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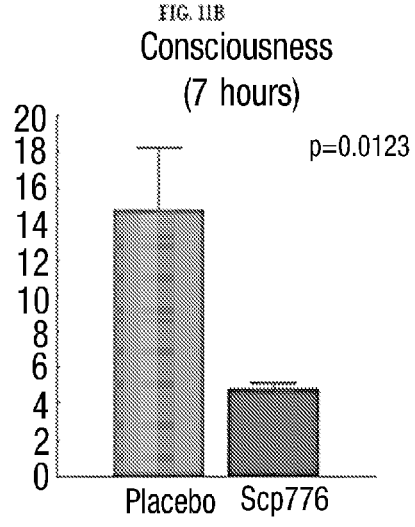
(54) Title: CHIMERIC PROTEINS AND METHODS OF USE FOR TREATMENT OF CENTRAL NERVOUS SYSTEM DISORDERS

(57) Abstract: Aspects of the present disclosure relate generally to kits and methods for treating acute central nervous systems disorders with chimeric proteins and pharmaceutical compositions comprising such chimeric proteins.

FIG. 11B



WO 2022/026902 A3

CHIMERIC PROTEINS AND METHODS OF USE FOR TREATMENT OF CENTRAL NERVOUS SYSTEM DISORDERS

RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Application No. 63/058,888, filed July 30, 2020, and U.S. Provisional Patent Application No. 17/390,206, filed July 30, 2021, the content of which are incorporated herein by reference in their entirety.

REFERENCE TO SEQUENCE LISTING

[0002] This specification includes a sequence listing submitted herewith, which includes the file entitled 132463-010401_ST25.txt having the following size: 70,958 bytes which was created July 29, 2021, the contents of which are incorporated by reference herein.

TECHNICAL FIELD

[0003] Aspects of the present disclosure relates generally to chimeric proteins and pharmaceutical compositions comprising such chimeric proteins, and methods for using such chimeric proteins for treating central nervous system disorders in a subject in need thereof.

BACKGROUND

[0004] Brain injuries and central nervous system disorders are common medical condition that can result in disabling and irreversible degradation of a person's cognitive and sensorimotor capacity. Brain injuries and central nervous system disorders that can result in nerve cell death and damage range from ischemic stroke to degenerative disorders.

SUMMARY

[0005] Methods and kit for treating central nervous system injury or neurodegenerative disease are provided.

[0006] In some embodiments, methods of treating acute central nervous system injury are provided. In some embodiments, the method comprises administering by bolus injection to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein and a pharmaceutically acceptable carrier, wherein the chimeric protein comprises (a) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding

to R63, K70, K101, E138, D139, N160, and combinations thereof; (b) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (c) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof. Administration of the pharmaceutical composition results in at least one of the following: mitigation of oxidative damage to cells of the cerebral cortex, repair or acceleration of repair of blood brain barrier, reduction of oedema, reduction of infarct volume, targeted stimulation of the phosphorylation of serine/threonine protein kinase B (AKT) pathway by selective activation of the IGF-1 receptor in cells of injured brain tissue, targeted delivery of pro-survival signals to injured brain tissue, increase in musculoskeletal coordination following stroke, improvement to consciousness following stroke, improvement to neurologic function following stroke, and improvement in motor function following stroke.

[0007] In some embodiments, the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1. In some embodiments, the variant of IGF-1 comprises E3R and Y31A substitutions relative to wild type human IGF-1.

[0008] In some embodiments, the variant of human Annexin 5 comprises the amino acids 2-320 corresponding to wild type human Annexin 5 and comprises R63A, K70A, K101A, E138A, D139G, N160A, C316A substitutions relative to wild type human Annexin 5.

[0009] In some embodiments, the variant of human serum albumin comprises the amino acids 26-609 corresponding to wild type human serum albumin and comprises C58S and N527Q substitutions relative to wild type human serum albumin.

[0010] In some embodiments, the chimeric protein is IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316A).

[0011] In some embodiments, the linker lk7 comprises or consist of -Gly-Ser-Gly-Gly-Gly-Ser-Gly.

[0012] In some embodiments, the chimeric protein is selectively targeted to cells comprising a target molecule phosphatidylserine and exhibits activation of the IGF-1 receptor at least twice as strong on cells containing the target molecule compared to cells that do not contain the target molecule as measured by phosphorylation of serine/threonine protein kinase B (AKT).

[0013] In some embodiments, the acute CNS injury is ischemic stroke. In some embodiments, the acute CNS injury is traumatic brain injury. In some embodiments, the acute CNS injury is hemorrhagic stroke. In some embodiments, the acute CNS injury is acquired brain injury. In some embodiments, the acute CNS injury is spinal cord injury. In some embodiments, the acute CNS injury is subarachnoid hemorrhage. In some embodiments, the acute CNS injury is iatrogenic in nature.

[0014] In some embodiments, the method comprises administering the pharmaceutical composition within 72 hours of diagnosis of the acute CNS injury. In some embodiments, the method comprises administering the pharmaceutical composition within 48 hours of diagnosis of the acute CNS injury.

[0015] In some embodiments, the method comprises administering daily from about 0.01 mg/kg to about 20 mg/kg of the chimeric protein to the subject in need thereof.

[0016] In some embodiments, the method comprises administering a total dose of from about 5 mg/kg to about 20 mg/kg of the chimeric protein to the subject in need thereof over a period of 4 to 7 days. In some embodiments, the method comprises administering a total dose of from about 5 mg/kg to about 20 mg/kg of the chimeric protein to the subject in need thereof over a period of 7 days.

[0017] In some embodiments, the method comprises administering descending doses of the chimeric protein to the subject in need thereof. In some embodiments, the method comprises administering to the subject in need thereof a first dose comprising from about 2 mg/kg to about 6 mg/kg of the chimeric protein on day 1, and a dose comprising from about 1 mg/kg to about 2 mg/kg one each of the following days.

[0018] In some embodiments, a kit for practicing the methods of the disclosure is provided. In some embodiments, the kit comprises a plurality of individual containers, each individual container comprising about 20 mg to about 1,000 mg of the chimeric protein.

[0019] In some embodiments, a kit for treating acute central nervous system injury is provided. In some embodiments, the kit comprises (a) a plurality of individual containers, each individual container comprising a volume of a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is formulated for bolus injection, wherein the chimeric protein comprises (i) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding to R63,

K70, K101, E138, D139, N160, and combinations thereof; (ii) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (iii) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof; wherein each of the plurality of the individual containers comprises a volume ranging from about 1 ml to about 50 ml, wherein each of the plurality of the individual containers comprises an amount of chimeric protein ranging from about 20 mg to about 1,000 mg; and (b) instructions for use in treating acute central nervous system injury.

[0020] In some embodiments, the plurality of containers of the kit comprise a total dose from about 5 mg/kg to about 20 mg/kg.

[0021] In some embodiments, the instructions for use comprise instructions to administer intravenously, intraarterially or intrathecally the pharmaceutical composition over a period of 4 to 7 days.

[0022] In some embodiments, the instructions for use comprise instructions to administer intravenously, intraarterially or intrathecally descending doses of the chimeric protein.

DESCRIPTION OF THE SEQUENCE LISTING

[0023] SEQ ID NO: 1 is the amino acid sequence of wild-type human IGF-1 (mature form).

[0024] SEQ ID NO: 2 is the amino acid sequence of a variant of wild-type human IGF-1 variant comprising E3R and Y31A substitutions.

[0025] SEQ ID NO: 3 is the amino acid sequence a variant of human IGF-1 (IGF-1 LONG).

[0026] SEQ ID NO: 4 is the amino acid sequence a variant of human IGF-1 (IGF1 E3R).

[0027] SEQ ID NO: 5 is the amino acid sequence of a variant of human IGF-1(IGF-1Des 1-3).

[0028] SEQ ID NO: 6 is the amino acid sequence of a variant of human IGF-1 (IGF-1 LR3).

[0029] SEQ ID NO: 7 is the amino acid sequence a variant of human IGF-1 (IGF1 R37X).

[0030] SEQ ID NO: 8 is the amino acid sequence of human a variant of human IGF-1 with deletion of residues 68-70 (IGF1 3X).

[0031] SEQ ID NO: 9 is the amino acid sequence of wild type human annexin A5 (AnxV).

[0032] SEQ ID NO: 10 is the amino acid sequence of a variant of wild-type human annexin 5 comprising the amino acids 2-320 of wild type annexin 5 and the R63A, K70A, K101A, E138A, D139G, N160A and C316A substitutions.

[0033] SEQ ID NO: 11 is the amino acid sequence of non-internalizing variant of human annexin A5 (ni-AnxV).

[0034] SEQ ID NO: 12 is the amino acid sequence of wild type Human Serum Albumin (HSA).

[0035] SEQ ID NO: 13 is the amino acid sequence of Human Serum Albumin variant mHSA (C34S, N503Q substitutions).

[0036] SEQ ID NO: 14 is the amino acid sequence of Human Serum Albumin variant mHSA7 (C34S, N503Q, E505G and V547A substitutions).

[0037] SEQ ID NO: 15 is the amino acid sequence of a variant human serum albumin comprising the amino acids 26-609 of wild type human serum albumin and the C58S and N527Q substitutions.

[0038] SEQ ID NO: 16 is the amino acid sequence of a peptide linker.

[0039] SEQ ID NO: 17 is the amino acid sequence of human transferrin (Tf).

[0040] SEQ ID NO: 18 is the amino acid sequence of Human Alpha Fetoprotein (AFP).

[0041] SEQ ID NO: 19 is the amino acid sequence of Human Vitamin D Binding Protein (VDBP).

[0042] SEQ ID NO: 20 is the amino acid sequence of Human Transthyretin (TTR).

[0100] SEQ ID NO: 21 is the amino acid sequence of a motif PASylation.

[0101] SEQ ID NO: 22 is the amino acid sequence of the albumin-binding domain human antibody (aldudAB).

[0043] SEQ ID NO: 23 is the amino acid sequence of a peptide linker lk7.

[0044] SEQ ID NO: 24 is the amino acid sequence of chimeric protein scp776.

[0045] SEQ ID NO: 25 is the amino acid sequence of chimeric protein IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316S).

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0047] FIG. 1 is a graph showing that chimeric protein scp776 protects against oxidative neuronal cell death in vitro (percent released LDH readout).

[0048] FIG. 2 is a graph showing that chimeric protein scp776 protects against oxidative neuronal cell death in vitro (percent neuronal cell death readout).

[0049] FIG. 3 is a schematic representation of filament placement in the rat tMCAO model of acute ischemic stroke.

[0050] FIG. 4 is a graph showing that intravenous administration of chimeric protein scp776 protects blood brain barrier integrity after ischemic stroke in rats.

[0051] FIG. 5 is a graph showing that intravenous administration of chimeric protein scp776 reduces hemispheric swelling after ischemic stroke in rats.

[0052] FIG. 6 is a graph showing that intravenous administration of chimeric protein scp776 reduces infarct size after ischemic stroke in rats.

[0053] FIG. 7 is a graph showing that chimeric protein scp776 accumulates in rat brain tissue following ischemic stroke

[0054] FIG. 8 is a graph showing that chimeric protein scp776 treatment enhances recovery of function following ischemic stroke in rats.

[0055] FIG. 9 shows IGF-1 residues identified via application of an algorithm that considers solvent accessibility, hydrophobicity, and temperature factor. Represented by X are residue position that can be mutated.

[0056] FIG. 10 shows hydrophobic core positions of IGF-1.

[0057] FIG. 11A shows the neurological deficit score (NDS)- motor system score at 48 hours (day 2) for placebo treated and scp776 treated animals.

[0058] FIG. 11B shows the NDS- consciousness score at day 7 for placebo treated and scp776 treated animals.

[0059] FIG. 11C shows the NDS - musculoskeletal coordination score at day 1, 2, 3, 7, 10, and 14 for placebo treated (top curve) and scp776 treated (bottom curve) animals.

[0060] FIG. 11D shows the total NDS score at day 1, 2, 3, 7, 10, and 14 for placebo treated (top curve) and scp776 treated (bottom curve) animals.

[0061] FIG. 12 shows the results of the MRI analysis of the lesion size (mm^2) at 8 hours, 72 hours and 14 days for placebo treated and scp776 treated animals.

DETAILED DESCRIPTION

[0062] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments of the disclosure only and is not intended to be limiting.

[0063] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one having ordinary skill in the art to which the disclosure pertains.

[0064] All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Definitions

[0065] As used in this specification and the appended claims, the singular forms "a, "an" and "the" include plural referents unless the content clearly dictates otherwise.

[0066] The term “peptide,” “polypeptide” and “protein” are used interchangeably to denote a sequence polymer of at least two amino acids covalently linked by an amide bond (also referred herein as peptide bond).

[0067] As used herein the term “target molecule” refers to any molecule that is associated with a tissue (e.g. “at risk”, diseased or damaged tissue). A “target cell” is meant to be a cell to which a protein or targeting domain thereof can specifically bind.

[0068] “Binding” or “specific binding” are used interchangeably herein and indicates that a protein (or the targeting polypeptide domain thereof or the activator domain thereof) exhibits substantial affinity for a specific molecule (e.g., targeting domain exhibits substantial affinity for a target molecule, or an activator domain exhibits substantial affinity for a molecule associated with the surface of a cell such as a growth factor receptor) or a cell or tissue bearing the molecule and is said to occur when the protein (or the targeting polypeptide domain thereof or the activator domain thereof) has a substantial affinity for a specific molecule and is selective in that it does not exhibit significant cross-reactivity with other molecules.

[0069] "Identity," as known in the art, is a relationship between two or more polypeptide or protein sequences, as determined by comparing the sequences. In the art, "identity" also refers to the degree of sequence relatedness between polypeptides or proteins, as determined by the match between strings of such sequences. "Identity" can be readily calculated by any bioinformational methods known in the art.

[0070] The term "parent polypeptide" refers to a wild-type polypeptide and the amino acid sequence or nucleotide sequence of the wild-type polypeptide is part of a publicly accessible protein database (e.g., EMBL Nucleotide Sequence Database, NCBI Entrez, ExPasy, Protein Data Bank and the like).

[0071] The term "mutant polypeptide" or "polypeptide variant" refers to a form of a polypeptide, wherein its amino acid sequence differs from the amino acid sequence of its corresponding wild-type (parent) form, naturally existing form or any other parent form. A mutant polypeptide can contain one or more mutations, e.g., substitution, insertion, deletion, addition etc. ..which result in the mutant polypeptide. Generally, variants are overall closely similar, and, in many regions, identical to the reference polypeptide. As used herein, "variant" refers to a polypeptide, differing in sequence from a native protein but retaining at least one functional and/or therapeutic property thereof as described elsewhere herein or otherwise known in the art.

[0072] The term "corresponding to a parent polypeptide" is used to describe a polypeptide of the disclosure, wherein the amino acid sequence of the polypeptide differs from the amino acid sequence of the corresponding parent polypeptide only by the presence of at least one amino acid variation. Typically, the amino acid sequences of the variant polypeptide and the parent polypeptide exhibit a high percentage of identity. In one example, "corresponding to a parent polypeptide" means that the amino acid sequence of the variant polypeptide has at least about 50% identity, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% identity or at least about 99% identity to the amino acid sequence of the parent polypeptide. In another example, the nucleic acid sequence that encodes the variant polypeptide has at least about 50% identity, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% identity or at least about 99% identity to the nucleic acid sequence encoding the parent polypeptide.

[0073] The term "substantial identity" or "substantial similarity," as used herein, when referring to a nucleic acid or fragment thereof, indicates that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95% to 99% of the sequence. The

term “substantial identity” or “substantial similarity,” as used herein, when referring to a protein or fragment thereof, indicates that when optimally aligned there is an amino acid sequence identity in at least about 95% to 99% of the sequence.

[0074] The term “damaged cell” or "damaged tissue," as used herein, means and includes biological cell or tissue; for example, but not limited to, neurons, glia or nervous tissue damaged or injured by, but not limited to, trauma or chemical insult, ischemic tissue, cell or tissue damaged by any means which results in interruption of normal blood flow to the tissue.

[0075] The term “therapeutically effective amount,” as used herein, means the amount of the protein or agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

[0076] The term “pharmaceutically acceptable,” or “physiologically acceptable” as used herein, means the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[0077] The term "targeting moiety", “targeting domain,” “targeting polypeptide” or “targeting module” are used herein interchangeably and refer to molecules that selectively localize the chimeric protein in a particular tissue or region of the body. The localization can be mediated by specific recognition of molecular determinants, molecular size of the targeting domain, ionic interactions, hydrophobic interactions and the like. As used herein, the terms "therapeutic moiety," “activator domain,” “activator polypeptide”, “signaling arm” and “effector module” are used herein interchangeably and refers to any agents useful for therapy and that are non-toxic, do not have a cytotoxic effect or are not detrimental to the cells. Such agents can include, but not limited to, growth factors.

Chimeric Proteins

[0078] Chimeric proteins provided herein are capable of specific binding to two or more different specific molecules. In some embodiments, the chimeric protein comprises a targeting domain having a binding specificity to a first specific target molecule, an activator domain having a binding specificity to a second target molecule, and a half-life modulator.

[0079] In some aspects, the activator domain has a binding specificity to a tyrosine kinase receptor at the cell surface. In some embodiments, the binding of the activator to the tyrosine kinase receptor activates intracellular signaling pathways associated with cell survival. In some

aspects, the activator domain has a binding specificity to a receptor that modulates/promotes tissue regeneration.

[0080] In some embodiments, the targeting domain serves to target the chimeric protein to a target cell or tissue while the activator domain serves to activate the intracellular signaling pathway associated with cell survival.

[0081] In some embodiments, the half-life modulator extends the half-life of the chimeric protein.

[0082] In some embodiments, the chimeric proteins are fusion proteins having a targeting polypeptide connected or linked to a half-life modulator and to an activator polypeptide. In some embodiments, the engineered proteins are chimeric proteins having a targeting polypeptide connected or linked to a half-life modulator and a growth factor or mutated growth factor.

[0083] In some embodiments, the mutated growth factor (e.g. IGF-1 variant) is engineered to reduce potency while retaining the ability to activate the cognate growth factor receptor. In some embodiments, wild type growth factors can be used as activator domains.

[0084] The targeting domain is generally used to target the chimeric proteins to a target cell. In some embodiments the target cell is undergoing apoptosis. The binding of the targeting domain to its target molecule does not induce a significant biological effect in the target cell. The activator domain binds to a receptor on a cell surface. The binding of the activator domain to its receptor is intended to modulate a specific biological effect, such as, activate the intracellular signaling pathway associated with cell survival. In some embodiments, binding of the activator domain to its receptor is intended the positively regulate survival of the targeted cells or tissue. In particular, the activator domain of chimeric protein can promote survival signaling.

[0085] In some embodiments, the *in vivo* activity of the chimeric protein can be assessed by detecting signaling changes in molecules that are regulated by the activator domain, including but not limited to cell surface receptor phosphorylation status or downstream mediators such as phospho-AKT or phospho-ERK (as detected by flow cytometry, immunofluorescence, ELISA, phospho-labeling, Western analysis of treated tissues, or any other methodology known in the art.) In some embodiments, a chimeric protein functions in vivo if it induces a significant (e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50% or more)

change in the level, functional activity, or phosphorylation of the regulated molecule detected by the assay.

Activator Domain

[0086] The activator domain can be any polypeptide that detectably modulates the activity of a cellular network. In some embodiments, the activator domain is capable of activating signal transduction pathways by binding to a receptor at the surface a cell. In some embodiments, certain activator domains are growth factor polypeptides, or any agonist of the receptor. It will be apparent that such modulation may be an increase in the activity of the cellular network such as induction of proliferation of cells, induction of cell growth, promotion of cell survival and/or inhibition of apoptosis.

[0087] An activator domain for a particular application may be selected based on the desired therapeutic outcome. For example, to increase survival and neuroprotection, activator domains that comprise IGF-1 (or variant or fragment thereof), NRG1 (or variant or fragment thereof), FGF2 (or variant or fragment thereof), FGF9 (or variant or fragment thereof), CCL4 (or variant or fragment thereof), Hepatocyte Growth Factor (NK1 or fragment thereof), Vascular Endothelial Growth Factor A (or variant or fragment thereof), BMP2 (or variant or fragment thereof), can be used.

[0088] In some embodiments, the activator domain comprises a change in the amino acid sequence, the three-dimensional structure of the protein, and/or the activity of the protein, relative to the wild-type form of the protein.

[0089] In some embodiments, the activator domain comprises or consists of a growth factor having amino acid sequence modification relative to the wild-type growth factor (e.g. IGF-1) to decrease its binding to its natural receptor (e.g. IGF-1 receptor), to decrease its binding to binding proteins (e.g. IGF binding proteins) and/or decrease its activation of its natural receptor (e.g. IGF-1 receptor). In some embodiments, the activator domain is a growth factor having amino acid sequence modification that reduce (e.g., for about 1- 5%, 5-10%, 10%-20%, about 20%-40%, about 50%, about 40%-60%, about 60%-80%, about 80%-90%, 90-95%) the binding to its natural receptor (e.g. IGF-1 receptor).

[0090] A growth factor polypeptide detectably modulates activation of a growth factor receptor. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains at least about 0.01 % of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth

factor, variant or fragment thereof that retain at least about 0.1 %, at least about 1%, at least about 10%, of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 0.01% to about 0.1% of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 0.01% to about 1% of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 0.01% to about 10% of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 0.1% to about 1% of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 0.1% to about 10% of wild-type biological activity. In some embodiments, the activator domain of the bi-specific protein is a growth factor, variant or fragment thereof that retains between about 01% to about 10% of wild-type biological activity. Biological activity in some embodiments can be determined by measuring activation of the corresponding growth factor receptor in appropriate cells. In some embodiments, activation may be assessed, for example, by measuring phosphorylation of receptor kinase or downstream effector proteins, such as, but not limited to, AKT, S6, ERK, JNK, mTOR, etc.

Insulin-like growth factors (IGFs) and derivatives thereof

[0091] The insulin-like growth factors (IGFs) constitute a family of proteins having insulin-like and growth stimulating properties. The IGFs Human IGF-1 is a 70 amino acids basic peptide having the protein shown in SEQ ID NO: 1, respectively. IGF-1 and extracellular tyrosine kinase receptor (e.g. IGF-1 receptor) are important for cellular processes such as cell proliferation and survival. Binding of IGF-1 or variant thereof to the IGF-1 receptor stimulates kinase activity, leading to phosphorylation of multiple substrate, thereby initiating signaling cascades. The chimeric proteins disclosed herein can maintain the ability to signal through the extracellular receptor, for example IGF-1 receptor. The activator domain IGF-1 stimulates cell proliferation and survival through activation of the AKT pathway. Upon binding of IGF-I to the IGF-1 receptor, a tyrosine kinase, phosphorylates tyrosine residues on two major substrates, IRS-1 and Shc, which subsequently signal through the Ras/Raf and PI 3-kinase/AKT pathways.

[0092] The interaction of IGF-1 (and IGF-2) with the IGF-1 receptor is regulated by IGF binding Proteins (IGFBPs). All six IGFBPs (particularly IGFBP5) have been shown to inhibit IGF action, but in some instances a stimulatory effect has been observed. At least 99% of the IGF in the circulation is normally bound to IGFBPs.

[0093] In some embodiments, the activator domain is a variant of the human IGF-1 or fragment thereof. In some embodiments, the variant of IGF-1 or fragment thereof is capable of maintaining selectivity to the IGF-1 receptor. FIG. 9 shows residues identified via application of an algorithm that considers solvent accessibility, hydrophobicity, and temperature factor. Represented by X are residue position that can be mutated. Collectively, these residues represent the hydrophobic core of IGF-1. Hydrophobic core positions of IGF-1 are depicted as spheres (pdb model 1H02) at FIG. 10.

[0094] In some embodiments, the IGF-1 variant is modified to reduce binding to IGF-1 binding proteins (IGFBPs) relative to wild-type IGF-1 while maintaining its ability to activate the AKT pathway. In some embodiments, the IGF-1 variant can activate the IGF-1 receptor with a decreased potency for non-target cells, as assessed by pAKT EC50. EC50 is defined as the concentration needed to achieve the half maximal level of pAKT signaling.

[0095] In some embodiments, the IGF-1 variant comprises a substitution at one or more of the tyrosine residues. In some embodiments, the IGF-1 variant comprises one or more substitutions at position Y24, Y31 and Y60. In an exemplary embodiment, the IGF-1 variant can comprise a single tyrosine substitution at position Y31, or Y24, or Y60. In an exemplary embodiment, the IGF-1 variant can comprise a single tyrosine substitution at position Y24 and Y31, Y24 and Y60, Y31 and Y60, or Y24 and Y60. In another exemplary embodiment, the IGF-1 variant can comprise one or more of the following substitutions, Y24L, Y31A, and Y60L relative to wild type IGF-1. For example, the IGF-1 variant can comprise the Y24L substitution and the Y31A substitution or the IGF-1 variant can comprise the Y24L substitution, the Y31A substitution and the Y60L. In some embodiments, one or more tyrosine residues (Y24, Y31, Y60 or combinations thereof) can be substituted for a short aliphatic amino acid. In some embodiments, one or more tyrosine residues (Y24, Y31, Y60 or combinations thereof) can be substituted for a polar amino acid. In some embodiments, one or more tyrosine residues (Y24, Y31, Y60 or combinations thereof) can be substituted for leucine, alanine, isoleucine, serine, threonine or any other amino acid.

