
Joint swelling (0-3)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
IVIg
AAT-Fc-2
AAT-Fc-3

Fig. 7B

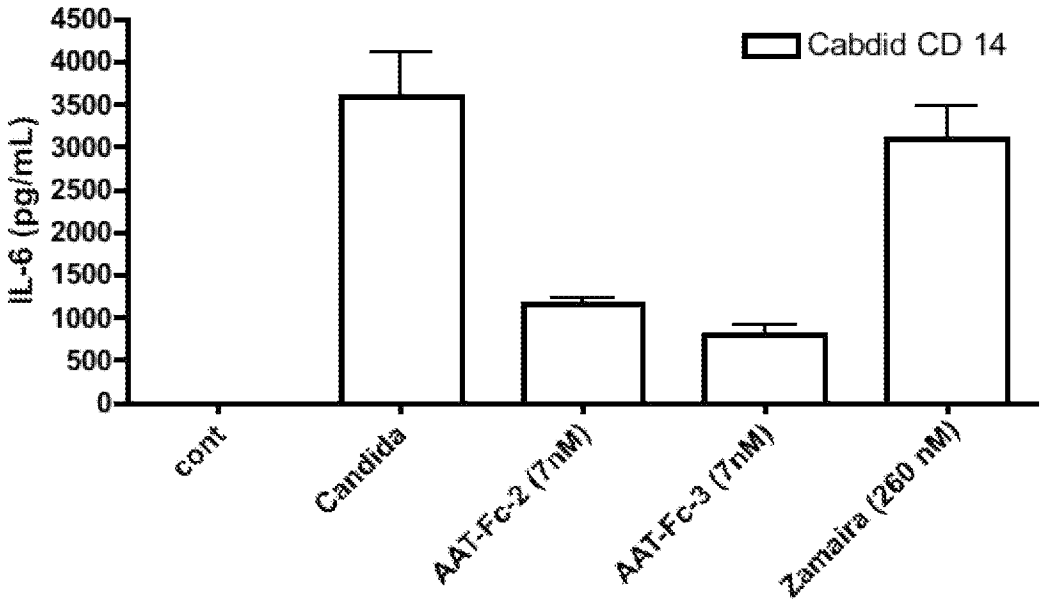

IL-6 (pg/mL)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Cabdid CD 14
cont
Candida
AAT-Fc-2 (7nM)
AAT-Fc-3 (7nM)
Zamaira (260 nM)

