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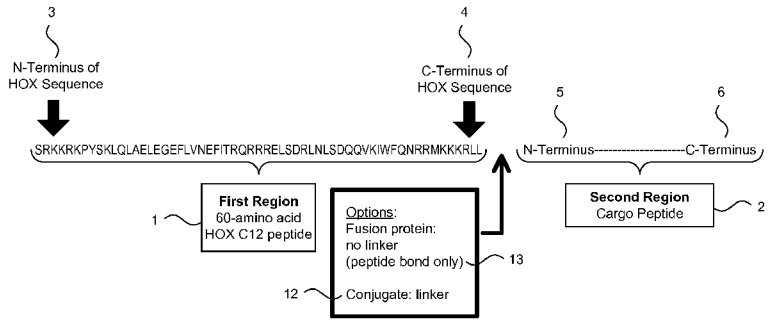


FIG. 1

(57) Abstract: Embodiments disclosed herein provide compositions for conjugates, including fusion proteins, and methods of using them to treat a variety of conditions. In some embodiments, the conjugates and/or fusion proteins incorporate a 60-amino acid human homeodomain (e.g., peptides derived from human HOX genes), to translocate functional and regulatory peptides and proteins or other biologically active molecules such as nucleic acids, which are not naturally associated with the human homeodomain, across cell and nuclear membranes to intended sites of action without provoking an unwanted immune response that may reduce exposure to the conjugate and/or result in a clinical adverse event. In further embodiments, disclosed conjugates and fusion proteins can pass through the blood-brain barrier to allow entry into the CNS. In various embodiments, the disclosed compositions are suitable for delivery into a cell (i) the expression product of a gene of interest and/or (ii) novel peptides or polynucleotides to regulate gene function.

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STRUCTURE, MANUFACTURING AND USES OF HUMAN-DERIVED CELL-PERMEABLE PEPTIDES CONJUGATED WITH SPECIFIC BIOLOGICALLY ACTIVE CARGO PEPTIDES

TECHNICAL FIELD

[0001] The embodiments disclosed herein relate generally to compositions and methods for treating a variety of conditions or diseases through administration of cell permeable peptide conjugates, particularly, but not exclusively, in the form of a fusion protein or protein/nucleic acid complex utilizing peptides derived from the amino acid homeodomain sequences coded for by human homeobox (HOX) genes and other human genes containing a homeodomain with cell permeabilizing properties. More particularly, the embodiments relate to a delivery system comprising a human homeodomain or variant or portion thereof, linked to one or more functional or regulatory peptides or proteins. These peptides facilitate entry into tissues, cells and the nucleus of cells thus allowing the fusion protein or conjugate containing a biologically active second region or "cargo" to reach its site of action for therapeutic purposes.

BACKGROUND

[0002] In diseases with manifestations due to aberrant gene function or deficient function, genes may be regulated through the direct delivery of biologically active molecules, such as nucleic acids, peptides and proteins, to their intracellular and intranuclear sites of action to influence gene expression either directly or indirectly through interference with transcription, translation or transcription factor production and action and also missing or defective protein products may be replaced to provide these types of molecules in individuals with germ-line or somatic mutations. The direct replacement of biologically important proteins in genetically deficient individuals is hampered by both (i) the inability of these proteins to reach intracellular sites and tissue sites such as the central nervous system (CNS) where they normally function and (ii) by the immunogenicity of these proteins.

SUMMARY

[0003] The embodiments disclosed herein together with a range of modifications provide compositions for conjugates, including fusion proteins, and methods of using them to treat a variety of conditions. The conjugates or fusion proteins incorporate a human HOX gene-derived 60 amino acid sequence or variant, or other human homeodomain sequence or variant or portion thereof to translocate functional and regulatory molecules, which are not naturally associated with the human homeodomain or variant or portion thereof, across cell and nuclear membranes to their intended sites of action without provoking an unwanted immune response that may reduce exposure and/or effectiveness of the conjugate, or which may produce an adverse clinical event. Further, such conjugates and fusion proteins also allow entry into the CNS by facilitating passage through the blood-brain barrier and then entry into cells in the CNS, such as microglia and neurons; they also allow for engagement of these functional or regulatory molecules to their intra-cytoplasmic and intranuclear targets. The ability to deliver directly into a cell (i) the expression product of a gene of interest or (ii) novel molecules able to regulate gene function has wide applicability in the medical field.

[0004] In addition to facilitating delivery of biologically active molecules to local (topical) and systemic intracellular and intranuclear targets, peptide sequences from human HOX genes also facilitate transport across the blood-brain barrier thus providing for CNS intracellular exposure to molecules containing these HOX gene sequences. Exploiting the technology's application of delivery of treatments across what are currently barriers to the delivery of drugs (such as the blood brain barrier), systemic administration can target cancers of the central nervous system by delivering drugs across the blood brain barrier. Examples of such applications are HOX gene derived peptides conjugated with adriamycin, doxorubicin or monoclonal antibodies directed at cancer cell targets such as Her2 (e.g., trastuzumab) for treatment of brain metastasized Her2 positive breast cancer. In addition, anti-bacterial and anti-viral agents that otherwise do not achieve therapeutic levels in the CNS are able to penetrate the blood brain barrier and successfully treat intracellular and extracellular bacterial and viral CNS infections in animal models.

[0005] In one embodiment, the conjugate comprises a first region, having a sequence described in any of SEQ ID Nos. 1-19 or variant or portion thereof,

conjugated to a second region not naturally associated with the first region, wherein the second region is a polypeptide having a sequence set forth in any one of SEQ ID Nos. 20, 23, 26, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, and 72 or a variant or variant or portion thereof. In one embodiment, the first region is derived from the human gene.

[0006] In one embodiment the conjugate comprises:

(a) a first region comprising a 60-amino acid domain or variant portion thereof of one of two HOX genes, HOX C12 and HOX D12; and

(b) a second region not naturally associated with the first region; and wherein at least the first region is non-denatured.

[0007] According to one embodiment, the second region may comprise a nucleic acid. This may be associated via the specific 1,3 dipolar Huisgen cycloaddition reaction known as 'click-reaction' between azide and alkyne groups and is employed for the synthesis of peptide–oligonucleotide conjugates. Alternatively, this embodiment may be seen as a protein/nucleic acid complex where the second region may comprise a nucleic acid binding domain, capable of binding nucleic acids as part of the complex.

[0008] According to another embodiment, the first and second regions are associated via the specific 1,3 dipolar Huisgen cycloaddition reaction (i.e., 'click-reaction'), wherein the oligonucleotides act as miRNAs or antagonists to miRNAs in order to affect gene expression. These oligonucleotides may comprise DNA, RNA, LNA (locked nucleic acid), PNA (peptide nucleic acid) or other nucleic acids.

[0009] According to a further embodiment, the first and second regions are connected via standard peptide bonds wherein nucleobases are attached to a peptide backbone rather than to a phosphodiester backbone. Some or all of these nucleobases are capable of hybridizing to other nucleic acids in the cell and affecting gene expression. These nucleobases may be PNA, γPNA or contain other side chains with similar properties.

[0010] In yet another embodiment, peptide or peptide-nucleic acid conjugates can be used to affect the activity of miRNAs including, but not limited to, antagonizing miRNA-132 for inhibition of the Ras pathway and cancer, antagonizing

miR-21 for inhibiting cancer or the NF-κB pathway and inflammation, or affecting the function of other miRNAs and pathways that may have detrimental and/or undesirable effects on health.

[0011] According to a further embodiment the conjugate is in the form of a fusion protein. In this embodiment, the second region is a functional or regulatory protein.

[0012] In another embodiment a conjugate can comprise:

(a) a first region comprising a human homeodomain with cell permeabilizing capabilities linked to

(b) a second region not naturally associated with the first region comprising a protein such as a functional enzyme.

[0013] In another embodiment a composition comprises a conjugate or fusion protein that may take any of the following forms: an inhalable composition; an eye drop or other ophthalmic composition for local or injectable use; an enema; a topical composition; or an injectable composition, including an injectable implant for sustained release.

[0014] Another embodiment disclosed is a method comprising steps that permit a conjugate or fusion protein containing one or more biologically active peptides or proteins to be translocated to cells of the CNS.

[0015] Other embodiments comprise methods to prevent immunogenicity of otherwise antigenic proteins delivered as conjugates or fusion proteins to their intended cytosolic or nuclear sites of action.

[0016] These and other embodiments will be disclosed in further detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] FIG. 1 shows an exemplary 60-amino acid human HOX C12 homeodomain (first region) or variant or portion thereof at the N-terminus of a Cargo peptide.

[0018] FIG. 2 shows an exemplary cargo peptide (second region) at the N-terminus of 60-amino acid human HOX D12 homeodomain or variant or portion thereof.

[0019] FIG. 3a shows an exemplary 60-amino acid human HOX D12 homeodomain (first region) or variant or portion thereof at its C-terminus with Cargo peptides (second and third regions), where the third region may be used to target the entire structure to specific tissues or cell types.

[0020] FIG. 3b shows an exemplary 60-amino acid human HOX D12 homeodomain (first region) or variant or portion thereof at its N-terminus with Cargo peptides (second and third regions), where the third region may be used to target the entire structure to specific tissues or cell type.

[0021] FIG. 4 shows an exemplary 60-amino acid human HOX C12 homeodomain (first region) or variant or portion thereof at the N-terminus of a Cargo peptide (second region), and His-tail conjugated to C-terminus through GS linker.

[0022] FIG. 5 shows an exemplary 60-amino acid human HOX D12 homeodomain (first region) or variant or portion thereof at N-terminus of NBD Cargo peptide (second region), and His-tail conjugated to C-terminus through GS linker.

[0023] FIG. 6 shows an exemplary sequence for plasmid pJ411:129925, showing a 6 HIS leader, with a transition sequence of SAA followed by the TEV cleavage site (ENLYFQS) to produce SEQ. ID No. 21 (also referred to as PPL-002) with the appropriate N-terminus.

[0024] FIG. 7 shows an exemplary sequence for plasmid pJ411:129926, showing a 6 HIS leader, with a transition sequence of SAA followed by the TEV cleavage site (ENLYFQS) to produce SEQ ID No. 22 (also referred to as PPL-003) with the appropriate N-terminus.

[0025] FIG. 8a shows inhibition of NF-κB activation in murine RAW 264.7 cells (LPS 4-hour stimulation). Error bars represent SEM.

[0026] FIG. 8b shows inhibition of NF-κB activation in Human Embryonic Kidney (HEK) 293 cells (TNF-α 4-hour stimulation). Error bars represent SEM.

[0027] FIG. 9 shows inhibition of TNF-α-stimulated NF-κB activation in HEK 293 NF-κB Luciferase Reporter Cells by NBD cargo containing cell permeable peptides FIG. 10: Timeline for determining *in vivo* activity of NBD cargo-containing cell permeable peptides: Mice were pretreated with peptides, challenged by injection of

lipopolysaccharide (LPS) and blood samples were taken for cytokine assays at the indicated times

[0028] FIG. 11a shows TNF-α response following LPS injection and peptide or dexamethasone (Dex) pretreatment. Each datapoint represents the value for a single mouse.

[0029] FIG. 11b shows IP-10 response following LPS injection and peptide or dexamethasone (Dex) pretreatment. Each datapoint represents the value for a single mouse.

[0030] FIG. 12 shows IL-10 response following LPS injection and peptide or dexamethasone (Dex) pretreatment. Each datapoint represents the value for a single mouse.

[0031] FIG. 13 shows PPL-003 concentration in plasma following dosing at 500, 1000, and 2000 microgram.

[0032] FIG. 14 shows PPL-003 C_{max} in plasma by dose, showing average and median at C_{max} (30 min.).

[0033] FIGS. 15a and 15b show amyloid A protein (SAA) response at 2 hours following LPS challenge with pretreatment of2000 microgram of PPL-003 at 0, 30- and 60-minutes prior to LPS stimulation. Error bars represent SEM in FIG 15a and data points in FIG 15b are individual mice, horizontal lines are median values.

[0034] FIGS. 16a and 16b show amyloid A protein (SAA) response to LPS following PPL-003 pretreatment at doses of 500, 1000, and 2000 micrograms. Error bars represent SEM in FIG 16a, and data points in FIG 16b are individual mice, horizontal lines are median values. Percentages are percent inhibition relative to vehicle/LPS.

[0035] FIGS. 17a and 17b show IL-10 and Mouse Kc responses, respectively, following 2000 microgram PPL-003 dose at 0, 30- and 60-minutes prior to LPS stimulation. Error bars represent SEM.

[0036] FIGs. 18a and 18b show inhibition of GM-CSF response following 2000 microgram PPL-003 or PPL-004 dose, respectively, at 0, 30- and 60-minutes prior to LPS stimulation. Error bars represent SEM.

DETAILED DESCRIPTION

[0037] The human HOX genes and other homeodomain coding genes are important in embryonic development and include small regions homologous to the gene antennapedia (Antp) but where the majority of amino acids are included in uniquely human sequences that facilitate their function. The protein sequence of antennapedia is characterized by the presence of a 60-amino acid motif (homeodomain) that binds to specific DNA target elements. The Antp homeodomain (see US Pat. No. 7,968,512) and much smaller sequences from the Antp gene have been shown to facilitate the entry of biologically active ("cargo") peptides into tissues and cells to reach their site of action for therapeutic purposes.

[0038] While the homeodomain and smaller regions of Antp can be used to translocate proteins, including functional and regulatory proteins in vitro and in animal models, peptide sequences from human HOX genes and certain other human homeodomain sequences can also facilitate transport of biologically active molecules such as peptides, proteins and nucleotides to their intracellular and intranuclear sites of action (i) without provoking an unwanted immune response that may reduce exposure and/or effectiveness of the conjugate or may produce an adverse event, and (ii) with greater efficiency than Antp conjugates and fusion proteins. The ability to deliver biologically active molecules including the expression product of a gene of interest directly into a cell has wide applicability, particularly in the medical field. HOX and other human homeodomain peptides are also able to translocate nucleic acids. This is especially advantageous for applications utilizing a mechanism of gene regulation.

[0039] Several of the disclosed embodiments and their modifications relate to novel conjugates, which may take the form of fusion proteins, each comprising a 60-amino acid peptide or variant or portion thereof (**1** e.g. as shown in FIG. 1) derived from human HOX genes or from other human genes containing a homeodomain with cell permeabilizing properties, linked (e.g. **12** or **13** as shown in FIG. 1) to a second region (**2**): at least one of the biologically active "cargo" peptides disclosed herein. FIG. 1 shows one embodiment comprising the 60-amino acid human HOX C12 homeodomain ("first region") (**1**) linked on its C-terminus (**4**) to the N-terminus (**5**) of a biologically-active cargo peptide ("second region") (**2**). FIG. 2 shows an alternative embodiment comprising the 60-amino acid human HOX D12 homeodomain ("first

region") (**1**) is linked on its N-terminus (**3**) to the C-terminus (**6**) of a biologically-active cargo peptide ("second region") (**2**). The links in both embodiments may be a simple peptide bond (**13**) or a linker sequence known in the art (**12**) provided that function of the fusion protein or conjugate is not compromised by its addition.

[0040] The term "compromised" refers to reduced function of the conjugate embodiments as reflected in reduced efficacy of treatment for any of the outcomes discussed herein, e.g., compromised function could be reflected in an attenuated reduction in immunogenicity as compared to the reduction in immunogenicity observed with (1) the conjugate in the form of a fusion protein, or (2) the conjugate comprising an alternative linker.

[0041] Cargo peptides include both small synthetic peptides and larger proteins such as antibodies or the binding regions of antibodies into cells for therapeutic purposes. (Schutze-Redelmeier M-P et al. "Introduction of exogenous antigens into the MHC class 1 processing and presentation pathway by *Drosophila* antennapedia homeodomain primes cytotoxic T cells *in vivo*. J. Imunol. 157(2):650-55, July 15, 1996; M.J. May et al. "Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex" Science 289:1550-54, Sept. 1, 2000; I Strickland and S Ghosh. "Use of cell permeable NBD peptides for suppression of inflammation" Ann. Rheum. Dis. 65(Suppl 3):iii75-iii82, 2006; C. Avignolo et al. "Internalization *via* Antennapedia protein transduction domain of an scFv antibody toward c-Myc protein" FASEB J. 22:1237-45, 2008.)

[0042] The terms "peptide(s)," "protein(s)," and "polypeptide(s)" are used synonymously.

[0043] The term "human homeodomain" refers to (1) human HOX-derived homeodomains such as the HOX C12 and HOX D12 sequences shown in Table 3 as SEQ ID No. 1 and SEQ ID No. 2, respectively, or variants or portions thereof; and (2) any other human homeodomain that has cell permeabilizing activity, such as SEQ ID Nos. 3 through 19, or variants or portions thereof.

[0044] The phrase "not naturally associated with" means that entire sequence of the conjugate or fusion protein is not found in nature, and that the entire sequence is not encoded for by a single gene found in nature.

[0045] The phrase "operably linked" means that the first and second region are linked such that the second region is able to translocate a cell membrane. Such linkage may be produced via application of “click” chemistry methods or other methods known in the art or may be incorporated as a fusion protein with a peptide bond between regions.

[0046] A person of ordinary skill in the molecular biology/biotechnology art would appreciate that numerous variations of the sequences shown in Tables 3 and 4 would fall within the embodiments disclosed herein. As used herein, homology refers to identity or near identity of nucleotide or amino acid sequences. As is understood in the art, nucleotide mismatches can occur at the third or wobble base in the codon without causing amino acid substitutions in the translated polypeptide sequence. Also, minor nucleotide modifications (e.g., substitutions, insertions or deletions) in certain regions of the gene sequence can be tolerated whenever such modifications result in changes in amino acid sequence that do not alter functionality of the final gene product. Homologs of specific DNA sequences may be identified by those skilled in the art using the test of cross-hybridization of nucleic acids under conditions of stringency as is well understood m the art (as described in Hames et al., Nucleic Acid Hybridisation, (1985) IRL Press, Oxford, UK). Extent of homology is often measured in terms of percentage of identity between the sequences compared.

[0047] The term "variant" refers to a polypeptide or protein that differs from a reference polypeptide or protein, but retains essential properties. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall (homologous) and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more modifications (e.g., substitutions, additions, and/or deletions). A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally.

[0048] Modifications and changes can be made in the structure of the polypeptides and proteins of this disclosure and still result in a molecule having similar characteristics as the polypeptide (e.g., a conservative amino acid substitution). For example, certain amino acids can be substituted for other amino

acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's or protein's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide or protein sequence and nevertheless obtain a polypeptide or protein with like properties.

[0049] Amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take one or more of the foregoing characteristics into consideration are well known to those of skill in the art and include, but are not limited to (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tip: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure, therefore, consider functional or biological equivalents of a polypeptide or protein as set forth above. In particular, embodiments of the polypeptides and proteins can include variants having about 50%, 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide and protein of interest.

[0050] "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 known bioinformational methods.

[0051] *First Region*: The first region of the conjugate or fusion protein embodiments disclosed may comprise a natural or synthetic 60-amino acid peptide or variant or portion thereof derived from the HOX C12 or HOX D12 gene or any other human homeodomain that has cell permeabilizing activity, such as SEQ ID Nos. 3 through 19, or variants or portions thereof.

[0052] In one embodiment, the HOX C12 (SEQ ID No. 1) amino acid sequence is:

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

[0053] In a another embodiment, the HOX D12 (SEQ ID No. 2) amino acid sequence is:

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

[0054] Alternatively, other embodiments will include any one of 17 additional human 60-amino acid homeodomain sequences or variants or portions thereof with permeabilizing properties to allow for intracellular and intranuclear delivery of conjugates and fusion proteins. Further, these additional sequences each provide for CNS penetration of molecules that otherwise would not penetrate the blood brain barrier. Examples of amino acid sequences of these conjugates and fusion proteins with HOX C12 and HOX D12 homeodomains in the first region are shown in Table 3 (SEQ ID Nos. 21-22, 24-25, 27-28, 31-32, 34-35, 37-38, 40-41, 43-44, 46-47, 49-50, 52-53, 55-56, 58-59, 61-62, 64-65, 67-68. 70-71, and 73-74, or variants or portions thereof) and any of the other human homeodomains or variants or portions thereof (SEQ ID Nos. 3-19) and variations on these sequences may be substituted for either of the HOX C12 and D12 homeodomains. Each of the human homeodomain sequences is less than 50% identical to the 60-amino acid Antp sequence and thus is structurally distinct and has unique human properties. Additionally, because all of the homeodomain sequences shown in the table are of human origin they will not be antigenic in humans. Each of the human homeodomain sequences is listed below in Table 3 along with the abbreviation of the name of the human gene from which it is derived (SEQ ID Nos. 1 through 19). Cargo sequences may or may not be of human origin depending on their intended function.

[0055] In addition synthetic variants may be used provided that they retain the ability to translocate the membrane. Synthetic variants will generally differ from the naturally-occurring proteins by substitution, particularly conservative substitution. The phrase “conservative amino acid changes” herein means replacing an amino acid from one of the amino acid groups, namely hydrophobic, polar, acidic or basic, with an amino acid from within the same group. An example of such a change is the replacement of valine by methionine and vice versa. Other examples of conservative substitutions may be seen by reference to Table 1 below:

[0056] Table 1: Conservative Amino Acid Substitutions.

ALIPHATIC	Non-polar	GAP	ILV
	Polar-uncharged	CSTM	NQ
	Polar - charged	DE	RK
AROMATIC			HFWY
OTHER			N

[0057] Such variants may be synthesized directly or prepared using standard recombinant DNA techniques such as site-directed mutagenesis. Where insertions are to be made, synthetic DNA encoding the insertion together with 5' and 3' flanking regions corresponding to the naturally-occurring sequence either side of the insertion site. The flanking regions will contain convenient restriction sites corresponding to sites in the naturally-occurring sequence so that the sequence may be cut with the appropriate enzyme(s) and the synthetic DNA ligated into the cut. The DNA is then expressed to make the encoded protein. These methods are only illustrative of the numerous standard techniques known in the art for manipulation of DNA sequences and other known techniques may also be used. Variants that retain at least 50% sequence identity with the claimed 60-amino acid sequences or variants or portions thereof derived from HOX-C12 and HOX-D12 will likely maintain their cell permeability characteristics and retain their human characteristics resulting in low immunogenicity potential. The ability of a naturally occurring or synthetic HOX sequence to translocate the membrane may be tested by routine methods known in the art. Any polynucleotide which encodes the amino acid of SEQ ID Nos. 1-19 (Table 3) or variant or portion thereof can be used in a fusion protein or conjugate herein.

[0058] *Second Region Peptides or Proteins*: The second region of the conjugate or fusion protein embodiments disclosed may comprise any peptide or protein sequence not naturally associated with the first region. The gene encoding the first region may or may not also encode the second region. The second region also may or may not be from the same species as the first region, but the first and second regions will be present in the conjugate or fusion protein embodiments in a manner different from the natural situation.

[0059] The second region of the fusion protein or conjugate embodiments may be a peptide or protein of any length as long as it is biologically active on its target when included in the fusion protein or conjugate.

[0060] *Second Region Nucleic Acids*: The second region may include any nucleic acid that may be therapeutically active. In one embodiment, the nucleic acid can be DNA or RNA. In another embodiment, the nucleic acid is an oligonucleotide or a PNA.

[0061] In one embodiment, the second region may include any peptide nucleic acid such as PNAs in which nucleobases replace standard amino acids and the second region may be therapeutically active, including acting via antagonizing or activating miRNAs, in order *to affect gene expression or oth*er biological process.

[0062] In another embodiment, the second region may include any peptide nucleic acid such as PNAs and wherein the second region may be therapeutically active, including acting via antagonizing or activating mRNAs, by activating or repressing splicing or translation in order to affect gene expression or other biological process.

[0063] In certain embodiments comprising a protein/nucleic acid complex, the complex can further comprise a nucleic acid or PNA as part of the second region that, when inside a cell, binds to a RISC complex or an miRNA. For example, binding to miR132 miRNA may inhibit cancer or NF-κB. Such binding to an miRNA molecule can form a stable complex, wherein the miRNA molecule can become incapable of affecting the expression of genes as would normally be the case for an unbound miRNA.

[0064] In some embodiments comprising a protein/nucleic acid complex, the complex can further comprise a nucleic acid or PNA as part of the second region that, when inside a cell, binds to genomic DNA. For example, binding to DNA at transcription factor binding sites may prevent transcription factor binding and/or activity and thus affect gene transcription.

[0065] In further embodiments comprising a protein/nucleic acid complex, the complex can further comprise a nucleic acid, LNA or PNA as part of the second region that, when inside a cell, binds to an mRNA. For example, binding to DYRK1b mRNA can down-regulate DYRK1b expression and/or translation.

[0066] In additional embodiments comprising a protein/nucleic acid complex, the complex can further comprise a nucleic acid (or DNA) binding domain as part of the second region. In one embodiment, the nucleic acid binding domain may serve to mediate the specific, high affinity and non-covalent interaction of the protein component with the nucleic acid or PNA component.