[0096] In some embodiments, the IGF-1 variant comprises a substitution replacing Arg for a Glu, Lys, Met, Val, Ala, Leu, Ile, Gly, Ser, or Thr at the 3 position of the polypeptide. In some embodiments, the IGF-1 variant comprises a substitution replacing Arg for a Glu at the 3 position of the polypeptide (E3R).

[0097] In some embodiments, the IGF-1 variant comprises a substitution at the position 3 and 31. For example, the IGF-1 variant comprises E3R and Y31A substitutions. In some embodiments, the activator domain has an amino acid sequence having SEQ ID NO: 2.

[0098] In some embodiments, the activator domain is a derivative of the human IGF-1 comprising one or more of the following modifications: a N-terminal 13-residue extension (IGF-1 LONG), a deletion of amino acids 1-3 (Des-1-3), a substitution replacing Arg for a Glu at the 3 position of the polypeptide (E3R), no Arginine at position 37 (R37X), a deletion of amino acids 68-70 (3X), an N-terminal 13-residue extension and a substitution replacing Arg for a Glu at the 3 position of the wild -type polypeptide (LR3), substitutions of one or more of tyrosine residues (Y24, Y31, Y60 or combinations thereof (e.g. Y24L, Y31A, Y60L substitutions or combinations thereof).

[0099] In some embodiments, the activator domain is variant of the human IGF-1 comprising a mutation (e.g. substitution, deletion) at one or more residues 24 to 37.

[00100] In some embodiments, the activator domain is a derivative of the human IGF-1 and comprises an N-terminal 13-residue extension (also referred as IGF-1 LONG, SEQ ID NO: 3), a mutation E3R (SEQ ID NO: 4) or a combination thereof (LONG E3R, also referred as LR3, SEQ ID NO: 6). In some embodiments, the IGF-1 variant comprises the E3R substitution, an N-terminal 13-residue extension, deletion of amino acids 1-3 ((Des1-3), SEQ ID NO: 5) or a combination thereof to decrease the binding of the activator domain to the IGF binding proteins which are present in the serum and other body fluid.

[00101] In some embodiments, the activator domain is a derivative of the human IGF-1 and comprises one or more of the following modifications: an N-terminal 13-residue extension (SEQ ID NO: 3), a deletion of amino acids 1-3 (SEQ ID NO: 5), a substitution replacing Arg for a Glu at the 3 position of the polypeptide (SEQ ID NO: 4), no Arginine at position 37 (R37X, SEQ ID NO: 7), a deletion of amino acids 68-70 (3X, SEQ ID NO: 8), or an N-terminal 13-residue extension and a substitution replacing Arg for a Glu at the 3 position of the wild -type polypeptide (SEQ ID NO: 6).

[00102] It is believed that the bi-specific proteins that contain the variant of IGF-1 described herein (e.g. E3R, IGF-1 LONG, IGF-1 LONG E3R (referred to as IGF-1(LR3)) or IGF1 Des1-3), have decreased affinity for IGF binding proteins relative to wild-type IGF-1. In some embodiments, the IGF-1 variants of the bi-specific proteins described herein can activate the signaling pathway while having a substantially decreased interaction with the IGF-1 binding proteins relative to wild-type IGF-1.

[00103] In some embodiments, the IGF-1 variant can be modified by glycosylation of one or more glycosylation site present in the IGF-1 variant.

[00104] In some embodiments, the chimeric proteins that contain the IGF-1 variants described herein have a potency for non-target cells that is less than wild-type IGF-1 for non-target cells.

[00105] Certain activator domains that bind to growth factor receptors are provided herein in SEQ ID NOs: 1-8.

[00106] Additional peptide sequence modifications can be included, such as variations, deletions, substitutions or derivatizations of the amino acid sequence of the sequences disclosed herein, so long as the peptide has substantially the same activity or function as the unmodified peptides. Notably, a modified peptide will retain activity or function associated with the unmodified peptide, the modified peptide will generally have an amino acid sequence "substantially homologous" with the amino acid sequence of the unmodified sequence.

[00107] In some embodiments, the IGF-1 variant can have an amino acid sequence having at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 97%, at least about 98% identity or at least about 99% identity to the amino acid sequence provided in SEQ ID NOs: 1-8. In some embodiments, the IGF-1 variant can have an amino acid sequence having from about 85% to about 90%, from about 90% to about 95%, from about 95% to about 98%, from about 98% identity to about 99% identity to the amino acid sequence provided in SEQ ID NOs: 1-8. In some embodiments, the IGF-1 variant can comprise 10, 20, 30, 40, 50, 60 or more consecutive amino acids of any one of amino acids in SEQ ID NOs: 1-8. In some embodiments, the IGF-1 variant can have an amino acid sequence recited in any one of SEQ ID NOs: 1-8. In some embodiments, the IGF-1 variant can have an amino acid sequence recited in any one of SEQ ID NOs: 2-8 In some embodiments, the IGF-1 variant can have an amino acid sequence recited in SEQ ID NO: 2.

[00108] In some embodiments, the bi-specific protein comprises an activator domain having a growth factor variant that is selected to give the bi-specific protein at least an order of magnitude lower EC50 in damaged tissue than in healthy tissue. For example, the bi-specific protein domain comprises a growth factor variant and has an EC50 in damaged tissue that is at least 10 times lower, at least 15 times lower, at least 20 times lower, at least 25 times lower, at least 30 times lower, at least 35 times lower, at least 40 times lower, at least 45 times lower, at least 50 times lower, at least 55 times lower, at least 60 times lower, at least 65 times lower, at least 70 times lower, at least 75 times lower, at least 80 times lower, at least 85 times lower, at least 90 times lower, at least 95 times lower, at least 100 times lower, at least 110 times lower than the EC50 in healthy tissue.

[00109] In some embodiments, the bi-specific proteins that contain the IGF-1 variants have a half maximal effective concentration (EC50) that is lower in damaged tissue than in healthy tissue. In some embodiments, the bi-specific proteins that contain the IGF-1 variants have a half maximal effective concentration (EC50) that is at least 10 times lower, at least 15 times lower, at least 20 times lower, at least 25 times lower, at least 30 times lower, at least 35 times lower, at least 40 times lower, at least 45 times lower, at least 50 times lower, at least 55 times lower, at least 60 times lower, at least 65 times lower, at least 70 times lower, at least 75 times lower, at least 80 times lower, at least 85 times lower, at least 90 times lower, at least 95 times lower, at least 100 times lower, at least 110 times lower in damaged tissue than in healthy tissue.

[00110] In some embodiments, the chimeric proteins provided herein having such variant growth factors have a higher specificity to the damaged tissue targeted.

Target molecules

[0102] In some aspects, target molecules are exposed or enriched on the exterior of a target cell. In some embodiments, the target molecule is associated with a damaged cell, early apoptotic or apoptotic cell, the target molecule being intracellular in a viable or undamaged cell and being exposed to the extracellular space in a damaged cell. Such molecules include, for example, molecules that are exposed in cells that undergo necrosis (such as DNA) or apoptosis (e.g., phosphatidylserine), myosin (including the tissue type-specific subtypes thereof), ICAM-1 or P-selectin. Yet in other embodiments, the target molecule is a molecule that is present or enriched at the surface of a diseased or dysfunctional cell or tissue as

compared to the level detected in a healthy or functional cell or tissue. In some embodiments, the target cell is not a tumor or cancerous cell.

[0103] Cells are bounded by a plasma membrane (or cell membrane) comprising a lipid bilayer. The cell membrane may be considered to have a surface facing the cytosol (cytosolic side or interior of the cell) and a surface facing the exterior of the cell, or the extracellular space. Trans-bilayer movement of anionic phospholipids from the inner to the outer leaflet of the plasma membrane occurs during apoptosis. The anionic phospholipid-binding protein, such as Annexin A5, synaptotagmin I or lactadherin can be used to detect the presence of phosphatidylserine on the outer leaflet of the cell membrane. Phosphatidylserine is a phospholipid, that is usually restricted to the cytosolic side of the membrane in viable or undamaged cells, and that becomes exposed on the outer cell surface or to the extracellular space in damaged cells or apoptosis.

[0104] In some embodiments, the target molecule is an “ischemia-associated molecule”. An "ischemia-associated molecule" is any molecule that is detected at a level that is significantly higher (e.g., at least 1.5 higher, at least 2-fold higher, at least 3-fold higher, at least 4-fold higher, at least 5-fold higher) following ischemia (which results in hypoxia) or hypoxia than in a cell of the same tissue that has not undergone an ischemic event (i.e., the molecule is specific to or enriched in the post-ischemic tissue). Ischemia occurs when there is insufficient blood flow to provide adequate oxygenation, which results in tissue hypoxia (reduced oxygen) or anoxia (absence of oxygen) as the most severe form of hypoxia, and ultimately tissue necrosis, and apoptosis.

Targeting Domain

[00111] In some embodiments, the targeting domain has a specific binding affinity to a target molecule associated with a tissue (for example, an ischemia-associated molecule). In some embodiments, the targeting domain has a specific binding affinity for a target molecule presented on the surface of early apoptotic cells. The targeting domain may be any polypeptide sequence that serves this function. In some embodiments, binding of the targeting domain to the target molecule does not have or does not modulate a biological activity. As used herein, “biological activity” refers to a defined, known activity performed by exposure of a molecule to a domain of the protein.

[00112] In some embodiments, the targeting domain can be a non-antibody polypeptide, fragment thereof or variant thereof having a binding affinity to the target molecule. Yet in

other embodiments, the targeting polypeptide domain comprises one or more antibody variable regions (e.g. scFv).

Annexin A5 and variants thereof

[00113] In some embodiments, the targeting domain comprises annexin, a variant thereof or a fragment thereof. The term "annexin" refers to any protein capable of binding to phospholipids, especially phosphatidylserine (PS), and member of the annexin family. In some embodiments, the annexin is Annexin A5 but other annexins can equally be used. In some embodiments, the targeting domain is human Annexin A5, a functional fragment thereof, or a variant thereof. A variant of Annexin A5 comprises at least one amino acid in at least one position in which this amino acid is not found in the parent wild type Annexin A5 polypeptide (SEQ ID NO: 9). The annexin variants according may comprise one or more amino acid substitutions, deletions, additions, or combinations thereof wherein the amino acid substitutions, deletions, or additions do not substantially affect the ability of the Annexin A5 variant of the chimeric protein to bind to at least one phospholipid, such as PS. In some embodiments, the Annexin A5 variant can have an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98% identity or at least about 99% identity to the amino acid sequence provided in SEQ ID NO: 9. In some embodiments, the Annexin A5 variant can comprise 50, 80, 100, 110, 200, 300, or more consecutive amino acid having at least about 85%, at least about 90%, at least about 95%, at least about 98% identity or at least about 99% identity to the amino acids in SEQ ID NO: 9.

[00114] In some embodiments, the variant of Annexin A5 is modified to substitute cysteine at position 315 (corresponding to C316) with serine or alanine to reduce dimer formation. For example, the cysteine can be substituted to an alanine or a serine. As used herein, the term "corresponding to" is used to designate the position/identity of an amino acid residue in a polypeptide (e.g., Annexin A5). Those of ordinary skill will appreciate that, for purposes of simplicity, a canonical numbering system (based on wild-type Annexin A5) is utilized herein, so that an amino acid "corresponding to" a residue at position 316, for example, need not actually be the 316th amino acid in a particular amino acid chain but rather corresponds to the residue found at position 316 in a for example Annexin A5 before the post-translational removal of the N-terminal methionine; those of ordinary skill in the art readily appreciate how to identify corresponding amino acids. In particular, it is noted that the amino

acid sequence of wild-type Annexin A5 (SEQ ID NO: 9) do not start with a Methionine as the Methionine residue is cleaved during processing.

[00115] In some embodiments, the variant of Annexin A5 has an amino acid sequences that has been mutated to reduce internalization of Annexin A5 or the chimeric protein comprising the variant of Annexin A5 into a cell while maintaining binding affinity to phosphatidylserine (PS). In some embodiments, the variant of Annexin A5 or the chimeric protein comprising the variant of Annexin A5 has a binding affinity to phosphatidylserine, and is not internalized into a cell or is internalized at a slower rate than wild-type annexin A5. In some embodiments, the targeting domain is a non-internalizing variant of Annexin A5, (also referred as ni-Annexin A5 or ni-AnxV, SEQ ID NO: 11). In some embodiments, the variant of Annexin A5 has an amino acid set forth in SEQ ID NO: 10. In some embodiments, the non-internalizing mutant of Annexin A5 has an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98% identity or at least about 99% identity to the amino acid sequence provided in SEQ ID NO: 11. In some embodiments, the non-internalizing mutant of Annexin A5 can have an amino acid sequence having from about 85% to about 90%, from about 90% to about 95%, from about 95% to about 98%, from about 98% to about 99% identity to the amino acid sequence provided in SEQ ID NO: 11. In some embodiments, the Annexin A5 variant can comprise 50, 80, 100, 110, 200, 300, or more consecutive amino acid of any one of amino acids in SEQ ID NO: 11. Any variation of Annexin A5 that results in substantially no internalization is envisioned.

[00116] It should be appreciated that the non-internalizing variant of annexin A5 can confer an extended half-life to the chimeric protein as compared to a chimeric protein that contains wild-type A5. In some embodiments, the variants of annexin A5 that results in substantially no internalization, or chimeric proteins containing variants of annexin A5 that results in substantially no internalization, can have an extended half-life of 1.1 to 1.2, 1.1 to 1.3, 1.1. to 1.4, 1.1 to 1.5, 1.1 to 1.6, 1.1 to 1.7, 1.1 to 1.8, 1.1 to 1.9, 1.1 to 2 or greater as compared to wild-type annexin A5, or chimeric proteins containing wild-type annexin A5.

[00117] The terms “non-internalizing” and "substantially no internalization," as used herein, refer to a lack of internalization of a substantial amount of the chimeric protein disclosed herein. For example, the phrase "substantially no internalization" will be understood as less than 50% of the chimeric protein being internalized by a cell to which the chimeric protein is bound, or less than 25% of the chimeric protein being internalized by a cell to which the

chimeric protein is bound, or less than 10% of the chimeric protein being internalized by a cell to which the chimeric protein is bound, or less than 5% of the chimeric protein being internalized by a cell to which the chimeric protein is bound, or less than 3% of the chimeric protein being internalized by a cell to which the chimeric protein is bound, or less than 1 % of the chimeric protein being internalized by a cell to which the bi-specific protein is bound.

[00118] In some embodiments, the non-internalizing mutant of Annexin A5 can have an amino acid sequence having at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% identity or at least about 99% identity to human Annexin A5. In some embodiments, the non internalizing variant of Annexin A5 comprises a substitution at position 315 (corresponding to C316) wherein the cysteine residue is substituted with serine (Ser), alanine (Ala), leucine (Leu), phenylalanine (Phe), methionine (Met) or tryptophan (Trp). In some embodiments, the non-internalizing mutant of Annexin A5 can have an amino acid sequence having at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% identity or at least about 99% identity to human Annexin A5. In some embodiments, the non internalizing variant of Annexin A5 comprises a substitution at position 315 (corresponding to C316) wherein the cysteine residue is substituted with serine (Ser) or alanine (Ala). In some embodiments, the non-internalizing mutant of Annexin A5 can have an amino acid sequence having at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% identity or at least about 99% identity to human Annexin A5 modified to substitute cysteine at position 315 (corresponding to C316) with serine or alanine.

[00119] In some embodiments, Annexin A5 or Annexin A5 variants (for example variant having a substitution at position C316) are modified to comprise one or more substitutions at the following positions: R62, K69, K100, E137, D138, N159, L313 (corresponding to R63, K70, K101, E138, D139, N160, L314 relative to wild type human Annexin A5).

[00120] In some embodiments, the Annexin A5 variant comprises:

R62A, R62E, R62D, R62M, R62L, R62I, R62Y (corresponding to R63A, R63E, R63D, R63M, R63L, R63I, R63Y relative to wild type human Annexin A5);

K69A, K69E, K69D, K69M, K69L, K69I, K69Y (corresponding to K70A, K70E, K70D, K70M, K70L, K70I, K70Y relative to wild type human Annexin A5);

E137A, E137K, E137R, E137M, E137L, E137I, E137Y (E138A, E138K, E138R, E138M, E138L, E138I, E138Y relative to wild type human Annexin A5);

D138G, D138K, D138R, D138M, D138L, D138I, D138Y (corresponding to D139G, D139K, D139R, D139M, D139L, D139I, D139Y relative to wild type human Annexin A5)

N159A, N160M, N160L, N160I, N160V, N160Y (corresponding to N160A, N160M, N160L, N160I, N160V, N160Y relative to wild type human Annexin A5)

L313E L313D, L313K, L313R, L313H, L313Q, L313N, L313Y (corresponding to L314E L314D, L314K, L314R, L314H, L314Q, L314N, L314Y relative to wild type human Annexin A5);

or any combinations of the foregoing.

[00121] In some embodiments, Annexin A5 or Annexin A5 variants comprise one or more substitutions at position D143 and/or E227. In some embodiments, Annexin A5 variant comprises:

D142G, D142A, D142K, or D142R (corresponding to D143G, D143A, D143K, or D143R) substitution, and/or

E226G, E226A, E226K, or E226R (corresponding to E227G, E227A, E227K, E227R) substitution

[00122] In some embodiments, Annexin A5 or Annexin A5 variants (for example having a substitution at C316, D143 and/or E227) are modified to comprise one or more of the following substitutions R62A, K69A, K100A, E137A, D138G, N159A, L313E (corresponding to R63A, K70A, K101A, E138A, D139G, N160A, L314E). For example, Annexin A5 having SEQ ID NO: 9 can be modified to have C315A or C315S substitution (corresponding to C316A or C316S relative to wild type Annexin A5) and one or more of the following substitutions R62A, K69A, K100A, E137A, D138G, N159A, L313E (corresponding to R63A, K70A, K101A, E138A, D139G, N160A, L314E relative to wild type Annexin A5).

[00123] In some embodiments, human Annexin A5 (SEQ ID NO: 9) are modified to comprise one or more of the following substitutions R62A, K69A, K100A, E137A, D138G, N159A, D143N, E227A, C315S or C315A (corresponding to R63A, K70A, K101A, E138A, D139G, D144N, N160A, E228A, C316S or C316A relative to wild type Annexin A5).

[00124] In some embodiments, the targeting domain is Annexin A5 which has been engineered to have R63A, K70A, K101A, E138A, D139G, N160A and C316A or C316S substitutions relative to wild type Annexin A5. For example, the targeting domain can have the amino acid sequence of SEQ ID NO: 10.

[00125] In some embodiments, the Annexin A5 variant comprises one, two, , three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen nineteen, twenty or more substitutions in different regions, in order to further decrease the internalization of the annexin in a cell. For example, the Annexin A5 variants may comprise R62A and K69A, R62A and K100A, R62A and E137A, R62A and D138G, R62A and N159A, R62A and K69A and K100A, R62A and K69A and E137A, R62A, K69A and K100A, R62A, K69A, K100A, and E137A etc…

[00126] The annexin variants according may further comprise one or more amino acid substitutions, deletions, or additions, wherein the amino acid substitutions, deletions, or additions do not substantially affect the ability of the Annexin A5 variant of the chimeric protein to bind to at least one phospholipid, such as PS.

[00127] Native polypeptide can be used as targeting domains. It will be apparent, however, that portions of such native sequences and polypeptides having altered sequences may also be used, provided that such polypeptides retain the ability to bind the target molecule with an appropriate binding affinity (Kd) as described in more details below.

Antibody targeting domain:

[00128] In some embodiments, an anti-phosphatidylserine antibody can be used as a targeting domain. As used herein, term "antibody" includes but is not limited to: (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) F(ab)2 and F(ab')2 fragments, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a scFv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Such antibodies may be produced from intact antibodies using methods known in the art, or may be produced recombinantly, using standard recombinant DNA and protein expression technologies.

Binding of Targeting domain

[00129] In some embodiments, the chimeric protein binds to the target molecule with a K_d of less than 10^{-6} M, preferably less than 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M.

Half-Life Modulator

[00130] One skilled in the art would appreciate that proteins used in therapeutic applications may not exhibit optimal serum half-lives due to their relatively low molecular weight. In some therapeutic applications, it may therefore be desirable to extend the half-life of the proteins. In some embodiments, to achieve accumulation of the chimeric protein to the diseased injured or damaged area of an organ, the chimeric protein is conjugated operatively associated or fused with a half-life modulator. Preferably, the half-life modulator is non-immunogenic polypeptide.

[00131] For example, short half-life is the most limiting attribute of wild-type growth factors as therapeutics. Intravenous administered IGF-1 has a serum half-life in humans of less than 1 hour. The extended half-life of chimeric proteins disclosed herein compared to IGF-1, for example, allows for 1) equivalent efficacy with less frequent dosing; 2) equivalent exposure at a lower dose; 3) lower Cmax at an equivalent exposure level, reducing the risk of Cmax-related toxicity.

[00132] In some embodiments, the half-life modulators can increase the *in vivo* half-life of the chimeric proteins. For example, the half-life of the chimeric proteins comprising the half-life modulator is about 1 hour, 2 hour, 3 hours, 4 hours, 5 hours, 6 hours or greater. For example, the half-life of the chimeric proteins can be about 8 hours or more when tested in cynomolgus monkey. In some embodiments, the half-life of the chimeric proteins comprising the half-life modulator is about 24 hours, or greater. In some embodiments, the half-life of the chimeric proteins comprising the half-life modulator is about a week or greater.

[00133] In some embodiments, the half-life modulator is non-immunogenic in humans.

[00134] In some embodiments, the half-life modulator is a polypeptide that interacts with cellular machinery that promote evasion of lysosomal degradation pathways (e.g. – FcRn receptor-mediated recycling).

[00135] In some embodiments, the half-life modulator is designed to extend the half-life of the chimeric protein through binding to serum components such as Human Serum Albumin (HSA). HSA is the most abundant protein in the blood and has a demonstrated safety in humans.

[00136] In some embodiments, the half-life modulator is a HSA variant. In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human serum albumin amino acid sequence (wtHSA, SEQ ID NO: 12). In some embodiments, the half-life modulator comprises at least 200 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human serum albumin amino acid sequence. In some embodiments, the half-life modulator comprises at least 300 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human serum albumin amino acid sequence. In some embodiments, the half-life modulator comprises at least 400 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human serum albumin amino acid sequence. In some embodiments, the half-life modulator comprises at least 500 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human serum albumin amino acid sequence.

[00137] In some embodiments, the HSA variant can have one of more of the following substitutions:

cysteine C58 can be substituted, for example, with serine (C58S), alanine (C58A), asparagine (C58N), leucine (C58L), or glutamine (C58Q),

lysine K420 can be substituted for example, with glutamic acid (K420E), aspartic acid (K420D), a leucine (K420L) or methionine (K410M),

asparagine N527 can be substituted for example, with glutamine (N527Q), aspartic acid (N527D), histidine (N527H), or tyrosine (N527Y),

glutamic acid E505 can be substituted for example, with glycine (E505G), alanine (E505A), leucine (E505L), lysine (E505K), valine (E505V), isoleucine (E505I), methionine (E505M), or glutamine (E505Q),

valine V547 can be substituted for example, with alanine (V547A), glycine (V547G), leucine (V547L), lysine (V547K), isoleucine (V547I), methionine (V547M),

asparagine N503 can be substituted for example, with a Glutamine (N527Q), aspartic acid (N503D), histidine (N503H), or tyrosine (N503Y), or glutamine (V547Q).

[00138] In some embodiments, the HSA variant can have amino acids 26-609 and have one of more of the following substitutions:

cysteine C58 can be substituted for example, with serine (C58S), alanine (C58A), asparagine (C58N), leucine (C58L), or glutamine (C58Q),
lysine K420 can be substituted for example, with glutamic acid (K420E), aspartic acid (K420D), a leucine (K420L) or methionine (K410M),
asparagine N527 can be substituted for example, with glutamine (N527Q), aspartic acid (N527D), histidine (N527H), or tyrosine (N527Y),
glutamic acid E505 can be substituted for example, with a glycine G (E505G), alanine (E505A), leucine (E505L), lysine (E505K), valine (E505V), isoleucine (E505I), methionine (E505M), or glutamine (E505Q),
valine V547 can be substituted for example, with an alanine (V547A), glycine (V547G), leucine (V547L), lysine (V547K), isoleucine (V547I), methionine (V547M),
asparagine N503 and/or N527 can be substituted for example, with Glutamine (N503Q and/or N527Q), aspartic acid (N503D and/or N527D), histidine (N503H and/or N527H), or tyrosin (N503Y and/or N527Y).

[00139] In some embodiments, the HSA variant (referred herein as mHSA) has the following substitutions: C34S, N503Q (SEQ ID NO: 13). In some embodiments, the HSA variant (referred herein as mHSA7) has the following substitutions C34S, N503Q, E505G and V547A (SEQ ID NO: 14). In some embodiments, the HSA variant has amino acids 26-609 and the following substitutions C58S and N527Q (SEQ ID NO: 15).