Fig. 7C

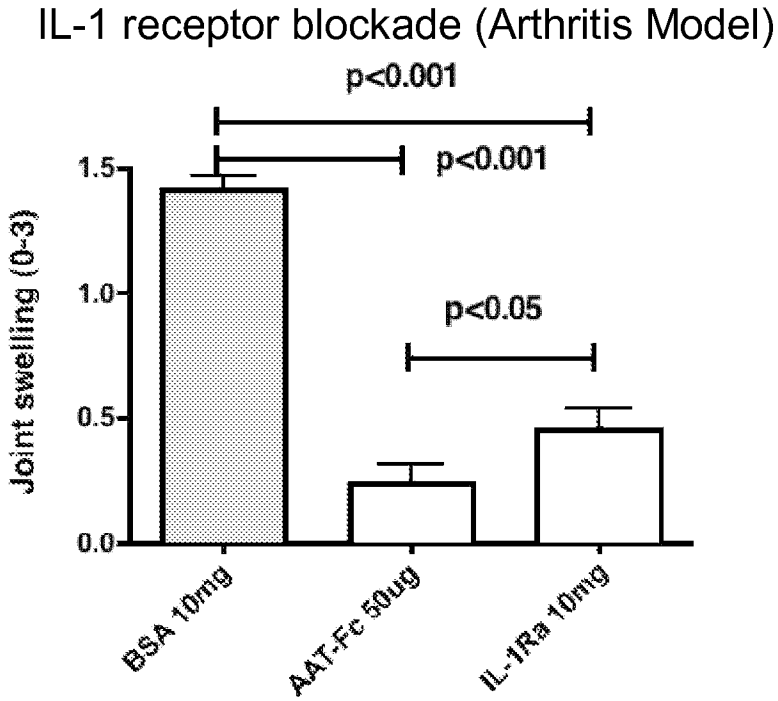

IL-1 receptor blockade (Arthritis Model)
p<0.001
p<0.001
p<0.05
Joint swelling (0-3)
1.5
1.0
0.5
0.0
BSA 10mg
AAT-Fc 50ug
IL-1Ra 10mg

Fig. 7D

Time course: Fc-AAT2 pretreatment intraperitoneally before instillation of monosodium urate (MSU) crystals into the knee joint of mouse.