[0067] The nucleic acid binding domain may be an RNA or DNA binding domain, e.g., the DNA binding domain of a transcription factor, particularly a yeast or human transcription factor. For example, a GAL4-derivable domain, mediates the selective binding of the protein to the DNA sequence CGGAGGACAGTCCTCCG (Cavey et al J Mol Biol 209:423, 1989). Further, the DNA binding domain consists of GAL4 amino acids 2 to 147. A DNA binding domain may bind to single-stranded or to a double-stranded DNA on the second domain.

[0068] Other applications for the conjugate or fusion protein embodiments disclosed include development of antibacterial and antiviral measures. For example, HOX gene-derived or other human homeodomain-derived cell permeable peptides may be used to transport in the cytoplasm of infected cells recombinant antibodies, naturally occurring innate immunity effecter molecules or DNA binding molecules and which destroy bacteria, parasites or interfere with a crucial step of bacterial or viral replication.

[0069] Suitable peptides and proteins include those that are of therapeutic and/or diagnostic application such as, but are not limited to: cytokines, chemokines, hormones, antibodies, engineered immunoglobulin-like molecules, a single chain antibody, conjugates, enzymes, immune co-stimulatory molecules, immunomodulatory molecules, anti-sense RNA, a transdominant negative mutant of a target protein, a toxin, such as endotoxin A, Colicin A, d-endotoxin, diphtheria toxin, Bacillus anthrox toxin, Cholera toxin, Pertussis toxin, E. coli toxins, Shigatoxin or a Shiga-like toxin, a conditional toxin, an antigen, a tumor suppressor protein and growth factors, membrane proteins, vasoactive proteins and peptides, anti-viral proteins and ribozymes, and derivatives. When included, such coding sequences may be typically operatively linked to a suitable promoter, which may be a promoter driving expression of a ribozyme(s), or a different promoter or promoters.

[0070] One or more embodiments may provide a pharmaceutical composition for treating an individual by gene therapy in animals or humans, wherein the composition comprises a therapeutically effective amount of the conjugate or fusion protein. Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular individual.

[0071] Fusion protein or conjugate embodiments delivering one or more therapeutic molecules such as genes or proteins may be used alone or in combination with other treatments or components of the treatment. Diseases which may be treated include, but are not limited to: cancer, neurological diseases, inherited diseases, heart disease, stroke, arthritis, viral and bacterial infections, and diseases of the immune system. Suitable therapeutic genes include those coding for tumor suppressor proteins, enzymes, pro-drug activating enzymes, immunomodulatory molecules, antibodies, engineered immunoglobulin-like molecules, conjugates, hormones, membrane proteins, vasoactive proteins or peptides, cytokines, chemokines, anti-viral proteins, antisense RNA and ribozymes.

[0072] The conjugate may also contain one or more cytokine-encoding nucleic acids or cytokines. Suitable cytokines and growth factors include but are not limited to: ApoE, Apo-SAA, BDNF, Cardiotrophin-1, EGF, ENA-78, Eotaxin, Eotaxin-2. Exodus-2, FGF-acidic, FGF-basic, fibroblast growth factor-10 (Marshall 1998 Nature Biotechnology 16: 129).FLT3 ligand (Kimura et al. (1997), Fractalkine (CX3C), GDNF, G-CSF, GM-CSF, GF-[beta]1, insulin, IFN-[gamma], IGF-I IGF-II, IL-1[alpha], IL-1[beta], IL-2, IL-3. IL-4, IL-5, IL-6, IL-7, IL-8 (72 a.a.), IL-8 (77 a.a.), IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18 (IGIF), Inhibin [alpha], Inhibin [beta], IP-10, keratinocyte growth factor-2 (KGF-2), KGF,. Leptin, LIF, Lymphotactin, Mullerian inhibitory substance, monocyte colony inhibitory factor, monocyte attractant protein (Marshall 1998 ibid), M-CSF, MDC (67 a.a.), MDC (69 a.a.), MCP-1 (MCAF), MCP-2, MCP-3, MCP4, MDC (67 a.a), MDC (69 a.a.), MIG, MIP-1[alpha], MIP-1[beta], MIP-3[alpha], MIP-3[beta], MIP4, myeloid progenitor inhibitor factor-1 (MPIF-1), NAP-2, Neurturin, Nerve growth factor, [beta]-NGF, NT-3, NT-4, Oncostatin M, PDGF-AA, PDGF-AB, PDGF-BB, PF-4, RANTES, SDF1[alpha], SDF1[beta], SCF, SCGF, stem cell factor (SCF), TARC, TGF-[alpha], TGF-[beta], TGF-[beta]2, TGF-[beta]3, tumor necrosis factor (TNF), TNF-[alpha], TNF-[beta], TNF-1, TPO, VEGF, GCP-2

GRO/IMGSA, GRO-[beta], GRO-[gamma], HCC1, and 1-309. It is also contemplated that homologous cytokines and growth factors can be used as cargo in suitable animal systems for research or veterinary applications.

[0073] The fusion protein or conjugate embodiments disclosed may comprise further suitable domains known to those skilled in the art. For example, an endoplasmic reticulum retention signal functions to affect the intracellular routing of the internalized conjugate or protein/nucleic acid complex. A suitable endoplasmic retention signal may be a mammalian endoplasmic reticulum retention signal.

[0074] Also present may be a translocation domain which serves to enhance nucleic acid or protein escape from the cellular vesicle system and thus to augment nucleic acid transfer by this route. This domain may serve to reduce or avoid lysosomal degradation after internalization of the protein/nucleic acid into the target cell. Suitable translocation domains are derivable from toxins, particularly bacterial toxins, such as exotoxin A, Colicin A, d-endotoxin, diphtheria toxin, Bacillus anthrax toxin, Cholera toxin, Perussis toxin, E. coli toxin toxins, Shigatoxin or Shiga-like toxin.

[0075] The first binding domain may be modified to target cell sites other than the nucleus.

[0076] Additionally, or alternatively, a target cell-specific binding domain recognizing a cell surface structure may be present, such as a receptor protein or surface antigen on the target cell.

[0077] The term “conjugate” or “conjugates” herein comprises a category of structures, including fusion proteins, in which the first region, a 60-amino acid human homeodomain sequence or variant or portion thereof, is conjugated directly via a peptide bond or other type of bond including both covalent and non-covalent bonds. Conjugates may include a linker region that connects the homeodomain sequence to a second region, a functional or regulatory peptide or protein (“cargo” peptide) that is not naturally associated with the first region. Any of a wide variety of linkers (short, connecting sequences) known in the art may be utilized to form the conjugate provided that function of the conjugate is not compromised by its addition. Thus, translocation of the second region is enabled through a cellular or nuclear membrane. For example, see a wide variety of linkers known in the art in Chen et al.

"Fusion protein linkers: property, design and functionality." Advanced Drug Delivery Reviews. http://dx.doi.org/10.1016/J.addr.2012.09.039. In alternative embodiments the term "fusion protein" is used to refer to a particular subcategory of conjugate that exists when no such linkers are used to form the conjugate and the domains are linked entirely by peptide bonds.

[0078] The first (HOX or other human homeodomain) region and second (cargo) regions may be linked by a cleavable linker region this may be any region suitable for this purpose provided the function of the conjugate is not compromised by its addition. The cleavable linker region is a protease cleavable linker, although other linkers, cleavable for example by small molecules, may be used. These include Met-X sites, cleavable by cyanogen bromide, Asn-Gly, cleavable by hydroxylamine, Asp-Pro, cleavable by weak acid and Trp-X cleavable by, inter alia, NBS-skatole. Protease cleavage sites require milder cleavage conditions and are found in, for example, factor Xa, thrombin and collagenase. Any of these may be used. The precise sequences are available in the art and the skilled person will have no difficulty in selecting a suitable cleavage site. By way of example, the protease cleavage region targeted by Factor Xa is I E G R. The protease cleavage region targeted by Enterokinase is D D D D K. The protease cleavage region targeted by Thrombin is L V P R G. The cleavable linker region may be one that is targeted by endocellular proteases. Linkers may not be required for function but linkers may be included between first and second regions to allow targeted release of the second region without compromising function or to enhance biological activity of the second region with linker cleavage.

[0079] Table 2 - showing partial listing of linkers known in the art, adapted from Chen et al., 2012.

$(GGGGS)_n$, where n = 1, 2, 3, or 4 $(Gly)_n$, where n = 6 or 8 $(EAAAK)_n$ where n = 1 – 3 $A(EAAAK)_4ALEA(EAAAK)_4$ A PAPAP AEAAAKEAAAKA $(Ala\text{-}Pro)_n$ where n = 10 – 34 aa VSQTSKLTR↓AETVFPDV PLG↓LWAc RVL↓AEA; EDVVCC↓SMSY; GGIEGR↓GS TRHRQPR↓GWE; AGNRVRR↓SVG; RRRRRRR↓R↓R GFLG↓ $A(EAAAK)_4ALEA(EAAAK)_4A$
↓ = cleavable at this location

[0080] The embodiments disclosed allow for potent therapeutic action, including efficient translocation into intracellular and intranuclear sites of action of any of a number of identified peptides for the specified therapeutic treatments, wherein (i) the CNS is a target tissue; and (ii) regardless of the target tissue, such translocation does not provoke an unwanted immune response that may reduce the exposure and/or effectiveness of the conjugate or produce an adverse or undesirable clinical event. The biologically-active peptide of the "second region" is not naturally associated with the human homeodomain sequence or variant or portion thereof (the "first region") before they are joined.

[0081] Further, in a variety of embodiments the conjugate or fusion protein may comprise a third region also comprising a cargo peptide. FIGS. 3a and 3b show alternative embodiments comprising the 60-amino acid human homeodomain ("first region," shown abbreviated here) and an additional cargo protein that may target the fusion protein or conjugate to its cellular site of action. In FIG. 3a, the human HOX D12 homeodomain on the left (**1**), is linked on its C-terminus (**4**) to the N-terminus (**5**) of a biologically-active cargo peptide of interest ("second region") (**2**), while a third cargo peptide of interest ("third region") (**7**) is linked on its N-terminus (**8**) to the C-terminus (**6**) of the "second region" (**2**). This third region may remain linked or fused via peptide bonds to the entire fusion protein or may be designed for removal after uptake or binding to specific sites in targeted tissues.

[0082] FIG. 3b shows an alternative embodiment, with the human HOX D12 homeodomain on the right (**1**), linked through its N-terminus (**3**) to the C-terminus (**6**) of the second region (**2**), while a third region cargo peptide (**7**) is linked through the

N-terminus (**5**) of the second region (**2**). The links may be simple peptide bonds (**13**) or a linker sequences known in the art (**12**), or a combination of both, provided that the function of the conjugate is not compromised by the addition of a linker sequence. A *second* cargo peptide ("third region") (**7**) may target the conjugate or fusion protein to its cellular site of action. *See* Example 2.

[0083] Conjugate and fusion protein embodiments herein may be produced in accordance with any of the standard molecular biology techniques described in the literature. See, for example, Ausubel et al. (2002) Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, 5th Ed. John Wiley & Sons. Manufacturing methods including purification methods that may be used are also disclosed in U.S. Pat. No. 7,968,512, the entirety of which is incorporated herein by reference.

[0084] "Expression vectors" or "plasmids" (used interchangeably herein) may be used for producing conjugates or components thereof to introduce heterologous DNA into host cells, either for expression or replication. Selection by the artisan of the appropriate vector will depend on its intended use, i.e. (DNA amplification or DNA expression), the size of the DNA to be inserted into the vector, and the host cell to be transformed with the vector. Each vector contains various components depending on its intended use, which comprise one or more of: an origin of replication, one or more marker genes, an enhancer element, a promoter, a transcription termination sequence and a signal sequence.

[0085] Sources of nucleic acid may be ascertained by reference to published literature or databanks provided by organizations such as NCBI or EMBL. Identification of sequences of interest may be accomplished by using BLAST, BLAT, or other homology search algorithms. Further, nucleic acid encoding the desired first or second region may be obtained from academic or commercial sources where such sources are willing to provide the material or by synthesizing or cloning the appropriate sequence where only the sequence data are available. Generally, this may be done by reference to literature sources which describe the cloning of the gene in question. Alternatively, where limited sequence data are available or where it is desired to express a nucleic acid homologous or otherwise related to a known nucleic acid, exemplary nucleic acids can be characterized as those nucleotide sequences which hybridize to the nucleic acid sequences known in the art.

[0086] The phrase "stringency of hybridization" refers to conditions under which polynucleic acids hybrids are stable. Such conditions are evident to those of ordinary skill in the art. Also as understood by persons skilled in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrid which decreases approximately 1 to 1.5[deg.] C. with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. The hybridization reaction typically is performed under conditions of higher stringency, followed by washes of varying stringency.

[0087] As used herein, the phrase "high stringency" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 1 M Na+ at 65-68[deg.] C. High stringency conditions can be provided, for example, by hybridization in an aqueous solution containing 6*SSC, 5* Denhardt's, 1% SDS (sodium dodecyl sulphate), 0.1 Na+ pyrophosphate and 0.1 mg/ml denatured salmon sperm DNA as nonspecific competitor. Following hybridization, high stringency washing may be done in several steps, with a final wash (about 30 minutes) at the hybridization temperature in 0.2-0.1*SSC, 0.1% SDS.

[0088] The phrase "moderate stringency" refers to conditions equivalent to hybridization in the above described solution, except that the temperature is at about 60-62[deg.] C. In that case the final wash is performed at the hybridization temperature in 1*SSC, 0.1% SDS.

[0089] Low stringency refers to conditions equivalent to hybridization in the above described solution at about 50-52[deg.] C. In that case, the final wash is performed at the hybridization temperature in 2*SSC, 0.1% SDS.

[0090] It is understood that these conditions may be adapted and duplicated using a variety of buffers, e.g., formamide-based buffers, and temperatures. Denhardt's solution and SSC are well known to those of skill in the art as are other suitable hybridization buffers (see, e.g. Sambrook, et al., eds. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York or Ausubel, et al., eds. (1990) Current Protocols in Molecular Biology, John Wiley & Sons, Inc.). Optimal hybridization conditions must be determined empirically, as the length and the GC content of the probe also play a role.

[0091] In one embodiment, the conjugate or fusion protein may be produced through use of an expression vector comprising the nucleic acid sequence and a promoter for recombinant synthesis in, for example, plant cells (including algae), in bacteria such as *E. coli*, or in eukaryotic cells such as Chinese Hamster Ovary (CHO) cells or yeast cells.

[0092] In another embodiment, a host cell is transformed with the expression vector.

[0093] In yet another embodiment a nucleic acid sequence encodes the conjugate or fusion protein for the purposes of synthesis and manufacture by recombinant technology.

[0094] In some embodiments, the conjugate or fusion protein is non-denatured, meaning it may exist in its native state, the form in which the protein occurs in the intact cell in its three-dimensional structure.

[0095] The term "non-denatured" may also, but need not, imply a specific non-denaturing step. Denaturing alters the three-dimensional shape of the protein molecule without rupture of its peptide bonds; disulfide bonds may be ruptured, or certain groups in the protein may be chemically modified if such processes are also accompanied by changes in its overall three-dimensional structure.

[0096] In other embodiments, the conjugate or fusion protein is renatured, a process by which the denatured protein is returned to its original conformation prior to denaturation. For peptides, reversible denaturation is generally brought about by disulfide reducing agents and urea, and for nucleic acid, by heat and salts.

[0097] In one embodiment, the first region is at the N-terminus of the second region. In another embodiment the first region is at the C-terminus of the second region.

[0098] Purification methods known in the art may be used in the process of preparing conjugates and fusion proteins according to the embodiments disclosed, for example, as described in Zachariou, M. (2010) Affinity Chromatography: Methods and Protocols, 2nd Ed. Totoua, NJ:Humana Press. The conjugate or fusion protein can be obtained from bacterial or eukaryotic cell lysates , as denaturing reagents, small changes in pH, and differences in osmolarity may have an effect on the

translocation properties of the peptides. Conditions for obtaining and purifying the human homeodomain peptides, including HOX-derived peptides are disclosed herein.

[0099] The ability of HOX peptide-conjugates to translocate across the cell surface membrane may be dependent on the conformation of the recombinant proteins. For example, translocation of the polypeptide by using either bacteria cell extracts or purified proteins exposed to small amounts of detergent (ionic and non-ionic) or denaturating agents (urea or guanidinuim) may prevent or inhibit translocation. This conformation-dependent property may be preserved by purifying the HOX peptide-conjugate or other human homeodomain conjugate under native conditions.

[00100] In one embodiment, both the first (the human homeodomain or variant or portion thereof) and second (biologically active peptide or protein) regions are purified from a bacterial lysate. In other embodiments, the conjugate or fusion protein is purified from a plant cell lysate. In yet another embodiment, the conjugate or fusion protein is purified from an eukaryotic cell lysate ,culture medium or fermentation broth.

[00101] One embodiment is a method for preparing a conjugate or fusion protein, comprising:

(a) culturing the host cell under conditions which provide for the expression of the conjugate from the expression vector within the host cell; and

(b) recovering the conjugate, which recovery comprises

(i) fusing an amino acid tail or other specific ligand to the conjugate, which tail is capable of binding to at least one substrate and not to another substrate, and wherein the conjugate or fusion protein is caused to bind via the tail to at least one substrate, and wherein components of the host cell do not bind to this substrate, and

(ii) putting into contact the conjugate and remaining components of the host cell with the other substrate such that the conjugate is not bound and the remaining components of the host cell are bound to the other substrate.

[00102] Another embodiment is a method for preparing a conjugate or fusion protein comprising:

(i) culturing a host cell, transformed with an expression vector comprising nucleic acid, operably linked to a promoter, encoding a fusion protein where

(a) a first region comprises the HOX peptide or other human homeodomain peptide; and

(b) a second region not naturally associated with the first region comprises a peptide or protein, under conditions which provide for expression of the fusion protein from the expression vector within the host cell; and

(ii) recovering the fusion protein, which method comprises fusing an amino acid tail or other ligand to the conjugate, which tail is capable of binding to at least one substrate and not to another substrate, and wherein the conjugate is caused to bind via the tail to at least one substrate such that components of the host cell do not bind to this substrate; and the conjugate is contacted with the other substrate such that the conjugate is not bound and remaining components of the host cell are bound to the other substrate.

[00103] In another embodiment as part of the affinity purification process, the embodiments include the use of a tail or ligand that is attached to the conjugate or fusion protein; this allows for both positive and negative purification steps.

[00104] For all combined first and second region sequences, additional amino acid sequences can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (such as HHHHHH, HHHHHHGS, the latter utilizing a GS linker, see FIGs. 4 and 5), and other similar tags known to those in the art. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human homeodomain peptide first region. For any tag, a peptide linker known in the art may be used and removed after purification with a specific protease.

[00105] In one embodiment, the amino acid tail or ligand is fused to the C-terminus of the conjugate. For example, FIG. 4 shows an embodiment comprising a biologically-active cargo peptide (2), linked on its C-terminus (6) through either a peptide bond (13) or a linker sequence known in the art (12) to the N-terminus (3) of the human homeodomain "first region" shown abbreviated here (1). A GS linker (14) is shown attaching a His-tag ("tail") to the C-terminus (6) of the cargo peptide (2). Any linker may be used provided it does not compromise the function of the conjugate.

[00106] In one embodiment, the immobilized substrate is a nickel or cobalt column, avidin column, or an antibody column with affinity for the amino acid tail. In another embodiment, the conjugate's amino acid tail is serially brought into contact with at least two immobilized substrates with which the tail has affinity, in which case the nickel or cobalt column and/or avidin and/or antibody may be used in any order.

[00107] In another embodiment, the method of purifying a conjugate comprises fusing an amino acid tail or ligand to the conjugate or fusion protein, which tail is capable of binding to at least one substrate while impurities bind only to a second substrate; the conjugate is contacted with the other substrate such that the conjugate is not bound and remaining impurities are bound to the other substrate.

[00108] According to another embodiment, a method for producing and purifying a conjugate or fusion protein comprising the 60-amino acid human homeodomain or variant or portion thereof and a biologically-active peptide or protein comprises culturing the host cell for the expression of the conjugate or fusion protein from the expression vector and subsequently recovering the conjugate or fusion protein using affinity purification techniques known in the art.

[00109] When the second region is a DNA binding domain, a complex with nucleic acid may be formed by mixing the conjugate formed with the nucleic acid.

[00110] Further embodiments include pharmaceutical compositions comprising the conjugates or fusion proteins of the embodiments disclosed herein, and methods of use of the conjugates or fusion proteins in the preparation of a medicament for the treatment of a disease.

[00111] In one embodiment, a conjugate or fusion protein comprises a biologically active peptide or protein that is a functional enzyme, linked to the human homeodomain or variant or portion thereof.

[00112] Other embodiments comprise any of a variety of formulations for treating conditions or diseases identified.

[00113] **Upstream Production of SEQ ID No. 21 and SEQ ID No. 22**. A bacterial expression construct was generated for two homeobox candidate proteins PPL-002 (SEQ ID No. 21, Table 3) and PPL-003 (SEQ ID No. 22, Table 3) capable of regulated expression of the target protein with a TEV cleavable N-terminal His tag in the T7 vector system. The T7 expression system is suitable for producing material for animal efficacy studies; however, other expression vectors/system are also contemplated and are known by one of ordinary skill in the molecular biology/biotechnology art.

[00114] Synthetically-created, codon-optimized gene sequences were cloned into the vector for inducible expression of the selected protein with kanamycin selection. The DNA and corresponding amino acid sequences for homeobox proteins PPL-002 (e.g., plasmid pJ411:129925) and PPL-003 (e.g., pJ411:129926) are shown in FIG. 6 and FIG. 7, respectively. Each expressed protein contains a 6 HIS leader, with a transition sequence of SAA, followed by the TEV cleavage site (ENLYFQS) to produce the PPL-002 or PPL-003 sequence with the appropriate N termimus.

[00115] Upon transformation into DH5α, six clones for each construct were tested for presence of the insert by restriction digest. The synthesized DNA sequence was sequenced for confirmation. Verified expression constructs for each protein were used to transform bacterial strain E. coli BL21 (DE3), a commercially available strain from Invitrogen (www.lifetechnologies.com/us/en/home/brands/invitrogen), and a PD glycerol cell stock was prepared for each best expressing clone.

[00116] Preliminary expression optimization studies were performed in shake flask cultures. Upon moving to small scale fermenters, further optimization of cell growth was performed. Early oxygen supplementation and a pH set point of about 7.1 were shown to be optimal. Induction temperature was optimized at about 18 °C.

[00117] Composition embodiments disclosed herein may comprise a pharmaceutically acceptable carrier, diluent or excipient. The term "pharmaceutically

acceptable carrier diluent or excipient" refers to any substance, not itself a therapeutic agent, used as a carrier or vehicle for delivery of a therapeutic agent to a subject or added to a pharmaceutical composition to improve its handling or storage properties or to permit or facilitate formation of a unit dose of the composition, and that does not produce unacceptable toxicity or interaction with other components in the composition.

[00118] The choice of pharmaceutically acceptable carrier, excipient or diluent may be selected based on the formulation and the intended route of administration, as well as standard pharmaceutical practice. Such compositions may comprise any agents that may aid, regulate, release or increase entry into the body compartment, tissue, intracellular or intranuclear target site, such as binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilizing agent(s), or other agents. An injectable implant for the sustained release of the protein may also be used to obtain prolonged exposure and action. The term "sustained release" refers to formulations from which the conjugate is released at a slow rate allowing for a longer period of exposure at active concentrations.

[00119] The compositions comprising one or more conjugates or fusion proteins disclosed herein can be administered, depending on condition to be treated or other considerations, in any number of ways, for example without limitation, by any one or more of the following: (1) inhalation; (2) in the form of a suppository or pessary; (3) in the form of a topical lotion, solution, cream, ointment or dusting powder; (4) by use of a skin patch; (5) orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavoring or coloring agents; (6) injected parenterally, for example intracavernosally, intravenously, intramuscularly or subcutaneously; (7) for ophthalmic diseases, they may be formulated as eye drops or for intraocular injection; (8) for parenteral administration, they may be in the form of a sterile aqueous solution or injectable implant which may contain other substances, for example, with adequate salt or monosaccharide content to make the solution isotonic with blood or substances that allow slow release; and (9) for buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

[00120] In one embodiment, the conjugate comprises:

a first region comprising a structure derived from (1) the human HOX genes, or (2) other human genes having a sequence described in any of SEQ ID Nos. 1 - 19, wherein 1 – 20, 1 – 15, or 1 - 10 amino acid residues are substituted, deleted, added, and/or inserted; and

a second region is a functional or regulatory polypeptide or protein not naturally associated with the first region. In various embodiments, the second region is selected from the group consisting of NBD peptide, PC1 CTT-derived p200, PC1 CTT-derived p21, glucocerebrosidase, alpha-L-iduronidase, iduronidate-2-sulfatase, retinal membrane guanylyl cyclase, the zinc finger peptide construct, ZF6xHunt-Kox-1, the zinc finger peptide construct, ZF12xHunt-Kox-1, and the zinc finger peptide construct, ZF18xHunt-Kox-1.