[00140] In some embodiments, the asparagine at position 503 and/or 527 of HSA, which may be deamidated and decrease half-life, can be removed by the N503Q substitution and/or the N527Q. In some embodiments, the cysteine C34 of HSA may be substituted to serine or alanine (S or A) to remove the free cysteine and minimize alternate disulfide-bond formation. In some embodiments, the half-life modulator is a modified version of the domain III (mHSA_dIII) of a modified HSA with the N503Q substitution and an additional terminal glycine. Such a modified version retains the HSA property of binding to FcRn and increased serum half-life.

[00141] In some embodiments, the half-life modulator is Fc domain of an antibody or a single chain constant fragment. In some embodiments, the half-life modulator comprises Fc regions of an immunoglobulin molecule (e.g. IgG). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a human Fc amino acid sequence. The Fc domain of an antibody has a natural capability to bind FcRn, resulting in an

extended half-life. In some embodiments, the Fc domain of an antibody is engineered not to bind Fc(gamma)R In an exemplary embodiment, the Fc domain is engineered to substitute N297 with Q (N297Q variant). In some embodiments, the half-life modulator is a monomeric variant form of Fc (scFc). For example, the subset of IgG heavy chain which naturally dimerizes to form Fc is hinge-CH2-CH3. In some embodiments, the Fc domain is engineered to form a single chain by linking the hinge-CH2-CH3 with a flexible linker such as GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 16) to create a hinge-CH2-CH3-linker-hinge-CH2-CH3 chain. In an exemplary embodiment, the single chain Fc (scFc) is engineered to substitute N297 with Q and C220 with S (N297Q, C220S).

[00142] In some embodiments, the half-life modulator is a single chain variable fragment (scFv) of an antibody targeted to albumin or other circulating protein. In some embodiments, the half-life modulator comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to scFv amino acid sequence directed to a specific antigen, such as, but not limited to, albumin. In some embodiments, the half-life modulator comprises at least 50, at least 100, at least 150, at least 200, at least 250 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a scFv amino acid sequence directed to a specific antigen, such as, but not limited to, albumin.

[00143] In some embodiments, the half-life modulator is transferrin such as human transferrin (Tf, SEQ ID NO: 17). In some embodiments, the half-life modulator comprises an amino acid sequence that is at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to human transferrin amino acid sequence. In some embodiments, the half-life modulator comprises at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 650 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a human transferrin amino acid sequence.

[00144] In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human alpha-fetoprotein amino acid sequence (AFP, SEQ ID NO: 18). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human alpha-fetoprotein (AFP) amino acid

sequence. In some embodiments, the N-linked glycosylation site of the AFP is removed by the N251Q substitution.

[00145] In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical wild-type vitamin D-binding protein amino acid sequence (VDBP, SEQ ID NO: 19). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical wild-type vitamin D-binding protein (VDBP) amino acid sequence. In some embodiments, the N-linked glycosylation site of the VDBP can be removed by the N288Q or N288T substitution.

[00146] In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild-type human transthyretin amino acid sequence (TTR, SEQ ID NO: 20). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to wild type human transthyretin (TTR) amino acid sequence. In some embodiments, the transthyretin is modified to remove the N118 N-glycosylation site. In some embodiments, the half-life modulator is a monomeric form of TTR.

[00147] In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a PASylation amino acid sequence. PASylation are proline-, alanine-, and/or serine-rich sequences that mimic PEGylation (see WO/2008/155134). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a PASylation amino acid sequence. PASylation are proline-, alanine-, and/or serine-rich sequences that mimic PEGylation. Polypeptide stretches of proline, alanine, and/or serine form semi-structured three-dimensional domains with large hydrodynamic radius, thereby reducing clearance of fusion proteins. In some embodiments, the PASylation amino acid sequence is about 200, 300, 400, 500 or 600 amino acids long. For example, the PASylation is a 20 times repeat of the amino acid sequence ASPAAPAPASPAAPAPSAPA (SEQ ID NO: 21).

[00148] In some embodiments, the half-life modulator comprises the attachment of polyethylene glycol (PEG) chain or chains to the fusion proteins through chemical attachment either to the N- and/or C-terminus and/or to an amino acid side chain (e.g., PEG-maleimide attachment to cysteines). PEG chains form semi-structured three-dimensional domains with large hydrodynamic radius, thereby reducing clearance of fusion proteins.

[00149] In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an albumin-binding domain human antibody (albudAb) amino acid sequence (SEQ ID NO: 22). In some embodiments, the half-life modulator comprises at least 100 consecutive amino acids that are about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to an albumin-binding domain human antibody (albudAb) amino acid sequence. Albumin-binding domain antibodies can increase the fusion protein half-life by binding non-covalently to serum albumin (see WO2008/096158 which is incorporated herein by reference in its entirety). In some embodiments, the albumin-binding domain human antibody is engineered to remove the C-terminal arginine to remove the Lys-Arg Kex2 protease site.

[00150] Representative such half-life modulators include those recited in any one of SEQ ID NOs: 12-15, 17-22.

[00151] In some embodiments, the half-life modulators can be modified to substitute the cysteine residues to serine or alanine residues to reduce the ability to form disulfide bonds.

[00152] In some embodiments, the targeting domain and activator domain can be joined via a half-life modulator. Accordingly, the half-life modulator can have two termini, an N-terminus and a C-terminus. In some embodiments, the half-life modulator is joined at one terminus via a peptide bond to the targeting polypeptide domain and is joined at the other terminus via a peptide bond to the activator domain. In certain embodiments, the half-life modulator is joined at the N-terminus to the C-terminus of the targeting polypeptide domain and at the C-terminus to the N-terminus of the activator domain. In other embodiments, the half-life modulator is joined at the C-terminus to the targeting polypeptide domain and at the N-terminus to the activator domain. Yet, in other embodiments, the half-life modulator is joined at one of the termini of the bi-specific protein. For example, in some embodiments, the half-life modulator is joined at the C-terminus to the N-terminus of the activator domain. In other embodiments, the half-life modulator is joined at the N-terminus to the C-terminus of the

targeting domain. In other embodiments, the half-life modulator can be joined at the N-terminus to the C-terminus of the activator domain. Yet in other embodiments, the half-life modulator can be joined at the N-terminus to the C-terminus of the targeting domain.

Peptide Linkers

[00153] In some embodiments, the activator domain, half-life modulator, and targeting domain are linked by peptide linker (e.g., from 2 to 40, 2-50, 2-100 amino acid residues) such that upon target recognition and engagement by the targeting domain, the presentation of the activator domain is optimized for binding to and activation of extracellular receptors on the surface of cells that present the target at a given surface density (e.g. – 5 x10^2 molecules / 1,000 Å2).

[00154] Targeted delivery of the activator domain for example IGF-1 for the activation of receptors on cells or tissues displaying a specific target requires appropriate presentation of both the activator domain and the targeting domain. In some embodiments, the flexibility of the linker is optimized for proper geometry of the engaged chimeric protein. Some of the principal determinants of the geometric constraints are the distances from the cell surface for the target and the receptor.

[00155] Additional optimization can be driven by the relative number of receptors and target molecules. At high ratios of Receptor : Target molecule, the engagement of both domains is reaction-limited. When the target molecule is more abundant than the receptor, the occupancy of both domains is diffusion-limited. Under the reaction-limit, optimal delivery of the activator domain is attained via short and rigid linkers. Under the diffusion limit, long and flexible linkers allow the activator domain to access a larger surface area. For cells with complex shapes (i.e. - bodies and neuronal processes) and receptor distributions, appropriate design of linker flexibility can enable precise targeting to sub-cellular regions.

[00156] In some embodiments, the peptide linker is present at the N-terminus, at the C-terminus or at both the N-terminus and the C-terminus of the half-life modulator at one or both ends. Suitable short connector polypeptides for use at the N-terminal end of the linker include, for example, dipeptides such as –Gly-Ser- (GS), –Gly-Ala- (GA) and –Ala-Ser- (AS). Suitable peptide linkers for use at the C-terminal end of the linker include, for example, dipeptides such as –Leu-Gln- (LQ) and –Thr-Gly- (TG). In some embodiments, the peptide linkers are longer than 2 amino acids. For example, the peptide linkers are 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids long or longer. In some embodiments, the peptide linkers are 20

or more 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, 100 or more amino acids long. Preferably, such peptide linkers are flexible (for example glycine-rich) or structured (e.g., alpha-helix rich). In some embodiments, the linker comprises or consist of amino acids – Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 23).

[00157] It will be apparent that elements in addition to those described above may optionally be included in the proteins provided herein. Such elements may be present for a variety of purposes, including to facilitate expression, preparation or purification of the bi-specific fusion protein, or to perform targeting functions.

Representative chimeric protein

[00158] In some embodiments, a representative bi-specific fusion proteins comprise (from N-terminal to C-terminal):

(a) a targeting polypeptide domain comprising or consisting of a non-internalizing human annexin V variant (e.g., comprising or consisting of amino acids 2-320 of wt human Annexin 5 and a substitution at C316, R63, K40, K101, E138, D139, N160);

(b) a linker peptide (e.g., – Gly-Ser-Gly-Gly-Gly-Ser-Gly);

(c) a half-life modulator (e.g., HSA variant comprising or consisting of amino acids 26-609 of wt human HSA and comprising substitution at C58 and N527);

(d) linker peptide (e.g., – Gly-Ser-Gly-Gly-Gly-Ser-Gly);

(e) an activator domain comprising or consisting of an IGF-1 variant (e.g., comprising a substitution at E3 and Y31.

[00159] In some embodiments, the chimeric protein comprises or consists of IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316A) (also referred herein as scp776). In some embodiments, the chimeric protein has and amino acid sequence as set forth in SEQ ID NO: 24.

[00160] In some embodiments, the chimeric protein comprises or consists of IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316S). In some embodiments, the chimeric protein has an amino acid sequence as set forth in SEQ ID NO: 25.

Nucleic acids

[00161] Provided herein are polynucleotides encoding the chimeric proteins that may be in the form of RNA or in the form of DNA, which DNA includes cDNA and synthetic DNA.

The DNA may be double-stranded or single-stranded. The coding sequences that encode the variants of the present disclosure may vary as a result of the redundancy or degeneracy of the genetic code.

Pharmaceutical Compositions-Methods of Treatment

[00162] Methods of treatment of central nervous system (CNS) disorders and pharmaceutical compositions for use in the treatment of CNS disorders are provided. In some embodiments, the CNS disorder is brain injury. Injuries to brain due for example to reduced blood flow such as stroke, trauma, concussion, or neurodegenerative disorders can lead to neurologic deficit, loss of behavioral function or death. Stroke is one of the main causes of death in the Unites States. Stroke can be categorized into "ischemic stroke" (about 87%) and "hemorrhagic stroke" (about 10%). Ischemic stroke occurs when the blood supply to the brain is suddenly interrupted, generally caused by thrombus lodging or forming in one of the blood vessels supplying the brain. Hemorrhagic stroke happens when a blood vessel located the around the brain bursts leading to the leakage and accumulation of blood directly in the brain tissue. Interventional treatments must occur within hours of the onset of symptoms otherwise ischemia may lead to permanent neurologic deficit or death. Traumatic brain injury (TBI) leads mostly to a focal ischemia that occurs when an external force injures the brain. Mild and moderate TBIs lead mainly to brain contusions of different degrees causing edema associated brain ischemia. Moderate and severe TBIs lead to polytraumatic injuries such as vessel destruction, intracranial bleeding, brain tissue destruction, which can be associated with ischemic conditions in the affect brain regions.

[00163] Acute CNS injury in neurological and cognitive deficits. More than 65% of moderate to severe TBI patients report long-term problems with cognitive functioning. Such deficits can have a profound long-term effect on patients' outcomes.

[00164] In some embodiments, methods of treatment of acute CNS injury are provided. In some embodiments, the acute CNS injury is ischemic stroke. In some embodiments, the acute CNS injury is traumatic brain injury (TBI). In some embodiments, the acute CNS injury is hemorrhagic stroke. In some embodiments, the acute CNS injury is acquired brain injury. In some embodiments, the acute CNS injury is spinal cord injury. In some embodiments, the acute CNS injury is subarachnoid hemorrhage. In some embodiments, the acute CNS injury is iatrogenic in nature. In some embodiments, methods for acute CNS injury neuroprotection are provided. In some embodiments, the method results in at least one or more of the following:

improvement in neurological function, improvement in mental function, improvement in cognitive function, and increased survival rate. Measures of neurological function can be assessed with Rankin Score, modified Rankin Score, Barthel Index, modified Barthel Index, NIH stroke score, and clinical neurologic examinations.

[00165] In some embodiments, methods of treatment and/or neuroprotection for chronic neurodegenerative diseases (e.g., Alzheimer, Parkinson's Disease, ALS). In some embodiments, methods of treatment of Alzheimer disease are provided. In some embodiments, methods of treatment of Parkinson disease are provided. In some embodiments, methods of treatment of Amyotrophic Lateral Sclerosis are provided. In some embodiments, the method results in at least one or more of the following: improvement in neurological function, improvement in mental function, and increased survival rate. Measures of neurological function can be assessed with Unified Parkinson's Disease Rating Scale (UPDRS), the Sandoz Clinical Assessment-Geriatric (SCAG) scale, and ALS functional rating score, or variations of these scores.

[00166] Pharmaceutical compositions comprising a therapeutically effective amount of at least one chimeric protein as described herein, together with at least one physiologically acceptable carrier, are provided. Such compositions may be used for treating patients who are suffering from, or at risk for, tissue damage, in order to prevent tissue damage, or to repair or regenerate damaged tissue, for example nervous system tissue. The chimeric proteins and the pharmaceutical compositions described herein can have a neuroprotective action and prevent or halt neuronal death. Such patients include, for example, patients who have experienced an ischemic stroke, hemorrhagic stroke, brain trauma or neurodegenerative disorders. If desired, other active ingredients may also be included within the pharmaceutical composition, such as stem cells or other agents that facilitate repair of damaged tissue.

[00167] A "patient" or a "subject" is a mammal, preferably a human. The term "treating" (or "treat" or "treatment") means slowing, reducing, or reversing the progression or severity of a symptom, disorder, condition, or disease.

[00168] The term "therapeutically effective amount" refers to the amount or dose of chimeric proteins described herein which, upon single or multiple dose administration to a patient, provides the desired treatment.

[00169] In some embodiments, the compositions or chimeric proteins described herein localizes to the central nervous system (CNS) tissue(s) subjected to ischemic injury whether

by traversing the blood brain barrier (BBB) or due to permeabilization of the blood brain barrier.

[00170] In some embodiments, the compositions or chimeric proteins described herein delivers pro-survival signals to neurons and glia of the subject.

[00171] In some embodiments, the pharmaceutical compositions or chimeric proteins described herein accelerates repair processes to the blood brain barrier of the subject in need thereof. In some embodiments, the pharmaceutical compositions or chimeric proteins described herein protects the blood brain barrier of the subject in need thereof.

[00172] In some embodiments, the pharmaceutical compositions or chimeric proteins described herein can be administered following the diagnosis of acute CNS injury. In some embodiments, acute CNS injury is acute spinal cord injury or acute brain injury. In some embodiments, the pharmaceutical compositions or chimeric proteins described herein is administered at the first presentation of neurovascular disease or acute CNS injury. In some embodiments, the pharmaceutical compositions or chimeric proteins described herein can be administered following the first imaging assessment that confirms occlusion of an arterial vessel that supplies blood to the principal organ of the CNS. In some embodiments, acute CNS injury can be diagnosed by clinical examination, neurological examination, imaging, biochemical markers indicative of acute CNS injury, or any diagnostic technology known in the art. In some embodiments, the acute CNS injury can be diagnosed using magnetic resonance imaging (MRI), computer tomography (CT) scan or a combination thereof. A MRI and CT imaging can show fractures, brain hemorrhage, hematomas, contusions or brain tissue swelling. Brain tissue swelling can also be assessed by using an intracranial pressure probe that is inserted through the skull.

[00173] In some embodiments, the compositions or chimeric proteins described herein can be administered to a subject in need thereof at the time of interventional reperfusion in the subject, wherein the subject suffers from thromboembolism.

[00174] In some embodiments, the pharmaceutical compositions or chimeric proteins described herein can be administered within 30 minutes of diagnosis, within 1 hour of diagnosis, within 2 hours of diagnosis, within 3 hours of diagnosis, within 4 hours of diagnosis, within 5 hours of diagnosis, within 6 hours of diagnosis, within 7 hours of diagnosis, within 8 hours of diagnosis, within 10 hours of diagnosis, within 12 hours of diagnosis, within 14 hours of diagnosis, within 16 hours of diagnosis, within 18 hours of diagnosis, within 20 hours of

diagnosis, within 22 hours of diagnosis, within 24 hours of diagnosis, within 30 hours of diagnosis, within 36 hours of diagnosis, within 42 hours of diagnosis, within 48 hours of diagnosis, or within 72 hours of diagnosis.

[00175] In some embodiments, a first dose of the pharmaceutical composition comprising the chimeric proteins can be administered within 30 minutes of diagnosis, within 1 hour of diagnosis, within 2 hours of diagnosis, within 3 hours of diagnosis, within 4 hours of diagnosis, within 5 hours of diagnosis, within 6 hours of diagnosis, within 7 hours of diagnosis, within 8 hours of diagnosis, within 10 hours of diagnosis, within 12 hours of diagnosis, within 14 hours of diagnosis, within 16 hours of diagnosis, within 18 hours of diagnosis, within 20 hours of diagnosis, within 22 hours of diagnosis, within 24 hours of diagnosis, within 30 hours of diagnosis, within 36 hours of diagnosis, within 42 hours of diagnosis, within 48 hours of diagnosis, or within 72 hours of diagnosis.

[00176] In some embodiments, the pharmaceutical compositions or chimeric proteins described herein can be administered following therapies that increase oxygen delivery to the injured issued, and in combination with other therapeutics that have neuroprotection properties.

[00177] In some embodiments, the pharmaceutical compositions or the chimeric protein can be administered to a subject in need thereof to protect or treat tissue or organ degeneration. For example, the pharmaceutical compositions can be used to treat central nervous system disorders such as, but not limited to, Acute Ischemic Stroke, Hemorrhagic Stroke, Transient Ischemic Attack, Iatrogenic Stroke, Traumatic Brain Injury (TBI), Concussion, acquired brain injury, spinal cord injury, subarachnoid hemorrhage, Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease, Diabetic Neuropathy, and Spinal Muscular Atrophy. In some embodiments, the acute CNS injury is subarachnoid hemorrhage. In some embodiments, the acute CNS injury is iatrogenic in nature.

[00178] The pharmaceutical compositions can also be used to treat ocular disorders such as, but not limited to, Retinopathy, Macular Degeneration, Glaucoma, Cataract, and Acute Retinal Arterial Ischemia.

[00179] In some embodiments, the pharmaceutical compositions can be administered in combination with or as a co-medication to existing treatments known in the art (i.e., tissue plasminogen activators, urokinase plasminogen activators, etc.).

[00180] As used herein, the term "physiologically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the bi-specific fusion protein is administered. Physiologically acceptable carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin (e.g., peanut oil, soybean oil, mineral oil, or sesame oil). Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water and ethanol. The pharmaceutical composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

[00181] In some embodiments, the pharmaceutical composition is a liquid formulation formulated for intravenous (IV) injection. In some embodiments, the composition is formulated for IV bolus injection.

[00182] In some embodiments, the pH of the pharmaceutical composition is from 7 to 8, for example about 7.5. In some embodiments, the pharmaceutical composition comprises in an alkalinizing agent, such as tromethamine or dibasic sodium phosphate. In some embodiments, the alkalinizing can be at a concentration of 10 to 50 mM, for example 20 mM. In some embodiments, the pharmaceutical composition comprises a surfactant. In some embodiments, the surfactant is a non-ionic surfactant such as polysorbate 80 or polysorbate 20 may be present in a concentration of 0.01%, 0.02%. 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, or 1% (w/v). In some embodiments, the pharmaceutical composition comprises sucrose. In some embodiments, the sucrose may be present in a concentration of 1%, 2%. 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% (w/v).

[00183] In some embodiments, the pharmaceutical composition comprising the chimeric protein comprises alkalinizing agent, a surfactant, sucrose or a combination thereof.

[00184] The pharmaceutical compositions of the disclosure may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological

conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents, preservatives and the like.

[00185] In some embodiments, the pharmaceutical composition comprises from about 1 to about 100 g/l chimeric protein, from about 10 mM to about 50 mM tromethamine, from about 2 to about 15 % (w/v) sucrose, from about 0.001% to about 0.04% (w/v) Polysorbate 80, at pH 7.5

[00186] In some embodiments, the chimeric protein (from about 1 to about 100 g/l) is formulated in 20 mM tromethamine, 7.5% sucrose, and 0.02% polysorbate 80 at pH 7.5 (via addition of HCl).

[00187] In some embodiments, the liquid composition is sterilized by conventional sterilization techniques, or sterile filtered. In some embodiments, the liquid composition is in a vial.

[00188] In some embodiments, the pharmaceutical composition is administered intravenously or intraarterially to a subject in need thereof via bolus injection. A bolus injection comprises, e.g., fast intravenous injection, for example less than 10 seconds (or less than 20, 30, 40, 50, 60 second), or intravenous infusion over less than approximately 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes or 10 minutes. In some embodiments, the pharmaceutical composition is administered intravenously to a subject in need thereof. In some embodiments, the composition is administered as an I.V. push. In some embodiments, the administration is over 5 min. or less, 4 min. or less, 3 min. of less, 2 min. or less, 1 min or less (for example 50 s, 40 s, 30 s, 20 s or any administration time therebetween). In other embodiments, the composition is administered as a slow I.V. injection.

[00189] In some embodiments, the pharmaceutical composition is administered intravenously to a subject using a syringe injection pump at a rate of 0.5 – 25 mL/min.

[00190] In some embodiments, the pharmaceutical composition is administered intraarterially to a subject using a syringe injection pump at a rate of 0.05 – 10 mL/min.

[00191] In some embodiments, the pharmaceutical composition is administered intrathecally to a subject using techniques known in the art.

[00192] In some embodiments, a therapeutically effective amount generally is in the range of about 0.01 mg/kg to about 100.0 mg/kg per dose. In some embodiments, a therapeutically effective amount of a protein disclosed herein can be, e.g., about 0.01 mg/kg to

about 0.1 mg/kg per dose, 0.01 mg/kg to about 1 mg/kg per dose, 0.01 mg/kg to about 10 mg/kg per dose, 0.01 mg/kg to about 100.0 mg/kg per dose, 0.1 mg/kg to about 1 mg/kg per dose, 0.1 mg/kg to about 10 mg/kg per dose, 0.1 mg/kg to about 100 mg/kg per dose, 1 mg/kg to about 100 mg/kg per dose or 10 mg/kg to about 100 mg/kg per dose.

[00193] In some embodiments, a therapeutically effective amount generally is in the range of about 0.01 mg/kg to about 20.0 mg/kg per dose. In some embodiments, a therapeutically effective amount cam be range from about 0.01 mg/kg to about 10.0 mg/kg per dose.

[00194] In some embodiments, a therapeutically effective amount of a protein disclosed herein can be from about 0.01 mg/kg to about 0.02 mg/kg per dose, about 0.01 mg/kg to about 0.03 mg/kg per dose, about 0.01 mg/kg to about 0.04 mg/kg per dose, about 0.01 mg/kg to about 0.05 mg/kg per dose, about 0.01 mg/kg to about 0.06 mg/kg per dose, about 0.01 mg/kg to about 0.07 mg/kg per dose, about 0.01 mg/kg to about 0.08 mg/kg per dose, about 0.01 mg/kg to about 0.09 mg/kg per dose, about 0.01 mg/kg to about 0.1 mg/kg per dose, about 0.01 mg/kg to about 0.2 mg/kg per dose, about 0.01 mg/kg to about 0.3 mg/kg per dose, about 0.01 mg/kg to about 0.4 mg/kg per dose, about 0.01 mg/kg to about 0.5 mg/kg per dose, about 0.01 mg/kg to about 0.6 mg/kg per dose, about 0.01 mg/kg to about 0.7 mg/kg per dose, about 0.01 mg/kg to about 0.8 mg/kg per dose, about 0.01 mg/kg to about 0.9 mg/kg per dose, about 0.01 mg/kg to about 1 mg/kg per dose, about 0.01 mg/kg to about 2 mg/kg per dose, about 0.01 mg/kg to about 3 mg/kg per dose, about 0.01 mg/kg to about 4 mg/kg per dose, about 0.01 mg/kg to about 5 mg/kg per dose, about 0.01 mg/kg to about 6 mg/kg per dose, about 0.01 mg/kg to about 7 mg/kg per dose, about 0.01 mg/kg to about 8 mg/kg per dose, about 0.01 mg/kg to about 9 mg/kg per dose, about 0.01 mg/kg to about 10 mg/kg per dose, about 0.01 mg/kg to about 11 mg/kg per dose, about 0.01 mg/kg to about 12 mg/kg per dose, about 0.01 mg/kg to about 13 mg/kg per dose, about 0.01 mg/kg to about 14 mg/kg per dose, about 0.01 mg/kg to about 15 mg/kg per dose, about 0.01 mg/kg to about 16 mg/kg per dose, about 0.01 mg/kg to about 17 mg/kg per dose, about 0.01 mg/kg to about 18 mg/kg per dose, about 0.01 mg/kg to about 19 mg/kg per dose, about 0.01 mg/kg to about 20 mg/kg per dose.