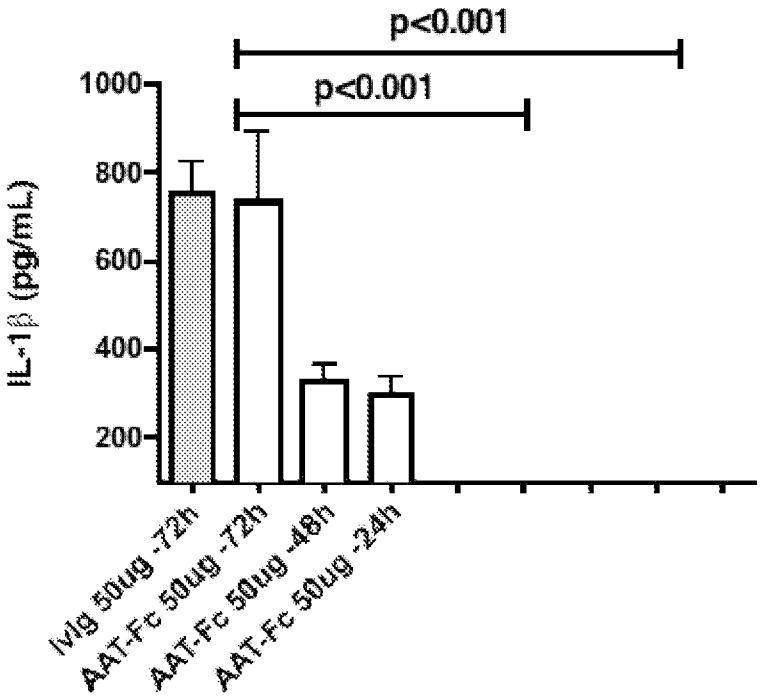


Fig. 8

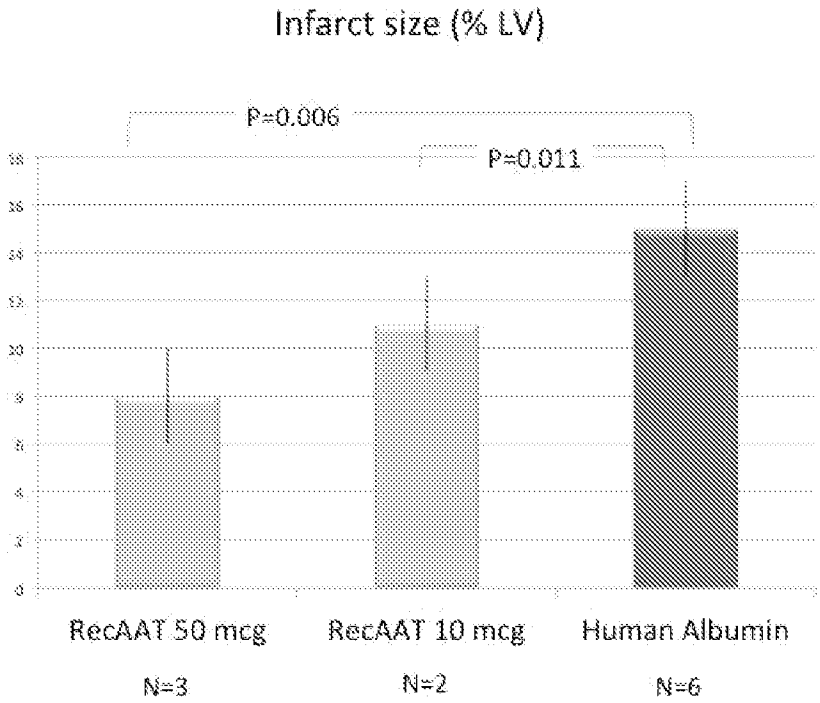
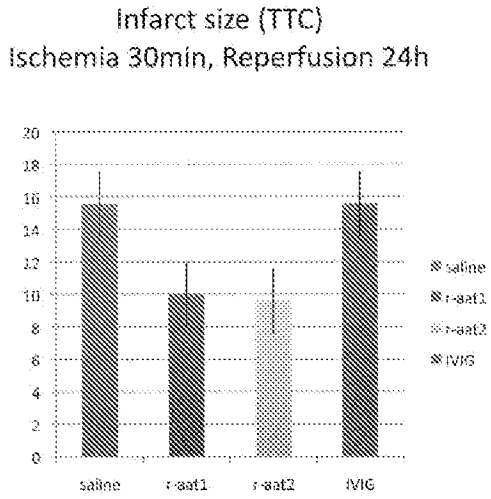
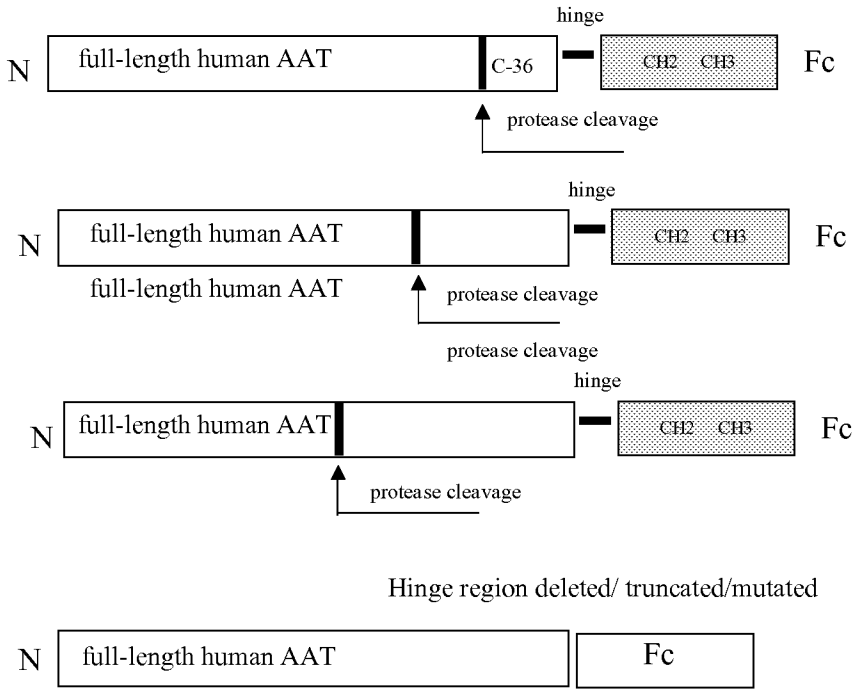

Infarct size (% LV)
P=0.006
P=0.011
RecAAT 50 mcg
N=3
RecAAT 10 mcg
N=2
Human Albumin
N=6

Fig.9



r-aat1: P<0.001 vs saline or IVIG
r-aat2: P<0.001 vs saline or IVIG

Fig. 10


hinge
N
full-length human AAT
C-36
CH2 CH3
Fc
protease cleavage
hinge
N
full-length human AAT
CH2 CH3
Fc
full-length human AAT
protease cleavage
protease cleavage
hinge
N
full-length human AAT
CH2 CH3
Fc
protease cleavage
Hinge region deleted/ truncated/mutated
N
full-length human AAT
Fc