[00121] In one embodiment, the conjugate comprises:

a first region comprising a structure derived from (1) the human HOX genes, or (2) other human genes having a sequence described in any of SEQ ID Nos. 1 - 19, wherein 1 – 20, 1 – 15, or 1 - 10 amino acid residues are substituted, deleted, added, and/or inserted and wherein said amino acid has (i) has human homeodomain activity; and (ii) maintains the cell permeabilizing activity of the helical regions of the human HOX genes or other human genes containing a homeodomain with cell permeabilizing properties; and

a second region is a functional or regulatory polypeptide or protein not naturally associated with the first region. In various embodiments the second region is selected from the group consisting of NBD peptide, PC1 CTT-derived p200, PC1 CTT-derived p21, glucocerebrosidase, alpha-L-iduronidase, iduronidate-2-sulfatase, retinal membrane guanylyl cyclase, the zinc finger peptide construct, ZF6xHunt-Kox-1, the zinc finger peptide construct, ZF12xHunt-Kox-1, and the zinc finger peptide construct, ZF18xHunt-Kox-1.

[00122] In one embodiment, the active concentration of fusion protein or conjugate in cell culture is less than about 115 μM, less than about 100 μM, less than about 90 μM, less than about 80 μM, less than about 70 μM, less than about 65 μM, less than about 60 μM, less than about 55 μM, less than about 50 μM, less than about 45 μM, less than about 40 μM, less than about 35 μM, less than about 30 μM, less than about 25 μM, less than about 20 μM, less than about 15 μM, less than

about 10 μM, less than about 5 μM, or less than about 1 μM for example, less than about 1 μM to about 3 μM, less than about 1 μM to about 6 μM, less than about 1 μM to about 8 μM, less than about 1 μM to about 15 μM, less than about 1 μM to about 25 μM, less than about 1 μM to about 50 μM, less than about 1 μM to about 70 μM, less than about 1 μM to about 85 μM, less than about 1 μM to about 110 μM, less than about 10 μM to about 110 μM, less than about 15 μM to about 70 μM, less than about 15 μM to about 60 μM, less than about 20 μM to about 55 μM, or less than about 25 μM to about 45 μM.

[00123] In other embodiments, the dosage delivered (daily or as required) in mouse models (per 20 g mouse) through (1) an intravenous (i.v.) or intraperitoneal (i.p.) injection, (2) a topical formulation, or (3) an inhaled formulation is at least 500 μg, at least 450 μg, at least 400 μg, at least 350 μg, at least 300 μg, at least 250 μg, at least 200 μg, at least 150 μg, at least 100 μg, at least 80 μg, at least 70 μg, at least 60 μg, at least 50 μg, at least 40 μg, at least 30 μg, at least 20 μg, at least 10 μg, or at least 1 μg, for example, about 1 μg to about 500 μg, about 10 μg to about 450 μg, about 20 μg to about 400 μg about 30 μg to about 350 μg, about 30 μg to about 200 μg, about 30 μg to about 100 μg, about 40 μg to about 300 μg, about 40 μg to about 200 μg, about 50 μg to about 100 μg, about 50 μg to about 90 μg, about 55 μg to about 85 μg, about 60 μg to about 80 μg, about 60 μg to about 100 μg; about 1 μg to about 200 μg; about 1 μg to about 100 μg, about 1 μg to about 90 μg, about 1 μg to about 80 μg, about 1 μg to about 70 μg, about 1 to about 60 μg, about 1 to about 50 μg, about 1 to about 40 μg, about 1 to about 30 μg, about 1 to about 20 μg, about 1 to about 15 μg, about 1 to about 12 μg, about 1 to about 10 μg, about 1 to about 8 μg about 1 to about 6 μg, about 1 to about 4 μg, or about 1 to about 3 μg.

[00124] In other embodiments, the dosage delivered (daily or as required) through an intravenous (i.v.) or intraperitoneal (i.p.) injection in a mouse model is at least 100 mg/kg, less than about 80 mg/kg, less than about 45 mg/kg, less than about 40 mg/kg, less than about 30 mg/kg, less than about 25 mg/kg, less than about 20 mg/kg, less than about 15 mg/kg, less than about 12 mg/kg, less than about 10 mg/kg, less than about 8 mg/kg, less than about 4 mg/kg, less than about 2 mg/kg, or less than about 1 mg/kg, for example, less than about 1 mg/kg to about 50 mg/kg, about 5 mg/kg to about 40 mg/kg, about 8 mg/kg to about 30 mg/kg, about 10 mg/kg to about 20 mg/kg, or about 12 mg/kg to about 15 mg/kg, about 8 mg/kg to about 12

mg/kg, about 5 mg/kg to about 9 mg/kg, about 3 mg/kg to about 6 mg/kg, about 2 mg/kg to about 5 mg/kg, about 2 mg/kg to about 4 mg/kg, about 1 mg/kg to about 3 mg/kg, about 1 mg/kg to about 2.0 mg/kg.

[00125] In other embodiments, the dosage delivered (daily or as required) through an inhaled formulation in humans (70 kg weight) is at least about 600 mg, at least about 500 mg, at least about 450 mg, at least about 400 mg, at least about 350 mg, at least about 300 mg, at least about 250 mg, at least about 200 mg, at least about 150 mg, at least about 125 mg, at least about 100 mg, at least about 75 mg, at least about 50 mg, at least about 25 mg, at least about 20 mg, at least about 15 mg, at least about 10 mg, at least about 5 mg, at least about 1 mg, at least about 500 µg, at least about 450 µg, at least about 400 µg, at least about 350 µg, at least about 300 µg, at least about 250 µg, at least about 200 µg, at least about 150 µg, at least about 100 µg, at least about 80 µg, at least about 70 µg, at least about 60 µg, at least about 50 µg, at least about 40 µg, at least about 30 µg, at least about 20 µg, at least about 10 µg, or at least about 1 µg, for example, between about 1 µg to about 750 µg; about 1 µg to about 500 µg, about 10 µg to about 450 µg, about 20 µg to about 400 µg about 30 µg to about 350 µg, about 30 µg to about 200 µg, about 30 µg to about 100 µg, about 40 µg to about 300 µg, about 40 µg to about 200 µg, about 50 µg to about 100 µg, about 50 µg to about 90 µg, about 55 µg to about 85 µg, about 60 µg to about 80 µg, about 60 µg to about 100 µg; about 1 µg to about 200 µg; about 1 µg to about 100 µg, about 1 µg to about 90 µg, about 1 µg to about 80 µg, about 1 µg to about 70 µg, about 1 to about 60 µg, about 1 to about 50 µg, about 1 to about 40 µg, about 1 to about 30 µg, about 1 to about 20 µg, about 1 to about 15 µg, about 1 to about 12 µg, about 1 to about 10 µg, about 1 to about 8 µg about 1 to about 6 µg, about 1 to about 4 µg, about 1 µg to about 3 µg, about 1 µg to about 1 mg, about 1 µg to about 2 mg, about 1 µg to about 5 mg; about 1 µg to about 10 mg; about 1 mg to about 10 mg, about 1 mg to about 15 mg; about 2 mg to about 20 mg, about 3 mg to about 30 mg, about 4 mg to about 40 mg, about 5 mg to about 50 mg, about 5 mg to about 80 mg, about 5 mg to about 110 mg, about 10 mg to about 150 mg, about 10 mg to about 80 mg, about 20 mg to about 70 mg, about 20 mg to about 60 mg, about 30 mg to about 60 mg, about 120 mg to about 190 mg, about 130 mg to about 180 mg, about 130 mg to about 200 mg, about 140 mg to about 250 mg, about 180 mg to about 300 mg, about 190 mg to about 350 mg, about 220 mg to

about 400 mg, about 250 mg to about 425 mg, about 280 mg to about 460 mg, about 300 mg to about 480 mg, about 350 mg to about 490 mg, about 380 mg to about 550 mg, about 400 mg to about 580 mg, about 480 mg to about 590 mg, or about 520 mg to about 600 mg.

[00126] In other embodiments, the dosage delivered (daily or as required) through topical formulation in humans and in mouse models is less than about 5% wt/vol, less than about 4.5% wt/vol, less than about 3.5% wt/vol, less than about 2.5% wt/vol, less than about 1.5% wt/vol, less than about 0.5% wt/vol, less than about 0.4% wt/vol, less than about 0.3% wt/vol, less than about 0.2%, less than about 0.1% wt/vol, less than about 0.09% wt/vol, less than about 0.08% wt/vol, less than about 0.07% wt/vol, less than about 0.06% wt/vol, less than about 0.05% wt/vol, less than about 0.04% wt/vol, less than about 0.03% wt/vol, less than about 0.02% wt/vol, less than about 0.01% wt/vol, less than about 0.008% wt/vol, less than about 0.006% wt/vol, less than about 0.004% wt/vol, or less than about 0.002% wt/vol, for example between about 0.002% wt/vol and about 5% wt/vol, about 0.01% wt/vol and about 4% wt/vol, about 0.05% wt/vol and about 3% wt/vol, about 0.02% wt/vol and about 2.5% wt/vol, about 0.03% wt/vol and about 2% wt/vol, about 0.05% wt/vol and about 1% wt/vol, about 0.06% wt/vol and about 0.9% wt/vol, about 0.07% wt/vol and about 0.6% wt/vol, about 0.08% wt/vol and about 0.4% wt/vol, about 0.09% wt/vol and about 0.2% wt/vol or about 0.09 wt/vol and about 0.1% wt/vol.

[00127] In other embodiments the dosage delivered (daily or as required) through a topical formulation in humans (70 kg weight) is less than about 70 µg, less than about 50 µg, less than about 45 µg, less than about 40 µg, less than about 30 µg, less than about 25 µg, less than about 20 µg, less than about 15 µg, less than about 12 µg, less than about 10 µg, less than about 8 µg, less than about 4 µg, less than about 2 µg, or less than about 1 µg, for example, about 1 µg to about 50 µg, about 5 µg to about 40 µg, about 8 µg to about 30 µg, about 10 µg to about 20 µg, or about 12 µg to about 15 µg, about 8 µg to about 12 µg, about 5 µg to about 9, about 3 µg to about 6 µg, about 2 µg to about 5 µg, or less than about 1 µg to about 3 µg.

[00128] In another embodiment, the systemic dosage delivered (daily or as required) through an intravenous, subcutaneous or intramuscular injection or an injectable implant for sustained release formulations in humans is less than about 100 mg/kg, less than about 80 mg/kg, less than about 45 mg/kg, less than about 40

mg/kg, less than about 30 mg/kg, less than about 25 mg/kg, less than about 20 mg/kg, less than about 15 mg/kg, less than about 12 mg/kg, less than about 10 mg/kg, less than about 8 mg/kg, less than about 4 mg/kg, less than about 2 mg/kg, less than about 1 mg/kg, less than about 0.1 mg/kg, or less than about 0.01 mg/kg, for example, less than about 0.01 mg/kg to about 50 mg/kg, less than about 5 mg/kg to about 40 mg/kg, less than about 8 mg/kg to about 30 mg/kg, less than about 10 mg/kg to about 20 mg/kg, or less than about 12 mg/kg to about 15 mg/kg, less than about 8 mg/kg to about 12 mg/kg, less than about 5 mg/kg to about 9 mg/kg, less than about 3 mg/kg to about 6 mg/kg, less than about 2 mg/kg to about 5 mg/kg, less than about 2 mg/kg to about 4 mg/kg, less than about 1 mg/kg to about 3 mg/kg, less than about 0.2 mg/kg to about 2.0 mg/kg, less than about 0.1 mg/kg to about 1.5 mg/kg, or less than about 0.01 mg/kg to about 2.00 mg/kg.

[00129] In other embodiments, the dosage delivered (daily or as required) to humans (based on 70 kg weight) through any formulation other than an intravenous, subcutaneous, or intramuscular injection or injectable implant for the sustained release, inhaled or topical formulation is at least about 600 mg, at least about 500 mg, at least about 450 mg, at least about 400 mg, at least about 350 mg, at least about 300 mg, at least about 250 mg, at least about 200 mg, at least about 150 mg, at least about 125 mg, at least about 100 mg, at least about 75 mg, at least about 50 mg, at least about 25 mg, at least about 20 mg, at least about 15 mg, at least about 10 mg, at least about 5 mg, at least about 1 mg, at least about 500 µg, at least about 450 µg, at least about 400 µg, at least about 350 µg, at least about 300 µg, at least about 250 µg, at least about 200 µg, at least about 150 µg, at least about 100 µg, at least about 80 µg, at least about 70 µg, at least about 60 µg, at least about 50 µg, at least about 40 µg, at least about 30 µg, at least about 20 µg, at least about 10 µg, or at least about 1 µg, for example, between about 1 µg to about 750 µg; about 1 µg to about 500 µg, about 10 µg to about 450 µg, about 20 µg to about 400 µg about 30 µg to about 350 µg, about 30 µg to about 200 µg, about 30 µg to about 100 µg, about 40 µg to about 300 µg, about 40 µg to about 200 µg, about 50 µg to about 100 µg, about 50 µg to about 90 µg, about 55 µg to about 85 µg, about 60 µg to about 80 µg, about 60 µg to about 100 µg; about 1 µg to about 200 µg; about 1 µg to about 100 µg, about 1 µg to about 90 µg, about 1 µg to about 80 µg, about 1 µg to about 70 µg, about 1 to about 60 µg, about 1 to about 50 µg, about 1 to about 40 µg, about

1 to about 30 µg, about 1 to about 20 µg, about 1 to about 15 µg, about 1 to about 12 µg, about 1 to about 10 µg, about 1 to about 8 µg about 1 to about 6 µg, about 1 to about 4 µg, about 1 µg to about 3 µg, about 1 µg to about 1 mg, about 1 µg to about 2 mg, about 1 µg to about 5 mg; about 1 µg to about 10 mg; about 1 mg to about 10 mg, about 1 mg to about 15 mg; about 2 mg to about 20 mg, about 3 mg to about 30 mg, about 4 mg to about 40 mg, about 5 mg to about 50 mg, about 5 mg to about 80 mg, about 5 mg to about 110 mg, about 10 mg to about 150 mg, about 10 mg to about 80 mg, about 20 mg to about 70 mg, about 20 mg to about 60 mg, about 30 mg to about 60 mg, about 120 mg to about 190 mg, about 130 mg to about 180 mg, about 130 mg to about 200 mg, about 140 mg to about 250 mg, about 180 mg to about 300 mg, about 190 mg to about 350 mg, about 220 mg to about 400 mg, about 250 mg to about 425 mg, about 280 mg to about 460 mg, about 300 mg to about 480 mg, about 350 mg to about 490 mg, about 380 mg to about 550 mg, about 400 mg to about 580 mg, about 480 mg to about 590 mg, or about 520 mg to about 600 mg.

[00130] In one embodiment, the formulation is administered systemically or locally (e.g. intraocular injection, enema formulation, inhalation) at intervals of 6 hours, 12 hours, daily or every other day or on a weekly or monthly basis to elicit the desired benefit or otherwise provide a therapeutic effect. In another embodiment, the formulation is administered as required to elicit the desired benefit or otherwise provide a therapeutic effect.

[00131] In another embodiment, the improvement in post-myocardial infarction (MI) cardiac output will be assessed by ultrasound. In another embodiment, the improvement in post-stroke neurological function will be assessed by determining the percent of patients with a NIHSS of less than 17 one month after presentation. In another embodiment, immunogenicity of the human homeodomain or variant or portion thereof conjugates or fusion proteins will be assessed by determining the incorporation of 3H-thymidine by lymphocytes. In another embodiment, immunogenicity of the human homeodomain or variant or portion thereof conjugates or fusion proteins will be assessed by determining the level of specific antibodies produced by the recipient animal or human. In another embodiment, the extent of ischemia-reperfusion injury will be assessed using known histopathology techniques to examine the area of necrotic tissue in a stroke model. In yet another embodiment, the GAG accumulation will be assessed using known histopathology techniques. In

another embodiment behavioral and motor deficits in a mouse model of Huntington's Disease will be assessed using published methods evaluating grip strength in the forepaws and balance ability on a rotorod balance beam. In other embodiments each outcome will be assessed by known methods.

[00132] In one embodiment, upon treatment of one or more human or animal subjects with any of the fusion protein or conjugate embodiments disclosed, the subject(s) will exhibit one or more of the following outcomes:

(a) a reduction in shortness of breath or difficulty breathing;

(b) a reduction in cytokine production;

(c) a reduction of TNF-α concentration in blood;

(d) a reduction of IL-1 concentration in blood;

(e) a reduction of IL-6 concentration in blood;

(f) a reduction in bronchoalveolar lavage fluid (BALF) inflammatory cell numbers;

(g) a reduction in BALF cytokine levels (e.g. TNF- α, IL-1, IL-6, IL-8);

(h) Improvement of pulmonary function (e.g. FEV1);

(i) Improvement in functional (including exercise) capacity;

(j) a reduction in exacerbations of COPD requiring emergent care or hospitalization;

(k) restoration of tear secretion towards normal levels;

(l) a reduction in hair loss;

(m) an increase in hair growth;

(n) a reduction in the area of necrotic brain tissue by MRI;

(o) a reduction in C-reactive protein (CRP);

(p) a reduction in acute mortality (i.e., at one month);

(q) an improvement in post-myocardial infarction (MI) cardiac output as measured by ultrasound;

(r) an improvement in post-stroke neurological function as measured by the determining the percent of patients with a NIHSS of less than 17 one month after presentation;

(s) an inhibition in kidney growth;

(t) reduced allergic responses and infusion reactions to the 60-amino acid human homeodomain (or variant or portion thereof) conjugates or fusion proteins compared to previous treatments with the cargo second region alone;

(u) reduced anti-cargo peptide specific antibody levels;

(v) a reduction in the accumulation of a GCase substrate, glycosylceramide;

(w) a reduction in the urinary excretion of dermatan sulfate;

(x) a reduction in the urinary excretion of heparan sulfate;

(y) restoration of electroretinogram (ERG) function;

(z) restoration of visual acuity as determined through standard clinical methodology;

(aa) normalization or improvement in motor deficits, as assessed using the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score; and/or other clinically validated scoring methods;

(bb) normalization of skeletal development as assessed by standard growth and development pediatric techniques;

(cc) normalization of brain development as assessed by standard pediatric techniques;

(dd) a reduced progression of renal cyst size and number assessed by MRI;

(ee) an increase in the level of anti-inflammatory interleukin-10 (IL-10) in blood;

(ff) an increase in the level of anti-inflammatory interleukin-10 (IL-10) in BALF;

(gg) a reduction in the level of IP-10 in blood;

(hh) a reduction in the level of IP-10 in BALF; and/or

(ii) a reduction in amyloid A protein (SAA) in blood;

(jj) a reduction in mouse Kc or human IL-8 in blood or BALF;

(kk) a reduction in GM-CSF in blood or BALF;

(ll) prevention of an increase in graded vitreous haze and/or graded anterior chamber cells;

(mm) prevention of deterioration of visual acuity;

(nn) reduced tumor cell growth and/or reduced tumor growth;

(oo) reduced expression of DYRK1b; and/or

(pp) reduced activation of NF-κB regulated genes.

[00133] In another embodiment, the patient will be treated over a period, for example, of about 1 day through the lifetime of the patient, over a period of about 1 day to about 200 weeks, about 1 day to about 100 weeks, about 1 day to about 80 weeks, about 1 day to about 50 weeks, about 1 day to about 40 weeks, about 1 day to about 20 weeks, about 1 day to about 15 weeks, about 1 day to about 12 weeks, about 1 day to about 10 weeks, about 1 day to about 5 weeks, about 1 week to about 4 weeks, about 2 weeks to about 3 weeks, about 1 day to about 2 weeks, about 1 week, about 1 to 5 days, about 1 to 3 days, or about 1 to 2 days.

[00134] In another embodiment comprising a fusion protein or conjugate formulation utilized in any of the proposed studies in the examples provided, in other research and treatment, including animal research for human and animal applications, and veterinary treatment, the treatment group members, or the treatment group(s) will exhibit one or more of the following outcomes, each compared to baseline or control, unless otherwise indicated:

(a) an inhibition of NF-κB transcriptional activity;

(b) a reduction in cytokine production;

(c) an inhibition of TNF-α concentration in blood;

(d) an inhibition of IL-1 concentration in blood;

(e) an inhibition of IL-6 concentration in blood;

(f) a reduction in bronchoalveolar lavage fluid (BALF) inflammatory cell numbers;

(g) a reduction in bronchoalveolar lavage fluid (BALF) cytokine levels (e.g. TNF-α, IL-1, IL-6, IL-8);

(h) a reduction in goblet cell infiltration in the epithelial cornea;

(i) an attenuation of the loss of goblet cells in the conjunctiva;

(j) reduced lymph-angiogenesis;

(k) restoration of tear secretion towards normal levels;

(l) a reduction in hair loss or an increase in hair growth;

(m) a reduction in the area of necrotic brain tissue as assess with MRI;

(n) a reduction in C-reactive protein (CRP);

(o) a reduction in acute mortality (i.e., at one month);

(p) an improvement in post-myocardial infarction (MI) cardiac output as measured by ultrasound;

(q) an improvement in post-stroke neurological function as measured by the percent of patients with a NIHSS of less than 17 one month after presentation;

(r) an inhibition in kidney growth;

(s) an attenuated change in concentration of osteocalcin in blood, as compared to the change elicited by treatment with the human homeodomain-p200 fusion protein or conjugate, e.g. HOX C12-p200 or HOX D12-p200;

(t) reduced allergic responses and infusion reactions to the 60-amino acid human homeodomain (or variant or portion thereof) conjugates or fusion proteins, as assessed through incorporation of 3H-thymidine by lymphocytes;

(u) a reduction in anti-cargo peptide specific antibody levels

(v) a reduction in the accumulation of a GCase substrate, glycosylceramide;

(w) a reduction in the accumulation of α-synuclein/ubiquitin aggregates in the brain;

(x) a reduction in the urinary excretion of dermatan sulfate;

(y) a reduction in the urinary excretion of heparan sulfate;

(z) a reduction in dermatan sulfate as determined through histopathology;

(aa) a reduction in heparan sulfate as determined through histopathology;

(bb) restoration of electroretinogram (ERG) function;

(cc) restoration of visual acuity as determined through standard clinical methodology;

(dd) a reduction or repression of the mutant Huntingtin gene expression in the HD murine model;

(ee) normalization or improvement in motor deficits in patients, as assessed using the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score;

(ff) normalization or improvement in motor deficits in mice as assessed using published methods evaluating grip strength in the forepaws and balance ability on a rotorod balance beam;

(gg) a reduction in neuronal loss and/or reduced striatal cell volume as assessed by molecular methods and histological analysis in the HD murine model;

(hh) an increase in the level of anti-inflammatory interleukin-10 (IL-10) in blood;

(ii) an increase in the level of anti-inflammatory interleukin-10 (IL-10) in BALF;

(jj) a reduction in the level of IP-10 in blood;

(kk) a reduction in the level of IP-10 in BALF;

(ll) a reduction in serum amyloid A protein (SAA) in blood;

(mm) a reduction in or human IL-8 in blood or BALF;

(nn) a reduction in GM-CSF in blood or BALF;

(oo) prevention of an increase in graded vitreous haze and/or graded anterior chamber cells;

(pp) prevention of deterioration of visual acuity;

(qq) reduced tumor cell growth and/or reduced tumor growth;

(rr) reduced expression of DYRK1b; and/or

(ss) reduced activation of NF-κB regulated genes.

[00135] In another embodiment, the treatment with a formulation comprising a fusion protein and/or conjugate embodiment disclosed in clinical studies will extend over a period, for example, of about 1 day to about 52 weeks, about 1 day to about 26 weeks, about 1 day to about 16 weeks, about 1 day to about 12 weeks, about 1 day to about 10 weeks, about 1 day to about 5 weeks, about 1 week to about 4 weeks, about 2 weeks to about 3 weeks, about 1 day to about 2 weeks, about 1 week, about 1 to 6 days, about 1 to 4 days, or about 1 to 2 days.