[00195] In some embodiments, a therapeutically effective amount of a protein disclosed herein can be from about 0.01 mg/kg to about 0.05 mg/kg per dose, about 0.05 mg/kg to about 0.1 mg/kg per dose, about 0.1 mg/kg to about 0.5 mg/kg per dose, about 0.05 mg/kg to about 1 mg/kg per dose, about 1 mg/kg to about 2 mg/kg per dose, about 2 mg/kg to about 3 mg/kg

per dose, about 3 mg/kg to about 4 mg/kg per dose, about 4 mg/kg to about 5 mg/kg per dose, about 5 mg/kg to about 6 mg/kg per dose, about 6 mg/kg to about 7 mg/kg per dose, about 7 mg/kg to about 8 mg/kg per dose, about 8 mg/kg to about 9 mg/kg per dose, about 9 mg/kg to about 10 mg/kg per dose, about 10 mg/kg to about 11 mg/kg per dose, about 11 mg/kg to about 12 mg/kg per dose, about 12 mg/kg to about 13 mg/kg per dose, about 13 mg/kg to about 14 mg/kg per dose, about 14 mg/kg to about 15 mg/kg per dose, about 15 mg/kg to about 16 mg/kg per dose, about 16 mg/kg to about 17 mg/kg per dose, 17 mg/kg to about 18 mg/kg per dose, about 18 mg/kg to about 19 mg/kg per dose, about 19 mg/kg to about 20 mg/kg per dose.

[00196] In some embodiments, a therapeutically effective amount of a protein disclosed herein can be about 0.01 mg/kg per dose, about 0.02 mg/kg per dose, about 0.03 mg/kg per dose, about 0.04 mg/kg per dose, about 0.05 mg/kg per dose, about 0.06 mg/kg per dose, about 0.07 mg/kg per dose, about 0.08 mg/kg per dose, about 0.09 mg/kg per dose, about 0.1 mg/kg per dose, about 0.2 mg/kg per dose, about 0.3 mg/kg per dose, about 0.4 mg/kg per dose, about 0.5 mg/kg per dose, about 0.6 mg/kg per dose, about 0.7 mg/kg per dose, about 0.8 mg/kg per dose, about 0.9 mg/kg per dose, about 1 mg/kg per dose, about 1.1 mg/kg per dose, about 1.2 mg/kg per dose, about 1.3 mg/kg per dose, about 1.4 mg/kg per dose, about 1.5 mg/kg per dose, about 1.6 mg/kg per dose, about 1.7 mg/kg per dose, about 1.8 mg/kg per dose, about 1.9 mg/kg per dose, about 2 mg/kg per dose, about 2.1 mg/kg per dose, about 2.2 mg/kg per dose, about 2.3 mg/kg per dose, about 2.4 mg/kg per dose, about 2.5 mg/kg per dose, about 2.6 mg/kg per dose, about 2.7 mg/kg per dose, about 2.8 mg/kg per dose, about 2.9 mg/kg per dose, about 3 mg/kg per dose, about 3.1 mg/kg per dose, about 3.2 mg/kg per dose, about 3.3 mg/kg per dose, about 3.4 mg/kg per dose, about 3.5 mg/kg per dose, about 3.6 mg/kg per dose, about 3.7 mg/kg per dose, about 3.8 mg/kg per dose, about 3.9 mg/kg per dose, about 4 mg/kg per dose, about 4.1 mg/kg per dose, about 4.2 mg/kg per dose, about 4.3 mg/kg per dose, about 4.4 mg/kg per dose, about 4.5 mg/kg per dose, about 4.6 mg/kg per dose, about 4.7 mg/kg per dose, about 4.8 mg/kg per dose, about 4.9 mg/kg per dose, about 5 mg/kg per dose, about 5.1 mg/kg per dose, about 5.2 mg/kg per dose, about 5.3 mg/kg per dose, about 5.4 mg/kg per dose, about 5.5 mg/kg per dose, about 5.6 mg/kg per dose, about 5.7 mg/kg per dose, about 5.8 mg/kg per dose, about 5.9 mg/kg per dose, about 6 mg/kg per dose, about 6.1 mg/kg per dose, about 6.2 mg/kg per dose, about 6.3 mg/kg per dose, about 6.4 mg/kg per dose, about 6.5 mg/kg per dose, about 6.6 mg/kg per dose, about 6.7 mg/kg per dose, about 6.8 mg/kg per dose, about 6.9 mg/kg per dose, about 7 mg/kg per dose, about 7.1 mg/kg per dose, about 7.2 mg/kg per dose, about 7.3 mg/kg per dose, about 7.4 mg/kg per dose, about 7.5 mg/kg per dose, about 7.6 mg/kg per dose,

about 7.7 mg/kg per dose, about 7.8 mg/kg per dose, about 7.9 mg/kg per dose, about 8 mg/kg per dose, about 8.1 mg/kg per dose, about 8.2 mg/kg per dose, about 8.3 mg/kg per dose, about 8.4 mg/kg per dose, about 8.5 mg/kg per dose, about 8.6 mg/kg per dose, about 8.7 mg/kg per dose, about 8.8 mg/kg per dose, about 8.9 mg/kg per dose, about 9 mg/kg per dose, about 9.1 mg/kg per dose, about 9.2 mg/kg per dose, about 9.3 mg/kg per dose, about 9.4 mg/kg per dose, about 9.5 mg/kg per dose, about 9.6 mg/kg per dose, about 9.7 mg/kg per dose, about 9.8 mg/kg per dose, about 9.9 mg/kg per dose, about 10 mg/kg per dose, about 11 mg/kg per dose, about 12 mg/kg per dose, about 13 mg/kg per dose, about 14 mg/kg per dose, about 15 mg/kg per dose, about 16 mg/kg per dose, about 17 mg/kg per dose, about 18 mg/kg per dose, about 19 mg/kg per dose, about 20 mg/kg per dose.

[00197] In some embodiments, a therapeutically effective amount generally is in the range of about 1 mg/kg to about 10.0 mg/kg per dose. In some embodiments, an effective amount of a protein disclosed herein can be, e.g., about 1 mg/kg to about 10 mg/kg per dose, 1 mg/kg to about 9 mg/kg per dose, 1 mg/kg to about 8 mg/kg per dose, 1 mg/kg to about 7 mg/kg per dose, 1 mg/kg to about 6 mg/kg per dose, 1 mg/kg to about 5 mg/kg per dose, 1 mg/kg to about 4 mg/kg per dose, 1 mg/kg to about 3 mg/kg per dose or 1 mg/kg to about 2 mg/kg per dose.

[00198] Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For instance, treatment of a nervous system disorder may comprise a one-time administration of an effective dose of the pharmaceutical composition disclosed herein. As a non-limiting example, an effective dose of the composition disclosed herein can be administered once to a patient, e.g., as a single injection or bolus. Alternatively, treatment of a nervous system disorder may comprise multiple administrations of an effective dose of the pharmaceutical composition disclosed herein carried out over a range of time periods, such as, e.g., four times daily, three times daily, twice daily, daily, once every few days, weekly, monthly or yearly. As a non-limiting example, a combination disclosed herein can be administered once or twice weekly to a patient. The timing of administration can upon such factors as the severity of the patient's symptoms. For example, an effective dose of the composition disclosed herein can be administered to a patient once a month for an indefinite period of time, or until the mammal no longer requires therapy.

[00199] In some embodiments, a therapeutically effective amount generally is in the range of about 0.01 mg/kg to about 200.0 mg/kg per day. In some embodiments, an effective

amount of a protein disclosed herein can be, e.g., .g., about 0.01 mg/kg to about 0.1 mg/kg per day, 0.01 mg/kg to about 1 mg/kg per day, 0.01 mg/kg to about 10 mg/kg per day, 0.01 mg/kg to about 100.0 mg/kg per day, 0.01 mg/kg to about 200.0 mg/kg per day, 0.1 mg/kg to about 1 mg/kg per day, 0.1 mg/kg to about 10 mg/kg per day, 0.1 mg/kg to about 100 mg/kg per day, 0.1 mg/kg to about 200 mg/kg per day, 1 mg/kg to about 100 mg/kg per day, 1 mg/kg to about 200 mg/kg per day, 10 mg/kg to about 100 mg/kg per day or 10 mg/kg to about 100 mg/kg per day.

[00200] In some embodiments, a therapeutically effective amount generally is in the range of about 0.01 mg/kg to about 20.0 mg/kg per day. In some embodiments, a therapeutically effective amount generally is in the range of about 0.01 mg/kg to about 10.0 mg/kg per day. . In some embodiments, a therapeutically effective amount generally is in the range of about 1 mg/kg to about 20.0 mg/kg per day. In some embodiments, a therapeutically effective amount generally is in the range of about 1 mg/kg to about 10.0 mg/kg per day. In some embodiments, an effective amount of a protein disclosed herein can be, e.g., about 1 mg/kg to about 10 mg/kg per day, 1 mg/kg to about 9 mg/kg per day, 1 mg/kg to about 8 mg/kg per day, 1 mg/kg to about 7 mg/kg per day, 1 mg/kg to about 6 mg/kg per day, 1 mg/kg to about 5 mg/kg per day, 1 mg/kg to about 4 mg/kg per day, 1 mg/kg to about 3 mg/kg per day or 1 mg/kg to about 2 mg/kg per day.

[00201] In some embodiments, a therapeutically effective amount of a chimeric protein disclosed herein can be from about 0.01 mg/kg to about 0.02 mg/kg per day, about 0.01 mg/kg to about 0.03 mg/kg per day, about 0.01 mg/kg to about 0.04 mg/kg per day, about 0.01 mg/kg to about 0.05 mg/kg per day, about 0.01 mg/kg to about 0.06 mg/kg per day, about 0.01 mg/kg to about 0.07 mg/kg per day, about 0.01 mg/kg to about 0.08 mg/kg per day, about 0.01 mg/kg to about 0.09 mg/kg per day, about 0.01 mg/kg to about 0.1 mg/kg per day, about 0.01 mg/kg to about 0.2 mg/kg per day, about 0.01 mg/kg to about 0.3 mg/kg per day, about 0.01 mg/kg to about 0.4 mg/kg per day, about 0.01 mg/kg to about 0.5 mg/kg per day, about 0.01 mg/kg to about 0.6 mg/kg per day, about 0.01 mg/kg to about 0.7 mg/kg per day, about 0.01 mg/kg to about 0.8 mg/kg per day, about 0.01 mg/kg to about 0.9 mg/kg per day, about 0.01 mg/kg to about 1 mg/kg per day, about 0.01 mg/kg to about 2 mg/kg per day, about 0.01 mg/kg to about 3 mg/kg per day, about 0.01 mg/kg to about 4 mg/kg per day, about 0.01 mg/kg to about 5 mg/kg per day, about 0.01 mg/kg to about 6 mg/kg per day, about 0.01 mg/kg to about 7 mg/kg per day, about 0.01 mg/kg to about 8 mg/kg per day, about 0.01 mg/kg to about 9 mg/kg per day, about 0.01 mg/kg to about 10 mg/kg per day, about 0.01 mg/kg to about 11 mg/kg per

day, about 0.01 mg/kg to about 12 mg/kg per day, about 0.01 mg/kg to about 13 mg/kg per day, about 0.01 mg/kg to about 14 mg/kg per day, about 0.01 mg/kg to about 15 mg/kg per day, about 0.01 mg/kg to about 16 mg/kg per day, about 0.01 mg/kg to about 17 mg/kg per day, about 0.01 mg/kg to about 18 mg/kg per day, about 0.01 mg/kg to about 19 mg/kg per day, or about 0.01 mg/kg to about 20 mg/kg per day.

[00202] In some embodiments, a therapeutically effective amount of a chimeric protein disclosed herein can be from about 0.01 mg/kg to about 0.05 mg/kg per day, about 0.05 mg/kg to about 0.1 mg/kg per day, about 0.1 mg/kg to about 0.5 mg/kg per day, about 0.05 mg/kg to about 1 mg/kg per day, about 1 mg/kg to about 2 mg/kg per day, about 2 mg/kg to about 3 mg/kg per day, about 3 mg/kg to about 4 mg/kg per day, about 4 mg/kg to about 5 mg/kg per day, about 5 mg/kg to about 6 mg/kg per day, about 6 mg/kg to about 7 mg/kg per day, about 7 mg/kg to about 8 mg/kg per day, about 8 mg/kg to about 9 mg/kg per day, about 9 mg/kg to about 10 mg/kg per day, about 10 mg/kg to about 11 mg/kg per day, about 11 mg/kg to about 12 mg/kg per day, about 12 mg/kg to about 13 mg/kg per day, about 13 mg/kg to about 14 mg/kg per day, about 14 mg/kg to about 15 mg/kg per day, about 15 mg/kg to about 16 mg/kg per day, about 16 mg/kg to about 17 mg/kg per day, 17 mg/kg to about 18 mg/kg per day, about 18 mg/kg to about 19 mg/kg per day, or about 19 mg/kg to about 20 mg/kg per day.

[00203] In some embodiments, a therapeutically effective amount of a chimeric protein disclosed herein can be about 0.01 mg/kg per day, about 0.02 mg/kg per day, about 0.03 mg/kg per day, about 0.04 mg/kg per day, about 0.05 mg/kg per day, about 0.06 mg/kg per day, about 0.07 mg/kg per day, about 0.08 mg/kg per day, about 0.09 mg/kg per day, about 0.1 mg/kg per day, about 0.2 mg/kg per day, about 0.3 mg/kg per day, about 0.4 mg/kg per day, about 0.5 mg/kg per day, about 0.6 mg/kg per day, about 0.7 mg/kg per day, about 0.8 mg/kg per day, about 0.9 mg/kg per day, about 1 mg/kg per day, about 1.1 mg/kg per day, about 1.2 mg/kg per day, about 1.3 mg/kg per day, about 1.4 mg/kg per day, about 1.5 mg/kg per day, about 1.6 mg/kg per day, about 1.7 mg/kg per day, about 1.8 mg/kg per day, about 1.9 mg/kg per day, about 2 mg/kg per day, about 2.1 mg/kg per day, about 2.2 mg/kg per day, about 2.3 mg/kg per day, about 2.4 mg/kg per day, about 2.5 mg/kg per day, about 2.6 mg/kg per day, about 2.7 mg/kg per day, about 2.8 mg/kg per day, about 2.9 mg/kg per day, about 3 mg/kg per day, about 3.1 mg/kg per day, about 3.2 mg/kg per day, about 3.3 mg/kg per day, about 3.4 mg/kg per day, about 3.5 mg/kg per day, about 3.6 mg/kg per day, about 3.7 mg/kg per day, about 3.8 mg/kg per day, about 3.9 mg/kg per day, about 4 mg/kg per day, about 4.1 mg/kg per day, about 4.2 mg/kg per day, about 4.3 mg/kg per day, about 4.4 mg/kg per day, about 4.5 mg/kg

per day, about 4.6 mg/kg per day, about 4.7 mg/kg per day, about 4.8 mg/kg per day, about 4.9 mg/kg per day, about 5 mg/kg per day, about 5.1 mg/kg per day, about 5.2 mg/kg per day, about 5.3 mg/kg per day, about 5.4 mg/kg per day, about 5.5 mg/kg per day, about 5.6 mg/kg per day, about 5.7 mg/kg per day, about 5.8 mg/kg per day, about 5.9 mg/kg per day, about 6 mg/kg per day, about 6.1 mg/kg per day, about 6.2 mg/kg per day, about 6.3 mg/kg per day, about 6.4 mg/kg per day, about 6.5 mg/kg per day, about 6.6 mg/kg per day, about 6.7 mg/kg per day, about 6.8 mg/kg per day, about 6.9 mg/kg per day, about 7 mg/kg per day, about 7.1 mg/kg per day, about 7.2 mg/kg per day, about 7.3 mg/kg per day, about 7.4 mg/kg per day, about 7.5 mg/kg per day, about 7.6 mg/kg per day, about 7.7 mg/kg per day, about 7.8 mg/kg per day, about 7.9 mg/kg per day, about 8 mg/kg per day, about 8.1 mg/kg per day, about 8.2 mg/kg per day, about 8.3 mg/kg per day, about 8.4 mg/kg per day, about 8.5 mg/kg per day, about 8.6 mg/kg per day, about 8.7 mg/kg per day, about 8.8 mg/kg per day, about 8.9 mg/kg per day, about 9 mg/kg per day, about 9.1 mg/kg per day, about 9.2 mg/kg per day, about 9.3 mg/kg per day, about 9.4 mg/kg per day, about 9.5 mg/kg per day, about 9.6 mg/kg per day, about 9.7 mg/kg per day, about 9.8 mg/kg per day, about 9.9 mg/kg per day, about 10 mg/kg per day, about 11 mg/kg per day, about 12 mg/kg per day, about 13 mg/kg per day, about 14 mg/kg per day, about 15 mg/kg per day, about 16 mg/kg per day, about 17 mg/kg per day, about 18 mg/kg per day, about 19 mg/kg per day, or about 20 mg/kg per day.

[00204] In some embodiments, the effective dose is administered day to the subject in need thereof over a period of 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or more. In some embodiments, the effective dose is administered day to the subject having an acute CNS injury over a period of a minimum 2 days to a period of 14 days, for example 4, 5, 6, 7 days.

[00205] In some embodiments, the effective dose is administered day to the subject having neurovegetative disease over a period of a minimum 2 days to at least 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12, years, 13 years, 14 years, 15 years, 16, years, 17 years, 18 years, 19 years or 20 years.

[00206] In some embodiments, the effective amount is administered once a day (or every 24 hours) to the subject in need thereof. In some embodiments, the effective amount is administered once a day to the subject in need thereof over a period of 2 days, 3 days, 4 days,

5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or more. In some embodiments, the effective amount is administered once a day to the subject in need thereof over a period of up to 2 days, up to 3 days, up to 4 days, up to 5 days, up to 6 days, up to 7 days, up to 8 days, up to 9 days, up to 10 days, up to 11 days, up to 12 days, up to 13 days, up to 14 days. In some embodiments, the effective amount is administered once a day to the subject in need thereof over a period of 7 days. In some embodiments, the effective amount is administered two, three or more times a day to the subject in need thereof. In some embodiments, the effective amount is administered two, three or more times a day to the subject in need thereof over a period of 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or more. In some embodiments, the effective amount is administered two, three or more times a day to the subject in need thereof over a period of up to 2 days, up to 3 days, up to 4 days, up to 5 days, up to 6 days, up to 7 days, up to 8 days, up to 9 days, up to 10 days, up to 11 days, up to 12 days, up to 13 days, up to 14 days. In some embodiments, the effective amount is administered two, three or more times a day to the subject in need thereof over a period of 7 days.

[00207] In some embodiments, a total dose of about 5 to 100 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 100 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 90 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 90 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 80 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 80 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments ,a total dose of about 5 to 70 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 70 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 60 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 60 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 50 mg/kg of the chimeric proteins described herein is

administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments a total dose of about 5 to 50 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 40 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 40 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 30 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 30 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 20 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 5 to 20 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 5 to 20 mg/kg of the chimeric proteins described herein is administered over a period of 4 days.

[00208] In some embodiments, a total dose of about 100 to 500 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 100 to 500 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 100 to 400 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments a total dose of about 100 to 400 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 100 to 300 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 100 to 300 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 100 to 200 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 100 to 200 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 100 to 150 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total dose of about 100 to 150 mg/kg of the chimeric proteins described herein is administered over a period of 7 days. In some embodiments, a total dose of about 140 mg/kg of the chimeric proteins described herein is administered over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, a total

dose of about 140 mg/kg of the chimeric proteins described herein is administered over a period of 7 days.

[00209] In some embodiments, the treatment regimen comprises a descending dosage regimen. In descending dosage regimen, the amount of the dose of the chimeric protein of the disclosure administered in the subject is reduced over the course of the duration of the treatment. In some embodiments, the dose administered on the second day is lower than the dose administered the second day. In other embodiments, the dose administered on the second day is the same than the dose administered the second day, and the dose administered the third day is lower than the dose administered the second day. In some embodiments, the treatment regimen provides for a total dose of about 2 mg/kg to about 200 mg/kg, of about 2 mg/kg to about 20 mg/kg, of about 2 mg/kg to about 10 mg/kg, of about 100 mg/kg to about 200 mg/kg, of about 100 mg/kg to about 150 mg/kg over a predetermined period of time (e.g. 4, 5, 6, or 7 days). In some embodiments, the treatment regimen provides for a total dose of about 5 mg/kg, of about 10 mg/kg, or of about 20 mg/kg over a predetermined period of time (e.g. 4, 5, 6, or 7 days).

[00210] In some embodiments, a first daily dose is administered the first day, a second daily dose that comprises from about 85% to about 95% of the amount of the chimeric protein present in the first dose is administered on day 2, a third daily dose that comprises from about 65% to about 85% of the amount of the chimeric protein present in the second dose and an amount that is lower than the second dose is administered on day 3, a fourth dose that comprises from about 45% to about 65% of the amount of the chimeric protein present in the first dose and an amount that lower than the third dose is administered on day 4, a fifth dose that comprises from about 35% to about 45% of the amount of the chimeric protein present in the first dose and an amount that lower than the fourth dose is administered on day 5, a sixth dose that comprises from about 25% to about 35% of the amount of the chimeric protein present in the first dose and an amount that lower than the fifth dose is administered on day 6, and a seventh dose that comprises from about 15% to about 25% of the amount of the chimeric protein present in the first dose and an amount that lower than the sixth dose is administered on day 7. In some embodiments, the descending treatment regimen comprises administering a first daily dose on day 1, a second daily dose corresponding to about 90% of the first daily dose on day 2, a third daily dose corresponding to about 70% of the first daily dose on day 3, a fourth daily dose corresponding to about 50% of the first daily dose on day 4, a fifth daily dose corresponding to about 40% of the first daily dose on day 5, a sixth daily dose corresponding

to about 30% of the first daily dose on day 6 and a seventh daily dose corresponding to about 20% of the first daily dose on day 7.

[00211] In some embodiments, a first daily dose is administered the first day, a second daily dose that comprises from about 85% to about 95% (e.g. about 90%) of the amount of the chimeric protein present in the first dose is administered on day 2, a third daily dose that comprises from about 65% to about 85% (e.g. about 70%) of the amount of the chimeric protein present in the second dose and an amount that is lower than the second dose is administered on day 3, a fourth dose that comprises from about 45% to about 65% (e.g. about 50%) of the amount of the chimeric protein present in the first dose and an amount that lower than the third dose is administered on day 4.

[00212] In some embodiments, a first daily dose administered the first day and the second daily dose administered on the second day are the same, a third daily dose comprising from about 65% to about 90% (e.g. about 75%) of the amount of the chimeric protein present in the first dose is administered on day 3, a fourth daily dose that comprises from about 45% to about 65% (e.g. about 50%) of the amount of the chimeric protein present in the second dose and an amount that is lower than the second dose is administered on day 4.

[00213] In some embodiments, the treatment regimen or descending treatment regimen provides a total dose of from about 2 mg/kg to about 20 mg/kg over a period of 7 days. In some embodiments, the treatment regimen provides a total dose of from about 2 mg/kg to about 20 mg/kg over a period of 4 days. In some embodiments, the treatment regimen or descending treatment regimen provides a total dose of from about 2 mg/kg to about 10 mg/kg over a period of 7 days. In some embodiments, the treatment regimen or descending treatment regimen provides a total dose of from about 2 mg/kg to about 10 mg/kg over a period of 4 days. In some embodiments, the treatment regimen or descending treatment regimen provides a total dose of about 5 mg/kg, about 10 mg/kg, or about 20 mg/kg over a period of 7 days. In some embodiments, the treatment regimen or descending treatment regimen provides a total dose of about 5 mg/kg, about 10 mg/kg, or about 20 mg/kg over a period of 4 days.

[00214] In some embodiments, the treatment regimen comprises a 5 days course of intravenous administration of the chimeric protein, such as scp776. In some embodiments, the treatment regimen comprises a 7 days course of intravenous administration of the chimeric protein, such as scp776. In some embodiments, the treatment regimen comprises a 4 days course of intravenous administration of the chimeric protein, such as scp776. In some

embodiments, the treatment regimen comprises a 3 days course of intravenous administration of the chimeric protein, such as scp776. In some embodiments, the treatment regimen comprises a 2 days course of intravenous administration of the chimeric protein, such as scp776. In some embodiments, the treatment regimen comprises administering intravenously a first dose at from about 2 mg/kg to about 6 mg/kg on day 1, and one dose of about 1 mg/kg to about 2 mg/kg one each of the following days. In some embodiments, the first and second dose are the same (e.g. 2 mg/kg), and the third dose is lower than the first dose. For example, the first dose can be 2 mg/kg, the second dose can be 2 mg/kg, the third dose can be 1.5 mg/kg and the fourth dose can be 1 mg/kg, in a 4 days treatment course. In some embodiments, the second dose is lower than the first dose and the third dose is lower than the second dose etc... For example, the first dose can be 2 mg/kg, the second dose can be 1.8 mg/kg, the third dose can be 1.4 mg/kg and the fourth dose can be 1 mg/kg, in a 4 days treatment course. In some embodiments, the second dose is lower than the first dose and the third dose is the same as the second dose, etc... For example, the course can comprise a first dose of about 5.2 mg/kg on day 1, and one dose of about 1.3 mg/kg on day 2, days 3, day 4 and day 5, if the course is a 5 days course.