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/21057

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/00; C07H 21/04 (2013.01)
USPC - 435/320.1; 536/23.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC- 435/320.1; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/134.1; 514/20.3, 514/44R,536/23.1 530/402, 530/387.3, 530/350, 530/363

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
US PTO website, Google, Google Patent, Google Scholar: AAT-Fc, hinge deletion, truncation, M type, RCL
GenCore 6.4.1: SEQ ID NO: 1, 39

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0203580 A1 (Dinarello, et al.) 13 August 2009 (13.08.2009) Abstract, para [0171], [0173], claims 5, 7	1-4, 6-13
Y	US 2003/0120059 A1 (Tao, et al.) 26 June 2003 (26.06.2003) Abstract, para [0136]-[0138]	1-4, 6-13
Y	US 7,138,370 B2 (Oliner, et al.) 21 November 2006 (21.11.2006) col 32, ln 10-50; claims 16, 24; SEQ ID NO: 60	10
Y	Blanco, et al. Efficacy of alpha1-antitrypsin augmentation therapy in conditions other than pulmonary emphysema. Orphanet Journal of Rare Diseases 2011, 6:14; Abstract	6
A	Tawara, et al. Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci U S A. Epub 27 December 2011, 109(2):564-569	1-4, 6-13
A	US 2011/0077383 A1 (Dall'acqua, et al.) 31 March 2011 (31.03.2011)	1-4, 6-13
A	WO 2009/015345 A1 (Treuheit, et al.) 29 January 2009 (29.01.2009) claims 1, 15	1-4, 6-13
A	US 2011/0053787 A1 (Brulliard, et al.) 03 March 2011 (03.03.2011) SEQ ID NO 1564, amino acids 1-426	1-4, 6-13

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 June 2013 (02.06.2013)	19 JUN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/21057

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 19/1
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 19/1 is not searchable under Article 34(4)(a)(ii) as being unclear, because a nucleic acid construct of claim 1 is not amenable to purification via protein A affinity chromatography.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-13, 25, 26, 39, drawn to a construct comprising: a nucleic acid encoding alpha-I antitrypsin (AAT) or fragment or peptide cleavage molecule thereof; and a nucleic acid encoding an immune fragment or a fragment capable of being joined to itself, the nucleic acid further manipulated to truncate or eliminate the immune fragment hinge region. The first invention (claims 1-4, 6-13) is restricted to SEQ ID NO:1 and 49. Should an additional fee(s) be paid, Applicant is invited to elect an additional SEQ ID NO(s) to be searched. The exact claims searched will depend on Applicant's election. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 6-13, restricted to SEQ ID NO:1 and 49

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/21057

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking:

Group II: claims 14-18, 19/14 (in part), 20-24, 27-38, 40, drawn to a fusion protein comprising consecutive amino acids which, beginning at the amino terminus of the protein, correspond to consecutive amino acids present in (i) alpha-l antitrypsin or carboxyterminal fragment thereof, (ii) a peptide linker, and (iii) an Fc immune fragment having a deleted or truncated hinge region, wherein the consecutive amino acids (I) remain bound to (iii) when purified.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I+ include the special technical feature of a construct comprising: a nucleic acid encoding alpha-l antitrypsin (AAT) or fragment or peptide cleavage molecule thereof; and a nucleic acid encoding an immune fragment or a fragment capable of being joined to itself, the nucleic acid further manipulated to truncate or eliminate the immune fragment hinge region, not required by Group II.

The invention of Group II includes the special technical feature of a fusion protein comprising consecutive amino acids which, beginning at the amino terminus of the protein, correspond to consecutive amino acids present in (i) alpha-l antitrypsin or carboxyterminal fragment thereof, (ii) a peptide linker, and (iii) an Fc immune fragment having a deleted or truncated hinge region, wherein the consecutive amino acids (I) remain bound to (iii) when purified, not required by Group I+.