[00136] In another embodiment, upon treatment with a formulation comprising a fusion protein and/or conjugate embodiments disclosed, the (1) patient(s) or (2) treatment group(s) as disclosed in the studies in the examples, including

experimental animals such as mice in animal models, exhibit one or more of the following outcomes compared to controls:

(a) an inhibition in NF-κB transcriptional activity of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(b) a reduction in cytokine production and/or concentration in blood and/or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(c) a reduction of TNF-α concentration in blood and/or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(d) a reduction of IL-1 concentration in blood and/or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least

about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(e) a reduction of IL-6 concentration in blood and/or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(f) a reduction in bronchoalveolar lavage fluid (BALF) inflammatory cell numbers of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(g) a reduction in bronchoalveolar lavage fluid (BALF) cytokines (e.g., TNF-α, IL-1, IL-6, IL-8) of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or

about 10% to about 80% (actual % change or median % change compared to baseline or control);

(h) a reduction in goblet cell infiltration in the epithelial cornea of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(i) an attenuation of the loss of goblet cells in the conjunctiva of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(j) reduced lymph-angiogenesis of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(k) restoration of tear secretion towards normal levels as measured by the Schirmer test, with an increase in Schirmer wetting of more than 20 mm, more than 15 mm, more than 10 mm, more than 5 mm, for example between 5 mm and 20 mm, 10 mm

and 20 mm, 5 mm and 15 mm, 5mm and 10 mm or 15 mm to 20 mm (actual % change or median % change compared to baseline or control);

(l) a reduction in hair loss or an increase in hair growth of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(m) a reduction in the area of necrotic brain tissue of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(n) a reduction in serum C-reactive protein (CRP) of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(o) a reduction in acute mortality (i.e., at one month) of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least

about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(p) an improvement in post-myocardial infarction (MI) cardiac output, as measured by ultrasound, of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control)

(q) an improvement in post-stroke neurological function as measured by the percent of patients with a NIHSS of less than 17, one month after presentation, of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(r) an inhibition in kidney growth of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about

5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(s) an attenuated change in concentration of osteocalcin using the HOX C12-p21, HOX D12-p21, or other p21-containing fusion protein or conjugate in blood comprising a human homeodomain with cell membrane permeabilizing properties of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to the change elicited by treatment with, e.g., the HOX C12-p200 fusion protein or conjugate or other p200-containing fusion protein or conjugate comprising a human homeodomain with cell membrane permeabilizing properties);

(t) reduced allergic responses and infusion reactions of the 60-amino acid human homeodomain (or variant or portion thereof) conjugates or fusion proteins compared to their cargo proteins alone, as assessed through incorporation of 3H-thymidine by lymphocytes of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change from baseline or control);

(u) a reduction in anti-cargo peptide specific antibody levels after treatment with 60-amino acid human homeodomain (or variant or portion thereof) conjugates or fusion proteins compared to treatment with their cargo proteins alone, of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about

35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change from baseline or control);

(v) reduction in the accumulation of a GCase substrate, glycosylceramide of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(w) reduction in the accumulation of α-synuclein/ubiquitin aggregates in the brain of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(x) reduction in the urinary excretion of dermatan sulfate of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(y) reduction in the urinary excretion of heparan sulfate of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(z) reduction in dermatan sulfate as determined through histopathology of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(aa) reduction in heparan sulfate as determined through histopathology of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(bb) the restoration of electroretinogram (ERG) function of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40%

to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(cc) the restoration of visual acuity as determined through standard clinical methodology of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(dd) a reduction or repression of the mutant Huntingtin gene expression in the HD murine model of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(ee) normalization or improvement in motor deficits in patients, as assessed using the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score by an absolute amount of at least about 4 points, at least about 3 points, at least about 2 points, at least about 1.5 points, at least about 1 point, at least about 0.5 point, at least about 0.4 point, at least about 0.3 point, or at least about 0.2 point, for example about 0.5 to about 2 points, about 1 to about 2 points, or about 1.5 to about 2.5 points, and/or an improvement in total motor score of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least

about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, about 10% to about 80%, about 10% to about 30%, about 10% to about 20%, about 20% to about 40%, about 30% to about 50% (compared to baseline and untreated control groups);

(ff) normalization or improvement in motor deficits in mice as assessed using published methods evaluating grip strength in the forepaws and balance ability on a rotorod balance beam of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, about 10% to about 80%, about 10% to about 30%, about 10% to about 20%, about 20% to about 40%, about 30% to about 50% (compared to baseline and untreated control groups);

(gg) a reduction in neuronal loss and/or reduced striatal cell volume as assessed by molecular methods and histological analysis in the HD murine model of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(hh) an increase in IL-10 in plasma (blood) and/or BALF of at least about 3,000%, at least about 2,000%, at least about 1,000%, at least about 500%, at least about 380%, at least about 360%, at least about 340%, at least about 320%, at least about 300%, at least about 280%, at least about 260%, at least about 240%, at least about 220%, at least about 200%, at least about 180%, at least about 160%, at least about

140%, at least about 120%, at least about 110%, at least about 100%, at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 400% to about 500%, about 450% to about 500%, about 420% to about 480%, about 300% to about 450%, about 350% to about 430%, about 320% to about 380%, about 200% to about 350%, about 250% to about 330%, about 220% to about 280%, about 100% to about 250%, about 150% to about 230%, about 120% to about 180%, about 50% to about 150%, about 60% to about 130%, about 80% to about 110%, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(ii) an increase in IL-10 in blood or BALF to least about 300 pg/mL, to at least about 400 pg/mL, to least about 500 pg/mL, to least about 600 pg/mL, to least about 800 pg/mL, to least about 1000 pg/mL, to least about 1200 pg/mL, to least about 1400 pg/mL, to least about 1600 pg/mL, to least about 1800 pg/mL, to least about 2000 pg/mL, to least about 2200 pg/mL, to least about 2400 pg/mL, to least about 2600 pg/mL, to least about 2800 pg/mL, to least about 3000 pg/mL, to at least about 4000 pg/ml, for example, about 300 mg/mL to about 3900 pg/mL, about 380 pg/mL to about 2900 pg/mL, about 400 pg/mL to about 2800 pg/mL, about 450 pg/mL to about 2500 pg/mL, about 500 pg/mL to about 2400 pg/mL, about 800 pg/mL to about 3000 pg/mL, about 900 pg/mL to about 2900 pg/mL, about 1200 pg/mL to about 2900 pg/mL, about 1400 pg/mL to about 2900 pg/mL, about 1800 pg/mL to about 2900 pg/mL, about 2000 pg/mL to about 2900 pg/mL, about 2200 pg/mL to about 2900 pg/mL, about 2200 pg/mL to about 3000 pg/mL, about 2500 pg/mL to about 3000 pg/mL, or about 2500 pg/mL to about 3000 pg/mL.

(jj) a reduction in the level of IP-10 in blood and BALF to at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about

70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control),

(kk) a reduction in serum amyloid A protein (SAA) in blood (plasma) of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(ll) a reduction in Mouse Kc or human IL-8 in blood and/or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(mm); a reduction in GM-CSF in blood or BALF of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control);

(nn) prevention of a clinically significant increase in graded vitreous haze and/or graded anterior chamber cells based on commonly accepted clinical criteria known to those expert in the field.

(oo) prevention of deterioration of a clinically significant change in visual acuity based on commonly used and accepted criteria known to those expert in the field;

(pp) reduced tumor cell growth and/or reduced tumor growth of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control); and/or

(rr) reduced expression of DYRK1b of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control); and/or

(ss) reduced activation of NF-κB regulated genes of at least about 99%, at least about 95%, at least about 90%, at least about 80%, at least about 70%, at least about 60%, at least about 50%, at least about 40%, at least about 35%, at least about 30%, at least about 20%, at least about 15%, at least about 10%, or at least about 5%, for example, about 30% to about 99%, about 80% to about 90%, about 70% to about 90%, about 60% to about 90%, about 50% to about 90%, about 40% to about 90%, about 35% to about 90%, about 30% to about 90%, about 25% to about 90%, about 5% to about 85%, or about 10% to about 80% (actual % change or median % change compared to baseline or control).

[00137] According to the embodiments disclosed and their modifications, the conjugates or fusion proteins may be used alone or in combination with other treatments or components of other treatments. Diseases and disorders or conditions that may be treated include, but are not limited to: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, hemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumor growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischemia, ischemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis; psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endoscierosis, cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumor immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration: inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilizing specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating hemophilia and stroke); antiinflammatory activity (for treating e.g. septic shock or Crohn's disease); as antimicrobials: modulators of e.g. metabolism or behavior; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine; macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere

to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scieritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome,

Sydenham chora, myasthenia gravis, pseudo-tumor cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases, e.g. leukemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

[00138] Table 3: Peptide Sequence Identities.

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
1	**HOX C12**	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLL
2	**HOX D12**	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVV
3	**OTP**	QKRHRTRFTPAQLNELERSFAKTHYPDIFMREELAL RIGLTESRVQVWFQNRRAKWKKRK
4	**TLX1**	KKKPRTSFTRLQICELEKRFHRQKYLASAERAALAK ALKMTDAQVKTWFQNRRTKWRRQT
5	**TLX3**	RKKPRTSFSRVQICELEKRFHRQKYLASAERAALAK SLKMTDAQVKTWFQNRRTKWRRQT
6	**TLX2**	RKKPRTSFSRSQVLELERRFLRQKYLASAERAALAK ALRMTDAQVKTWFQNRRTKWRRQT
7	**NKX6-1**	RKHTRPTFSGQQIFALEKTFEQTKYLAGPERARLAY SLGMTESQVKVWFQNRRTKWRKKH
8	**NKX6-2**	KKHSRPTFSGQQIFALEKTFEQTKYLAGPERARLAY SLGMTESQVKVWFQNRRTKWRKRH
9	**NKX6-3**	KKHTRPTFTGHQIFALEKTFEQTKYLAGPERARLAY SLGMTESQVKVWFQNRRTKWRKKS
10	**NKX3-2**	KKRSRAAFSHAQVFELERRFNHQRYLSGPERADLA ASLKLTETQVKIWFQNRRYKTKRRQ
11	**BARHL1**	PRKARTAFTDHQLAQLERSFERQKYLSVQDRMELA ASLNLTDTQVKTWYQNRRTKWKRQT
12	**BARHL2**	PRKARTAFSDHQLNQLERSFERQKYLSVQDRMDLA AALNLTDTQVKTWYQNRRTKWKRQT

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
13	**BARX1**	GRRSRTVFTELQLMGLEKRFEKQKYLSTPDRIDLAE SLGLSQLQVKTWYQNRRMKWKKIV
14	**BARX2**	PRRSRTIFTELQLMGLEKKFQKQKYLSTPDRLDLAQ SLGLTQLQVKTWYQNRRMKWKKMV
15	**BSX**	RRKARTVFSDSQLSGLEKRFEIQRYLSTPERVELAT ALSLSETQVKTWFQNRRMKHKKQL
16	**DLX2**	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKFKKMW
17	**DLX3**	VRKPRTIYSSYQLAALQRRFQKAQYLALPERAELA AQLGLTQTQVKIWFQNRRSKFKKLY
18	**DBX1**	GMLRRAVFSDVQRKALEKMFQKQKYISKPDRKKL AAKLGLKDSQVKIWFQNRRMKWRNSK
19	**DBX2**	GILRRAVFSEDQRKALEKMFQKQKYISKTDRKKLAI NLGLKESQVKIWFQNRRMKWRNSK
20	**NEMO binding domain ("NBD")** (second region peptide only)	TALDWSWLQTE
21	**HOX C12-NBD (**Entire sequence: combined SEQ ID Nos. 1 and 20)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLLTALDWSWLQT E

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
22	**HOX D12-NBD** (Entire sequence: combined SEQ ID Nos. 2 and 20)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVVTALDWSWLQ TE
23	**p200 peptide derived from the PC1 CTT** (second region peptide only)	VILRWRYHALRGELYRPAWEPQDYEMVELFLRRL RLWMGLSKVKEFRHKVRFEGMEPLPSRSSRGSKVS PDVPPPSAGSDASHPSTSSSQLDGLSVSLGRLGTRCE PEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLH SLQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRG VDLATGPSRTPLRAKNKVHPSST
24	**HOX C12-p200** (Entire sequence: combined SEQ ID Nos. 1 and 23, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLVILRWRYHA LRGELYRPAWEPQDYEMVELFLRRLRLWMGLSKV KEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGS DASHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQAV FEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSSR APAGSSRGPSPGLRPALPSRLARASRGVDLATGPSR TPLRAKNKVHPSST
25	**HOX D12-p200** (Entire sequence: combined SEQ ID Nos. 2 and 23, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVVILRWRYH ALRGELYRPAWEPQDYEMVELFLRRLRLWMGLSK VKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAG SDASHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQA VFEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSS RAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPS RTPLRAKNKVHPSST
26	**p21 peptide** (alternate second region peptide for PKD structures)	IRRIRLWMGLSKVKEFRHKVR

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
27	**HOX C12-p21 (**Entire sequence: combined SEQ ID Nos. 1 and 26, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLIRRIRLWMG LSKVKEFRHKVR
28	**HOX D12-p21 (**Entire sequence: combined SEQ ID Nos. 2 and 26, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVIRRIRLWM GLSKVKEFRHKVR
29	**VRQ397-** (noncompetitive V2 receptor antagonist. that can be linked to protein structures for targeting p21 and p200 containing HOX or Human Homeodomain fusion peptides)	CRAVKY

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
30	**glucocerbrosidase amino acid sequence ("GCase")** (second region peptide only)	ARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTF SRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQ KFQKVKGFGGAMTDAAALNILALSPPAQNLLLKSY FSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLH NFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPWTSP TWLKTNGAVNGKGSLKGQPGDIYHQTWARYFVKF LDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGF TPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRL LLPHWAKVVLTDPEAAKYVHGIAVHWYLDFLAPA KATLGETHRLFPNTMLFASEACVGSKFWEQSVRLG SWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEG GPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSK FIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVV VLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRR Q
31	**HOX C12-GCase** (Entire sequence: combined SEQ ID Nos. 1 and 30, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLARPCIPKSFG YSSVVCVCNATYCDSFDPPTFPALGTFSRYESTRSG RRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGF GGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYN IIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDT KLKIPLIHRALQLAQRPVSLLASPWTSPTWLKTNGA VNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHK LQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKV VLTDPEAAKYVHGIAVHWYLDFLAPAKATLGETH RLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQ YSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRN FVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQR VGLVASQKNDLDAVALMHPDGSAVVVVLNRSSKD VPLTIKDPAVGFLETISPGYSIHTYLWRRQ

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
32	**HOX D12-GCase** (Entire sequence: combined SEQ ID Nos. 2 and 30, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVARPCIPKSF GYSSVVCVCNATYCDSFDPPTFPALGTFSRYESTRS GRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKG FGGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGY NIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEED TKLKIPLIHRALQLAQRPVSLLASPWTSPTWLKTNG AVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEH KLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAK VVLTDPEAAKYVHGIAVHWYLDFLAPAKATLGET HRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGM QYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVR NFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQ RVGLVASQKNDLDAVALMHPDGSAVVVVLNRSSK DVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
33	**RetGC-1** (second region peptide only)	AVFTVGVLGPWACDPIFSRARPDLAARLAAARLNR DPGLAGGPRFEVALLPEPCRTPGSLGAVSSALARVS GLVGPVNPAACRPAELLAEEAGIALVPWGCPWTQA EGTTAPAVTPAADALYALLRAFGWARVALVTAPQ DLWVEAGRSLSTALRARGLPVASVTSMEPLDLSGA REALRKVRDGPRVTAVIMVMHSVLLGGEEQRYLL EAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALA NSSQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQER RELPSDLNLQQVSPLFGTIYDAVFLLARGVAEARAA AGGRWVSGAAVARHIRDAQVPGFCGDLGGDEEPP FVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHF PRGGSAPGPDPSCWFDPNNICGGGLEPGLVFLGFLL VVGMGLAGAFLAHYVRHRLLHMQMVSGPNKIILT VDDITFLHPHGGTSRKVAQGSRSSLGARSMSDIRSG PSQHLDSPNIGVYEGDRVWLKKFPGDQHIAIRPATK TAFSKLQELRHENVALYLGLFLARGAEGPAALWEG NLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLL DLIKGIRYLHHRGVAHGRLKSRNCIVDGRFVLKITD HGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPAL ERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEE VVQRVRSPPPLCRPLVSMDQAPVECILLMKQCWAE QPELRPSMDHTFDLFKNINKGRKTNIIDSMLRMLEQ YSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVA EALKTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPI EVVDLLNDLYTLFDAIIGSHDVYKVETIGDAYMVA SGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMP EVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVN TASRMESTGLPYRIHVNLSTVGILRALDSGYQVELR GRTELKGKGAEDTFWLVGRRGFNKPIPKPPDLQPG SSNHGISLQEIPPERRRKLEKARPGQFS

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
34	**HOX C12-RetGC-1** (Entire sequence: combined SEQ ID Nos. 1 and 33, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLAVFTVGVLG PWACDPIFSRARPDLAARLAAARLNRDPGLAGGPR FEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPA ACRPAELLAEEAGIALVPWGCPWTQAEGTTAPAVT PAADALYALLRAFGWARVALVTAPQDLWVEAGRS LSTALRARGLPVASVTSMEPLDLSGAREALRKVRD GPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTD GSLVFLPFDTIHYALSPGPEALAALANSSQLRRAHD AVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQ VSPLFGTIYDAVFLLARGVAEARAAAGGRWVSGA AVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAAG DRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPD PSCWFDPNNICGGGLEPGLVFLGFLLVVGMGLAGA FLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPHG GTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIG VYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQELR HENVALYLGLFLARGAEGPAALWEGNLAVVSEHC TRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLH HRGVAHGRLKSRNCIVDGRFVLKITDHGHGRLLEA QKVLPEPPRAEDQLWTAPELLRDPALERRGTLAGD VFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRSPP PLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMD HTFDLFKNINKGRKTNIIDSMLRMLEQYSSNLEDLI RERTEELELEKQKTDRLLTQMLPPSVAEALKTGTPV EPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDL YTLFDAIIGSHDVYKVETIGDAYMVASGLPQRNGQ RHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGL HSGPCVAGVVGLTMPRYCLFGDTVNTASRMESTG LPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKG AEDTFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQEI PPERRRKLEKARPGQFS

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
35	**HOX D12-RetGC-1** (Entire sequence: combined SEQ ID Nos. 2 and 33, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVAVFTVGVL GPWACDPIFSRARPDLAARLAAARLNRDPGLAGGP RFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNP AACRPAELLAEEAGIALVPWGCPWTQAEGTTAPAV TPAADALYALLRAFGWARVALVTAPQDLWVEAGR SLSTALRARGLPVASVTSMEPLDLSGAREALRKVR DGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLT DGSLVFLPFDTIHYALSPGPEALAALANSSQLRRAH DAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQ QVSPLFGTIYDAVFLLARGVAEARAAAGGRWVSG AAVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAA GDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGP DPSCWFDPNNICGGGLEPGLVFLGFLLVVGMGLAG AFLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPH GGTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNI GVYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQEL RHENVALYLGLFLARGAEGPAALWEGNLAVVSEH CTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYL HHRGVAHGRLKSRNCIVDGRFVLKITDHGHGRLLE AQKVLPEPPRAEDQLWTAPELLRDPALERRGTLAG DVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRSP PPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSM DHTFDLFKNINKGRKTNIIDSMLRMLEQYSSNLEDL IRERTEELELEKQKTDRLLTQMLPPSVAEALKTGTP VEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLND LYTLFDAIIGSHDVYKVETIGDAYMVASGLPQRNG QRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIG LHSGPCVAGVVGLTMPRYCLFGDTVNTASRMEST GLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGK GAEDTFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQ EIPPERRRKLEKARPGQFS
36	**ZF6xHunt-Kox-1** (second region peptide only) showing 6 zinc finger repeats	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRN FSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHT GEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGG GSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEG MDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTA QQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKG EEPWLVEREIHQETHPDSETAFEIKSSV

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
37	**HOX C12-ZF6xHunt-Kox-1** (Entire sequence: combined SEQ ID Nos. 1 and 36, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQC RICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQR HIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTA WSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNV MLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVER EIHQETHPDSETAFEIKSSV
38	**HOX D12-ZF6xHunt-Kox-1** (Entire sequence: combined SEQ ID Nos. 2 and 36, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTG SERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTA WSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNV MLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVER EIHQETHPDSETAFEIKSSV
39	**ZF12xHunt-Kox-1** (second region peptide only) showing 12 zinc finger repeats	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRN FSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHT GEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGG GSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI HTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPF ACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMR NFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATL QRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSP QHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVF VDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLG YQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETA FEIKSSV

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
40	**HOX C12-ZF12xHunt-Kox-1** (entire sequence, combined SEQ ID Nos. 1 and 39, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQC RICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQR HIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTH TGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLR QKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGI IKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEW KLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVI LRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV
41	**HOX D12-ZF12xHunt-Kox-1** (entire sequence, combined SEQ ID Nos. 2 and 39, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTG SERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTH TGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLR QKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGI IKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEW KLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVI LRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
42	**ZF18xHunt-Kox-1** (second region peptide only) showing 18 zinc finger repeats	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRN FSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHT GEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGG GSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI HTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPF ACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMR NFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATL QRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAE RPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDIC GRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQR ATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTK IHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPF ACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGG GSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKS LTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYR NVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLV EREIHQETHPDSETAFEIKSSV
43	**HOX C12-ZF18xHunt-Kox-1** (entire sequence, combined SEQ ID Nos. 1 and 42, also showing initiating methionine residue)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQC RICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQR HIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTH TGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLR QKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICM RNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRAT LQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRT HTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPF QCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRK FAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGS QLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRT LVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLEN YKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQE THPDSETAFEIKSSV

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
44	**HOX D12-ZF12xHunt-Kox-1** (entire sequence, combined SEQ ID Nos. 2 and 42, also showing initiating methionine residue)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTG SERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSG GGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTH TGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQ CRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKF AQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLR QKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICM RNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRAT LQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRT HTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPF QCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRK FAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGS QLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRT LVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLEN YKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQE THPDSETAFEIKSSV
45	**Alpha-L-iduronidase** (second region 653 amino acid second region peptide including the 27 amino acid signal sequence)	MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVD AARALWPLRRFWRSTGFCPPLPHSQADQYVLSWD QQLNLAYVGAVPHRGIKQVRTHWLLELVTTRGST GRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASG HFTDFEDKQQVFEWKDLVSSLARRYIGRYGLAHVS KWNFETWNEPDHHDFDNVSMTMQGFLNYYDACS EGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCH DGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKV VAQQIRQLFPKFADTPIYNDEADPLVGWSLPQPWR ADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLS NDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLL RKPVLTAMGLLALLDEEQLWAEVSQAGTVLDSNH TVGVLASAHRPQGPADAWRAAVLIYASDDTRAHP NRSVAVTLRLRGVPPGPGLVYVTRYLDNGLCSPDG EWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPA GGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLRA LPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKA YTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALDYW ARPGPFSDPVPYLEVPVPRGPPSPGNP

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
46	**HOX C12-Alpha-L-iduronidase** (Entire sequence , combined SEQ ID Nos. 1 and 45, also showing initiating methionine residue	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLMRPLRPRAA LLALLASLLAAPPVAPAEAPHLVHVDAARALWPLR RFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVG AVPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFTH LDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQQ VFEWKDLVSSLARRYIGRYGLAHVSKWNFETWNE PDHHDFDNVSMTMQGFLNYYDACSEGLRAASPAL RLGGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGEA GVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPK FADTPIYNDEADPLVGWSLPQPWRADVTYAAMVV KVIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHP FAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLA LLDEEQLWAEVSQAGTVLDSNHTVGVLASAHRPQ GPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRG VPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPT AEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRL PSLLLVHVCARPEKPPGQVTRLRALPLTQGQLVLV WSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTF NLFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVP YLEVPVPRGPPSPGNP
47	**HOX D12-Alpha-L-iduronidase** (Entire sequence , combined SEQ ID Nos. 2 and 45, also showing initiating methionine residue	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVMRPLRPRA ALLALLASLLAAPPVAPAEAPHLVHVDAARALWPL RRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYV GAVPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFT HLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQ QVFEWKDLVSSLARRYIGRYGLAHVSKWNFETWN EPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPA LRLGGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGE AGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFP KFADTPIYNDEADPLVGWSLPQPWRADVTYAAMV VKVIAQHQNLLLANTTSAFPYALLSNDNAFLSYHP HPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGL LALLDEEQLWAEVSQAGTVLDSNHTVGVLASAHR PQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLR GVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFP TAEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALR LPSLLLVHVCARPEKPPGQVTRLRALPLTQGQLVLV WSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTF NLFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVP YLEVPVPRGPPSPGNP

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
48	**Iduronate-2-sulfatase** (second region 550 amino acid sequence including the 25 amino acid signal sequence)	MPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTD ALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHS LLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNS YWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGIS SNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGEL HANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMK TSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLA PDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPY GPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDL QLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATH VPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEP GRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHV ELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQ YPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTV WVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMY NDSQGGDLFQLLMP
49	**HOX C12-Iduronate-2-sulfatase** (Entire sequence, combined SEQ ID nos. 1 and 48, also showing initiating methionine)	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELS DRLNLSDQQVKIWFQNRRMKKKRLLMPPPRTGRG LLWLGLVLSSVCVALGSETQANSTTDALNVLLIIVD DLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQ AVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNF STIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYS WSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVD VLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAV GYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLP PVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKI RQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFT SDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGR TASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLV ELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKN LLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQ WNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEF LANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLF QLLMP

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
50	**HOX D12-Iduronate-2-sulfatase** (Entire sequence, combined SEQ ID nos. 2 and 48, also showing initiating methionine)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKEL SNRLNLSDQQVKIWFQNRRMKKKRVVMPPPRTGR GLLWLGLVLSSVCVALGSETQANSTTDALNVLLIIV DDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQ QAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGN FSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPY SWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPV DVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLA VGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDG LPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQR KIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAF TSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPG RTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDL VELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGK NLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIP QWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPD EFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGD LFQLLMP
51	**Peptide One of Four spanning the Ag85A 99-118 Epitope** (second region 15 amino acid sequence, TB Vaccine)	ETFLTSELPGWLQAN
52	**HOX C12-Ag85A 99-118 Epitope Peptide One of Four** (Entire sequence, combined SEQ ID nos. 1 and 51, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLLETFLTSELPGW LQAN