[00215] In some embodiments, the course of treatment comprises administration of the effective amount over 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days or more. In some embodiments, the course of treatment comprises administration of the effective amount over 7 days. In some embodiments, the course of treatment comprises administration of the effective amount over 4 days. In some embodiments, the course of treatment comprises administration of the effective amount over consecutive days. In some embodiments, the course of treatment comprises administration of the effective amount every day, every 2nd day, every 3rd day or every 4th day.

[00216] In some embodiments, the subject in need thereof treated with the chimeric protein or pharmaceutical compositions described herein is continuously infused with dextrose solution to sustain euglycemia. In some embodiments, the dextrose solution comprises from about 5% (w/v) dextrose to about 50% (w/v) dextrose. In some embodiments, the dextrose solution is in sterile water or normal saline solution. For example, the subject in need thereof can be administered i.e., 5% Dextrose in normal saline or 10% Dextrose in sterile water to sustain euglycemia. In some embodiments, the subject in need thereof is administered the chimeric protein or pharmaceutical compositions described herein via bolus injection or intrathecally and is administered a dextrose solution by continuous infusion. In some

embodiments, the subject is infused with the dextrose solution for 48 hours. In some embodiments, the subject is infused with the dextrose solution up to the end of the treatment with the chimeric protein. In some embodiments, the subject is infused with the dextrose solution up to 24 hours after the end of the treatment with the chimeric protein. For example, the subject can be treated for 4 days with the pharmaceutical composition described herein and the subject can be infused for up to 4 or 5 days with the dextrose solution.

[00217] In some embodiments, the human dose regimen can be calculated based on allometric scaling of the Non-Human Primate to Human dose regimen.

[00218] An estimate of the human equivalent dose (HED) to the efficacious dosing regimen can be obtained via allometric scaling (USDHHS, FDA, CDER, Guidance for Industry, 2005). Allometric scaling treats the problem of differential metabolic rates between species by applying a correction factor based on body surface area to the dose of interest in a given species. Allometric scaling is most frequently used in estimating safe starting doses for first-in-human studies, but it is also commonly applied in translating effective doses from animals to man. The FDA-recommended correction factor for converting dosages in 3 kg rhesus monkeys to humans is 3.1 (USDHHS, FDA, CDER, Guidance for Industry, 2005). In some embodiments, the estimated HED of the efficacious dose regimen comprises an initial dose of 16 mg/kg ÷ 3.1 = 5.2 mg/kg, followed by additional doses at 24 hour intervals of 4 mg/kg ÷ 3.1 = 1.3 mg/kg.

[00219] In some embodiments, the pharmaceutical compositions described herein can further include one or more additional bioactive or therapeutic agents or components to aid in the treatment of damaged tissue or cells and/or facilitate the tissue regenerative process. In some embodiments, the additional therapeutic agent is a thrombolytic agent. In some embodiments, the thrombolytic agent is recombinant tissue plasminogen activator (rt-PA). In some embodiments, the additional therapeutics agent comprise a neuroprotective agent. In some embodiments, the additional therapeutic agent comprises or consists of Glucocorticoids, FK506, Cyclosporin, Sirolimus, nerinetide, oxygen carrying therapeutics (perfluorocarbons) or the like.

[00220] In some embodiments, the method comprises administering two or more (e.g. two, there or more) pharmaceutical compositions. The different pharmaceutical compositions may be administered to the subject in any order and in any suitable interval. For example, in some embodiments, the one or more some embodiments compositions are administered simultaneously or near simultaneously. In some embodiments, the method comprises a

staggered administration of the two or more some embodiments compositions, where a first some embodiments composition is administered and a second some embodiments composition administered at some later time point. Any suitable interval of administration which produces the desired therapeutic effect may be used.

[00221] In certain embodiments, the method has an additive effect, wherein the overall effect of the administering a combination of therapeutic agents or procedures is approximately equal to the sum of the effects of administering each therapeutic agent or procedure alone. In other embodiments, the method has a synergistic effect, wherein the overall effect of administering a combination of therapeutic agents or procedures is greater than the sum of the effects of administering each therapeutic agent or procedure alone.

[00222] In some embodiments, the pharmaceutical compositions and chimeric proteins described herein, when administered to a subject having an acute CNS injury, result in at least one of more of the following: mitigation of oxidative damage to cells of the cerebral cortex, repair or acceleration of repair of blood brain barrier, reduction of oedema, reduction of infarct volume, reduction in blood brain barrier permeability, targeted delivery of pro-survival signals to injured brain tissue, increase in musculoskeletal coordination following stroke, improvements to consciousness following stroke, improvements to neurologic function following stroke, and increase in motor function following stroke.

[00223] In some embodiments, the pharmaceutical compositions and chimeric proteins described herein, when administered to a subject having a neurodegenerative disease, result in improvement in motor functions and decrease in disease symptoms, such as decrease in tremors in subjects having PD, decreases in autonomic dysfunction in subjects having ALS, or decrease in AD symptoms such as memory loss and confusion in subjects having AD.

[00224] In some embodiments, a therapeutically effective amount of the pharmaceutical composition comprising the chimeric protein reduces at least one symptom associated with a nervous system disorder by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In some embodiments, a therapeutically effective amount of the chimeric protein reduces at least one symptom associated with a nervous system disorder by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In some embodiments, a therapeutically effective amount of the chimeric protein disclosed herein reduces at least one symptom associated with a nervous system disorder by,

e.g., about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 20%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%. In some embodiments, a therapeutically effective amount of the chimeric protein reduces at least one symptom associated with a nervous system disorder for, e.g., at least one week, at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, or at least twelve months.

[00225] In some embodiments, a therapeutically effective amount of the pharmaceutical composition comprising the chimeric protein results in an improvement of at least one neurological or cognitive function by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% or about 100%. In some embodiments, a therapeutically effective amount of the chimeric protein results in an improvement of at least one neurological or cognitive function by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In some embodiments, a therapeutically effective amount of the chimeric protein disclosed herein results in an improvement of at least one neurological or cognitive function by, e.g., about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 20%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%. In some embodiments, a therapeutically effective amount of the chimeric protein results in an improvement of at least one neurological or cognitive function for, e.g., at least one week, at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, or at least twelve months.

[00226] In some embodiments, administration of the effective amount to a subject having an acute CNS injury results in a significant improvement in neurologic function as

assessed by a decrease in the Neurologic Deficit Scoring (NDS) such as musculoskeletal coordination, consciousness, motor system; or improvement as assessed by any other neurologic function scale known in the art (e.g. NIH stroke scale, modified Rankin Scale, modified Barthel Index, or Glasgow coma scale, MDS-Unified Parkinson's Disease Rating Scale, or Hunt-Hess Classification).

[00227] In some embodiments, administration of the effective amount to a subject having an acute CNS injury results in a significant reduction in lesion volume, perfusion deficit, blood brain barrier permeability, or any other imaging assessment of injury severity known in the art (e.g., Fisher scale). In some embodiments, the reduction is by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 99% or about 100%.

[00228] In some embodiments, administration of the effective amount to a subject having an acute CNS injury results in a significant improvement in cognitive function as assessed by for example, but not limited to, NIH stroke score, modified Rankin Score, and/or modified Barthel Index.

[00229] In some embodiments, administration of the effective amount to a subject having a neurodegenerative disease results in a significant improvement in cognitive function as assessed by, for example, but not limited to, USPDRS, SCAG, and ALS FRS.

[00230] Methods of treatment of central nervous system (i.e. brain and spinal cord) disorders are provided. In some embodiments, the methods comprise administering a therapeutically effective amount of the composition or chimeric proteins described herein by bolus injection to a subject in need thereof, wherein administration results in at least one of more of the following: mitigation of oxidative damage to cells of the cerebral cortex, repair or acceleration of repair of blood brain barrier, reduction of oedema, reduction of infarct volume, reduction of blood brain barrier permeability, targeted delivery of pro-survival signals to injured brain tissue, increase in musculoskeletal coordination following stroke, improvements to consciousness following stroke, improvement to neurologic function following stroke, and improvement in motor function following stroke.

[00231] Methods of treating a brain, central nervous system injury and ocular disease are provided.

[00232] In some embodiments, the method of treating a brain or central nervous system injury comprises administering a therapeutically effective amount of a chimeric protein by

bolus injection to a subject in need thereof. In some embodiments the method of treating a brain or central nervous system injury comprises administering a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein by bolus injection to a subject in need thereof. In some embodiments, the the chimeric protein comprises (a) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding to R63, K70, K101, E138, D139, N160, and combinations thereof; (b) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (c) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof. In some embodiments, administration of the chimeric protein results in at least one of the following: mitigation of oxidative damage in primary cortical cultures, repair or acceleration of repair of blood brain barrier, reduction of oedema, reduction of infarct volume, reduction of blood brain barrier permeability, localization to injured brain tissue, increase in motor function following stroke.

[00233] In some embodiments, the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1. In some embodiments, the variant of IGF-1 comprises E3R and Y31A substitutions relative to wild type human IGF-1.

[00234] In some embodiments, the variant of human Annexin 5 comprises the amino acids 2-320 corresponding to wild type human Annexin 5 and comprises R63A, K70A, K101A, E138A, D139G, N160A, C316A substitutions relative to wild type human Annexin 5.

[00235] In some embodiments, the variant of human serum albumin comprises the amino acids 26-609 corresponding to wild type human serum albumin and comprises C58S and N527Q substitutions relative to wild type human serum albumin.

[00236] In some embodiments, the chimeric protein is IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316A). In some embodiments, the linker lk7 comprises – Gly-Ser-Gly-Gly-Gly-Ser-Gly.

[00237] In some embodiments, the chimeric protein is selectively targeted to cells comprising a target molecule phosphatidylserine and exhibits activation of the IGF-1 receptor

at least twice as strong on cells containing the target molecule compared to cells that do not contain the target molecule as measured by phosphorylation of serine/threonine protein kinase B (AKT).

[00238] In some embodiments, the brain injury is an ischemic stroke. In some embodiments, administration is at the first presentation of a neurovascular disease.

[00239] In some embodiments, the subject in need thereof has Alzheimer disease.

[00240] In some embodiments, 0.01 mg/kg to 100 mg/kg per dose is administered to the subject in need thereof. In some embodiments, up to 200 mg/kg of the chimeric protein is administered to the subject in need thereof.

Kits

[00241] Aspects of the disclosure relates to unit doses and kits. In some embodiments, the kit comprises a supply of individual unit doses comprising the chimeric protein; and instructions for use. In some embodiments, each unit dose is supplied in a suitable container or prefilled package, such as syringes, ampoules or vials. In some embodiments, each kit has a sufficient number of containers or unit dose to treat an individual patient for the suitable numbers of days for a course of treatment.

[00242] In some embodiments, the ingredients of the kit are supplied either separately or mixed together in unit dose, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule, vial, syringe or sachette indicating the quantity of active agent. Where the active agent(s) are lyophilized, an ampoule of sterile water or saline for injection can be provided so that the active agent(s) may be mixed prior to injection.

[00243] In some embodiments, the kit comprises a plurality of suitable containers, such as vials, and instructions for use. The vials may be fitted with a rubber septum to allow syringe entry for withdrawal of the vial contents. In some embodiments, each vial comprises between 1 and 50 ml extractable volume of the liquid formulation or any volume therebetween. In some embodiments, each vial comprises between 1 and 10 ml extractable volume of the liquid formulation. In some embodiments, each vial comprises 5 ml extractable volume of the liquid formulation. In some embodiments, the kit comprises a plurality of vials with different volume of the liquid formulation, wherein one or more of the plurality of vials comprises a different amount of the chimeric protein. In some embodiments, the plurality of containers comprises a liquid formulation comprising the chimeric protein to be administered in a volume ranging from

1 ml to 50 ml, or any volume therebetween. In some embodiments, the solution comprises about 20 mg/ml of chimeric protein. In some embodiments, the plurality of containers comprises a liquid formulation comprising from 20 mg to about 1g of the chimeric protein to be administered in a volume ranging from 1 ml to 50 ml or any volume therebetween. In some embodiments, the liquid formulation is ready for injection. In some embodiments, the liquid formulation is diluted into standard IV fluids (e.g., normal saline, 5% dextrose in normal saline, or 10% dextrose in water).

[00244] In some embodiments, the volume of the vial in the kit is between 1 mL to 50 mL, corresponding to 20 mg to 1 g of chimeric protein, such as scp776.

[00245] The kits described herein may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein.

[00246] In some embodiments, the instructions for use states to intravenously or intraarterially administer a unit dose from the supply once a day from the supply. In some embodiments, the instructions for use states to intravenously or intraarterially administer a unit dose from the supply two, three or more times a day from the supply. In some embodiments, the instructions for use states to intrathecally administer a unit dose from the supply once a day from the supply. In some embodiments, the instructions for use states to intrathecally administer a unit dose from the supply two, three or more times a day from the supply.

[00247] In some embodiments, the supply supports a single course of treatment. In some embodiments, the supply comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more individual unit dose (e.g. vials). In some embodiments, the supply comprises 8 individual unit dose (e.g. vials)for a four day treatment course to account for variation in weight of patients and for convenience. In some embodiments, the supply comprises 14 individual unit dose (e.g. vials) for a seven days treatment course to account for variation in weight of patients and for convenience.

[00248] In some embodiments, the individual unit dose can be contained in one or more package and are optionally labelled “Dose 1”, “Dose 2”, “Dose 3” etc… or “Day 1”, “Day 2”, Day 3” etc…

[00249] In some embodiments, the unit dose that is to be administered on day 2 (referred herein as second dose comprises from about 85% to about 95% (e.g. about 90%) of the amount of the chimeric protein present in the unit dose that is to be administered on day 1 (referred

herein as first dose), the unit dose that is to be administered on day 3 (referred herein as third dose) comprises from about 65% to about 85% (e.g. about 70%) of the amount of the chimeric protein present in the second dose and an amount that is lower than the second dose, the unit dose that is to be administered on day 4 comprises from about 45% to about 65% (e.g. about 50%) of the amount of the chimeric protein present in the first dose and an amount that lower than the third dose, the unit dose that is to be administered on day 5 comprises from about 35% to about 45% (e.g. about 40%) of the amount of the chimeric protein present in the first dose and an amount that lower than the fourth dose, the unit dose that is to be administered on day 6 comprises from about 25% to about 35% (e.g. about 30%) of the amount of the chimeric protein present in the first dose and an amount that lower than the fifth dose, and the unit dose that is to be administered on day 7 comprises from about 15% to about 25% (e.g. about 20%) of the amount of the chimeric protein present in the first dose and an amount that lower than the sixth dose

[00250] In some embodiments, the kit comprises a total dose of about 5 to 100 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 100 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 90 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 90 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 80 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 80 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 70 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 70 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 60 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 60 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 50 mg/kg of the chimeric proteins

described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 50 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 40 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 40 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 30 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 30 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 20 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 20 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 10 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 to 10 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 to 10 mg/kg of the chimeric proteins described herein for administration over a period of 4 days. In some embodiments, the kit comprises a total dose of about 10 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 10 mg/kg of the chimeric proteins described herein for administration over a period of 4 days. In some embodiments, the kit comprises a total dose of about 10 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 5 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 5 mg/kg of the chimeric proteins described herein for administration over a period of 4 days.

[00251] In some embodiments, the kit comprises a total dose of about 100 to 500 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6

days, or 7 days. In some embodiments, the kit comprises a total dose of about 100 to 500 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 100 to 400 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 100 to 400 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 100 to 300 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 100 to 300 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 100 to 200 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments the kit comprises a total dose of about 100 to 200 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 100 to 150 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 100 to 150 mg/kg of the chimeric proteins described herein for administration over a period of 7 days. In some embodiments, the kit comprises a total dose of about 140 mg/kg of the chimeric proteins described herein for administration over a period of 4 days, 5 days, 6 days, or 7 days. In some embodiments, the kit comprises a total dose of about 140 mg/kg of the chimeric proteins described herein for administration over a period of 7 days.

[00252] In some embodiments, the instructions for use comprises dosing schedule treatment in a readable medium such as pamphlet or written information. In some embodiments, the readable medium comprises specifications of the active agents, preparation instructions, dosage variations, storage instructions, administration instructions, side effects of the pharmaceutical ingredients, contraindications for administration, stability data or any other information relevant to the use of the composition described herein for patient care or physician information.

[00253] Aspects of the disclosure relate to the use of pharmaceutical composition for the treatment of acute central nervous system injury, the pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein described herein and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is administered in the form of bolus injection. Other aspects of the disclosure relate to the use of pharmaceutical composition for the

treatment of neurovegetative disease, the pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein described herein and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is administered in the form of bolus injection. In some embodiments, the chimeric protein comprises (a) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding to R63, K70, K101, E138, D139, N160, and combinations thereof; (b) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (c) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof.

EXAMPLES

[00254] The following Examples are offered by way of illustration and not by way of limitation. Unless otherwise specified, all reagents and solvents are of standard commercial grade and are used without further purification. Using routine modifications, the procedures provided in the following Examples may be varied by those of ordinary skill in the art to make and use other bi-specific fusion proteins and pharmaceutical compositions within the scope of the present disclosure.

Example 1. Scp776-mediated In Vitro Protection of Primary Cortical Cells from Oxidative Stress

[00255] Methods:

[00256] Preparation of Rat Mixed Cortical Cultures

[00257] Rat mixed cortical cultures were prepared from E18 Wistar rat embryos under sterile conditions. In summary, following anesthesia, the embryos were removed from the womb and decapitated. The cortices were dissected out and placed in a dish containing cold 10 mM HEPES and 10 µg/mL gentamicin (HBSS-A) buffer. The tissue was cut into small pieces, transferred to a 15 mL conical tube and digested with DNase and papain for 15 min at 37 oC.

The cells were collected by centrifugation (1500 rpm, 5 min RT). The supernatant was removed and 1 mL of MEM (2 g/L glucose) supplemented with 2 mM glutamine, 10 μg/mL gentamicin, 10 % heat-inactivated fetal bovine serum (FBS-HI) and 10 % heat-inactivated horse serum (HS-HI) was added to the tube and the tissue was triturated with a pipette. The cells were counted and then plated on poly-L-lysine coated 48-well plates, at a cell density of 400,000 cells/well in the same medium. The plates were placed in a humidified 37 °C/5 % CO2, 95 % air incubator. After 3-4 h, the medium was changed to MEM (2 g/L glucose) supplemented with 2 mM glutamine, 10 μg/mL gentamicin and 5 % HS-HI (MEM+). After three days in vitro, the medium was replaced with fresh medium containing MEM (2 g/L glucose) supplemented with 2 mM glutamine, 10 μg/mL gentamicin and 5 % FBS-HI and 5 % HS-HI. On day 6 in vitro, unwanted cell division was inhibited by adding cytosine arabinoside (10 μM final concentration in MEM+) for 24 h. The next day, (day 7 in vitro), the medium was replaced with fresh MEM+.

[00258] H2O2 Exposure

[00259] Culture wells that had a healthy cell monolayer were selected for experimental treatment on day 10 after isolation in vitro. The cell culture media was completely removed from the wells and scp776, positive control, or vehicle control (DPBS) were added into the wells in a volume of 270 μl in MEM+. After the pre-incubation period of 30 mins at 37 °C, 30 μl of 500 μM H2O2 (final 50 μM in wells) was added and cells were incubated for 60 min at 37 °C/ 5 % CO2, 95 % air) incubator. Afterwards, the H2O2 medium was removed and normal culture media (MEM+) containing the compounds or vehicle control (DBPS) were pipetted to the wells, and the plates were placed in a normoxic (37 °C/ 5 % CO2, 95 % air) incubator for an additional 23 h.

[00260] Assessment of Cell Death by LDH Measurement

[00261] Cell death was assessed by quantifying the levels of lactate dehydrogenase (LDH) released in the culture media following 23 h of H2O2 exposure. After 20 h the culture media from all wells were collected and possible cell debris was removed by centrifugation (13,000 rpm, 3 min, 4 °C). A 100 μL aliquot from each sample was pipetted into a 96 well micro titer plate as duplicates, and an equal volume of LDH substrate was pipetted into the wells. The absorbance at 340 nm was measured immediately using a 3 min kinetic measurement protocol in a Multiskan MS ELISA reader. The change in absorbance/min was determined, which is directly proportional to the released LDH.

[00262] Assessment of Neuronal Cell Counts by NeuN Immunocytochemistry

[00263] Neuronal cell counts were quantified by immunostaining the neurons with the neuronal specific antibody targeting NeuN. After removing the culture medium, the cells were fixed with 4 % formaldehyde in PBS for 30 min at RT then washed twice with PBS. The fixed cells were permeabilized and non-specific binding was blocked by 30 min incubation with blocking buffer (1 % bovine serum albumin and 0.3 % Triton-X-100 in PBS). The cells were incubated with the primary antibody, mouse anti-NeuN (Millipore, catalog # MAB377, 1:1,000 dilution), for 24 h at RT, followed by washing then a 2 h incubation with the secondary antibody, biotinylated goat anti-mouse (Vector Laboratories, catalog # BA-9200 1:200) at RT. The wells were washed twice with PBS and the cells were then incubated for 2 h with the avidin-HRP complex (Vector Laboratories, catalog # PK-6100 1:400) and the color was developed with nickel-enhanced DAB (Vector Laboratories, catalog # SK-4100). Each well was imaged at four random locations using a brightfield AxioVert A1 microscope (Carl Zeiss) using an LD A-Plan 20x objective (NA ∞/1.0 (PS)) (Carl Zeiss). The captured images were exported as tiff files using the ZEN software (Carl Zeiss). The tiff images were used for neuronal count analysis using ImageJ v1.48e (NIH). The images were manually thresholded to delimit the NeuN stained neurons that were counted according to their size by using the Counting Particles function available in the ImageJ software. The NeuN-positive neurons were counted by an experimenter blind to the treatments. Altogether, 4 fields for each well were counted.

[00264] Results

[00265] Primary mixed cortical cultures from E18 Wistar rat embryos were maintained under normal growth conditions for 10 days. The cells were then pre-treated with vehicle, positive control compound, or scp776 (10, 50 or 100 nM) for 30 minutes. Oxidative stress was then induced by addition of 50 μM H_2O_2. After 1 hour under H_2O_2-induced oxidative stress, the growth media was replaced with fresh media containing vehicle, positive control compound, or scp776 at different concentrations (10, 50 or 100 nM). After 23 hours, the media was removed and assayed for the presence of lactate dehydrogenase (LDH), an intracellular enzyme that is released into media upon cell death. FIG. 1 shows that a significant reduction in released LDH was observed in cultures treated with scp776 at 10, 50 or 100 nM compared to vehicle (H_2O_2 Only) (1-way ANOVA with Dunnett's multiple comparisons test; $p = 0.0023$ (10 nM), $p < 0.0001$ (50, 100 nM)).

[00266] Primary mixed cortical cultures from E18 Wistar rat embryos were maintained under normal growth conditions for 10 days. The cells were then pre-treated with vehicle, positive control compound, or scp776 at different concentrations (10, 50 or 100 nM) for 30 minutes. Oxidative stress was then induced by addition of 50 μM H_2O_2. After 1 hour under H_2O_2-induced oxidative stress, the growth media was replaced with fresh media containing vehicle, positive control compound, or scp776 (10, 50 or 100 nM). After 23 hours, the cells were fixed and stained for NeuN, a specific marker of neuronal nuclei, using immunocytochemical techniques. NeuN-positive cells were counted by light microscopy. FIG. 2 shows that a significant increase in NeuN positive cells (a reduction in cell death) was observed in cells treated with scp776 at 10, 50 or 100 nM compared to vehicle (H2O2 Only) (1-way ANOVA with Dunnett's multiple comparisons test; $p = 0.0007$ (10 nM), $p < 0.0001$ (50, 100 nM)).

[00267] Conclusion

[00268] Scp776 protected against oxidative neuronal and glial cell death in vitro. Treatment of rat primary mixed cortical cultures with scp776 before, during and after stimulation of oxidative stress with hydrogen peroxide reduced cell death as measured by LDH release into cell media and by intact neuronal nuclei counting.

Example 2. Scp776 Efficacy in the Rodent Model of Acute Ischemic Stroke (AIS)

[00269] Methods

[00270] The transient Middle Cerebral Artery Occlusion (tMCAO) model creates an ischemic injury to brain that is representative of acute ischemic stroke. The duration of ischemia is controlled by the placement of an occluding filament, and the moment of reperfusion is determined by the removal of the filament. This mechanical model is analogous to standard revascularization modalities (e.g. – IV tissue plasminogen activator, intra-arterial thrombolysis, mechanical thrombectomy).