The inventions of Group I+ share the technical feature of a construct of claim 1.
The inventions of Group I+ and Group II share the technical feature of a fusion protein comprising alpha-l antitrypsin (AAT) or fragment thereof, and an immune fragment having a deleted or truncated hinge region.

However, these shared technical features do not represent a contribution over prior art as being obvious over US 2009/0203580 A1 to Dinarello, et al. (herenafter "Dinarello") in view of US 2003/0120059 A1 to Tao, et al. (hereinafter "Tao") as follows:

Dinarello teaches a construct comprising a nucleic acid encoding alpha-l antitrypsin (AAT) (para [0172], [0174]-[0177], claims 5-7) or fragments thereof (para [0019]), and a nucleic acid encoding an immune fragment (para [0170]-[0172]; [0170]-"a fusion polypeptide may include AAT (e.g. mammalian .alpha.1-antitrypsin) or an analog thereof and a different amino acid sequence that may be heterologous to AAT or analog substance" which are [0172]- "produced by recombinant DNA techniques"), wherein the AAT is human AAT fusion polypeptides (para [0170]-[0176]) comprising IgG (para [0171], [0173]- "...a fusion protein can include a heterologous sequence derived from... a human immunoglobulin constant region such as a human IgG1 constant region...the FcR region of the immunoglobulin...the immunoglobulin heterologous sequence of the fusion protein can be mutated....) and the nucleic acid is further manipulated to truncate (para [0164]-[0165], [0168]-[0169]), but does not teach specific elimination of the immune fragment hinge region. However, it would have been obvious to a person having ordinary skill in the art to manipulate nucleic acid constructs to truncate or eliminate the immune fragment hinge region, because immunoglobulin heterologous sequence of the fusion protein can be mutated (Dinarello, para [0173]) or truncated (para [0164]-[0165]) that can be produced by recombinant DNA techniques (para [0172]), through recombinant DNA techniques to truncate or eliminate, to produce the desired immune fragment and the AAT- human IgG1 constant region (Fc) fusion protein.

Dinarello teaches AAT fragments can have intramolecular disulfide bonds (Dinarello, para [0191]), but does not specifically disclose an immune fragment capable of being joined to itself. Tao teaches that hinge region of IgG provides intermolecular disulfide bonds between heavy and light chains using critical cysteine residues (para [0046]). Accordingly, it would have been obvious to one of ordinary skill in the art that Fc region of IgG forms multimeric association (Tao, para [0046]) and accordingly, the immune fragments capable of being joined to itself can be used to produce fusion proteins using recombinant DNA technology (Dinarello, para [0172], Tao [0050]-[0062]).

Further, Tao discloses a nucleic acid encoding an IgG1 heavy chain manipulated to truncate or eliminate the immune fragment hinge region, specifically having a CHI-hinge deletion (para [0137], "CHI-hinge deletion (SEQ ID NO:4) capable of activating macrophages as revealed by an ADCC assay (para [0136, [0137], "CHI-hinge deletion (SEQ ID NO:4) gave similar levels of macrophage activation"), but lacked the complement activation capability (para 0138], " In summary, a novel human IgG1 Fc chimera has been constructed... The Fc receptor binding property was retained in the molecule, IgG1 Fc chimera the complement activation capability was absent").