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
53	**HOX D12-Ag85A 99-118 Epitope, Peptide One of Four** (Entire sequence, combined SEQ ID nos. 2 and 51, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVVETFLTSELPG WLQAN
54	**Peptide Two of Four spanning the Ag85A 99-118 epitope** (second region 15 amino acid sequence, TB Vaccine)	SELPGWLQANRHVKP
55	**HOX C12-Ag85A 99-118 Epitope, Peptide Two of Four** (Entire sequence, combined SEQ ID nos. 1 and 54, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLLSELPGWLQAN RHVKP

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
56	**HOX D12-Ag85A 99-118 Epitope, Peptide Two of Four** (Entire sequence, combined SEQ ID nos. 2 and 54, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVSELPGWLQANRHVKP
57	**Peptide Three of Four spanning the Ag85A 99-118 epitope** (second region 15 amino acid sequence, TB Vaccine)	WLQANRHVKPTGSAV
58	**HOX C12-Ag85A 99-118 Epitope, Peptide Three of Four** (Entire sequence, combined SEQ ID nos. 1 and 57, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLWLQANRHVKPTGSAV

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
59	**HOX D12-Ag85A 99-118 Epitope, Peptide Three of Four** (Entire sequence, combined SEQ ID nos. 2 and 57, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVVWLQANRHVK PTGSAV
60	**Peptide Four of Four spanning the Ag85A 99-118 epitope** (second region 15 amino acid sequence, TB Vaccine)	RHVKPTGSAVVGLSM
61	**HOX C12-Ag85A 99-118 Epitope, Peptide Four of Four** (Entire sequence, combined SEQ ID nos. 1 and 60, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLLRHVKPTGSAV VGLSM

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
62	**HOX D12-Ag85A 99-118 Epitope, Peptide Four of Four** (Entire sequence, combined SEQ ID nos. 2 and 60, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVRHVKPTGSAVVGLSM
63	**Peptide One of Three spanning the Ag85A 70-78 epitope** (second region 15 amino acid sequence, TB Vaccine)	QSGLSVVMPVGGQSS
64	**HOX C12-Ag85A 70-78 Epitope, Peptide One of Three** (Entire sequence, combined SEQ ID nos. 1 and 63, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLQSGLSVVMPVGGQSS

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
65	**HOX D12-Ag85A 70-78 Epitope, Peptide One of Three** (Entire sequence, combined SEQ ID nos. 2 and 63, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVQSGLSVVMPVGGQSS
66	**Peptide Two of Three spanning the Ag85A 70-78 Epitope** (second region 15 amino acid sequence, TB Vaccine)	VVMPVGGQSSFYSDW
67	**HOX C12-Ag85A 70-78 Epitope, Peptide Two of Three** (Entire sequence, combined SEQ ID nos. 1 and 66, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLVVMPVGGQSSFYSDW

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
68	**HOX D12-Ag85A 70-78 Epitope, Peptide Two of Three** (Entire sequence, combined SEQ ID nos. 2 and 66, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVVVMPVGGQSSFYSDW
69	**Peptide Three of Three spanning the Ag85A 70-78 Epitope** (second region 15 amino acid sequence, TB Vaccine)	GGQSSFYSDWYQPAC
70	**HOX C12-Ag85A 70-78 Epitope, Peptide Three of Three** (Entire sequence, combined SEQ ID nos. 1 and 69, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLGGQSSFYSDWYQPAC

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
71	**HOX D12-Ag85A 70-78 Epitope, Peptide Three of Three** (Entire sequence, combined SEQ ID nos. 2 and 69, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVVGGQSSFYSDW YQPAC
72	**Ag85A Epitope** (second region cargo)	AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA
73	**HOX C12-Ag85A** (Entire sequence, combined SEQ ID nos. 1 and 72, TB Vaccine)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSD RLNLSDQQVKIWFQNRRMKKKRLL AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA

Sequence ID No.	Descriptor or Gene from which the Sequence was Derived	Sequence
74	**HOX D12-Ag85A (**Entire sequence, combined SEQ ID nos. 2 and 72, TB Vaccine)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSN RLNLSDQQVKIWFQNRRMKKKRVV AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA

[00139] Various features and embodiments and select modifications will now be described by way of non-limiting examples. In all examples where an initiating methionine is included in a complete sequence to allow recombinant synthesis, the initiating methionine may be removed during purification to derive the active product with the indicated human homeodomain sequence. References to sequence numbers for complete conjugates in paragraphs describing their use are referring to the conjugate after removal of the initiating methionine.

EXAMPLE 1

[00140] Conjugates or fusion peptides or proteins comprised of the 60-amino acid human homeodomain and the cargo NBD peptide that inhibits NF-κB transcription factor generation are examples of embodiments that regulate gene expression through interference with protein-protein interactions.

[00141] In one embodiment, the HOX C12 amino acid sequence first region is SEQ ID No. 1:

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment, the HOX D12 amino acid sequence first region is SEQ ID No. 2:

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

In these two embodiments, this first region is linked via a simple peptide bond or, alternatively, a linker known in the art, (See Table 2), to the NF-κB essential modifier (NEMO), abbreviated NBD, for **N**EMO **b**inding **d**omain, the "cargo" peptide. Any linker may be used provided its addition does not compromise the function of the conjugate. The NBD amino acid sequence is SEQ ID No. 20, the second region:

TALDWSWLQTE

and the entire fusion protein sequence embodiment incorporating the HOX C12 first region (SEQ ID No. 1), containing only a peptide bond linking the two regions, is:

SEQ ID No. 21,

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLLTALDWSWLQTE

The entire fusion protein sequence embodiment incorporating the HOX D12 first region (SEQ ID No. 2), containing only a peptide bond linking the two regions, is:

SEQ ID No. 22:

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVVTALDWSWLQTE

[00142] The size of each of SEQ ID Nos 21 and 22 allows the entire fusion protein to be produced synthetically. If it is produced through recombinant synthesis SEQ ID Nos. 21 and 22, or variants or portions thereof may include an N-terminal methionine. Linkers are not required for function but linkers may be included in alternative embodiments between the first and second regions provided they do not compromise function of the conjugate. To that end, any linker known in the art (see, e.g., Table 2) may be used to produce embodiments of this conjugate, and would alter SEQ ID Nos. 21 and 22, at least in part, to the extent that the linker sequence would bridge these first and second regions.

[00143] In still further embodiments, additional amino acid sequences ("tails") can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (HHHHHH), and other similar tags known to those in the art. Such tags may or may not include linkers. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human homeodomain peptide first region. FIG. 5 shows one embodiment in which the HOX D12 homeodomain is conjugated to the NBD cargo peptide, and is further conjugated to a His-tag through a GS linker.

[00144] In various embodiments, SEQ ID Nos. 21 and 22 or a portion thereof may be formulated for systemic or local delivery for the treatment of inflammatory diseases. Embodiments of local delivery include examples such as an inhaled liquid or dry powder formulation for chronic obstructive lung disease (COPD), a colonic enema formulation for ulcerative colitis or Crohn's disease (inflammatory bowel disease) with colonic and/or rectal inment, an eye drop formulation for inflammatory eye diseases such as dry eyes (keratoconjuntivitis sicca), uveitis and scleritis and topical ointment and cream formulations for inflammatory and autoimmune skin diseases such as psoriasis and alopecia areata. Systemic formulations include intravenous, subcutaneous and intramuscular injection and injectable implant formulations for sustained release for the treatment of chronic inflammatory diseases such as rheumatoid arthritis, ulcerative colitis, Crohn's disease, psoriasis, etc., and

acute inflammatory conditions such as inflammatory atherosclerotic plaque instability for the prevention of acute myocardial infarction (coronary artery thrombosis) and stroke, prevention of ischemia-reperfusion injury in stroke and myocardial infarction (reduced infarct size and improved function) and in organ transplant patients (improved organ function). To the extent that activation of NF-κB inflammatory pathways are involved in the pathogenesis and progression of certain cancers, as reported widely in the literature, for example, in colon, lung and some skin cancers, systemic administration includes treatment of cancer.

[00145] SEQ ID No. 21 (labeled PPL-002 in the studies described) and SEQ ID No. 22 (labeled PPL-003 in the studies described) were chemically synthesized and their respective physical and biological properties were characterized and compared to two Antennapedia-based cell permeable membrane constructs with the same 11-amino acid NEMO binding domain (NBD) cargo (SEQ ID No. 20) that has been previously described (M.J. May et al. "Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex" Science 289:1550-54, Sept. 1, 2000; I Strickland and S Ghosh. "Use of cell permeable NBD peptides for suppression of inflammation" Ann. Rheum. Dis. 65(Suppl 3):iii75-iii82, 2006). One of these fusion peptides utilizes an N-terminal 17-amino acid sequence from the Antennapedia homeodomain and the 11-amino acid NBD peptide at its C-terminal end and was labeled PPL-004 in these studies. The other Antennapedia-based construct is a fusion peptide combining the 60 amino acid homeodomain of Antennapedia with the same 11-amino acid NBD cargo and is labeled PPL-001 in these studies.

[00146] **Physical characteristics:** SEQ ID No. 22,(PPL-003), differed in its solubility and salt interactions compared to SEQ ID No. 21 (PPL-002) that make it better suited for pharmaceutical development. It also differed in these characteristics compared to the published Antennapedia based NBD peptide (PPL-004) and the other Antennapedia based NBD peptide, PPL-001.

[00147] PPL-001, PPL-002 and PPL-004 peptides are soluble in water; however, they did not demonstrate solubility in salt containing buffers such a those commonly used as pharmaceutical vehicles or formulations. In 10 mM Tris buffered saline, pH 7.4, which is conventionally used pharmaceutical administration, PPL-003 was soluble as a clear solution even at the highest concentration tested, 5 mg/mL (0.566

mM), while PPL-002 (1 mg/mL) formed a cloudy solution possibly due to salt interactions with the charged groups on the peptide. The Antennapedia-based NBD peptides (PPL-001 and PPL-004) were also insoluble in this buffered saline solution. Using 1mM arginine, 0.01% and 0.1% Tween 20 or adding histidine to Tris buffered saline and using citrate buffered solutions did not improve the solubility characteristics of PPL-001, PPL-002 or PPL-004. The different solubility characteristics of PPL-003 compared to PPL-002 and the Antennapedia-based peptides was not predicted on the basis of structural differences. In addition only PPL-003 could be completely dissolved at 5 mg/mL in phosphate buffered physiological saline solutions at pH 7.4.

[00148] We tested the ability of certain embodiments of the human HOX C12 or HOX D12 homeodomain–NBD permeabilizing peptides (fusion proteins) disclosed to inhibit NF-κB activation and the formation of cytokines such as tumor necrosis factor alpha (TNF-α) when it is preincubated with cells prior to stimulation with endotoxin using techniques known in the art. We conducted *in vitro* studies utilizing embodiments comprising SEQ ID No. 21 and/or No. 22, or a portion thereof in which TNF-α-stimulated NF-κB transcriptional activities were measured and where endotoxin-stimulated cytokine production was studied.

[00149] Embodiments of the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain (SEQ ID Nos. 21 and/or 22 or variant or portion thereof) were active in both of these *in vitro* assays at various concentrations between about 50 μM to about 200 μM, and TNF-α-stimulated NF-κB transcriptional activity and/or cytokine production was measured by known methods and compared to the control group.

[00150] ***In Vitro* Activity using NF-κB luciferase reporter cell lines:** An *in vitro* assay measuring the inhibition of NF-κB activation utilizing a TNF-α stimulated human embryonic kidney cell line (HEK 293) and an endotoxin (LPS) stimulated murine macrophage-like cell line (RAW 264.7), both with an NF-κB luciferase reporter gene, were utilized to measure the bioactivity of embodiments of cell permeable peptides with the NBD cargo (Park et al. Phosphoinositide-dependent kinase 1 integrates T cell receptor and CD28 co-receptor signaling to effect NF-κB

induction and T cell activation. Nat Immunol. 10(2):158–166, Feb. 2009). (FIGs. 8a and 8b.)

[00151] KEY to FIGs. 8a, 8b and 9:

Unstimulated or Vehicle	Cells without endotoxin (LPS) or TNF-α stimulation
LPS	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 3-hour incubation with control buffer
Mutant:200 micromolar	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 3-hour incubation with the NBD mutant inactive peptide
NBD	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 30-hour incubation with commercially purchased peptide with a 17-amino acid Antennapedia-based homeodomain sequence and a C-terminal 11-amino acid NBD sequence (SEQ ID No. 020 is the NBD portion)
PPL-001	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 3-hour incubation with a Portage Pharmaceuticals-produced peptide with a 60-amino acid Antennapedia-based homeodomain sequence and a C-terminal 11-amino acid NBD sequence (SEQ ID No. 020).
PPL-002 (SEQ ID No. 21)	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 3-hour incubation with a Portage Pharmaceuticals-produced peptide with a 60-amino acid human HOX-C12 homeodomain sequence (SEQ ID No. 001) and a C-terminal 11-amino acid NBD sequence (SEQ ID No. 020)
PPL-003 (SEQ ID No. 22)	Cells stimulated with endotoxin (LPS) 10 ng/mL or human TNF-α at 10 ng/mL for 4 hours following a 3-hour

	incubation with a Portage Pharmaceuticals-produced peptide with a 60-amino acid human HOX-D12 homeodomain sequence (SEQ ID 002) and a C-terminal 11-amino acid NBD sequence (SEQ ID 020).

[00152] In initial experiments these peptides were first dissolved in DMSO and then diluted in phosphate buffered saline prior to addition to complete tissue culture medium containing the NF-κB luciferase reporter cell lines. In these studies PPL-003 was found to have activity similar to PPL-004 and a commercially available version of PPL-004 with the identical structure. In one test, a control mutant peptide with a change in the NBD sequence rendering it unable to bind to the NBD target sequence was inactive demonstrating the specificity of the test for the NBD sequence used as cargo.

[00153] When the final concentration of DMSO was adjusted to 1% for all peptides and their ability to inhibit human TNF-α-stimulated (5 ng/ml) NF-κB activation was measured using HEK 293 cells, there was a clear dose-response for all peptides. However, PPL-003 appeared to be more potent than the others at the same and lowest 50 micromolar concentration. FIG. 9 reports the luciferase signal as a percent of the TNF-α-stimulated cells with no peptide pretreatment.

[00154] In the rodent-systemic intravenous endotoxin challenge model, mice were pretreated using systemic intravenous (i.v.) or intraperitoneal (i.p.) administration of an embodiment formulations comprising the NBD cargo peptide conjugated to a human HOX C12 or HOX D12 homeodomain (SEQ ID Nos. 21 and/or 22) as described below and in future studies at a dose per 20 g mouse of 1 μg to at least 2,000 μg or more. Multiple inflammatory and anti-inflammatory cytokines and the acute phase protein, serum amyloid A (SAA) were measured in blood and results compared to the vehicle negative control group and to a dexamethasone pretreated positive control group.

[00155] ***In Vivo*** **activity of NBD cargo-containing cell permeable peptides:** Initial studies were conducted in mice pretreated by i.p. injection of peptides at a dose of 500 micrograms (μg) per mouse (10 mg/kg) or dexamethasone (3 mg/kg) 30 minutes prior to challenge by injection of endotoxin (LPS) at a dose of 50 micrograms/kg. The ability of peptide pretreatment to inhibit the inflammatory

cytokine response or stimulate the anti-inflammatory cytokine response to LPS was determined 2 hours after LPS injection (FIG. 10).

[00156] Cytokine levels in mouse plasma two hours after LPS injection were reduced in mice treated with NBD-containing peptides. (FIGs. 11a and 11b) For example, levels of TNF-α and IP-10 (CXCL 10) were convincingly reduced by pretreatment with PPL-003 with some mice demonstrating reduced levels of these cytokines similar to the dexamethasone-treated group. Interestingly PPL-003 was differentiated from PPL-004 with respect to their abilities to reduce the IP-10 response. While IP-10 levels were reduced by i.p. pretreatment with PPL-003 to levels similar to those measured after dexamethasone treatment, there was no effect of PPL-004 i.p. pretreatment on IP-10 plasma levels. These selective effects on specific cytokines may have important implications for PPL-003 as a therapeutic agent for inflammatory diseases.

[00157] Pretreatment with PPL-002 and PPL-003 embodiments also lead to increased levels of the anti-inflammatory cytokine Interleukin-10 (IL-10). Peptides were dosed both i.p. and i.v. at 500 micrograms per mouse (10 mg/kg) 30 minutes prior to challenge by injection of endotoxin (LPS) at a dose of 50 micrograms/kg. Dexamethasone dosed i.p. at 3 mg/kg had no effect on IL-10 levels while PPL-002 (SEQ ID No. 21) i.p. pretreatment led to a significant increase in IL-10 ($p<0.01$). PPL-002 (SEQ ID No. 21) and PPL-003 (SEQ ID No. 22) embodiment i.v. pretreatments also increased IL-10 levels. Interestingly this abilitity to increase IL-10 levels after LPS challenge was not seen in mice pretreated with PPL-004, the published NBD cell permeable peptide with an N-terminal 17-amino acid sequence from the Antennapedia homeodomain and the 11-amino acid NBD peptide at its C-terminal end (FIG. 12) where there was no detectable difference between PPL-004 and vehicle pretreated groups. .

[00158] **Activity and pharmacokinetics of E. coli-derived recombinant PPL-003**. A recombinant PPL-003 (SEQ ID No. 22) embodiment produced in E. coli and purified as described above (see *Upstream Production of SEQ ID No. 21 and SEQ ID No. 22*) was administered by intra-peritoneal injection in a 10 mM Tris buffered physiological saline solution, pH 7.4, to mice, and the plasma concentration of PPL-003 was measured by ELISA assay that utilized an affinity purified rabbit antibody to SEQ ID No. 20, the NBD cargo. Mice were injected with 500 µg [micrograms], 1,000

μg or 2,000 μg of PPL-003 and blood was sampled at the indicated time points (FIG. 13). Blood was anti-coagulated with EDTA and plasma separated by centrifugation. PPL-003 levels were measured by ELISA and calculated using a standard curve of chemically synthesized pure PPL-003. Plasma PPL-003 was measurable at 5 minutes post-dose and Cmax was at about 30 minutes with levels reaching the lower limit of detection at about 240 minutes after dosing (FIGs. 13-14).

[00159] As before mice were challenged with LPS after dosing with recombinant PPL-003 and multiple cytokine responses to the LPS injection were measured 2 hours after the LPS challenge. Dosing with PPL-003 was at the same time as LPS challenge, 30 minutes prior to LPS challenge or 60 minutes prior to LPS challenge. For comparison, dexamethasone was injected 30 minutes prior to LPS challenge.

[00160] LPS-stimulated plasma levels of amyloid A protein (SAA), TNFα, IP-10, GM-CSF, IL-6, KC (equivalent of human IL-8, CXCL1) and MCP-1 were reduced to levels lower than achieved with dexamethasone pre-treatment while anti-inflammatory cytokine IL-10 levels were significantly elevated. In general the greatest inhibition was with dosing 30 minutes prior to LPS challenge although SAA inhibition was greatest with simultaneous injection of PPL-003 and LPS (FIGs. 15a, 15b, 16a and 16b). The average percent reductions in SAA concentration are indicated above the bars in Figure 16a. IL-10 increases and Kc decreases were greatest with dosing 60 minutes prior to LPS injection. (FIGs. 17a and 17b)

[00161] FIGs. 18a and 18b. show inhibition of the GM-CSF response to the i.v. LPS (endotoxin) challenge in mice when PPL-003 was injected (i.p.). 60-and 30-minutes before LPS was administered, and at the same time LPS was administered (0 min.). GM-CSF reduction is greater than seen after pretreatment with dexamethasone, which provides only modest inhibition. PPL-003 is more potent than PPL-004 at the same microgram dosage, although the molar dosage of PPL-004 was about three times higher than that of PPL-003 at equivalent microgram doses.

[00162] Local delivery or topical delivery will prevent unwanted systemic activity that could result in susceptibility to infections in the case of the NBD peptide containing fusion protein. As an inhaled formulation for the treatment of inflammatory airway diseases such as chronic obstructive pulmonary disease

(COPD), one or more of the human HOX C12 or HOX D12 homeodomain-NBD conjugate or fusion protein embodiments disclosed (SEQ ID Nos. 21 or 22, or a portion thereof) will be evaluated for their ability to inhibit the airway inflammatory response in a well-described model in rodents (mice and rats) following pretreatment by inhalation of these compounds prior to inhalation of endotoxin. Specifically, we will conduct a study utilizing one or more human homeodomain-NBD fusion protein or conjugate embodiments at various concentrations, for example between about 1 μM to about 115 μM, and evaluate the corresponding maximal inhibition of the endotoxin-stimulated cellular and cytokine responses in BALF. In addition, studies are planned in humans challenged with small doses of inhaled endotoxin to evaluate (i) liquid formulation embodiments delivered by nebulizer; and (ii) dry powder formulation embodiments, each such formulation comprising SEQ ID Nos. 21 and/or 22, or a portion thereof. These formulation and delivery system embodiments will have efficacy in patients with inflammatory airway diseases such as COPD.

[00163] In the rodent-inhaled endotoxin challenge, administered to mice through an inhaled formulation, an embodiment comprising the NBD cargo peptide conjugated to a human HOX C12 or HOX D12 homeodomain (for example, SEQ ID Nos. 21 and/or 22, or a portion thereof) at a dose per mouse of at least 1 μg to at least about 200 μg or more. TNF-α and IL-6 concentrations in bronchoalveolar lavage fluid (BALF) fluid will be measured by known methods and results compared to the control group.

[00164] Also in the rodent-inhaled endotoxin challenge, administered to mice through an inhaled formulation, another embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (for example, SEQ ID Nos. 21 and/or 22, or a portion thereof) at a dose per mouse of at least 1 μg to at least about 200 μg or more. Bronchoalveolar lavage fluid (BALF) inflammatory cell numbers will be measured by known methods, and results compared to the control group.

[00165] In the human-inhaled endotoxin challenge, an inhaled formulation embodiment will be administered to healthy volunteers comprising the NBD cargo peptide conjugated to the human homeodomain or variant or portion thereof (for example, SEQ ID Nos. 21 and/or 22, or a portion thereof), at a dose per 70 kg person of at least 1 μg to at least about 600 mg or more. TNF-α, IL-6, and IL-1

concentrations and inflammatory cell numbers in bronchoalveolar lavage fluid (BALF) will be measured by known methods, and results compared to the control group.

[00166] Dry eyes, keratoconjunctivitis sicca, is a medical condition in which local inflammation leads to reduced tear production. In its extreme case, corneal damage occurs. Symptoms of dry eyes such as burning, pain, excess tearing reflex in dry air or windy conditions are very common and mild cases are treated with "artificial tears." However, topical immunosuppressive therapy is required for moderate to severe cases, for example, cyclosporine eye drops (Restasis®) made by Allergan. Recently the importance of Toll-like receptor (TLR) signaling has been implicated, and murine models have been developed (Redfern et.al. "Toll-Like Receptor Expression and Activation in Mice with Experimental Dry Eye" that appeared in Invest. Ophthalmol. Vis. Sci. 54(2):1554-63, Feb. 28, 2013). Initially, we will conduct a study in the murine experimental dry eye (EDE) model in which inflammatory biomarkers and pathological changes can be evaluated. For example, we will evaluate the effect of one or more embodiments of the NBD peptide-containing fusion protein or conjugate administered in a topical formulation on (1) goblet cell infiltration in epithelial cornea and loss in the conjunctiva; (2) tear secretion and (3) lymph-angiogenesis. and/or (4) inflammatory cell accumulation in lachrymal tissues.

[00167] In the EDE rodent model, chronically administered daily, every other day, or weekly with a topical formulation, another embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (SEQ ID Nos. 21 and/or 22, or a portion thereof), at a dose per mouse of at least 1 µg to at least about 500 µg or more if solubility permits. Tear production, goblet cell infiltration in epithelial cornea and loss of goblet cells in the conjunctiva will be assessed by known methods and the results compared to controls.

[00168] In more severe dry eye rodent models, administered daily, every other day, or weekly with a topical formulation, another embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D2 homeodomain or variant or portion thereof (for example, SEQ ID Nos. 21 and/or 22, or a portion thereof) at a dose per 20 g mouse of at least 1 µg to at least about 500 µg or more if

solubility permits. Lymph-angiogenesis and corneal pathology will be assessed by known methods, and the results compared to controls.

[00169] In humans, chronic daily, every other day, or weekly administration with a topical formulation, another embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (for example, SEQ ID Nos. 21 and/or 22, or a portion thereof) at dose per 70 kg person of less than 1 µg to about 100 µg changes in tear secretion towards normal levels compared to control subjects receiving a placebo treatment will be measured by the Schirmer test.

[00170] Alopecia areata is a common autoimmune skin disease resulting in the loss of hair on the scalp and elsewhere on the body. It usually starts with one or more small, round, smooth patches on the scalp and can progress to total scalp hair loss (alopecia totalis) or complete body hair loss (alopecia universalis). Alopecia areata affects approximately two percent of the population overall, including more than 5 million people in the United States alone. This common skin disease is highly unpredictable and cyclical. Hair can grow back in or fall out again at any time, and the disease course is different for each person.