[00271] Each rat was anesthetized in an induction chamber (2 – 5% Isoflurane, in 70% N2O and 30% O2; flow 300 mL/min) and placed on its back on a surgery platform on a heating pad. The heating pad was set to 37°C and a rectal probe was inserted into the animal's rectum with help of a lubricant. Anesthesia was maintained at 1.5 – 2.5%.

[00272] After midline skin incision, the right common carotid artery (CCA) was exposed, and the external carotid artery (ECA) was ligated distal from the carotid bifurcation. Filament with silicon-covered tip (Doccol filament 4-0, dia 0.185 mm, silicon 5 – 6 mm/dia 0.35 mm) was inserted 22 – 23 mm into the internal carotid artery (ICA) up to the origin of MCA (FIG. 3). After the operation, the rat was placed in a clean cage on a heating pad to wake up from the anesthesia. The filament was left in place for 90 min (120 minutes in some implementations).

[00273] For reperfusion, anesthesia was induced as described above, the animal was placed back on the heating pad and the rectal probe was inserted. The neck wound was carefully reopened, and the intraluminal filament was carefully removed to start reperfusion. After removal of the filament, the tissues were positioned back in place and the wound was sutured closed with the nylon thread. Pre-warmed 4 mL of 0.9% NaCl was given intra-peritoneally (I.P.) to rehydrate the rat. After the operation, the rat was placed in a clean cage on a heating pad to wake up from the anesthesia. Finally, the rat was returned to its home cage and allowed free access to food and water.

[00274] Sham rats underwent identical procedures, including anesthesia regime, but without the filament insertion, ligation, and actual tMCAO occlusion.

[00275] Analytical methods used to assess scp776 efficacy in this model included: T2-weighted MRI (Infarct Size, Hemispheric Swelling), Gd-Enhanced MRI (Blood Brain Barrier (BBB) Integrity, Infarct Size), Tissue Homogenate ELISA (scp776 Accumulation), Automated Kinematic Gait Analysis.

[00276] Dosage:

[00277] Rats were dosed with scp776 or vehicle in multiple dosing regimens. All dosing was intravenous (I.V.) bolus administration. In some implementations of the model, rats received a single I.V. dose of 25 mg/kg scp776 or vehicle 1–5 minutes prior to reperfusion. In other implementations, rats received 3 I.V. doses of 25 mg/kg scp776 or vehicle at the following times: 1–5 minutes prior to reperfusion, 4 hours post-reperfusion and 8 hours post-reperfusion.

[00278] Results

[00279] IV administration of scp776 protects blood brain barrier integrity after ischemic stroke in rats. Sprague Dawley rats were subjected to the tMCAO model of ischemic stroke or sham procedure. At the time of reperfusion, tMCAO rats were dosed with an IV bolus of scp776 (25 mg/kg) or vehicle; sham rats were dosed with vehicle. Blood brain barrier (BBB) integrity was assessed by gadolinium-enhancement MRI 2 hours post-reperfusion. FIG. 4 shows the BBB leakage (percent Gd enhancement readout). Individual data points and means ± SD are shown for each group. Unpaired t-test showed a significant improvement ($p = 0.014$) in BBB integrity in the scp776 treated group, compared to vehicle.

[00280] IV administration of scp776 reduces hemispheric swelling after ischemic stroke in rats. Sprague Dawley rats were subjected to the tMCAO model of ischemic stroke or sham procedure. At the time of reperfusion, tMCAO rats were dosed with an IV bolus of scp776 (25 mg/kg) or vehicle; sham rats were dosed with vehicle. Hemispheric swelling was assessed by T2-weighted MRI 2 hours post-reperfusion. FIG. 5 shows the hemispheric swelling (delta ischemic/healthy % readout). Individual data points and means ± SD are shown for each group. A trend of reduced hemispheric swelling was observed in animals that received scp776 compared to vehicle (Unpaired t-test; $p = 0.10$).

[00281] IV administration of scp776 reduces infarct size after ischemic stroke in rats (see FIG. 6). Sprague Dawley rats were subjected to the tMCAO model of ischemic stroke or sham procedure. At the time of reperfusion, tMCAO rats were dosed with an IV bolus of scp776 (25 mg/kg) or vehicle; sham rats were dosed with vehicle. Infarct size was assessed by T2-weighted MRI 2 hours post-reperfusion. Individual data points and means ± SD are shown for each group. A trend of reduced infarct size was observed in animals that received scp776 compared to vehicle (Unpaired t-test; $p = 0.17$).

[00282] Scp776 treatment enhances recovery of function following ischemic stroke in rats (see FIG. 7). Sprague Dawley rats were subjected to the tMCAO model of ischemic stroke or sham procedure. At the time of reperfusion, tMCAO rats were dosed with an IV bolus of scp776 (25 mg/kg) or vehicle; sham rats were dosed with vehicle. A group of animals that were subjected to tMCAO received additional 25 mg/kg doses of scp776 at 4 and 8 hours post-reperfusion (Multiple Dose group). Fine motor kinematic analysis was performed on Day 10 post-tMCAO. Rats were analyzed in the MotoRater test using walking behavioral tasks (Step, Stride, Stance, Swing analyses, Limb Coordination). On the day of testing, the rats were marked in appropriate points of body, such as joints of limbs and parts of tail to ease the data

analysis process. The movement data was captured using a high-speed camera (300 frames / second) from three different directions (from below and both sides). Different gait patterns and movements were analyzed using a custom-made automated analysis system. The analyzed parameters include: 1) general gait pattern parameters (stride time and speed, step width, stance and swing time during a stride, interlimb coordination), 2) body posture and balance (toe clearance, iliac crest and hip height, hind limb protraction and retraction, tail position and movement), and 3) fine motor skills (swing speed during a stride, jerk metric during swing phase, angle ranges and deviations of different joints, vertical and horizontal head movement). The analyzed parameters were combined into a single score per animal representing the deviation in overall movement from the average Sham (Healthy) animal; this parameter "Distance from Sham" is the y-axis of FIG. 8. The multiple dose group is labeled as scp776 in FIG. 8; the single dose group is not shown. FIG. 8 shows that the animals that received 3 doses of scp776 were significantly more similar to healthy animals than animals that received vehicle (1-way ANOVA with Tukey's Honest Significant Difference test; $p = 0.0147$).

[00283] Conclusion

[00284] Scp776 showed efficacy in the rat tMCAO model of stroke by several direct measures of injury physiology. During the acute injury phase, 2 hours post-reperfusion and dosing, scp776-treated animals showed improved BBB integrity, reduced swelling of the infarcted hemisphere and reduced infarct volumes compared to vehicle treated animals. Coupled with the apparent beneficial activity in the injured brain, scp776 showed enhanced accumulation in injured brains at both 2 and 24 hours post-injury.

[00285] Scp776 also showed a functional benefit to tMCAO rats 10 days post-injury. Automated kinematic gait analysis defines a rat's walking stride in 97 parameters using high-speed video. A software package is used to cluster related parameters and evaluate the difference between those clusters in healthy animals and tMCAO animals. Animals that underwent the tMCAO procedure and received 3 doses of scp776 showed a significantly improved overall gait profile compared to animals that received vehicle.

[00286] Localization to injured brain tissue was unexpected. Whether due to the permeabilization of BBB in injured brain or active transport to regions of apoptosis, the capacity to deliver the chimeric protein such as scp776 to injured brain may open many therapeutic opportunities to treat central nervous brain disorders. In particular, the functional

improvement in gait observed in scp776 treated animals 10 days following ischemic stroke was unexpected and surprising.

Example 3: Efficacy study of scp776 in a Non-Human Primate Model (NHP) of Ischemic Stroke

[00287] Methods

[00288] 1. Description of the transient middle cerebral artery occlusion (tMCAO) Ischemic Stroke (IS) Model in Cynomolgus fascicularis (Macaca fascicularis)

[00289] Under anesthesia, the right eye globe was removed. Under an operating microscope (Kom300, Konan Medical, Hogo, Japan), the orbital content was dissected and excised. A window of approximately 10 mm in diameter was opened just anterior to the foramen of the optic nerve at the base of the skull. The main trunk of the right middle cerebral artery (MCA) was visible through the window, which is beneath dura matter. After opening the dura matter, tMCAO was performed using two microvascular clips (Mini #81, Sugita Aneurysm Clips, Mizuho Medical Corp., Tokyo, Japan), one on the proximal part of the main MCA trunk and the other on the distal-to-orbitofrontal branch. These clips were removed four (4) hours after MCA occlusion. After visual confirmation of re-establishment of MCA blood flow, the burr hole was closed using Clearfil New Bond (Kuraray Noritake Dental, Inc., Tokyo, Japan) and the incision was sutured. A pair of new clips was used for each macaque.

[00290] Any abnormal observations during clip placement and/or removal, such as vessel rupture or bleeding, was noted and reported.

[00291] 2. Neurologic Deficit Scoring (NDS) Method

[00292] Twenty-four (24) hours (± 2 hrs.), 48 hrs. (± 2 hrs.), 72 hrs. (± 2 hrs.), 7, 10 and 14 days after occlusion, neurologic deficits were obtained according to the method described by Kito et al. (J. Neurosci. Meth., 2001; 105: 45-53).

[00293] FIG. 11A shows the NDS motor system score at 48 hours (day 2) of placebo treated and scp776 treated animals.

[00294] FIG. 11B shows the NDS consciousness score at day 7 of placebo treated and scp776 treated animals.

[00295] FIG. 11C shows the NDS musculoskeletal coordination score at day 1, 2, 3, 7, 10, and 14 of placebo treated and scp776 treated animals.

[00296] FIG. 11D shows the total NDS score at day 1, 2, 3, 7, 10, and 14 of placebo treated and scp776 treated animals.

[00297] 3. Magnetic Resonance Imaging (MRI) Method

[00298] Brain images using MRI were obtained 4 hrs. after reperfusion (i.e. 8 hrs. after occlusion), 72 hrs. (±2 hrs.), and 14 days after occlusion.

[00299] During imaging, macaques were anesthetized with propofol (12-20 mg/kg; Maruishi PharmaceuticalCo., Osaka, Japan). Animals were fixed on a MRI bed (Signa Explorer 1.5T, GE Healthcare, Milwaukee, WI), and serial coronal images (6 mm thickness, vertical plane against orbitomeatal line) were be obtained. All imaging sequences consisted of the following:

- Diffusion-weighted imaging (DWI);
- Arterial spin labeling (ASL);
- Apparent diffusion coefficient (ADC);
- T1;
- Fluid-attenuated inversion-recovery (FLAIR); and
- T2-weighted imaging.

[00300] For the MRI conducted at the 8 hr. time point, in order to visualize blood brain barrier (BBB) disruption following treatment, contrast-enhanced T1-weighted imaging consisted of T1-weighted whole brain volume acquired before and about 5 min. after intravenous injection of gadobutrol contrast agent (1.0 mmol/mL, 0.2 mL/kg; Gadovist; Bayer HealthCare Pharmaceuticals, Osaka, Japan). All animals received a dose volume of 0.2 mL/kg.

[00301] Apparent diffusion coefficient (ADC) maps, cerebral blood flow (CBF), and perfusion deficit was generated with software (READY View, GE Healthcare) available on the MR scanner console for all MRIs done in the study.

[00302] The lesion volume (mm^3) was calculated as the sum of the infarct area of each section and thickness (6 mm). Lesion volumes were calculated in cerebral cortex, white matter and basal ganglia and the total lesion volume was the sum of these volumes.

[00303] The area of BBB disruption (mm^2) was delineated from each contrast-enhanced T1-weighted image using OsiriX MD version 12.0.0. BBB disruption was manually delineate using trace contrast-enhanced T1-weighted images. The volume (mm^3) of BBB disruption was calculated as the sum of the areas of BBB disruption of each section (6 mm thickness).

[00304] Dosage

[00305] All animals received one (1) dose of 1 ml/kg thirty minutes prior to release of the clips (or 3.5 hours after clip placement) and an additional four doses separated by 24 hours for a total of five (5) doses via Intravenous (i.v.) slow push over approximately 30 seconds. Placebo corresponds to vehicle alone (20 mM Tris, 7.5% w/v Sucrose, 0.02% w/v polysorbate 80, pH 7.5).

[00306] All animals received a total of five (5) doses. The dosage regimen is shown the table below. The dose 16 mg/kg corresponds to 16 mg/ml and the dose 4 mg/kg correspond to 4 mg/ml of the chimeric protein. The interval between administrations was no less than 20 hours and no more than 28 hours. Any instances where the interval between administrations falls outside of this range (20 - 28 hours) was reported.

Placebo (n = 12)	Day:	1	2	3	4	5
	Treatment:	placebo	placebo	placebo	placebo	placebo
Divided Dose (n = 10)	Day:	1	2	3	4	5
	Treatment:	16 mg/kg scp776	4 mg/kg scp776	4 mg/kg scp776	4 mg/kg scp776	4 mg/kg scp776

[00307] Dextrose supplementation

[00308] In some embodiments, the animals being treated are continuously infused at a rate of 0.5 mL/kg/hr with dextrose to sustain euglycemia. Dextrose solution can comprise between about 5% to about 50% (w/v) dextrose in sterile water or saline solution.

[00309] NDS, MRI, and Survival Results Summary

[00310] Animals were assessed using NDS scoring: The four systems for the NDS scoring system are Motor, Consciousness, Musculoskeletal coordination, and Sensory. The Total NDS score is the sum of the four systems.

[00311] Treatment with scp776 significantly improves neurologic function in three of the four systems that are assessed in the NDS scoring when compared with treatment with placebo.

- Significant improvements to Consciousness were observed at day 3 (not shown) and day 7 (see FIG. 11B).
- Significant improvements to Motor System were observed at day 2 (see FIG. 11A).

- Significant improvements to Musculoskeletal Coordination were observed at day 1, 2, 3, 7, 10, and 14 (See FIG. 11C).
- Improvement to total NDS score were observed at all time points. Significant improvements to total NDS score were observed at day 1 and day 3 (see FIG. 11D).

[00312] At all time points evaluated, treatment with scp776 resulted in a smaller lesion size or area than treatment with placebo (see FIG. 12).

- The reduction in lesion volume achieved statistical significance in the cerebral cortex and total lesion volumes at 8 hrs.
- Total lesion volume was significantly reduced at 72 hours by scp776 treatment.
- The volume of lesion in the cerebral cortex was significantly reduced by scp776 treatment at 14 days.

[00313] Scp776 treatment resulted in a statistically significant survival benefit. In the placebo group, 50% of the animals died as a result of the stroke injury, while only 10% of animals that received scp776 died prior to study completion. This survival difference, when assessed by the log rank method and chi-squared distribution, reveals a p-value of 0.039 (Bewick et al. Critical Care 2004, 8:389-394).

Example 4 Treatment of Parkinson's Disease (PD), Amyotrophic lateral sclerosis (ALS), and Alzheimer's Disease (AD)

[00314] Parkinson's Disease (PD), Amyotrophic lateral sclerosis (ALS), and Alzheimer's Disease (AD) are characterized by progressive degeneration of the central or peripheral nervous systems. Each of these diseases is associated with impaired musculoskeletal coordination and motor system control. The model of Acute Ischemic Stroke in the non-human primates induces significant disfunction in both musculoskeletal coordination and motor control. The neuroprotectant activity of scp776 significantly reduces these disfunctions. The neuroprotectant activity of scp776 is independent of the type of insult that results in injured tissue. In chronic neurologic diseases, brain tissue is also injured and undergoes similar distress at the cellular level. Taken together, these results and the mode of disease progression, indicate that if scp776 were to be administered during symptomatic progression, the neuroprotective outcome observed in the model of acute ischemic stroke would translate to the chronic disease setting.

[00315] Experiments are conducted to examine the effect of scp776 on the treatment of Parkinson disease. Scp776 is dosed from about 0.01 mg/kg to about 20 mg/kg as often as daily.

Administration results in improvements of, for example but not limited to, the measures of motor function and decrease in neurodegenerative symptoms, such as decreases of tremors.

[00316] Experiments are conducted to examine the effect of scp776 on the treatment of Amyotrophic lateral sclerosis (ALS). Scp776 is dosed from about 0.01 mg/kg to about 20 mg/kg as often as daily. Administration results in improvements of, for example but not limited to, measures of motor function and decreases on ALS symptoms such as autonomic dysfunction.

[00317] Experiments are conducted to examine the effect of scp776 on the treatment of Alzheimer's Disease (AD). Scp776 is dosed from about 0.01 mg/kg to about 20 mg/kg as often as daily. Administration results in improvements of, for example but not limited to, measures of cognitive function and decreases on AD symptoms such as memory loss and confusion.

[00318] Specific examples of compositions, methods and kits have been described herein for purposes of illustration. These are only examples. The technology provided herein can be applied to systems other than the example systems described above. Many alterations, modifications, additions, omissions, and permutations are possible within the practice of this invention. This disclosure includes variations on described embodiments that would be apparent to the skilled addressee, including variations obtained by: replacing features, elements and/or acts with equivalent features, elements and/or acts; mixing and matching of features, elements and/or acts from different embodiments; combining features, elements and/or acts from embodiments as described herein with features, elements and/or acts of other technology; and/or omitting combining features, elements and/or acts from described embodiments.

INCORPORATION BY REFERENCE

[00319] All publications, patents and sequence database entries mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A method of treating acute central nervous system injury, the method comprising administering by bolus injection to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein and a pharmaceutically acceptable carrier,

wherein the chimeric protein comprises (a) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding to R63, K70, K101, E138, D139, N160, and combinations thereof; (b) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (c) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof,

wherein administration results in at least one of: mitigation of oxidative damage to cells of the cerebral cortex, repair or acceleration of repair of blood brain barrier, reduction of oedema, reduction of infarct volume, reduction of blood brain barrier permeability, targeted stimulation of the phosphorylation of serine/threonine protein kinase B (AKT) pathway by selective activation of the IGF-1 receptor in cells of injured brain tissue, targeted delivery of pro-survival signals to injured brain tissue, increase in musculoskeletal coordination following stroke, improvement to consciousness following stroke, improvement to neurologic function following stroke, and improvement in motor function following stroke.

2. The method of claim 1, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild type IGF-1.

3. The method of claim 1, wherein the variant of IGF-1 comprises E3R and Y31A substitutions relative to wild type human IGF-1.

4. The method of claim 1, wherein the variant of human Annexin 5 comprises the amino acids 2-320 corresponding to wild type human Annexin 5 and comprises R63A, K70A, K101A, E138A, D139G, N160A, C316A substitutions relative to wild type human Annexin 5.

5. The method of claim 1, wherein the variant of human serum albumin comprises the amino acids 26-609 corresponding to wild type human serum albumin and comprises C58S and N527Q substitutions relative to wild type human serum albumin.

6. The method of claim 1, wherein the chimeric protein is IGF1(E3R/Y31A)_lk7_HSA26-609(C58S/N527Q)_lk7_AnxV2-320(R63A/K70A/K101A/E138A/D139G/N160A/C316A).

7. The method of claim 6, wherein the linker lk7 comprises -Gly-Ser-Gly-Gly-Gly-Ser-Gly.

8. The method of claim 1, wherein the chimeric protein is selectively targeted to cells comprising a target molecule phosphatidylserine and wherein the chimeric protein exhibits activation of the IGF-1 receptor at least twice as strong on cells containing the target molecule compared to cells that do not contain the target molecule as measured by phosphorylation of serine/threonine protein kinase B (AKT).

9. The method of any of claims 1-8, wherein the acute central nervous system injury is an ischemic stroke, traumatic brain injury, hemorrhagic stroke, acquired brain injury, spinal cord injury, subarachnoid hemorrhage, or is iatrogenic in nature.

10. The method of any of claims 1-8, comprising administering within 72 hours of diagnosis of the acute CNS injury the pharmaceutical composition to the subject in need thereof.

11. The method of any of claims 1-8, comprising administering daily from about 0.01 mg/kg to about 20 mg/kg of the chimeric protein to the subject in need thereof.

12. The method of any of claims 1-8, comprising administering a total dose of from about 5 mg/kg to about 20 mg/kg of the chimeric protein to the subject in need thereof over a period of 4 to 7 days.

13. The method of any of claims 1-8, comprising administering descending doses of the chimeric protein to the subject in need thereof.

14. The method of claim 13, comprising administering to the subject in need thereof a first dose comprising from about 2 mg/kg to about 6 mg/kg of the chimeric protein on day 1, and a dose comprising from about 1 mg/kg to about 2 mg/kg one each of the following days.

15. The method of any of claims 1-8, comprising administering intravenously, intraarterially, or intrathecally the pharmaceutical composition.

16. A kit for practicing the method of claim 1, the kit comprising a plurality of individual containers, each individual container comprising about 20 mg to about 1,000 mg of the chimeric protein.

17. A kit for treating acute central nervous system injury, the kit comprising:

(a) a plurality of individual containers, each individual container comprising a volume of a pharmaceutical composition comprising a therapeutically effective amount of a chimeric protein and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is formulated for bolus injection,

wherein the chimeric protein comprises (i) a targeting domain comprising a variant of human Annexin 5 (AnxV) comprising one or more mutations, wherein the one or more mutations consist of a substitution at the position corresponding to C316 and optionally at one or more positions corresponding to R63, K70, K101, E138, D139, N160, and combinations thereof; (ii) an activator domain comprising a variant of human insulin-like growth factor IGF-1 comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to E3, Y24, Y31, Y60, and combinations thereof, wherein the variant of IGF-1 decreases activation of the IGF-1 receptor relative to the wild-type IGF-1, and (iii) a half-life modulator comprising a variant of human serum albumin (HSA) comprising one or more mutations, wherein the one or more mutations consist of a substitution at one or more positions corresponding to C58 and N527, and combinations thereof,

wherein each of the plurality of the individual containers comprises a volume ranging from about 1 ml to about 50 ml,

wherein each of the plurality of the individual containers comprises an amount of chimeric protein ranging from about 20 mg to about 1,000 mg; and

(b) instructions for use in treating acute central nervous system injury.

18. The kit of claim 17, wherein the plurality of containers comprises a total dose from about 5 mg/kg to about 20 mg/kg.

19. The kit of claim 17, wherein the instructions for use comprise instructions to administer intravenously or intrathecally the pharmaceutical composition over a period of 4 to 7 days.

20. The kit of claim 17, wherein the instructions for use comprise instructions to administer intravenously, intraarterially, or intrathecally descending doses of the chimeric protein.