Regarding the limitation that human IgG1 constant region (Fc) is capable of being joined to itself, Tao discloses that said CHI-hinge deletion Fc is capable of being joined to itself (para [0137], "The extracellular region of the transferrin receptor used to anchor Fc (residues 89-97) is able to substitute for native hinge. The hTR fragment used in this study is approximately equal in length, if not amino acid identity, to native IgG1 hinge, and may effectively provide functions similar to that of the hinge region. Another critical function of hinge is to provide intermolecular disulfide bonds between heavy and light chains using critical cysteine residues. The hTR (1-97), which contains at least one cysteine (C89) necessary for TR dimerization, is able to mimic hinge by allowing multimer association of the hTR-FcH monomers").
It would have been obvious to one of ordinary to combine Dinarello and Tao by introducing, in the course of routine experimentation and with a reasonable expectation of success, the CHI-hinge deletion of Tao in the AAT- Fc fusion protein of Dinarello, because Dinarello specifically discloses that "the FcR region of the immunoglobulin may be ... mutated. In certain embodiments, it may be desirable to utilize an immunoglobulin fusion protein that does not interact with an Fc receptor and does not initiate ADCC reactions. In such instances, the immunoglobulin heterologous sequence of the fusion protein can be mutated to inhibit such reactions" (para [0171]), while Tao explains that "antibody-dependent cell-mediated cytotoxicity (ADCC) has an important role in the destruction of many target cells... by macrophages" (para [0005]) and that "the lytic attack on the target cells is triggered by Fc receptor-mediated ADCC" (para [0005]), and that ADCC reactions are avoided by using the Fc having CHI-hinge deletion (para 0138]). As said construct would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

********** See the following Supplemental Sheet to continue **********

In Continuation of the Preceding Supplemental Sheet:

Another common technical feature of the inventions listed as Group I+ is a nucleic acid encoding specific amino acid sequence of AAT or fragments thereof and a fragment capable of being joined to itself. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2003/0073217 A1 to Barr, et al. (hereinafter "Barr"). Bar discloses a nucleic acid (para [0030], [0086], FIGs. 1-4, Tables 7, 9, 11) encoding the claimed amino acid SEQ ID NO: 1 (para [0017], [0174], [0181]- SEQ ID NO 2, 100% identity) or frangments thereof (para [0074]) and a fragment capable of being joined to itself (para [0174]-methods of formation of intra- and intermolecular disulfide bonds). Therefore, no significant structural similarities can readily be ascertained among the claimed nucleic acids encoding specific amino acid sequences, either known or unknown in the prior art. Without a shared special technical feature, the inventions lack unity with one another.

Further to the discussion above, the shared the technical feature of a fusion protein comprising alpha-I antitrypsin (AAT) or fragment thereof; and an immune fragment having a deleted or truncated hinge region of Group I+ and Group II, does not represent a contribution over prior art as being obvious over Dinarello.

Dinarello teaches a construct comprising a fusion protein alpha-I antitrypsin (AAT) (para [0172], [0174]-[0177]) or fragments thereof (para [0019]), and an immune fragment (para [0170]-[0172]; [0170]-"a fusion polypeptide may include AAT (e.g. mammalian .alpha.1-antitrypsin) or an analog thereof and a different amino acid sequence that may be heterologous to AAT or analog substance" which are [0172]- "produced by recombinant DNA techniques"), wherein the AAT is human AAT fusion polypeptides (para [0170]-[0176]) comprising IgG (para [0171], [0173]- "...a fusion protein can include a heterologous sequence derived from... a human immunoglobulin constant region such as a human IgG1 constant region...the FcR region of the immunoglobulin...the immunoglobulin heterologous sequence of the fusion protein can be mutated....) and further manipulated to truncate (para [0164]-[0165], [0168]-[0169]), but does not teach specific elimination of the immune fragment hinge region. However, it would have been obvious to a person having ordinary skill in the art to manipulate nucleic acid constructs to truncate or eliminate the immune fragment hinge region; because immunoglobulin heterologous sequence of the fusion protein can be mutated (Dinarello, para [0173]) or truncated (para [0164]-[0165]) that can be produced by recombinant DNA techniques (para [0172]), through recombinant DNA techniques to truncate or eliminate, to produce the desired immune fragment and the AAT- human IgG1 constant region (Fc) fusion protein.

As the common technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Therefore, Groups I+ and II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.