[00171] Formulated in an appropriate shampoo or topical formulation for application to the scalp and other involved areas, we will evaluate the human HOX C12 or HOX D12 homeodomain-NBD fusion protein embodiment or alternative NBD protein-containing conjugate embodiments to affect (1) the downstream inflammatory response to T-cell derived cytokines by skin biopsy, (2) hair loss, and (3) hair regrowth. In this double-blind study in alopecia areata patients, paired signal lesions at the same stage of hair loss development will be treated with placebo (topical formulation alone) and with the human HOX C12 or HOX D12 homeodomain-NBD peptide-containing formulation. Hair growth and hair follicle inflammation will be measured in both lesions and compared in untreated lesions. In other parallel group studies hair loss and growth of signal lesions and numbers of new lesions will be measured and compared between treatment groups.

[00172] In rodent models of alopecia areata, for example, the Dundee Experimental Bald Rat (DEBR) model, and in alopecia areata patients, chronic administration daily, every other day, or weekly with a topical formulation, an

embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (SEQ ID Nos. 21 and/or 22, or a portion thereof) at a dosage of less than about 0.01% wt/vol to less than about 5% wt/vol. Hair loss and hair growth will be assessed by known methods in treated versus untreated lesions.

[00173] Administered as an i.v. or i.p. formulation, we will evaluate the effect of pretreatment of rodents with embodiments of the human gene-derived permeabilizing NBD fusion protein or conjugate described (SEQ ID Nos. 21 and/or 22, or a portion thereof) on ischemia-reperfusion injury in well described animal models, including a stroke model. Specifically, we will examine by known histopathology techniques in this stroke model the area of necrotic tissue, an indicator of ischemia-reperfusion injury.

[00174] In the rodent stroke model, sub-acute administration beginning within 1 hour of the ischemic event and continued at intervals such as every 6 hours, 12 hours or daily for one to three days) through an i.v. or i.p. formulation, another embodiment comprising the NBD cargo peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (SEQ ID Nos. 21 and/or 22, or a portion thereof) at a dosage of less than about 0.1 mg/kg to less than about 100 mg/kg. The area of necrotic brain tissue in the treatment group will be assessed using known histopathology methods.

[00175] Uveitis is a common inflammatory condition of the eye. The many versions of uveitis can be sight-threatening, ocular inflammatory diseases. Experimental auto-immune uveoretinitis (EAU) is thought to be an animal model of human uveitis. Immunosuppressants used to treat human uveitis are effective in such an animal model. Furthermore, the NF-κB inflammatory pathway, a target of HOX C12 and HOX D12 homeodomain-NBD fusion proteins, is reported to be involved in EAU pathogenesis. Additionally, there is a particular form of familial granulomatous uveitis, Blau syndrome, which can be caused by specific mutations in the human NOD2 gene and is directly associated with NF-κB inflammation.

[00176] Formulated as a topical administration to the eye, a human HOX D12 homeodomain-NBD fusion protein embodiment will be evaluated for its ability to reduce inflammation of an animal model of uveitis. Mice deficient in NOD2 are given

an intravitreal injection of muramyl dipeptide (MDP) and the intravascular response can be measured within the iris and cellular infiltration can be assessed by intrvital microscopy and histology. Or, as a preliminary test of topical efficacy, the fusion proteins can be evaluated for their ability in mice to reduce ocular inflammation caused the administration of the LPS endotoxin. It is anticipated that following indication of effectiveness in animal models of uveitis, human trials could be considered in more common types of uveitis. Clinical endpoints used on uveitis clinical trials include mean change from baseline in graded vitreous haze and mean change from baseline in graded anterior chamber cells after 16 weeks of therapy. Prevention of deterioration shown by not observing an increase in vitreous haze of greater than or equal to 2 grades, shown by preventing an increase in anterior chamber cells greater than or equal to 2 grades or preventing deterioration of visual acuity of greater than or equal to 0.3 logMAR from baseline.

[00177] In one embodiment, the effect of treatment in human subjects with an intravenous formulation of the human-derived permeabilizing NBD conjugate described (SEQ ID Nos. 21 and/or 22, or a portion thereof) can be evaluated with respect to the clinical consequences of atherosclerotic plaque instability that that lead to intravascular thrombosis and unstable angina, acute myocardial infarction and acute stroke. In human subjects with symptoms of unstable angina, acute MI and/or acute stroke, the underlying pathology is associated with inflammatory atherosclerotic plaque instability with production of prothrombotic factors by macrophages within the atherosclerotic lesion that lead to thrombus formation and partial or complete vascular occlusion. Specifically, evaluation may begin at presentation to an acute care facility. Further evaluation can include observing/measuring the effects of acute and subacute treatment for one to five days on biomarkers of inflammation such as C-reactive protein (CRP) at 24 hours post presentation. Clinical outcomes, such as mortality, cardiac function (ejection fraction in the case of acute MI) and neurological function (in the case of acute stroke) measured with the National Institutes of Health Stroke Scale (NIHSS) can be assessed at approximately one month following presentation. The NIHSS is a systematic assessment tool that provides a quantitative measure of stroke-related neurologic deficit. For example, a stroke patient with a NIHSS value below 12-14 will have an 80% chance for a good or excellent outcome while a NIHSS value above

20-26 has less than a 20% chance for a good or excellent outcome. Therefore, in these studies, the percent of human subjects with a NIHSS score below 17 at one month may be used as an endpoint for neurological function.

[00178] In another embodiment, daily intravenous therapy of a human gene-derived cell-permeabilizing NBD conjugate (SEQ ID Nos. 21 and/or 22, or a portion thereof), at daily doses of about 0.1 mg/kg to less than about 100 mg/kg, can be evaulated. CRP concentrations at 24 hours will be assessed in serum by known methods. In addition, we will assess at one month post presentation: (1) post-stroke neurological function as measured by the percent of patients with a NIHSS of less than17; (2) post-MI cardiac output as measured by ultrasound; and (3) acute mortality levels. Results will be compared to controls.

[00179] Alternative embodiments comprising any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the NBD peptide include a variety of fusion proteins or conjugates comprising any one or more linkers known in the art, provided the function of the fusion protein or conjugate is not compromised by its addition.

EXAMPLE 2

PC1 Cytoplasmic C-terminal Tail Peptide for Polycystic Kidney Disease

[00180] Another embodiment of a human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) fusion protein that inhibits protein-protein interactions is one in which the second region is composed of a 200 amino acid peptide whose sequence is derived from the cytoplasmic C-terminal tail region of the PC1 protein (PC1 CTT), also referred to as p200 (Lal et al. "Polycystin-1 C-terminal tail associates with ß-catenin and inhibits canonical Wnt Signaling" Hum. Mol. Genet. 17(20):3105-17, Oct. 15, 2008, including supplemental materials). The PC1 gene and/or the PC2 gene are commonly mutated in polycystic kidney disease (PKD). These mutations are inherited or result from somatic mutation or a combination of these events and result in unregulated activation of genes by a transcription factor cleaved from the c-terminal cytoplasmic tail region of the PC1 protein. The p200 protein blocks the action of this transcription factor and may be able to restore normal renal tubular development in patients with PKD if it can be effectively delivered to its site of action inside renal tubular cells.

[00181] In one embodiment, the first region human HOX C12 homeodomain 60-amino acid sequence is:

SEQ ID No. 1 (Table 3):

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment the first region human HOX D12 homeodomain 60-amino acid sequence is:

SEQ ID No. 2 (Table 3):

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

and the linked second region is the p200 peptide derived from the PC1 CTT sequence; the amino acid sequence of this second portion is:

SEQ ID No. 23 (Table 3):

VILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGLSKVKEFRHKV RFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGR LGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSSRA PAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRAKNKVHPSST

In yet other embodiments, the second region is a variation on the cargo sequence shown here. With the addition of an initiating methionine, the entire fusion protein sequence embodiment containing HOX C12 is shown as SEQ ID No. 24 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ NRRMKKKRLLVILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGL SKVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQ LDGLSVSLGRLGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLH SLQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRA KNKVHPSST

and with the addition of an initiating methionine, the entire fusion protein sequence embodiment containing HOX D12 is shown as SEQ ID No. 25 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVVILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGLS
KVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQL
DGLSVSLGRLGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHS
LQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRAK
NKVHPSST

Linkers are not required for function but linkers may be included between SEQ ID Nos.1 and 23 or a portion thereof or between SEQ ID Nos. 2 and 23, or a portion thereof, without compromising function. A linker known in the art may be used, and SEQ ID Nos. 24 and 25 embodiments would be altered, at least in part, to the extent that the linker sequence would bridge the first and second regions of each conjugate.

[00182] In other embodiments, the p200 peptide may be conjugated to any of SEQ ID Nos. 3 through 19.

[00183] In still further embodiments, additional amino acid sequences can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (HHHHHH), and other similar tags known to those in the art. Such tags may or may not include linkers. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human HOX C12 or HOX D12 homeodomain.

[00184] The fusion protein embodiment that includes the 60-amino acid human HOX C12 or HOX D12 homeodomain or variant or portion thereof with the PC1 CTT p200 peptide in the second region will be included in systemic formulations including intravenous, subcutaneous, intramuscular and injectable implant formulations for sustained release. They may also be formulated to include specific structures that target them for reuptake by renal tubular cells after they are filtered by the glomerulus and enter the nephron or via specific receptors such as the vasopressin 2 receptor (V2R, see below) that can target renal cyst cells via the blood. See, e.g., FIG. 3, showing a third region representing such a structure. As noted the biological activity of the fusion protein can be demonstrated *in vitro* in renal tubule cell cultures with PKD mutations where cyst-like structure formation is inhibited, and also in

rodent models of PKD where the progression of cyst formation and kidney growth is inhibited. (Lal et.al. 2008). We will evaluate the effect of the fusion protein embodiments (SEQ ID Nos. 24 or 25, or a portion thereof without the initiating methionine, or an alternative PC1 CTT p200 peptide-containing conjugate embodiment) on cyst formation *in vitro* in these renal tubular cell cultures and also in animal models of PKD (e.g., Pkd1 transgenic mice (Pkd2W525/-)). In this PKD-1 transgenic mouse model we will measure by ultrasound inhibition of increase in kidney size (i.e., inhibition of kidney growth). In patients with PKD, using pharmaceutical formulations of one or more of the disclosed fusion protein and/or conjugate embodiments comprising the PC1 CTT p200 peptide in the second region, we will evaluate by MRI total kidney volume and the progression of cyst size and number.

[00185] In another embodiment of a PC1 CTT peptide-containing fusion protein or conjugate for treatment of PKD, the second region of the protein is p21, a portion of the p200 molecule that retains some but not all of the biological activities of p200. See Table 3, SEQ ID No. 26. The p21 peptide when genetically expressed in PKD renal tubule cell cultures described above restores the normal tubule-like phenotype to the cells in a manner similar to the effect of expressing the entire p200 peptide in these cells. Removal of the p21 amino acids from the p200 construct also eliminates the ability of p200 expression to correct the cyst-like phenotype of these cells in culture. Unlike p200, p21 does not activate the Wnt signaling pathway and hence may not induce osteoblastic activity. These differences can be measured in vivo using osteoblastic bone formation biomarkers like osteocalcin in mouse models of PKD where the beneficial effects on progression of renal cyst formation and kidney size can also be measured using ultrasound.

[00186] The amino acid sequence of the 60-amino acid human HOX C12 homeodomain first region (SEQ ID No. 1) is shown above. The amino acid sequence of this second region p21 (SEQ ID No. 26) is:

IRRIRLWMGLSKVKEFRHKVR.

In yet other embodiments, the second region is a variation on the cargo sequence shown here.

[00187] Certain embodiments such as fusion proteins containing HOX C12 and HOX D12, each with an initiating methionine, are shown in SEQ ID Nos. 27 and 28, respectively, below and in Table 3.

SEQ ID No. 27:

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLIRRIRLWMGLSKVKEFRHKVR

SEQ ID No. 28:

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVIRRIRLWMGLSKVKEFRHKVR

[00188] Linkers are not required for function but linkers may be included, for example, between SEQ ID Nos.1 and 27 or 28, and between SEQ ID Nos. 2 and 27 or 28 without compromising function. Any linker known in the art may be used, and SEQ ID Nos. 27 and 28 embodiments would be altered, at least in part, to the extent that the linker sequence would bridge these first and second regions.

[00189] In other embodiments, the p21 peptide may be conjugated to any of SEQ ID Nos. 3 through 19.

[00190] In the PKD-1 transgenic mouse model, we will evaluate using ultrasound the effect of chronic daily i.p. or i.v. injections of formulations including (1) our human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate embodiment and (2) our human HOX C12 or HOX D12 homeodomain-p21 fusion protein or conjugateembodiment on kidney size.

[00191] In the PKD-1 transgenic mouse model, an embodiment comprising the PC1 CTT cargo peptide (either p200 (SEQ ID No. 23) or p21 (SEQ ID No. 26)) conjugated to the human HOX C12 or HOX D12 homeodomain (as SEQ ID Nos. 27 and 28 in the case of p21, and as SEQ ID Nos. 24 and 25 in the case of p200), or an alternative PC1 CTT peptide-containing embodiment varying by the type of linker, if any, at a dose per mouse of at least about 1 µg to at least 500 µg or more if solubility permits. Kidney size and growth rate will be assessed by ultrasound, and results from the group receiving the human HOX C12 or HOX D12 homeodomain-p21 treatment formulation will be compared to those of the control group and the group

receiving the human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate treatment formulation.

[00192] In the PKD-1 transgenic mouse model, we will further evaluate the ability of another embodiment comprising the human HOX C12 or HOX D12 homeodomain-p21 and the human HOX C12 or HOX D12 homeodomain-p200 fusion proteins or conjugates to reduce kidney growth in comparison to the control group, and to compare effect of both human homeodomain-p21 and human homeodomain-p200 changes in osteoblastic bone biomarkers, such as osteocalcin in blood in comparison. The human HOX C12 or HOX D12 homeodomain-p21 and the human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate embodiments will be administered in a formulation at a dose per mouse of at least about 1 µg to at least 500 µg or more if solubility permits. Osteocalcin concentration in blood will be assessed by known methods, and results from the group receiving embodiments such as the human HOX C12 or HOX D12 homeodomain-p21 treatment formulation will be compared to those of the control group and the group receiving embodiments such as the human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate treatment formulation.

[00193] In patients with PKD treated systemically with chronic daily dosing of the formulations described above, embodiments comprising the PC1 CTT cargo peptide (either p200 (SEQ ID No. 23) or p21 (SEQ ID No. 26) or a portion thereof) conjugated through a peptide bond to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof (with, e.g., the full sequence embodiments as SEQ ID Nos. 27-28, or variant or portion thereof, in the case of p21, and as the full sequence embodiments as SEQ ID Nos. 24-25, or variant or portion thereof, in the case of p200) or an alternative PC1 CTT peptide-containing conjugate embodiment varying by the type of linker, if any, will be administered at a dosage of less than about 0.01 mg/kg to less than about 100 mg/kg. Kidney growth rate will be assessed by known methods, and results from in the group receiving the human HOX C12 or HOX D12 homeodomain-p21 treatment formulation embodiment will be compared to those of the control group and the group receiving the human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate treatment formulation embodiment.

[00194] **Targeting Renal Cysts:** Renal collecting ducts are thought to be the origin of some or most cysts in PKD. Collecting duct cells express the vasopressin 2 receptor (V2R). It may be possible to target fusion protein and conjugate embodiments with a human HOX C12 or HOX D12 homeodomain peptide or variant or portion thereof in the first region and p200 or p21 in the second region to the renal cysts of collecting duct origin by including in some embodiments a linked peptide V2R antagonist or by adding a peptide V2R antagonist as an additional segment of the fusion protein. In some embodiments a removable linker may be used if function of the conjugate is not compromised with its addition. An example of a non-competitive peptide V2R antagonist was reported by Rihakova, et al. "VRQ397 (CRAVKY): a novel noncompetitive V2 receptor antagonist" Am. J. Physiol. Integr. Comp. Physiol. 297:R1009-R1018, 2009. We intend to test the addition of VRQ397 (CRAVKY) (SEQ ID No. 29, shown in Table 3), a non-competitive peptide V2R antagonist, to the N-terminal or C-terminal of the human HOX C12 or HOX D12 homeodomain-p200 and/or the human HOX C12 or HOX D12 homeodomain-p21 fusion (conjugate) protein embodiments with a linker designed to unlink or dissolve once the exposed human homeodomain region has facilitated cell entry into the cytoplasm to evaluate the dosage needed for efficacy (such as change in kidney size), as well as the efficiency of delivery to the site of action. Further, we will examine whether the peptide V2R antagonist affects the efficacy of the fusion protein or conjugate embodiment via a different mechanism.

[00195] SEQ ID No. 29 (Table 3): Peptide V2R six amino acid antagonist: CRAVKY.

[00196] In various embodiments, targeted peptides for the treatment of PKD may be any of the following: CRAVKY-(linker)-p200-human homeodomain; CRAVKY-(linker)-p21-human homeodomain; human homeodomain-p200-(linker)-CRAVKY; human homeodomain-p21-(linker)-CRAVKY where the CRAVKY sequence is at the N-terminal of the protein in the first two examples and at the C-terminal of the protein in the second two examples.

[00197] The relative activity of embodiments comprising, for example, p21 and p200 peptides with and without the linked CRAVKY peptide can be evaluated *in vitro* in PKD cyst cell cultures as described above provided these cells are engineered to also express V2R on their surface. The *in vitro* dose response to the various

CRAVKY-containing fusion protein or conjugate embodiments described, for example, above can be compared to the same fusion protein or conjugate embodiments without CRAVKY for their ability to change the *in vitro* phenotype of the cell cultures from cyst-like to tubule-like. In addition, these constructs can also be tested in the murine PKD model described above for their relative ability to reduce the growth in kidney size.

[00198] If (1) the efficacy of the human HOX C12 or HOX D12 homeodomain-p21 and/or human HOX C12 or HOX D12 homeodomain-p200 fusion protein or conjugate formulation embodiments in the PKD cyst cell cultures is observed, and (2) greater potency (i.e., a shift in the dose-response curve to the left) in the PDK-1 transgenic mouse model described above is observed when targeted using the CRAVKY peptide, at least one of the sequence embodiments described above that comprises SEQ ID No. 29 will be tested in the PDK-1 transgenic mouse model as well as in affected patients instead of SEQ ID Nos. 24, 25, 27 and/or 28 (or variant or portion thereof), or their conjugate counterparts. Kidney growth rate will be assessed by known methods.

[00199] Alternative embodiments, including those comprising any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the (1) the p200 peptide or (2) the p21 peptide include a variety of fusion proteins or conjugates comprising any one or more linkers known in the art provided function is not compromised, and may further comprise the CRAVKY peptide.

EXAMPLE 3

Cell Permeable Peptide Enzyme Replacement Therapy for Lysosomal Storage Disease

[00200] There is strong evidence that cell permeable peptides can enable large "cargo" peptides or proteins such as antibodies to enter cells. Embodiments of conjugates or fusion proteins comprising a HOX-derived peptide or other human homeodomain cell permeable peptides such as those disclosed similarly can chaperone larger molecules across cell membranes to intracellular sites of action. They also reduce the immunogenicity of the otherwise antigenic cargo peptides and proteins, and allow them to cross the blood brain barrier and enter the CNS. The embodiments disclosed and their modifications therefore also include embodiments

in which the first region of the conjugate or fusion protein is the 60-amino acid human homeodomain or variant or portion thereof, and the second region is an active enzyme. These compounds will restore enzymatic function in patients with inherited enzyme deficiencies such as in lysosomal storage diseases. We include three examples of lysosomal storage disease and associated "cargo" structures ((1) glucocerebrosidase (GCase), for example, as shown in SEQ ID No. 30, for Gaucher Disease, (2), alpha-L-iduronidase, for example, as shown in SEQ ID No. 45, for Hurler Syndrome; and (3) iduronate-2-sulfatase, for example, as shown in SEQ ID No. 48, for Hunter Syndrome), the same principles apply to human homeodomain fusion proteins or conjugates with other "cargo" enzymes that are absent or defective in the other lysosomal storage diseases.

[00201] The lysosomal storage diseases are generally classified by the nature of the primary stored material involved, and can be broadly broken into the following: (ICD-10 codes are provided where available).

[00202] •(E75) lipid storage disorders, mainly sphingolipidoses (including Gaucher's and Niemann-Pick diseases (E75.0-E75.1) gangliosidosis (including Tay-Sachs disease (E75.2) leukodystrophies.

[00203] •(E76.0) mucopolysaccharidoses (including Hunter syndrome and Hurler disease)

[00204] •(E77) glycoprotein storage disorders

[00205] •(E77.0-E77.1) mucolipidoses

[00206] Also, Glycogen storage disease type II (Pompe disease) is also a defect in lysosomal metabolism, although it is otherwise classified into E74.0 in ICD-10.

Gaucher Disease

[00207] Gaucher Disease is an inherited lysosomal storage disorder caused by the absence or mutation of the gene GBA 1, which in turn leads to a deficiency in the GBA1 product, glucocerebrosidase (GCase). Intravenous administration of GCase relieves some symptoms of Gaucher in patients. A GCase deficiency also contributes to some inherited forms of Parkinson's Disease.

[00208] In one embodiment, the first region human HOX C12 homeodomain 60-amino acid sequence is:

SEQ ID No. 1 (Table 3):

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment the first region human HOX D12 homeodomain 60-amino acid sequence is:

SEQ ID No. 2 (Table 3):

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

and in some embodiments, the linked second portion is the GCase amino acid sequence:

SEQ ID No. 30 (Table 3):

ARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTFSRYESTRSGRRMEL SMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLK IPLIHRALQLAQRPVSLLASPWTSPTWLKTNGAVNGKGSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAV HWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRG MQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPIIVDITKDTFY KQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLNR SSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

In yet other embodiments, the second region is a variation on the cargo sequence shown here. In another embodiment with the addition of an initiating methionine, the entire peptide sequence of the fusion protein with HOX C12 is

SEQ ID No. 31 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTFSRYE
STRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAAL
NILALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLH
NFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPWTSPTWLKTNGAVNGKGSL
KGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQ
CLGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDP
EAAKYVHGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQ
SVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDS
PIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPD
GSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

With the addition of an initiating methionine in yet another embodiment, the entire peptide sequence of the fusion protein with HOX D12 is

SEQ ID No. 32 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTFSRYES
TRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNI
LALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNF
SLPEEDTKLKIPLIHRALQLAQRPVSLLASPWTSPTWLKTNGAVNGKGSLKG
QPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLG
FTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAA
KYVHGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVR
LGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPIIV
DITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS
AVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

[00209] Linkers may not be required for function but linkers may be included in certain embodiments, for example, between SEQ ID Nos. 1 and 30 or between SEQ ID Nos. 2 and 30 without compromising function. Any linker known in the art may be used provided the function of the conjugate is not compromised with its addition. SEQ ID Nos. 31 and 32 embodiments, or variants or portions thereof would be altered, at least in part, to the extent that the linker sequence would bridge the first and second regions in each.

[00210] Protein glycosylation may be altered in the final structure as well. Exposure of mannose at several glycosylation sites may enhance lysosomal targeting of embodiments including, for example, SEQ ID Nos. 31 and/or 32 or variants or portions thereof.

[00211] In still further embodiments, additional amino acid sequences can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (HHHHHH), and other similar tags known to those in the art. Such tags may or may not include linkers. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human homeodomain or HOX peptide first region.

[00212] Conjugate or fusion protein embodiments that include a human homeodomain or variant or portion thereof and,for example, the GCase sequence in the second region will be included in systemic formulations including intravenous, subcutaneous, intramuscular and injectable implant formulations for sustained release. In murine models of Gaucher Disease where human mutations are incorporated into the mouse GCase sequence, these fusion proteins prevent the accumulation of substrate in various tissues and prevent neurological manifestations of Gaucher Disease.

[00213] We will examine immunogenicity of the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugate or fusion protein embodiments using lymphocyte proliferation assays that are well known in the art. To study the general ability of, for example, the 60-amino acid human homeodomain or variant or portion thereof to eliminate the immunogenicity of otherwise immunogenic proteins, isolated human mononuclear cells from subjects immune to tetanus toxoid are incubated *in vitro* for three days with tetanus toxoid or human homeodomain (or variant or portion thereof) peptide-tetanus toxoid conjugates at various concentrations to be determined, ranging from about 1 μM to about 115 μM. 3H-thymidine incorporation by lymphocytes in the cultures will then be determined.

[00214] In addition, we will evaluate immunogenicity of the enzyme fusion proteins or conjugate embodiments *in vitro*. Specifically, mononuclear leukocytes will be isolated from patients with known infusion reactions and antibody formation to the enzyme. Using techniques well known in the art these cells will be incubated *in vitro* with media alone and with various concentrations of the standard enzyme formulation and with various concentrations of the human HOX C12 or HOX D12 homeodomain peptide fusion protein and/or conjugate embodiments such as the GCase sequence or variant or portion thereof, for example, between 1 μM and about 115 μM. The incorporation of 3H-thymidine by lymphocytes will be determined as a measure of the lymphocyte immune response.