FIG. 1

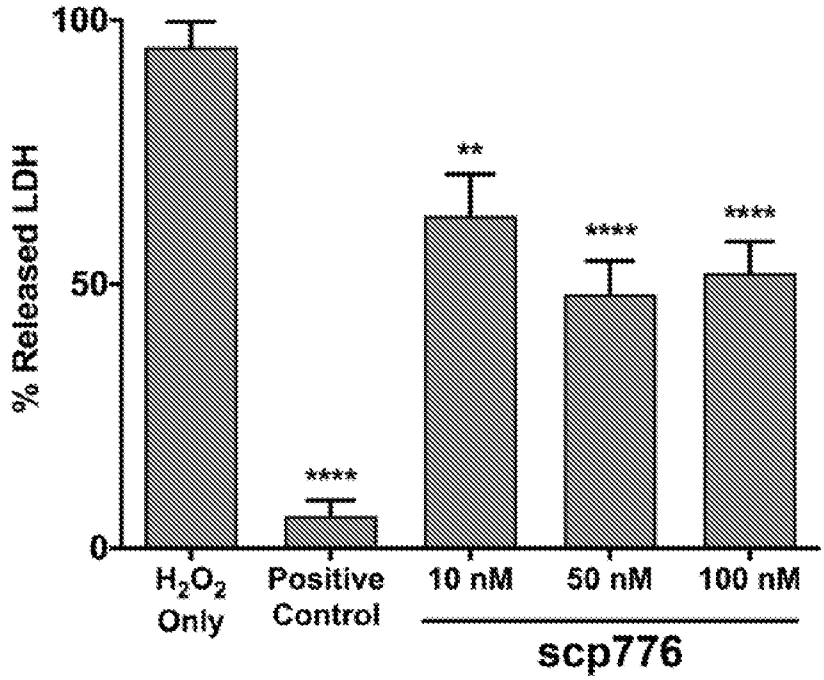

100
50
0
% Released LDH
**
****
****
****
H2O2 Only
Positive Control
10 nM
50 nM
100 nM
scp776

FIG. 2

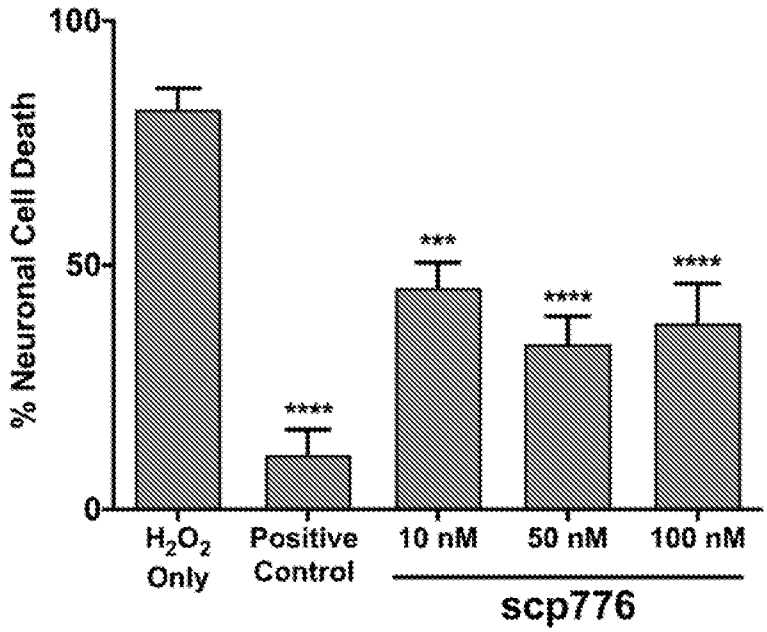

100
50
0
% Neuronal Cell Death
***
****
****
****
H2O2 Only
Positive Control
10 nM
50 nM
100 nM
scp776

FIG. 3

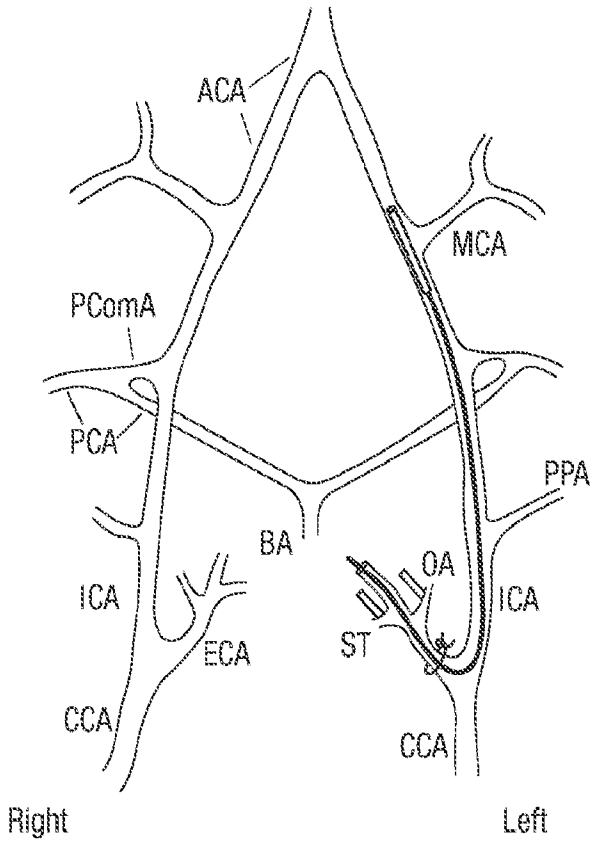

ACA
MCA
PComA
PCA
PPA
BA
OA
ICA
ICA
ECA
ST
CCA
CCA
Right
Left

FIG. 4

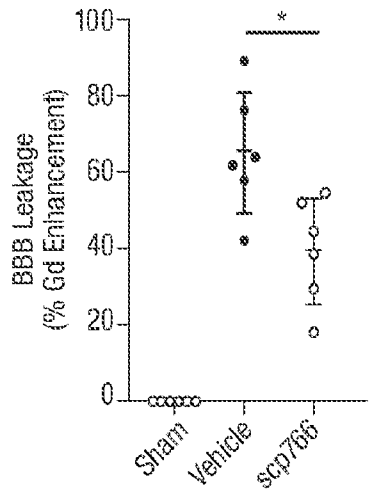

100
80
60
40
20
0
BBB Leakage
(% Gd Enhancement)
*
Sham
Vehicle
scp766

FIG. 5

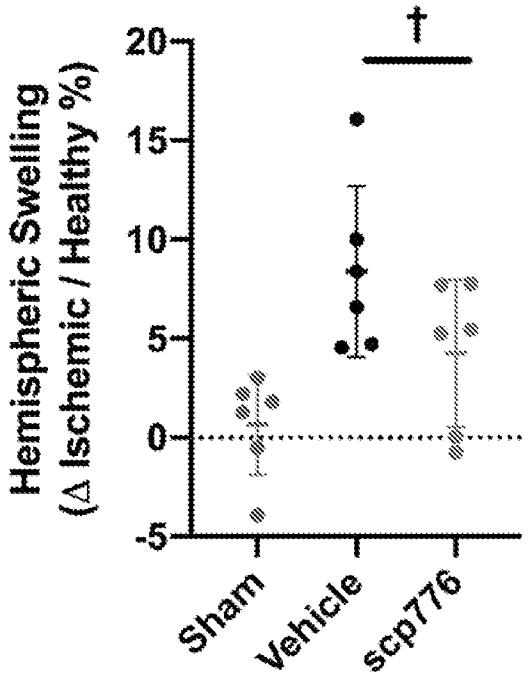

Hemispheric Swelling
(Δ Ischemic / Healthy %)
20
15
10
5
0
-5
†
Sham
Vehicle
scp776

FIG. 6

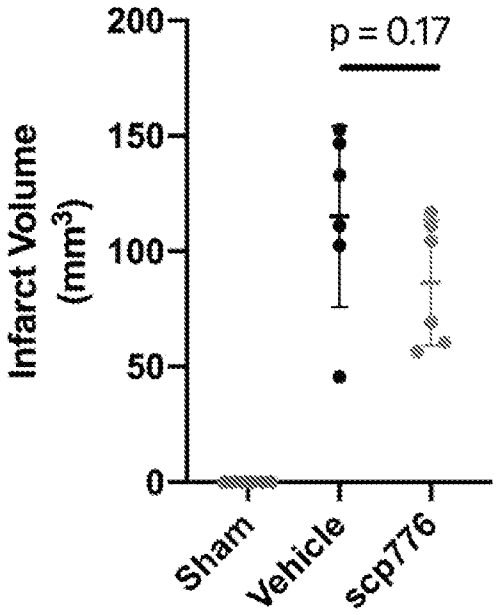

Infarct Volume
(mm3)
200
150
100
50
0
p = 0.17
Sham
Vehicle
scp776

FIG. 7

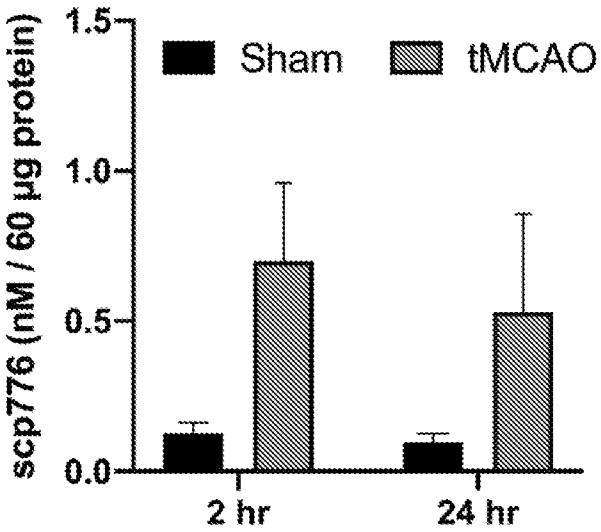

scp776 (nM / 60 µg protein)
1.5
1.0
0.5
0.0
Sham
tMCAO
2 hr
24 hr

FIG. 8

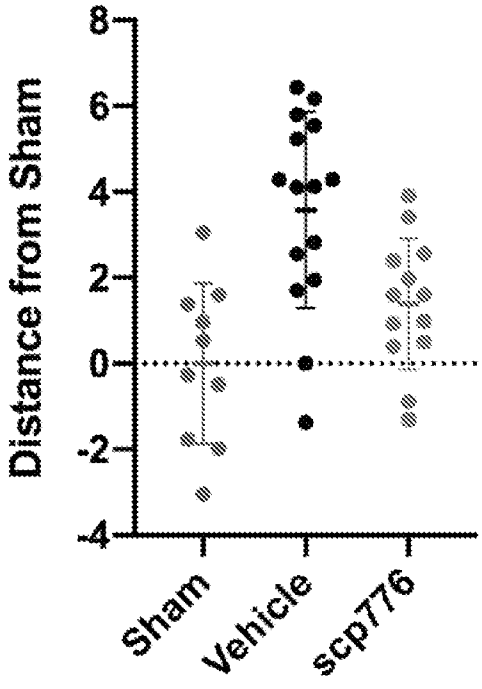

Distance from Sham
8
6
4
2
0
-2
-4
Sham
Vehicle
scp776

FIG. 9

GPETLCGAEL VDALQFVCGD RGFYFNKPTG YGSSSRRAPQ TGIVDECCFR SCDLRRLEMY CAPLKPAKSA (SEQ ID NO: 1)

XXXXLCXXXL XXXLXXXCXX XXFXFXXXXX XXXXXXXXXX XXIXXXCCXX XCXLXXLXXX CXXXXXXXXX

FIG. 10

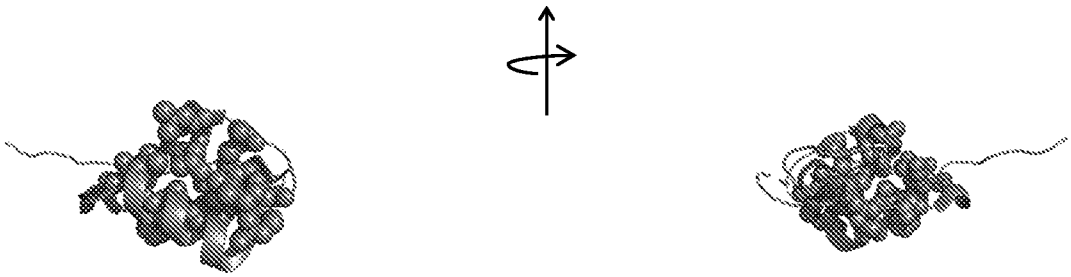


FIG. 11A

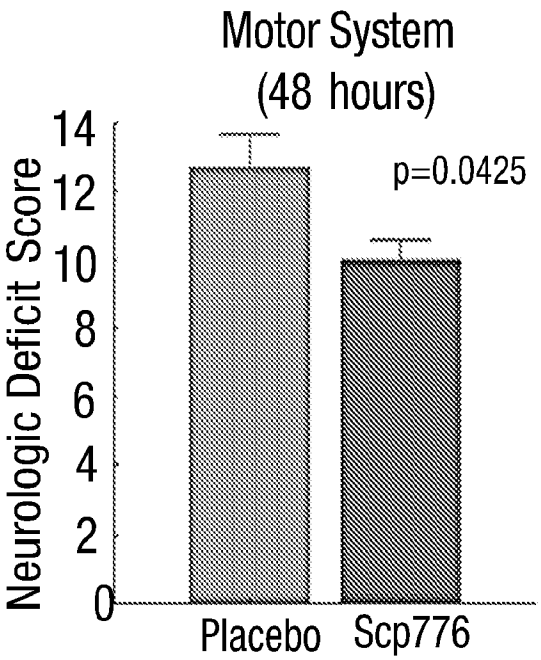


FIG. 11B

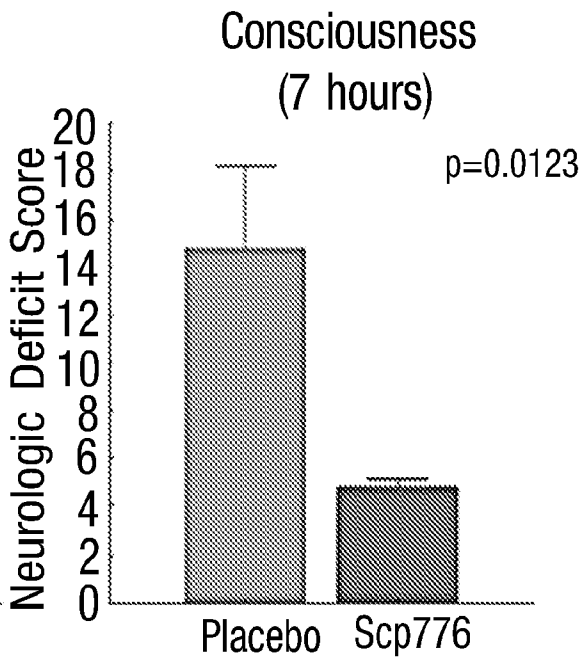


FIG. 11C

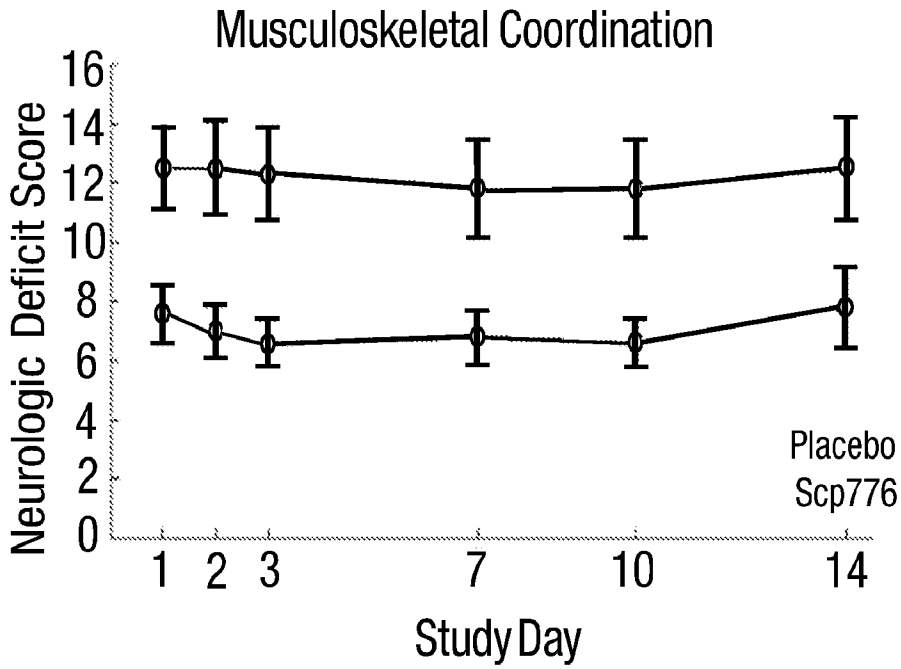
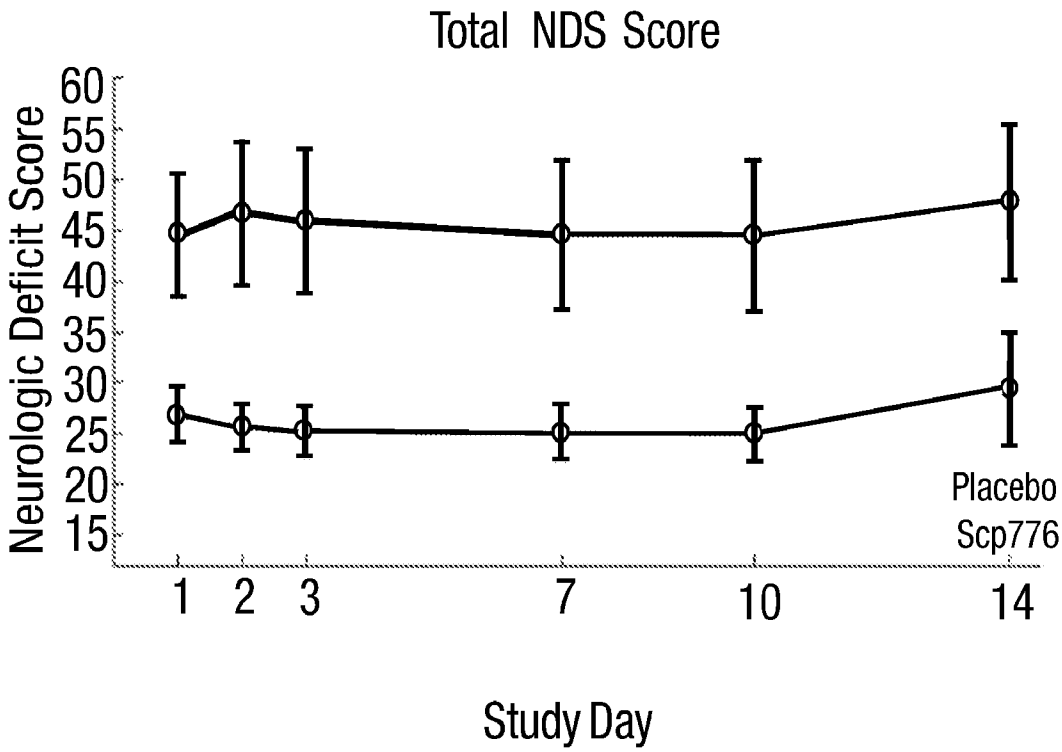


FIG. 11D


Total NDS Score
Neurologic Deficit Score
60
55
50
45
40
35
30
25
20
15
1
2
3
7
10
14
Placebo
Scp776
Study Day

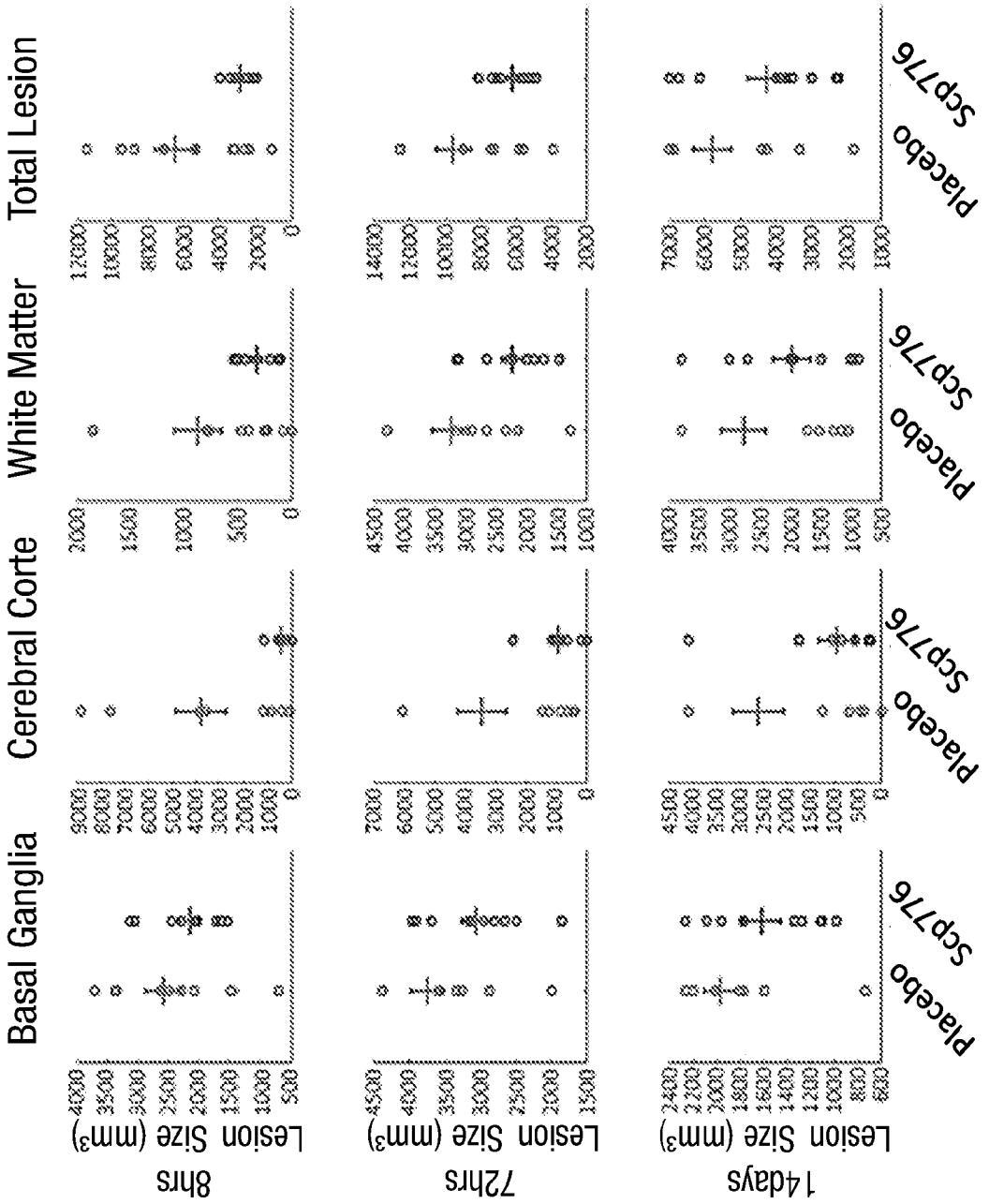

Basal Ganglia
Cerebral Corte
White Matter
Total Lesion
8hrs
72hrs
14days
Lesion Size (mm3)
Placebo
Scp776

FIG. 12

SEQUENCE LISTING

<110> Silver Creek Pharmaceuticals, Inc.

<120> CHIMERIC PROTEINS AND METHODS OF USE FOR TREATMENT OF CENTRAL NERVOUS SYSTEM DISORDERS

<130> 132463-010401

<150> 63/058,888
<151> 2020-07-30

<160> 25

<170> PatentIn version 3.5

<210> 1
<211> 70
<212> PRT
<213> Homo sapiens

<400> 1

Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60

Lys Pro Ala Lys Ser Ala
65 70

<210> 2
<211> 70
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 2

Gly Pro Arg Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Ala Gly
20 25 30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60

Lys Pro Ala Lys Ser Ala
65 70

<210> 3
<211> 83
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 3

Met Phe Pro Ala Met Pro Leu Ser Ser Leu Phe Val Asn Gly Pro Glu
1 5 10 15

Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
20 25 30

Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
35 40 45

Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
50 55 60

Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
65 70 75 80

Lys Ser Ala

<210> 4
<211> 70
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 4

Gly Pro Arg Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1 5 10 15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
20 25 30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
35 40 45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
50 55 60

Lys Pro Ala Lys Ser Ala
65 70

<210> 5
<211> 67
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 5

Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
1   5   10   15

Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
            20   25   30

Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser
        35   40   45

Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
    50   55   60

Lys Ser Ala Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
65   1   5   10

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
    15   20   25

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
30   35   40   45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
                50   55   60

Lys Pro Ala Lys Ser Ala
            65

<210> 6
<211> 83
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 6

Met Phe Pro Ala Met Pro Leu Ser Ser Leu Phe Val Asn Gly Pro Arg
1   5   10   15

Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe Val Cys Gly
            20   25   30

Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly Ser Ser Ser
        35   40   45

Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe Arg Ser

```
    50                  55                  60

Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys Pro Ala
65                  70                  75                  80

Lys Ser Ala
```

<210> 7
<211> 69
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 7

```
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1               5                   10                  15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30

Ser Ser Ser Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys Phe
        35                  40                  45

Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu Lys
    50                  55                  60

Pro Ala Lys Ser Ala
65
```

<210> 8
<211> 67
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 8

```
Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1               5                   10                  15

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
            20                  25                  30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60

Lys Pro Ala
65
```

<210> 9
<211> 319
<212> PRT
<213> Homo sapiens

<400> 9

```
Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp Glu
1               5                   10                  15


Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr
            20                  25                  30


Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala Gln
        35                  40                  45


Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Arg Asp Leu
    50                  55                  60


Leu Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile
65                  70                  75                  80


Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys
                85                  90                  95


His Ala Leu Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu Ile
            100                 105                 110


Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val Tyr
        115                 120                 125


Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp Val Val Gly Asp Thr
    130                 135                 140


Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn Arg
145                 150                 155                 160


Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala Gln
                165                 170                 175


Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys
            180                 185                 190


Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val
        195                 200                 205


Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile
    210                 215                 220


Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val
225                 230                 235                 240
```

```
Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr
                245                 250                 255

Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Met
            260                 265                 270

Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg
        275                 280                 285

Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser
    290                 295                 300

Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Glu Asp Asp
305                 310                 315
```

<210> 10
<211> 319
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 10

```
Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp Glu
1               5                   10                  15

Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr
            20                  25                  30

Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala Gln
        35                  40                  45

Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Ala Asp Leu
    50                  55                  60

Leu Asp Asp Leu Ala Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile
65                  70                  75                  80

Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys
                85                  90                  95

His Ala Leu Ala Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu Ile
            100                 105                 110

Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val Tyr
        115                 120                 125

Glu Glu Glu Tyr Gly Ser Ser Leu Ala Gly Asp Val Val Gly Asp Thr
    130                 135                 140

Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Ala Arg
145                 150                 155                 160
```

```
Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala Gln
                165                 170                 175


Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys
            180                 185                 190


Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val
        195                 200                 205


Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile
    210                 215                 220


Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val
225                 230                 235                 240


Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr
                245                 250                 255


Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Met
            260                 265                 270


Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg
        275                 280                 285


Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser
    290                 295                 300


Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Ala Gly Glu Asp Asp
305                 310                 315


<210>  11
<211>  319
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic construct

<400>  11

Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp Glu
1               5                   10                  15


Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr
            20                  25                  30


Asp Glu Glu Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala Gln
        35                  40                  45


Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Ala Asp Leu
    50                  55                  60


Leu Asp Asp Leu Ala Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile
65                  70                  75                  80
```

```
Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys
                85                  90                  95

His Ala Leu Ala Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu Ile
            100                 105                 110

Ile Ala Ser Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val Tyr
        115                 120                 125

Glu Glu Glu Tyr Gly Ser Ser Leu Ala Gly Asp Val Val Gly Asp Thr
    130                 135                 140

Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Ala Arg
145                 150                 155                 160

Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala Gln
                165                 170                 175

Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys
            180                 185                 190

Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val
        195                 200                 205

Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile
    210                 215                 220

Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val
225                 230                 235                 240

Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr
                245                 250                 255

Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Met
            260                 265                 270

Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg
        275                 280                 285

Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser
    290                 295                 300

Gly Asp Tyr Lys Lys Ala Leu Leu Leu Leu Cys Gly Glu Asp Asp
305                 310                 315
```