[00215] For example, in vitro testing of the fusion protein embodiments comprising the GCase cargo peptide conjugated to the 60-amino acid human HOX C12 or HOX D12 homeodomain or variant or portion thereof (e.g., embodiments comprising SEQ ID Nos. 24, 25, 27 and/or 28, or variants or portions thereof) will be evaluated. In one embodiment we will compare immunogenicity of the 60-amino acid human homeodomain conjugates or fusion proteins containing the GCase peptide with the immunogenicity of the enzyme alone using lymphocyte proliferation assays of isolated human mononuclear leukocytes from patients with known immune-mediated infusion reactions when treated with enzyme alone. Following incubation *in vitro* for three to five days at an optimal micromolar concentration to be determined. Immunogenicity of embodiments comprising the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugates or fusion proteins will be assessed by examining incorporation of 3H-thymidine by lymphocytes, and results compared to controls.

[00216] Later in clinical testing, various embodiments comprising human HOX C12 or HOX D12 homeodomain peptide fusion proteins or conjugates that include the GCase sequence will be evaluated on the basis of their abilities to restore function without either clinically significant infusion reactions or functionally important anti-GCase antibody production.

[00217] There are two examples of cellular assays that can be used to determine that an human homeodomain-GCase conjugate embodiment is effective, and at what concentration. In normal cells, for example, to fibroblasts in culture, the addition of the substrate for GCase results in activation of the enzyme and reduction of the

substrate (which accumulates in Gaucher Disease). In fibroblasts with defective or absent GBA 1 genes, the addition of the substrate for GCase does not lead to substrate turnover. However in cultures with these cells, the addition of increasing amounts of human homeodomain-GCase with exposed mannose residues or human homeodomain-GCase conjugates delivers more enzyme to the cell and decreases, for example, radio-labeled substrate. Alternatively, in cells taken from Gaucher patients, for example, leukocytes, a fluorometric beta-glucosidase assay determines relative GCase activity as a diagnosis of the disease, and is also usable as human cell assay to demonstrate the effectiveness of embodiments such as human homeodomain-GCase fusion protein or conjugate in restoring enzyme activity.

[00218] In the fibroblast cell cultures with Gba1 gene mutations, GCase cargo peptide (for example, SEQ ID No. 30) conjugated as a fusion protein to the human HOX C12 or HOX D12 homeodomain (for example, any of SEQ ID Nos. 1 and/or 2 or a portion thereof) will be active in this *in vitro* assay at various concentrations between about 1 μM to about 115 μM. We will measure the accumulation of the GCase substrate, glycosylceramide, compared to that in control cultures.

[00219] Point mutations in the GBA1 gene of mice have produced a reliable model of Gaucher Disease. We will evaluate for up to two months of daily i.v. or i.p. administration of an embodiment comprising the human homeodomain-GCase fusion protein or conjugate to reduce glucosylceramide (the GCase substrate) accumulation in this murine model. The GCase cargo peptide (for example, SEQ ID No. 30) conjugated as a fusion protein to the human HOX C12 or HOX D12 homeodomain (for example, any of SEQ ID Nos. 1 and/or 2 or a portion thereof) will be administered at a dose per mouse of between at least about 1 μg to at least about 500 μg or more. Again, we will assess the accumulation of the GCase substrate, glucosylceramide, compared to that in control group.

[00220] In another mouse model of Gaucher Disease with central nervous system involvement, accumulation of α-synuclein and tau inclusions occur in regions of the brain and are hallmarks of neurodegenerative diseases, such as Parkinson's Disease. For example, single point mutations in the murine Gba1 locus (Gba1D409V/D409V) result in accumulation of α–synuclein/ubiquitin aggregates in the CNS and a measureable deficit in hippocampal memory. In this mouse model we will evaluate the ability of embodiments comprising the human HOX C12 or HOX

D12 homeodomain-GCase fusion protein or conjugate to cross the blood brain barrier and deliver enzyme capable of preventing the structural associations of Parkinson's Disease, with the goal of reducing accumulation of α-synuclein/ubiquitin aggregates in the brain, compared to the administration of the pure enzyme systemically. In this mouse model of Gaucher disease, the GCase cargo peptide (for example, SEQ ID No. 30) conjugated as a fusion protein to the human homeodomain (for example, any of SEQ ID Nos. 1 through 19, or a portion thereof) will be administered intravenously or intraperitoneally for up to two months at a dose per mouse of between at least about 1 μg to at least about 500 μg. We will assess the accumulation of the α-synuclein/ubiquitin aggregates in the brain by known methods compared to that in control group.

[00221] Alternative embodiments such as any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the GCase peptide include a variety of fusion proteins or conjugates comprising one or more linkers known in the art, provided the function of the conjugate is not compromised.

Hurler Syndrome

[00222] Another lysosomal storage disease, Hurler Syndrome, mucopolysaccharidosis I (MPS I), is one of the mucopolysaccharidoses, a group of inherited disorders caused by a lack of specific lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs), or mucopolysaccharides. The accumulation of partially degraded GAGs causes interference with cell, tissue, and organ function. Hurler Syndrome is due to an inherited deficiency of alpha-L-iduronidase due to abnormal IDUA gene structure or IDUA gene regulation and can result in a wide range of phenotypic involvement with 3 major recognized clinical entities: Hurler (MPS IH), Scheie (MPS IS; 607016), and Hurler-Scheie (MPS IH/S) syndromes. Hurler and Scheie syndromes represent phenotypes at the severe and mild ends of the MPS I clinical spectrum, respectively, and the Hurler-Scheie syndrome is intermediate in phenotypic expression.

[00223] Mucopolysaccharidosis 1H (MPS1H) is a severe form of mucopolysaccharidosis type 1 characterized by progressive physical deterioration with urinary excretion of dermatan sulfate and heparan sulfate. Patients with MPS1H usually present, within the first year of life, a combination of

hepatosplenomegaly, skeletal deformities, corneal clouding and severe mental retardation. Obstructive airways disease, respiratory infection and cardiac complications usually result in death before 10 years of age.

[00224] Mucopolysaccharidosis 1H/S (MPS1H/S) is a form of mucopolysaccharidosis type 1 characterized by progressive physical deterioration with urinary excretion of dermatan sulfate and heparan sulfate. MPS1H/S represents an intermediate phenotype of the MPS1 clinical spectrum. It is characterized by relatively little neurological involvement, but most of the somatic symptoms described for severe MPS1 develop in the early to mid-teens, causing considerable loss of mobility.

[00225] Mucopolysaccharidosis 1S (MPS1S) is a mild form of mucopolysaccharidosis type 1 characterized by progressive physical deterioration with urinary excretion of dermatan sulfate and heparan sulfate. Patients with MPS1S may have little or no neurological involvement, normal stature and life span, but present development of joints stiffness, mild hepatosplenomegaly, aortic valve disease and corneal clouding.

Here we refer to all of these clinical phenotypes as Hurler syndrome or MPS I. It is more frequent than MPS II (Hunter syndrome), which has no corneal clouding and pursues a slower course (see below).

[00226] In one embodiment, the HOX C12 amino acid sequence first region is SEQ ID No. 1:

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment, the HOX D12 amino acid sequence first region is SEQ ID No. 2:

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

The second region may be, for example, SEQ ID No. 45 (Table 3), the 653 amino acid Alpha-L-iduronidase sequence (including the 27 amino acid signal peptide):

MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTG
FCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWLLELVTTRGS
TGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEW
KDLVSSLARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVSMTMQGFLNYY
DACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGEA
GVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEADPLVG
WSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLSNDNAFLSYH
PHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQLWAEVSQ
AGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTL
RLRGVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQFRRMRAAE
DPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLRALPLT
QGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLFVFSPDT
GAVSGSYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPGNP

In yet other embodiments, the second region is a variation on the cargo sequence shown here. And the complete sequence of this embodiment including the HOX C12 homeodomain with an initiating methionine may be, for example, SEQ ID No. 46 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLMRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALW
PLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQVRTH
WLLELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTD
FEDKQQVFEWKDLVSSLARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVS
MTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCH
DGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIY
NDEADPLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLS
NDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEE
QLWAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAH
PNRSVAVTLRLRGVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAE
QFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQ
VTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPST
FNLFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPG
NP

The complete sequence of another embodiment including the HOX D12 homeodomain with an initiating methionine may be, for example, SEQ ID No. 47 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVMRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALWP
LRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHW
LLELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFE
DKQQVFEWKDLVSSLARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVSMT
MQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCHDG
TNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYND
EADPLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLSND
NAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQL
WAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPN
RSVAVTLRLRGVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQF
RRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVT
RLRALPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFN
LFVFSPDTGAVSGSYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPGNP

[00227] In some embodiments, protein glycosylation may be altered in the final structure as well. Exposure of mannose at several glycosylation sites may enhance lysosomal targeting of an alpha-L-iduronidase-containing fusion protein or conjugate embodiment.

[00228] Fusion protein and conjugate embodiments comprised of the 60-amino acid human homeodomain or variant or portion thereof and the active alpha-L-iduronidase enzyme or an alternative alpha-L-iduronidase-containing fusion protein or conjugate embodiment with one or more linkers will be evaluated in a mouse model for their abilities to reach (1) sites of accumulated GAGs in (1) brain tissue, (2) chondrocytes within articular cartilage, and (3) the growth plate cartilage of long bones.

[00229] The MPS 1-H knock-in mouse model of Hurler Syndrome carries a mutation that is analogous to the mutation found in the Hurler Syndrome patients (IDUA-W402X); the MPS 1-H mice exhibit a phenotype of biochemical, metabolic and morphological abnormalities that correlate with abnormalities in previously utilized Hurler Syndrome animal models, and with abnormalities in the most severe form of alpha-L-iduronidase deficiency in these patients. Wang et al. "Characterization of an MPS 1-K knock-in mouse that carries a nonsense mutation analogous to the human IDUA-W402X mutation" Molecular Genetics and Metabolism 99:62-71, 2010.

[00230] In this model we will administer i.v. or i.p. injections of a formulation embodiment comprising the alpha-L-iduronidase enzyme conjugated to the human homeodomain or variant or portion thereof, or an alternative alpha-L-iduronidase-containing fusion protein or conjugate embodiment with one or more linkers at a dose per mouse of 1 µg to at least 500 µg, if solubility permits. We will assess the accumulation of GAGs (heparan sulfate and dermatan sulfate) using histopathology techniques, and compare these results to a control group's results.

[00231] In patients with Hurler Syndrome, we will evaluate the effects of fusion protein or conjugate embodiments comprising the active alpha-L-iduronidase enzyme on GAG accumulation by measuring the reduction of urinary excretion of GAGs, including heparan sulfate and dermatan sulfate. Specifically, patients with Hurler Syndrome will be treated systemically with chronic daily dosing of the formulation embodiments comprising alpha-L-iduronidase enzyme conjugated to a 60-amino acid human HOX C12 or HOX D12 homeodomain or variant or portion thereof, or an alternative alpha-L-iduronidase-containing fusion protein or conjugate embodiment with one or more linkers at a dose of less than about 0.01 mg/kg to less than about 100 mg/kg. GAG excretion in urine (heparan sulfate and dermatan sulfate) will be assessed by known methods, and results will be compared to patients who received a formulation embodiment comprising the alpha-L-iduronidase enzyme not in fusion protein or conjugate form. Further, we will evaluate skeletal and brain development assessed by standard growth and development pediatric techniques.

[00232] Various embodiments comprising the human HOX C12 or HOX D12 homeodomain-alpha-L-iduronidase fusion protein or conjugate will be tested *in vitro* in cultures of fibroblast cell lines with homozygous mutations of the IDUA gene as occurs in patients born with Hurler Syndrome. In these cultures, GAG turnover with and without a fusion protein or conjugate embodiment will be measured to confirm active enzyme and reduced lysosomal accumulation of dermatan sulfate and heparan sulfate.

[00233] In the fibroblast cell cultures with homozygous mutations of the IDUA gene, one embodiment comprising the human HOX C12 or HOX D12 homeodomain-alpha-L-iduronidase fusion protein, or alternative alpha-L-iduronidase-containing fusion protein or conjugate embodiment comprising one or more linkers, will be active in this *in vitro* assay at an optimal concentration between about 1 µM to about

115 μM. Again, we will measure the accumulation of GAG substrates, dermatan sulfate and heparin sulfate, and compare our results to control cultures.

[00234] We will further evaluate the immunogenicity of the human HOX C12 or HOX D12 homeodomain-alpha-L-iduronidase fusion protein or conjugate embodiments in lymphocyte cultures using peripheral blood mononuclear cells from MPS I patients with documented infusion reactions while receiving replacement enzyme therapy.

[00235] In one embodiment we will compare immunogenicity of the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugates or fusion proteins containing the alpha-L-iduronidase peptide with the immunogenicity of the enzyme alone using lymphocyte proliferation assays in isolated human mononuclear leukocytes from patients with known allergic (infusion) reactions when receiving standard alpha-L-iduronidase enzyme replacement therapy. 3H-thymidine incorporation is measured following incubation *in vitro* for three to five days at an optimal enzyme concentration between about 1 μM to about 115 μM. Immunogenicity of an embodiment comprising the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugate or fusion protein will be assessed by examining incorporation of 3H-thymidine by lymphocytes, and results will be compared to those of the treatment group receiving only the enzyme.

[00236] Immunogenicity of human HOX C12 or HOX D12 homeodomain-enzyme fusion protein and/or conjugate embodiments in the clinic will be compared to that of standard enzyme replacement proteins by comparing the occurrence of allergic infusion reactions and the production of IgG anti-enzyme antibody.

[00237] Alternative embodiments comprising, for example, any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the α-L-iduronidase peptide include a variety of fusion protein or conjugate embodiments, and can further comprise any one or more linkers known in the art provided the function of the conjugate is not compromised with the addition of one or more linker(s).

Hunter Syndrome

[00238] Hunter Syndrome or mucopolysaccharidosis II (MPS II) is a rare X-linked recessive disorder caused by deficiency of the lysosomal enzyme iduronate-2-

sulfatase, leading to progressive accumulation of glycosaminoglycans in nearly all cell types, tissues, and organs. Patients with MPS II excrete excessive amounts of chondroitin sulfate B (dermatan sulfate) and heparitin sulfate (heparan sulfate) in the urine. MPS II is a multisystem disorder. Most children with MPS2 have a severe form with early somatic abnormalities including skeletal deformities, hepatosplenomegaly, and progressive cardiopulmonary deterioration. A prominent feature is neurological damage that presents as developmental delay and hyperactivity but progresses to mental retardation and dementia. They die before 15 years of age, usually as a result of obstructive airway disease or cardiac failure. In contrast, those with a mild form of MPS2 may survive into adulthood, with attenuated somatic complications and often without mental retardation (Wraith, J. E. et al. "Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy" Europ. J. Pediat. 167: 267-277, 2008).

[00239] In one embodiment, the first region human HOX C12 homeodomain 60-amino acid sequence is:

SEQ ID No. 1 (Table 3):

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment the first region human HOX D12 homeodomain 60-amino acid sequence is:

SEQ ID No. 2 (Table 3):

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

And the second region may be, for example, SEQ ID No. 48, the Iduronate-2-sulfatase enzyme (550 amino acids including 25 amino acid signal sequence):

MPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLLIIVDDLRPSLG
CYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLY
DFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSW
SFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQ
AIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPD
GLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDT
QVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIF
YVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAG
LAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYS
QYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDI
HAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

In yet other embodiments, the second region is a variation on the cargo sequence shown here. The complete amino acid sequence embodiment of the combined first (HOX C12) and second region with an initiating methionine is, for example, SEQ ID No. 49:

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLMPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLLI
IVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTG
RRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNH
TDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTL
PDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITL
APDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYF
ASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDV
ATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVS
LFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGN
PRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDE
FLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

The complete amino acid sequence embodiment of the combined first (HOX D12) and second region with an initiating methionine is, for example SEQ ID No. 50:

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVMPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLLI
VDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTG
RRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNH
TDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTL
PDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITL
APDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYF
ASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDV
ATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVS
LFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGN
PRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDE
FLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

[00240] In some embodiments, protein glycosylation may be altered in the final structure as well. Exposure of mannose at several glycosylation sites may enhance lysosomal targeting of the human HOX C12 or HOX D12 homeodomain-iduronate-2-sulfatase fusion protein or conjugate embodiments.

[00241] In the fibroblast cell cultures with homozygous mutations of the IDS gene, an embodiment such as the human HOX C12 or HOX D12 homeodomain-iduronate-2-sulfatase fusion protein, or alternative iduronate-2-sulfatase-containing fusion protein or conjugate embodiment comprising one or more linkers, will be active in this *in vitro* assay at various concentrations between about 1 μM to about 115 μM. Again, we will measure the accumulation of GAG substrates, and compare our results to control cultures.

[00242] In a murine model (idsy/−) of MPSII (Hunter Syndrome) that has also been used to evaluate experimental gene therapy approaches, the onset of the gross morphological phenotype is manifest at 3–4 months of age, and becomes progressively more severe until death at age 60 to 70 weeks. This phenotype includes reduced weight gain, craniofacial and other skeletal abnormalities, with a short cranium and alopecia and thickening of the digits. Further, these mice show irregular gait, abnormal walking pattern and poor locomotor and exploratory abilities in the open-field test. IDS activity in this mouse model is undetectable in liver, spleen, lung, heart, kidney, skeletal muscle, brain and eye when compared with the activities of this enzyme in the wild-type tissues. However, IDS initiates the catabolism of the dermatan and heparan sulfate GAGs. The loss of IDS activity

causes GAG accumulation within all tissues after just several days of life, with a dramatic increase seen during adult life stages; it leads to progressive cellular vacuolization and the consequent cell death. MPSII mice at 3 and 9 months of age show high levels of GAG storage within the cells and, as shown in radiographic assays, progressive GAG accumulation caused skeletal deformation as well, which affected the performance in locomotor tests. (Polito, VA et al. "Correction of Hunter syndrome in the MPSII mouse model by AAV2/8-mediated gene delivery." Hum. Mol. Genet. 15(7):1225-36, April 1, 2006).

[00243] We will examine IDS activity by visualizing and quantifying GAG accumulation in protein extracts prepared from tissue homogenates of these MPSII mice using the fluorescent substrate 4-methylumbelliferyl-α-iduronate-2-sulfate. GAGs can be visualized in tissues by Alcian-blue staining of the paraffin-embedded sections of liver, spleen, lung, heart, kidney and skeletal muscle in these mice. In addition, GAG concentrations will be determined using a dimethyl-methylene blue assay with protein.

[00244] We will administer to male idsy/- mice i.v. or i.p. injections of a formulation embodiment comprising the iduronate-2-sulfatase peptide conjugated to the human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) at a dose per mouse of 1 µg to at least 500 µg or more, if solubility permits. We will assess the accumulation of GAGs (heparan sulfate and dermatan sulfate) using histopathology techniques, and compare these results to those of a control group.

[00245] In patients with Hunter Syndrome, we intend to evaluate the effect of treatment with formulation embodiments comprising the human HOX C12 or HOX D12 homeodomain-iduronate-2-sulfatase fusion protein or conjugate on systemic manifestations of the disease, including those in the CNS, in bone, and in joints, in relation to normal development. Patients with Hunter Syndrome will be treated systemically with chronic daily dosing of the formulation embodiments comprising, for example, iduronate-2-sulfatase enzyme conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof, or an alternative fusion protein or conjugate embodiment comprising the iduronate-2-sulfatase sequence and one or more linkers at a dose of less than about 0.01 mg/kg to less than about 100 mg/kg. GAG excretion in urine (heparan sulfate and dermatan sulfate) will be assessed by known methods, and results will be compared to patients who received a formulation

comprising the iduronate-2-sulfatase enzyme not in fusion protein or conjugate form. Further, we will evaluate skeletal and brain development assessed by standard growth and development pediatric techniques.

[00246] We will further evaluate the *in vitro* immunogenicity of human HOX C12 or HOX D12 homeodomain-iduronate-2-sulfatase fusion protein or conjugate embodiments by comparing the proliferative responses in the treatment versus control (placebo) groups. Lymphocyte cultures will be prepared using peripheral blood mononuclear cells from MPS II patients in whom there have been documented infusion reactions while receiving replacement enzyme therapy. Proliferative responses will be determined by measuring incorporation of 3H-thymidine in these cells after three to five days of culture. In one embodiment we will compare immunogenicity of the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugates or fusion proteins containing the human homeodomain-iduronate-2-sulfatase peptide with the immunogenicity of the enzyme alone using lymphocyte proliferation assays in isolated human mononuclear leukocytes following incubation *in vitro* for three to five days at an optimal concentration to be determined, in the range of about 1 μM to about 115 μM. Immunogenicity of embodiments comprising the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) conjugates or fusion proteins will be assessed by examining incorporation of 3H-thymidine by lymphocytes.

[00247] Alternative embodiments comprising, for example, any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the iduronate-2-sulfatase peptide include a variety of fusion proteins or conjugates, and can comprise any one or more linkers known in the art provided the function of the conjugate is not compromised by the addition of one or more linker(s).

EXAMPLE 4

Retinal membrane guanylyl cyclase (RetGC-1) Replacement Therapy in Congenital Blindness

[00248] Leber Congenital Amourosis type 1 (LCA1): Human RetGC-1 is an 1103 amino acid protein, including amino acids 52-1103 coding the enzyme alone without the signal peptide. RetGC-1 is the gene product of RPE65 which, when absent or mutated, causes blindness. In this disease residual cone-photoreceptor vision

correlates with biochemical properties of the mutants implying that vision restoration can be achieved if RetGC-1 enzyme activity in the retinal can be restored. Embodiments and their modifications disclosed herein therefore include fusion proteins or conjugates comprising the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) cell permeable peptides and the RetGC-1 enzyme as a cargo peptide, and can be administered in any number or formulation embodiments for periodic intraocular injection or topical administration.

[00249] In one embodiment, the first region human HOX C12 homeodomain 60-amino acid sequence is:

SEQ ID No. 1 (Table 3):

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment the first region human HOX D12 homeodomain 60-amino acid sequence is:

SEQ ID No. 2 (Table 3):

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

and the linked second region is, for example, a RetGC-1 enzyme sequence such as:

SEQ ID No. 33 (Table 3):

AVFTVGVLGPWACDPIFSRARPDLAARLAAARLNRDPGLAGGPRFEVALLP
EPCRTPGSLGAVSSALARVSGLVGPVNPAACRPAELLAEEAGIALVPWGCP
WTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDLWVEAGRSLSTA
LRARGLPVASVTSMEPLDLSGAREALRKVRDGPRVTAVIMVMHSVLLGGEE
QRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALANSSQLRRAHDA
VLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLAR
GVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDA
AGDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPDPSCWFDPNNICGG
GLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITF
LHPHGGTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIGVYEGDRVWL
KKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFLARGAEGPAALWEGN
LAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHHRGVAHGR
LKSRNCIVDGRFVLKITDHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPA
LERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRSPPPLCRPL
VSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNINKGRKTNIIDSMLR
MLEQYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVAEALKTGTPVEPE
YFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDVYKVETIGD
AYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGLHS
GPCVAGVVGLTMPRYCLFGDTVNTASRMESTGLPYRIHVNLSTVGILRALDS
GYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQ
EIPPERRRKLEKARPGQFS.

In yet other embodiments, the second region is a variation on the cargo sequence shown here. And in another embodiment with the addition of an initiating methionine, the entire peptide sequence of the fusion protein including HOX C12 is, for example:

SEQ ID No. 34 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLAVFTVGVLGPWACDPIFSRARPDLAARLAAARLNRDPGLAG
GPRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPAACRPAELLAEEA
GIALVPWGCPWTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDL
WVEAGRSLSTALRARGLPVASVTSMEPLDLSGAREALRKVRDGPRVTAVIM
VMHSVLLGGEEQRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALA
NSSQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLF
GTIYDAVFLLARGVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGD
EEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPDPS
CWFDPNNICGGGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVS
GPNKIILTVDDITFLHPHGGTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSP
NIGVYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFLAR
GAEGPAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGI
RYLHHRGVAHGRLKSRNCIVDGRFVLKITDHGHGRLLEAQKVLPEPPRAED
QLWTAPELLRDPALERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVV
QRVRSPPPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNI
NKGRKTNIIDSMLRMLEQYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSV
AEALKTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDAII
GSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRH
MPEVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRMESTGLPYRIH
VNLSTVGILRALDSGYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPKPP
DLQPGSSNHGISLQEIPPERRRKLEKARPGQFS

And in yet another embodiment with the addition of an initiating methionine, the entire peptide sequence of the fusion protein including HOX D12 is, for example:

SEQ ID No. 35 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVAVFTVGVLGPWACDPIFSRARPDLAARLAAARLNRDPGLAGG
PRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPAACRPAELLAEEAGI
ALVPWGCPWTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDLWV
EAGRSLSTALRARGLPVASVTSMEPLDLSGAREALRKVRDGPRVTAVIMVM
HSVLLGGEEQRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALANSS
QLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIY
DAVFLLARGVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGDEEPP
FVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPDPSCWF
DPNNICGGGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVSGPNK
IILTVDDITFLHPHGGTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIGVY
EGDRVWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFLARGAEGP
AALWEGNLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHH
RGVAHGRLKSRNCIVDGRFVLKITDHGHGRLLEAQKVLPEPPRAEDQLWTA
PELLRDPALERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRS
PPPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNINKGRK
TNIIDSMLRMLEQYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVAEALK
TGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDV
YKVETIGDAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVP
VRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRMESTGLPYRIHVNLST
VGILRALDSGYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPKPPDLQPG
SSNHGISLQEIPPERRRKLEKARPGQFS

[00250] Linkers may not be required for function but linkers may be included in a variety of embodiments, for example, between SEQ ID Nos. 1 and 33, or between SEQ ID Nos. 2 and 33, or variants or portions thereof, without compromising function. Any linker known in the art may be used, provided the function of the conjugate is not compromised by its addition. Embodiments, for example, such as SEQ ID Nos. 34 and/or 35 or variants or portions thereof would be altered, at least in part, to the extent that the linker sequence would bridge these first and second regions.