<210> 12
<211> 585
<212> PRT
<213> Homo sapiens

<400> 12

```
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1               5                   10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
```

```
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560
```

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
565 570 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
580 585

<210> 13
<211> 585
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 13

Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1 5 10 15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20 25 30

Gln Ser Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
115 120 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
130 135 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145 150 155 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
165 170 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
180 185 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu

```
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480
```

```
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Gln Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
```

<210> 14
<211> 585
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 14

```
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1               5                   10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Ser Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
```

```
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400
```

```
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Gln Ala Gly Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Ala Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
```

<210> 15
<211> 585
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 15

```
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1               5                   10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Ser Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
```

```
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320
```

```
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Gln Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
```

<210> 16
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 16

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1   5   10   15

Gly Gly Gly Ser
            20

<210> 17
<211> 698
<212> PRT
<213> Homo sapiens

<400> 17

Met Arg Leu Ala Val Gly Ala Leu Leu Val Cys Ala Val Leu Gly Leu
1   5   10   15

Cys Leu Ala Val Pro Asp Lys Thr Val Arg Trp Cys Ala Val Ser Glu
            20   25   30

His Glu Ala Thr Lys Cys Gln Ser Phe Arg Asp His Met Lys Ser Val
        35   40   45

Ile Pro Ser Asp Gly Pro Ser Val Ala Cys Val Lys Lys Ala Ser Tyr
    50   55   60

Leu Asp Cys Ile Arg Ala Ile Ala Ala Asn Glu Ala Asp Ala Val Thr
65   70   75   80

Leu Asp Ala Gly Leu Val Tyr Asp Ala Tyr Leu Ala Pro Asn Asn Leu
                85   90   95

Lys Pro Val Val Ala Glu Phe Tyr Gly Ser Lys Glu Asp Pro Gln Thr
            100   105   110

Phe Tyr Tyr Ala Val Ala Val Val Lys Lys Asp Ser Gly Phe Gln Met
        115   120   125

Asn Gln Leu Arg Gly Lys Lys Ser Cys His Thr Gly Leu Gly Arg Ser
    130   135   140

Ala Gly Trp Asn Ile Pro Ile Gly Leu Leu Tyr Cys Asp Leu Pro Glu
145   150   155   160

Pro Arg Lys Pro Leu Glu Lys Ala Val Ala Asn Phe Phe Ser Gly Ser
                165   170   175

```
Cys Ala Pro Cys Ala Asp Gly Thr Asp Phe Pro Gln Leu Cys Gln Leu
            180                 185                 190

Cys Pro Gly Cys Gly Cys Ser Thr Leu Asn Gln Tyr Phe Gly Tyr Ser
        195                 200                 205

Gly Ala Phe Lys Cys Leu Lys Asp Gly Ala Gly Asp Val Ala Phe Val
    210                 215                 220

Lys His Ser Thr Ile Phe Glu Asn Leu Ala Asn Lys Ala Asp Arg Asp
225                 230                 235                 240

Gln Tyr Glu Leu Leu Cys Leu Asp Asn Thr Arg Lys Pro Val Asp Glu
                245                 250                 255

Tyr Lys Asp Cys His Leu Ala Gln Val Pro Ser His Thr Val Val Ala
            260                 265                 270

Arg Ser Met Gly Gly Lys Glu Asp Leu Ile Trp Glu Leu Leu Asn Gln
        275                 280                 285

Ala Gln Glu His Phe Gly Lys Asp Lys Ser Lys Glu Phe Gln Leu Phe
    290                 295                 300

Ser Ser Pro His Gly Lys Asp Leu Leu Phe Lys Asp Ser Ala His Gly
305                 310                 315                 320

Phe Leu Lys Val Pro Pro Arg Met Asp Ala Lys Met Tyr Leu Gly Tyr
                325                 330                 335

Glu Tyr Val Thr Ala Ile Arg Asn Leu Arg Glu Gly Thr Cys Pro Glu
            340                 345                 350

Ala Pro Thr Asp Glu Cys Lys Pro Val Lys Trp Cys Ala Leu Ser His
        355                 360                 365

His Glu Arg Leu Lys Cys Asp Glu Trp Ser Val Asn Ser Val Gly Lys
    370                 375                 380

Ile Glu Cys Val Ser Ala Glu Thr Thr Glu Asp Cys Ile Ala Lys Ile
385                 390                 395                 400

Met Asn Gly Glu Ala Asp Ala Met Ser Leu Asp Gly Gly Phe Val Tyr
                405                 410                 415

Ile Ala Gly Lys Cys Gly Leu Val Pro Val Leu Ala Glu Asn Tyr Asn
            420                 425                 430

Lys Ser Asp Asn Cys Glu Asp Thr Pro Glu Ala Gly Tyr Phe Ala Ile
        435                 440                 445
```

```
Ala Val Val Lys Lys Ser Ala Ser Asp Leu Thr Trp Asp Asn Leu Lys
    450                 455                 460

Gly Lys Lys Ser Cys His Thr Ala Val Gly Arg Thr Ala Gly Trp Asn
465                 470                 475                 480

Ile Pro Met Gly Leu Leu Tyr Asn Lys Ile Asn His Cys Arg Phe Asp
                485                 490                 495

Glu Phe Phe Ser Glu Gly Cys Ala Pro Gly Ser Lys Lys Asp Ser Ser
            500                 505                 510

Leu Cys Lys Leu Cys Met Gly Ser Gly Leu Asn Leu Cys Glu Pro Asn
        515                 520                 525

Asn Lys Glu Gly Tyr Tyr Gly Tyr Thr Gly Ala Phe Arg Cys Leu Val
    530                 535                 540

Glu Lys Gly Asp Val Ala Phe Val Lys His Gln Thr Val Pro Gln Asn
545                 550                 555                 560

Thr Gly Gly Lys Asn Pro Asp Pro Trp Ala Lys Asn Leu Asn Glu Lys
                565                 570                 575

Asp Tyr Glu Leu Leu Cys Leu Asp Gly Thr Arg Lys Pro Val Glu Glu
            580                 585                 590

Tyr Ala Asn Cys His Leu Ala Arg Ala Pro Asn His Ala Val Val Thr
        595                 600                 605

Arg Lys Asp Lys Glu Ala Cys Val His Lys Ile Leu Arg Gln Gln Gln
    610                 615                 620

His Leu Phe Gly Ser Asn Val Thr Asp Cys Ser Gly Asn Phe Cys Leu
625                 630                 635                 640

Phe Arg Ser Glu Thr Lys Asp Leu Leu Phe Arg Asp Asp Thr Val Cys
                645                 650                 655

Leu Ala Lys Leu His Asp Arg Asn Thr Tyr Glu Lys Tyr Leu Gly Glu
            660                 665                 670

Glu Tyr Val Lys Ala Val Gly Asn Leu Arg Lys Cys Ser Thr Ser Ser
        675                 680                 685

Leu Leu Glu Ala Cys Thr Phe Arg Arg Pro
    690                 695

<210>  18
<211>  609
<212>  PRT
<213>  Homo sapiens
```

<400> 18

```
Met Lys Trp Val Glu Ser Ile Phe Leu Ile Phe Leu Leu Asn Phe Thr
1               5                   10                  15

Glu Ser Arg Thr Leu His Arg Asn Glu Tyr Gly Ile Ala Ser Ile Leu
            20                  25                  30

Asp Ser Tyr Gln Cys Thr Ala Glu Ile Ser Leu Ala Asp Leu Ala Thr
        35                  40                  45

Ile Phe Phe Ala Gln Phe Val Gln Glu Ala Thr Tyr Lys Glu Val Ser
    50                  55                  60

Lys Met Val Lys Asp Ala Leu Thr Ala Ile Glu Lys Pro Thr Gly Asp
65                  70                  75                  80

Glu Gln Ser Ser Gly Cys Leu Glu Asn Gln Leu Pro Ala Phe Leu Glu
                85                  90                  95

Glu Leu Cys His Glu Lys Glu Ile Leu Glu Lys Tyr Gly His Ser Asp
            100                 105                 110

Cys Cys Ser Gln Ser Glu Glu Gly Arg His Asn Cys Phe Leu Ala His
        115                 120                 125

Lys Lys Pro Thr Pro Ala Ser Ile Pro Leu Phe Gln Val Pro Glu Pro
    130                 135                 140

Val Thr Ser Cys Glu Ala Tyr Glu Glu Asp Arg Glu Thr Phe Met Asn
145                 150                 155                 160

Lys Phe Ile Tyr Glu Ile Ala Arg Arg His Pro Phe Leu Tyr Ala Pro
                165                 170                 175

Thr Ile Leu Leu Trp Ala Ala Arg Tyr Asp Lys Ile Ile Pro Ser Cys
            180                 185                 190

Cys Lys Ala Glu Asn Ala Val Glu Cys Phe Gln Thr Lys Ala Ala Thr
        195                 200                 205

Val Thr Lys Glu Leu Arg Glu Ser Ser Leu Leu Asn Gln His Ala Cys
    210                 215                 220

Ala Val Met Lys Asn Phe Gly Thr Arg Thr Phe Gln Ala Ile Thr Val
225                 230                 235                 240

Thr Lys Leu Ser Gln Lys Phe Thr Lys Val Asn Phe Thr Glu Ile Gln
                245                 250                 255

Lys Leu Val Leu Asp Val Ala His Val His Glu His Cys Cys Arg Gly
            260                 265                 270
```

```
Asp Val Leu Asp Cys Leu Gln Asp Gly Glu Lys Ile Met Ser Tyr Ile
        275                 280                 285

Cys Ser Gln Gln Asp Thr Leu Ser Asn Lys Ile Thr Glu Cys Cys Lys
    290                 295                 300

Leu Thr Thr Leu Glu Arg Gly Gln Cys Ile Ile His Ala Glu Asn Asp
305                 310                 315                 320

Glu Lys Pro Glu Gly Leu Ser Pro Asn Leu Asn Arg Phe Leu Gly Asp
                325                 330                 335

Arg Asp Phe Asn Gln Phe Ser Ser Gly Glu Lys Asn Ile Phe Leu Ala
            340                 345                 350

Ser Phe Val His Glu Tyr Ser Arg Arg His Pro Gln Leu Ala Val Ser
        355                 360                 365

Val Ile Leu Arg Val Ala Lys Gly Tyr Gln Glu Leu Leu Glu Lys Cys
    370                 375                 380

Phe Gln Thr Glu Asn Pro Leu Glu Cys Gln Asp Lys Gly Glu Glu Glu
385                 390                 395                 400

Leu Gln Lys Tyr Ile Gln Glu Ser Gln Ala Leu Ala Lys Arg Ser Cys
                405                 410                 415

Gly Leu Phe Gln Lys Leu Gly Glu Tyr Tyr Leu Gln Asn Ala Phe Leu
            420                 425                 430

Val Ala Tyr Thr Lys Lys Ala Pro Gln Leu Thr Ser Ser Glu Leu Met
        435                 440                 445

Ala Ile Thr Arg Lys Met Ala Ala Thr Ala Ala Thr Cys Cys Gln Leu
    450                 455                 460

Ser Glu Asp Lys Leu Leu Ala Cys Gly Glu Gly Ala Ala Asp Ile Ile
465                 470                 475                 480

Ile Gly His Leu Cys Ile Arg His Glu Met Thr Pro Val Asn Pro Gly
                485                 490                 495

Val Gly Gln Cys Cys Thr Ser Ser Tyr Ala Asn Arg Arg Pro Cys Phe
            500                 505                 510

Ser Ser Leu Val Val Asp Glu Thr Tyr Val Pro Pro Ala Phe Ser Asp
        515                 520                 525

Asp Lys Phe Ile Phe His Lys Asp Leu Cys Gln Ala Gln Gly Val Ala
    530                 535                 540
```

```
Leu Gln Thr Met Lys Gln Glu Phe Leu Ile Asn Leu Val Lys Gln Lys
545                 550                 555                 560


Pro Gln Ile Thr Glu Glu Gln Leu Glu Ala Val Ile Ala Asp Phe Ser
                565                 570                 575


Gly Leu Leu Glu Lys Cys Cys Gln Gly Gln Glu Gln Glu Val Cys Phe
            580                 585                 590


Ala Glu Glu Gly Gln Lys Leu Ile Ser Lys Thr Arg Ala Ala Leu Gly
        595                 600                 605


Val
```

<210> 19
<211> 474
<212> PRT
<213> Homo sapiens

<400> 19

```
Met Lys Arg Val Leu Val Leu Leu Leu Ala Val Ala Phe Gly His Ala
1               5                   10                  15


Leu Glu Arg Gly Arg Asp Tyr Glu Lys Asn Lys Val Cys Lys Glu Phe
            20                  25                  30


Ser His Leu Gly Lys Glu Asp Phe Thr Ser Leu Ser Leu Val Leu Tyr
        35                  40                  45


Ser Arg Lys Phe Pro Ser Gly Thr Phe Glu Gln Val Ser Gln Leu Val
    50                  55                  60


Lys Glu Val Val Ser Leu Thr Glu Ala Cys Cys Ala Glu Gly Ala Asp
65                  70                  75                  80


Pro Asp Cys Tyr Asp Thr Arg Thr Ser Ala Leu Ser Ala Lys Ser Cys
                85                  90                  95


Glu Ser Asn Ser Pro Phe Pro Val His Pro Gly Thr Ala Glu Cys Cys
            100                 105                 110


Thr Lys Glu Gly Leu Glu Arg Lys Leu Cys Met Ala Ala Leu Lys His
        115                 120                 125


Gln Pro Gln Glu Phe Pro Thr Tyr Val Glu Pro Thr Asn Asp Glu Ile
    130                 135                 140


Cys Glu Ala Phe Arg Lys Asp Pro Lys Glu Tyr Ala Asn Gln Phe Met
145                 150                 155                 160


Trp Glu Tyr Ser Thr Asn Tyr Gly Gln Ala Pro Leu Ser Leu Leu Val
                165                 170                 175
```

```
Ser Tyr Thr Lys Ser Tyr Leu Ser Met Val Gly Ser Cys Cys Thr Ser
            180                 185                 190

Ala Ser Pro Thr Val Cys Phe Leu Lys Glu Arg Leu Gln Leu Lys His
        195                 200                 205

Leu Ser Leu Leu Thr Thr Leu Ser Asn Arg Val Cys Ser Gln Tyr Ala
    210                 215                 220

Ala Tyr Gly Glu Lys Lys Ser Arg Leu Ser Asn Leu Ile Lys Leu Ala
225                 230                 235                 240

Gln Lys Val Pro Thr Ala Asp Leu Glu Asp Val Leu Pro Leu Ala Glu
                245                 250                 255

Asp Ile Thr Asn Ile Leu Ser Lys Cys Cys Glu Ser Ala Ser Glu Asp
            260                 265                 270

Cys Met Ala Lys Glu Leu Pro Glu His Thr Val Lys Leu Cys Asp Asn
        275                 280                 285

Leu Ser Thr Lys Asn Ser Lys Phe Glu Asp Cys Cys Gln Glu Lys Thr
    290                 295                 300

Ala Met Asp Val Phe Val Cys Thr Tyr Phe Met Pro Ala Ala Gln Leu
305                 310                 315                 320

Pro Glu Leu Pro Asp Val Glu Leu Pro Thr Asn Lys Asp Val Cys Asp
                325                 330                 335

Pro Gly Asn Thr Lys Val Met Asp Lys Tyr Thr Phe Glu Leu Ser Arg
            340                 345                 350

Arg Thr His Leu Pro Glu Val Phe Leu Ser Lys Val Leu Glu Pro Thr
        355                 360                 365

Leu Lys Ser Leu Gly Glu Cys Cys Asp Val Glu Asp Ser Thr Thr Cys
    370                 375                 380

Phe Asn Ala Lys Gly Pro Leu Leu Lys Lys Glu Leu Ser Ser Phe Ile
385                 390                 395                 400

Asp Lys Gly Gln Glu Leu Cys Ala Asp Tyr Ser Glu Asn Thr Phe Thr
                405                 410                 415

Glu Tyr Lys Lys Lys Leu Ala Glu Arg Leu Lys Ala Lys Leu Pro Asp
            420                 425                 430

Ala Thr Pro Thr Glu Leu Ala Lys Leu Val Asn Lys His Ser Asp Phe
        435                 440                 445
```

Ala Ser Asn Cys Cys Ser Ile Asn Ser Pro Pro Leu Tyr Cys Asp Ser
450 455 460

Glu Ile Asp Ala Glu Leu Lys Asn Ile Leu
465 470

<210> 20
<211> 147
<212> PRT
<213> Homo sapiens

<400> 20

Met Ala Ser His Arg Leu Leu Leu Leu Cys Leu Ala Gly Leu Val Phe
1 5 10 15

Val Ser Glu Ala Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu
20 25 30

Met Val Lys Val Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val
35 40 45

Ala Val His Val Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe
50 55 60

Ala Ser Gly Lys Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr
65 70 75 80

Glu Glu Glu Phe Val Glu Gly Ile Tyr Lys Val Glu Ile Asp Thr Lys
85 90 95

Ser Tyr Trp Lys Ala Leu Gly Ile Ser Pro Phe His Glu His Ala Glu
100 105 110

Val Val Phe Thr Ala Asn Asp Ser Gly Pro Arg Arg Tyr Thr Ile Ala
115 120 125

Ala Leu Leu Ser Pro Tyr Ser Tyr Ser Thr Thr Ala Val Val Thr Asn
130 135 140

Pro Lys Glu
145

<210> 21
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 21

Ala Ser Pro Ala Ala Pro Ala Pro Ala Ser Pro Ala Ala Pro Ala Pro
1 5 10 15

Ser Ala Pro Ala
            20

<210> 22
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 22

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Trp Ile Gly Ser Gln
            20   25   30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35   40   45

Met Trp Arg Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Glu Asp Phe Ala Thr Tyr Tyr Cys Ala Gln Gly Ala Ala Leu Pro Arg
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105

<210> 23
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 23

Gly Ser Gly Gly Gly Ser Gly
1   5

<210> 24
<211> 988
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic construct

<400> 24

Gly Pro Arg Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
1   5   10   15

```
Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Ala Gly
            20                  25                  30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60

Lys Pro Ala Lys Ser Ala Gly Ser Gly Gly Gly Ser Gly Asp Ala His
65                  70                  75                  80

Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
                85                  90                  95

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Ser Pro
            100                 105                 110

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
        115                 120                 125

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
    130                 135                 140

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
145                 150                 155                 160

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
                165                 170                 175

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
            180                 185                 190

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
        195                 200                 205

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
    210                 215                 220

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
225                 230                 235                 240

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
                245                 250                 255

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
            260                 265                 270

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
        275                 280                 285
```

```
Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
    290                 295                 300

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
305                 310                 315                 320

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
                325                 330                 335

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
            340                 345                 350

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
        355                 360                 365

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
    370                 375                 380

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
385                 390                 395                 400

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
                405                 410                 415

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
            420                 425                 430

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
        435                 440                 445

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
    450                 455                 460

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
465                 470                 475                 480

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
                485                 490                 495

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
            500                 505                 510

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
        515                 520                 525

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
    530                 535                 540

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
545                 550                 555                 560
```

```
Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
                565                 570                 575

Lys Glu Phe Gln Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
            580                 585                 590

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
        595                 600                 605

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
    610                 615                 620

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
625                 630                 635                 640

Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
                645                 650                 655

Gln Ala Ala Leu Gly Leu Gly Ser Gly Gly Gly Ser Gly Ala Gln Val
            660                 665                 670

Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp Glu Arg Ala Asp
        675                 680                 685

Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp Glu Glu
    690                 695                 700

Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala Gln Arg Gln Glu
705                 710                 715                 720

Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Ala Asp Leu Leu Asp Asp
                725                 730                 735

Leu Ala Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val Ala Leu
            740                 745                 750

Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His Ala Leu
        755                 760                 765

Ala Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu Ile Ile Ala Ser
    770                 775                 780

Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val Tyr Glu Glu Glu
785                 790                 795                 800

Tyr Gly Ser Ser Leu Ala Gly Asp Val Val Gly Asp Thr Ser Gly Tyr
                805                 810                 815

Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Ala Arg Asp Pro Asp
            820                 825                 830

Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala Gln Ala Leu Phe
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        835                 840                 845

Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe Ile Thr
    850                 855                 860

Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val Phe Asp Lys
865                 870                 875                 880

Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile Asp Arg Glu
                885                 890                 895

Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val Lys Ser Ile
            900                 905                 910

Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr Ala Met Lys
        915                 920                 925

Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Met Val Ser Arg
    930                 935                 940

Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys Asn Phe
945                 950                 955                 960

Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly Asp Tyr
                965                 970                 975

Lys Lys Ala Leu Leu Leu Leu Ala Gly Glu Asp Asp
            980                 985


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<211>  988
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic construct

<400>  25

Gly Pro Arg Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
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Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Ala Gly
            20                  25                  30

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
        35                  40                  45

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
    50                  55                  60

Lys Pro Ala Lys Ser Ala Gly Ser Gly Gly Gly Ser Gly Asp Ala His
65                  70                  75                  80
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Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu Glu Asn Phe
                85                  90                  95

Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln Gln Ser Pro
            100                 105                 110

Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu Phe Ala Lys
        115                 120                 125

Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys Ser Leu His
    130                 135                 140

Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu Arg Glu Thr
145                 150                 155                 160

Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro Glu Arg Asn
                165                 170                 175

Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu Pro Arg Leu
            180                 185                 190

Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His Asp Asn Glu
        195                 200                 205

Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg Arg His Pro
    210                 215                 220

Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg Tyr Lys Ala
225                 230                 235                 240

Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala Cys Leu Leu
                245                 250                 255

Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser Ser Ala Lys
            260                 265                 270

Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu Arg Ala Phe
        275                 280                 285

Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro Lys Ala Glu
    290                 295                 300

Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys Val His Thr
305                 310                 315                 320

Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp Arg Ala Asp
                325                 330                 335

Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser Ser Lys Leu
            340                 345                 350

Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His Cys Ile Ala
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        355                 360                 365

Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser Leu Ala Ala
    370                 375                 380

Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala Glu Ala Lys
385                 390                 395                 400

Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg Arg His Pro
                405                 410                 415

Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr Tyr Glu Thr
            420                 425                 430

Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu Cys Tyr Ala
        435                 440                 445

Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro Gln Asn Leu
    450                 455                 460

Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu Tyr Lys Phe
465                 470                 475                 480

Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro Gln Val Ser
                485                 490                 495

Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys Val Gly Ser
            500                 505                 510

Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys Ala Glu Asp
        515                 520                 525

Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His Glu Lys Thr
    530                 535                 540

Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser Leu Val Asn
545                 550                 555                 560

Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr Tyr Val Pro
                565                 570                 575

Lys Glu Phe Gln Ala Glu Thr Phe Thr Phe His Ala Asp Ile Cys Thr
            580                 585                 590

Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala Leu Val Glu
        595                 600                 605

Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu Lys Ala Val
    610                 615                 620

Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys Ala Asp Asp
625                 630                 635                 640
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Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val Ala Ala Ser
                645                 650                 655

Gln Ala Ala Leu Gly Leu Gly Ser Gly Gly Gly Ser Gly Ala Gln Val
            660                 665                 670

Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp Glu Arg Ala Asp
        675                 680                 685

Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly Thr Asp Glu Glu
    690                 695                 700

Ser Ile Leu Thr Leu Leu Thr Ser Arg Ser Asn Ala Gln Arg Gln Glu
705                 710                 715                 720

Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Ala Asp Leu Leu Asp Asp
                725                 730                 735

Leu Ala Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu Ile Val Ala Leu
            740                 745                 750

Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu Lys His Ala Leu
        755                 760                 765

Ala Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu Ile Ile Ala Ser
    770                 775                 780

Arg Thr Pro Glu Glu Leu Arg Ala Ile Lys Gln Val Tyr Glu Glu Glu
785                 790                 795                 800

Tyr Gly Ser Ser Leu Ala Gly Asp Val Val Gly Asp Thr Ser Gly Tyr
                805                 810                 815

Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Ala Arg Asp Pro Asp
            820                 825                 830

Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala Gln Ala Leu Phe
        835                 840                 845

Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu Lys Phe Ile Thr
    850                 855                 860

Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys Val Phe Asp Lys
865                 870                 875                 880

Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr Ile Asp Arg Glu
                885                 890                 895

Thr Ser Gly Asn Leu Glu Gln Leu Leu Leu Ala Val Val Lys Ser Ile
            900                 905                 910
```

```
Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr Tyr Ala Met Lys
        915                 920                 925

Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val Met Val Ser Arg
    930                 935                 940

Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe Arg Lys Asn Phe
945                 950                 955                 960

Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr Ser Gly Asp Tyr
                965                 970                 975

Lys Lys Ala Leu Leu Leu Leu Ser Gly Glu Asp Asp
            980                 985
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