[00251] In still further embodiments, additional amino acid sequences can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (HHHHHH), and other similar tags known to those in the art. Such tags may or

may not include linkers. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human homeodomain or HOX peptide first region.

[00252] Successful functional translocation of the enzyme into cells in which the enzyme is usually low or absent, for example, enzyme translocation in HEK293 cells can be determined by providing the enzyme's substrate and measuring guanylyl cyclase activity by standard methods. Evidence of increased guanylyl cyclase activity would be taken as experimental evidence that an embodiment such as a human HOX C12 or HOX D12 homeodomain with cell permeabilizing capabilities can deliver functional enzyme to human cells.

[00253] We will evaluate embodiments comprising, for example, a human HOX C12 or HOX D12 homeodomain-RetGC-1 enzyme fusion protein or conjugate in a mouse knock-out model. The animal model that best approximates LCA1 is a mouse knock-out of Gucy2e, a gene that encodes RetGC-1. This mouse exhibits nonresponsive cone photoreceptors and demonstrates the LCA1 phenotype. Jacobson et al. "Determining consequences of retinal membrane guanylyl cyclase (RetGC1) deficiency in human Leber congenital amaurosis en route to therapy: residual cone-photoreceptor vision correlates with biochemical properties of the mutants." Human Molecular Genetics 22(1):168-83, 2013.

[00254] We will administer an embodiment of a human homeodomain-RetGC-1 conjugate or fusion protein by retinal (retrobulbar) injection or by eye drops (i.e., topically) to the knock-out mice with evaluation of restored electroretinogram (ERG) function as a measure of returned vision. Results will be compared to those in a control group administered a buffer without the human HOX C12 or HOX D12 homeodomain-RetGC-1. In one embodiment, proteins will be administered to mice by retinal (retrobulbar) injection or by eye drops a formulation comprising the RetGC-1 peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof, or alternative RetGC-1-containing fusion protein or conjugate embodiments comprising one or more linkers at a dose per 20 g mouse of at least about 1 µg to at least about 500 µg. We will examine restoration of ERG function compared to that of an untreated control group.

[00255] In children with Leber Congenital Amourosis type 1 (LCA1), changes in visual acuity and electroretinogram function will be evaluated after chronic (daily, every other day, twice per week, weekly or less frequent) treatment with an ophthalmic formulation embodiment comprising the RetGC-1 peptide conjugated to the human HOX C12 or HOX D12 homeodomain or variant or portion thereof or alternative fusion protein or conjugate embodiments comprising the RetGC-1 sequence and one or more linkers at a dose of less than about 0.01 mg/kg to less than about 100 mg/kg. After various treatment durations such as 1 day, 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks or longer, Patients receiving the fusion protein or conjugate treatment will be compared to their pretreatment values to evaluate clinically meaningful improvements in visual function using standard clinical methods to assess visual acuity, including, without limitation, ERG function.

[00256] Alternative embodiments comprising any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to the RetGC-1 peptide include a variety of fusion proteins or conjugates comprising any one or more linkers known in the art, provided the function of the conjugate is not compromised by the addition.

EXAMPLE 5

A Regulatory Peptide for Huntington's Disease (HD)

[00257] Huntington's Disease (HD), a neurodegenerative genetic disorder that affects muscle coordination and leads to cognitive decline, is caused by the expression of a mutant Huntingtin gene (HTT). It often manifests in the mid-30s to early 40s, but may begin earlier with both physical and mental abilities declining over time; gradually coordinated movement becomes very difficult. Clinically, there are also a number of subtle defects seen in patients with HD, including oculomotor, speech and cognitive problems. Mutant HTT expression is specific to the striatum, a part of the brain which controls movements, and neuronal loss and/or inflammation here is thought to underlie the clinical signs of HD.

[00258] ZF6xHunt-Kox-1 is a specifically designed zinc finger protein which binds longer CAG repeats and represses mutant HTT gene expression. Other lengths of zinc finger proteins are also possible including but not limited to ZF12xHunt-Kox-1 and ZF18xHunt-Kox-1. Fusion protein or conjugate embodiments with the human HOX C12 or HOX D12 homeodomain cell permeable peptide will enable ZF6xHunt-

Kox-1, ZF12xHunt-Kox-1, ZF18xHunt-Kox-1, and related proteins to enter cells and the nucleus to effectively and selectively down regulate mutant HTT gene expression. We will evaluate the effect of a variety of embodiments comprising the 60-amino acid human HOX C12 or HOX D12 homeodomain-zinc finger fusion proteins or conjugates on neurological pathology. To this end we will administer intravenous formulations comprising the human homeodomain-zinc finger embodiments to Huntington's Disease patients. Specifically, we will evaluate the ability of the human HOX C12 or HOX D12 homeodomain peptide to cross the blood brain barrier and enter the central nervous system.

[00259] In one embodiment, the first region human HOX C12 homeodomain 60-amino acid sequence is:

SEQ ID No. 1 (Table 3):

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment the first region human HOX D12 homeodomain 60-amino acid sequence is:

SEQ ID No. 2 (Table 3):

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

and the linked second region is, for example, a ZF6xHunt-Kox-1 sequence;

SEQ ID No. 36 (Table 3):

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQ RATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG RKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVT QGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVM LENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSS V

In yet other embodiments, the second region is a variation on the cargo sequence shown here. And, in another embodiment with the addition of an initiating

methionine, the entire peptide sequence of the fusion protein including the HOX C12 peptide is, for example:

SEQ ID No. 37 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG
RKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTG
EKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVS
SLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDT
AQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHP
DSETAFEIKSSV

and in yet another embodiment with the addition of an initiating methionine, the entire peptide sequence of the fusion protein including the HOX D12 peptide is, for example:

SEQ ID No. 38 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR
KFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE
KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSS
LSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTA
QQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPD
SETAFEIKSSV

[00260] In a further embodiment or embodiments, additional numbers of zinc finger repeats may be included as shown in these examples of the second component with 12 and 18 repeats respectively:

[00261] ZF12xHunt-Kox-1, for example:

SEQ ID No. 39 (Table 3):

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ
RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQ
RATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG
RKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRI
CMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER
PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIH
TGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ
RHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGM
DAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL
GYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[00262] ZF18xHunt-Kox-1, for example:

SEQ ID No. 42 (Table 3):

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ
RHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQ
RATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG
RKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRI
CMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER
PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIH
TGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQ
RHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRA
TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRN
FSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCR
ICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKD
GGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRT
LVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVIL
RLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[00263] In further embodiments, these sequences with additional numbers of zinc finger repeats are shown with the human homeodomain peptide included, for example, in an embodiment comprising the sequence with an initiating methionine residue:

[00264] Human HOX C12-ZF12xHunt-Kox-1, for example:

SEQ ID No. 40 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG
RKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTG
EKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVG
TAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQR
HTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR
ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR
KFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVT
QGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVM
LENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSS
V

[00265] Human HOX D12-ZF12xHunt-Kox-1, for example:

SEQ ID No. 41 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR
KFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE
KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGT
AERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRA
TLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRK
FAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQ
GIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVML
ENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[00266] Human HOX C12-ZF18xHunt-Kox-1, for example:

SEQ ID No. 43 (Table 3):

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQ
NRRMKKKRLLTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICG
RKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTG
EKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVG
TAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQR
HTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR
ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR
KFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRIC
MRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPF
QCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTG
SERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDA
KSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY
QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[00267] Human HOX D12-ZF18xHunt-Kox-1, for example:

SEQ ID No. 44 (Table 3):

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQN
RRMKKKRVVTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR
KFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE
KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGT
AERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRA
TLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRK
FAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRIC
MRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPF
QCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTG
SERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDA
KSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGY
QLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[00268] In other embodiments, the second region may be a variation on any of the cargo sequences shown above.

[00269] Linkers may not be required for function but linkers may be included in a variety of embodiments, for example, between SEQ ID Nos. 1 and 36, 39, or 42 or between SEQ ID Nos. 2 and 36, 39, or 42 or a portion thereof without compromising function. Any linker known in the art may be used provided the function of the conjugate is not compromised by its addition; see, e.g., Table 2. Embodiments such as SEQ ID nos. 37, 38, 40, 41, 43 and/or 44 would be altered, at least in part, to the extent that the linker sequence would bridge these first and second regions.

[00270] In still further embodiments, additional amino acid sequences can be added to either the amino or carboxy termini in order to facilitate purification. Such sequences may include FLAG-tags (DYKDDDDK), myc-tags (EQKLISEEDL), His-tags (HHHHHH), and other similar tags known to those in the art. In addition, ligands such as the biotin-acceptor protein (GLNDIFEAQKIEWHE) together with the active BirA protein may be used. For sequences that include an N-terminal initiating methionine, if a N-terminal purification domain is added the methionine will be on the N-terminal of the purification domain instead of at the N-terminal of the human homeodomain or HOX peptide first region. Embodiments of the human HOX C12 or HOX D12 homeodomain-zinc finger fusion protein or conjugate will be tested in an appropriate cell line to determine that the constructs are functionally translocated and that the mutant HTT gene is repressed. Knock-in STHdh cells, where the first exon of the mouse HTT gene has been replaced by a human exon with 111 CAG repeats (STHdhQ111/HdhQ111 or STHdhQ7/HdhQ111) will be used.

[00271] In one embodiment, we will examine the translocation of the zinc finger cargo protein using the 60-amino acid human HOX C12 or HOX D12 homeodomain (or variant or portion thereof) containing fusion protein or conjugate present following incubation of knock-in STHdh cells in culture for various periods of time such as 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, or 24 hours at an optimal micromolar concentration to be determined, likely between about 1 μM to about 115 μM. We will evaluate expression of the mutant HTT gene by known methods and compare the results to control cultures.

[00272] R6/2 mice are the most widely used animal model of HD. This knock-in mouse model for HD overexpresses the human HTT gene in the brain at a location known to be effected in human patients, the striatum. R6/2 mice demonstrate motor

deficits phenotypically that are analogous to the signs and symptoms in HD patients, for example, the R6/2 mice lack the ability to grasp with their front claws.

[00273] In one embodiment, beginning at three or four weeks of age we will evaluate motor skills in R6/2 mice; half will be treated and the others will serve as a control group. We will administer to mice through i.v. or i.p. injections a formulation daily, every other day or every third day, a human HOX C12 or HOX D12 homeodomain-zinc finger fusion protein or conjugate embodiment comprising, for example, any of SEQ ID Nos. 37, 38, 40, 41, 42, or 43 or any portion thereof, for one to two weeks. At four to six weeks of age we will reevaluate motor skills just prior to sacrifice for histological analysis. Histological analysis will be in two parts. Firstly, using rtPCR, mutant HTT expression will be evaluated in each striatum of treated and control groups of mice. Secondly, evidence of striatal neuronal loss will be evaluated in the striata of treated vs. non-treated control mice. The reduction in neuronal loss and/or striatal cell volume will be assessed using techniques well known in neurohistology laboratories, and will be reported as density of neurons per standard microscope field at common magnification. Thus, we will evaluate both the expression of the mutant HTT gene and evidence of neuronal loss in the striatum of the brain by known methods, and after assessing motor deficits, compare the results of treated R6/2 mice to those of the untreated R6/2 controls; we will further compare each group's post-treatment motor deficits score to its own baseline score.

[00274] In clinical trials with Huntington's Disease patients motor deficits will be evaluated by using the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score, a clinical tool used to evaluate patients in HD therapy trials. It provides a quantitative assessment of treatment benefits in HD, providing a composite score based on a number of motor deficits. Huntington Study Group authors, "Unified Huntington's Disease Rating Scale: Reliability and Consistency" Movement Disorders 11(2):136-142, 1996. A change from pre- to post-treatment overall motor score of one point or more is considered clinically significant.

[00275] We will evaluate the progression of the disease as reflected in the change in motor skills in HD patients following administration of a formulation embodiment comprising an human HOX C12 or HOX D12 homeodomain-zinc finger fusion protein or conjugate comprising. For example, any of SEQ ID Nos. 37, 38, 40, 41, 42, or 43, or a portion thereof. First we will assess UHDRS scores in this treatment

group of these patients and then, over the course of six to eight weeks, administer the fusion protein or conjugate formulation embodiment by intravenous or subcutaneous injection on a daily basis as an optimal dose to be determined, ranging from at least 0.01 mg/kg to at least about 500 mg/kg body weight. Motor function and other HD scored deficits will be evaluated post-treatment based on the Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score; results will be compared to the baseline score. We will assess percent improvement in UHDRS relative to non-treated controls or historical controls followed for the same period.

[00276] Alternative embodiments comprising any of SEQ ID Nos. 1 through 19, or variants or portions thereof, conjugated to any of the zinc finger peptides discussed above include a variety of fusion proteins or conjugates comprising any one or more linkers known in the art, provided the function of the conjugate is not compromised with the addition.

EXAMPLE 6

[00277] **Cell-Penetrating Peptides and Vaccine Applications**. Cell penetrating peptides have been shown to facilitate the delivery of antigenic epitopes into the cytoplasm of antigen-presenting cells, such as dendritic cells, enabling antigen processing and presentation in a major histocompatiblity complex (MHC) class I or class II dependent manner to CD8 and CD4 T-cells respectively and provoke an effective immune response (Brooks, et al. Cell-penetrating peptides: Application in vaccine delivery, Biochimica et Biophysica Acta 1805:25–34, 2010). CPPs utilizing a furin-sensitive linker for conjugation with antigenic epitopes have been shown effective at provoking antigen specific cytolytic CD8 T-cell responses when administered with oligodeoxynucleotides containing CpG motifs (Lu et al. TAP-independent presentation of CTL epitopes by Trojan antigens, J. Immunol. 166:7063–7071, 2001.). Other methods of conjugation that allow for release of the antigenic cargo in antigen presenting cells include use of a disulfide linkage that is cleaved by cytoplasmic glutathione (Kim et al. Introduction of soluble proteins into the MHC class I pathway by conjugation to an HIV tat peptide, J. Immunol. 159:1666–68, 1997) and use of antigenic peptides treated with sulfosuccinimidyl 4-maleimidomethylcyclohexane carboxylate (SMCC) followed by reaction with a C-terminal cysteine modified CPP (Apostolopoulos et.al. Delivery of tumor associated

antigens to antigen presenting cells using penetratin induces potent immune responses, Vaccine 24:3191–202, 2006). CPP fusion proteins without linkers have also been successfully used to generate specific T-cell responses to tumor antigens (Batchu et.al. Protein transduction of dendritic cells for NY-ESO-1-based immunotherapy of myeloma, Cancer Res. 65:10041–49, 2005, Scheller et.al. Human cytomegalovirus protein pp65: an efficient protein carrier system into human dendritic cells, Gene Ther. 15:318–325, 2008, Viehl et.al. Tat mammaglobin fusion protein transduced dendritic cells stimulate mammaglobin-specific CD4 and CD8 T cells, Breast Cancer Res. Treat.91:271–78, 2005).

[00278] Traditionally vaccines have relied on adjuvants that are often ligands for toll-like receptors (TLRs) to target antigens to dendritic cells for enhanced immune responses. This approach has not worked well in the case of vaccines to prevent or improve outcomes in tuberculosis. Perhaps one reason is a TLR2-dependent inhibition of MHC class II dependent antigen presentation that has also been reported to be an important mechanism allowing persistent infection of mycobacteria in macrophages (Harding, C.V and Boom, W.H., Regulation of antigen presentation by Mycobacterim tuberculosis: a roll for Toll-like receptors. Nat Rev Microbiol. 8(4):296-307, 2010). Thus the use of CPPs may promote efficient dendritic cell antigen processing and presentation and avoid this undesirable effect of TLR2 agonists while many of the current tuberculosis vaccine candidates such as those that rely on adenovirus vectors (Appledorn et.al. Adenovirus Vector-Induced Innate Inflammatory Mediators, MAPK Signaling, As Well As Adaptive Immune Responses Are Dependent upon Both TLR2 and TLR9 In Vivo. J Immunol 181:2134-44, 2008) and adjuvants do not (Frick, M. The Tuberculosis Vaccines Pipeline, June 2013 http://www.pipelinereport.org/2013/tb-vaccine and Rowland, R. and McShane. Expert Rev Vaccines 10(5):645-58, 2011).

[00279] In one vaccine embodiment, the conjugate comprises a first region, the human homeodomain or variant or portion thereof having a sequence described in, for example, any of SEQ ID Nos. 1-19 or variant or portion thereof conjugated to a second region not naturally associated with the first region, wherein the second region is a peptide antigen or portion of the antigen that is an antigenic epitope. In a variety of embodiments, conjugation may be as a fusion protein (peptide linkage) or

may utilize a linker such as the furin-sensitive linker (RVKR), a disulfide linkage, or other types of protein to protein conjugation known in the art.

[00280] **Tuberculosis Vaccine Antigen 85A and its Epitopes.** The antigen 85 complex is a multigene family encoding several closely related major secreted proteins in mycobacteria. The signal peptide is cleaved in mature Ag85 proteins. Ag85A is a peptide of 338 amino acids with a molecular mass of 35.7 kDa and a predicted pI of 6.5. It is also called extracellular α-antigen and has mycolyltransferase activity important in cell wall synthesis. Analysis of the immunogenicity and protective activity of smaller peptide epitopes in Balb/c mice (Tchilian et. al. Immunization with different formulations of Mycobacterium tuberculosis antigen 85A induces immune responses with different specificity and protective efficacy. Vaccine 31:4624-31, 2013) after intranasal and systemic immunization of mice using a recombinant adenoviral vector, recombinant Ag85A itself or as a set of overlapping 15mer peptides (single Balb/c H-2D restricted epitopes) demonstrated superior protection against mycobacterium tuberculosis (Mtb) challenge after intranasal immunization. CD4 85A99-118 and CD8 85A70-78 responses were protective while response to CD8 85A145-152 was not protective.

[00281] Human vaccine trials using the adenovirus Ag85A vaccine to enhance the effectiveness of BCG vaccine in infants induced only modest immune responses and did not have any clinical efficacy (Tameris et.al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. The Lancet 381:1021-1028, 2013). This could be due to the inhibitory effects of TLR2 agonist properties of the adenovirus component shown to provoke IL-10 responses that down-regulate the immune response or due to non-protective responses to some Ag85A epitopes.

[00282] **Tuberculosis Vaccine.** The current embodiments for a protective human tuberculosis vaccine are conjugates or a mixture of conjugates administered by the inhaled, intranasal or parenteral injection route. In one embodiment the inhaled route provides local immunity in the lung.

[00283] In Balb/c mice the concept will be tested where a conjugate or conjugate mixture is composed of a first region, the human homeodomain or variant or portion thereof including but not limited to sequences described in any of SEQ ID Nos. 1-19

or variant or portion thereof conjugated to a second region not naturally associated with the first region. In certain embodiments, the second region is one or more of four peptides that span the Ag85A 99-118 epitope (See Table 3):

Peptide one of four of the Ag85A 99-118 epitope is SEQ ID No. 51: ETFLTSELPGWLQAN;

Peptide two of four of the Ag85A 99-118 epitope is SEQ ID No. 54: SELPGWLQANRHVKP;

Peptide three of four of the Ag85A 99-118 epitope is SEQ ID No. 57: WLQANRHVKPTGSA; and

Peptide four of four of the Ag85A 99-118 epitope is SEQ ID No. 60: RHVKPTGSAVVGLSM.

In yet other embodiments, the second region is a variation on one or more of these four peptides. The sequence ID numbers for several of these conjugates are shown in Table 3. For example, in one embodiment, the complete amino acid sequence of the combined first (HOX C12) and second region SEQ ID No. 51 of the Ag85A 99-118 epitope is SEQ ID No. 52: SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRM KKKRLLETFLTSELPGWLQAN. Likewise, in another embodiment, the complete amino acid sequence of the combined first (HOX D12) and second region SEQ ID No. 51 of the Ag85A 99-118 epitope is SEQ ID No. 53: ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRM KKKRVVETFLTSELPGWLQAN.

[00284] The second conjugate or conjugate mixture for testing in Balb/c mice is composed of a first region, the human homeodomain or variant or portion thereof having a sequence described in any of SEQ ID Nos. 1-19 or variant or portion thereof conjugated to a second region not naturally associated with the first region. In certain embodiments, the second region is one or more of three peptides that span the Ag85A 70-78 epitope (See Table 3):

Peptide one of three of the Ag85A 70-78 epitope is SEQ ID No. 63: QSGLSVVMPVGGQSS;

Peptide two of three of the Ag85A 70-78 epitope is SEQ ID No. 66: VVMPVGGQSSFYSDW; and

Peptide three of three of the Ag85A 70-78 epitope is SEQ ID No. 69: GGQSSFYSDWYQPAC.

In yet other embodiments, the second region is a variation on one or more of these three peptides. The sequence ID numbers for several of these conjugates are also shown in Table 3. For example, in one embodiment the complete amino acid sequence of the combined first (HOX C12) and second region SEQ ID No. 63 of the Ag85A 70-78 epitope is SEQ ID No. 64: SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRM KKKRLLQSGLSVVMPVGGQSS. Likewise, in another embodiment the complete amino acid sequence of the combined first (HOX D12) and second region SEQ ID No. 63 of the Ag85A 70-78 epitope is SEQ ID No. 65: ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRM KKKRVVQSGLSVVMPVGGQSS.

[00285] Thus, the entire vaccine to be tested in mice and later in humans is composed of mixtures of conjugates where each conjugate has, as its cargo in a variety of embodiments, one or more of these smaller peptides or variations thereof, and together they provide for a robust CD4 response to Ag85A 99-118 and a robust CD8 response to Ag85A 70-78.

[00286] In one human vaccine embodiment the vaccine will be composed of a first region, the human homeodomain or variant or portion thereof including but not limited toa sequence described in any of SEQ ID Nos. 1-19 or variant or portion thereof conjugated to a second region not naturally associated with the first region, wherein the second region is, in one embodiment, the entire Ag85A 338-amino acid peptide or an HLA-restricted epitope of Ag85A such as one or more specific HLA-restricted epitopes of Ag85A shown to provoke immune responses in appropriate human subjects (see Table 3). The Ag85A peptide, or its human epitopes, may be linked to the first region as described above. In another embodiment, the entire Ag85A sequence is linked to the first region to to provide a single vaccine construct for subjects with different HLA class I and class II genotypes. The Ag85A sequence is: MQLVDRVRGA VTGMSRRLVV GAVGAALVSG LVGAVGGTAT AGAFSRPGLP

VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA
FEWYDQSGLS ***V***VMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG
WLQANRHVKP TGSAVVGL***S***M AASSALTLAI YHPQQFVYAG AMSGLLDPSQ
AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV
WVYCGNGKPS DLGGNNLPAK FL***E***GFVRTSN IKFQDAYNAG GGHNGVFDFP
DSGT***H***SWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA:

[00287] Here the the longer region of the sequence in bold and underlined font above is the signal peptide that is cleaved when the mature Ag85A sequence is produced (the remaining 296 amino acid peptide). The fibronectin binding domain is shown in bold and italicized font just below it, and the three amino acids below that in bold, underlined and italicized font are in the catalytic domain.

[00288] In one embodiment, the second region amino acid sequence for Ag85A (without the signal peptide) would be: AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA The sequence ID number (SEQ. ID No. 72) for this Ag85A cargo peptide is shown in Table 3, along with two of many possible embodiments in which the Ag85A cargo peptide or a variation comprises the second region of the conjugate or fusion protein: those containing the first region HOX C12 (SEQ. ID No. 73) or HOX D12 (SEQ. ID No. 74) sequences. A person of ordinary skill in the molecular biology/biotechnology art would appreciate that numerous variations on any of these and other sequences shown in Table 3 would fall within the embodiments disclosed herein.

[00289] **Measurement of the Vaccine-Induced Immune Response.** In vitro whole blood cultures with specific antigens and measurement of type I cytokine responses was successfully used to measure the immune response to the adenovirus vector Ag85A vaccine in a phase I clinical trial (Smaill et.al. A Human Type 5 Adenovirus–Based Tuberculosis Vaccine Induces Robust T Cell Responses in Humans Despite Preexisting Anti-Adenovirus Immunity. Sci. Transl. Med. 5, 205: 1-11, 2013). This technique can be used to measure the immune response in

humans to CPP-based Ag85A epitope vaccine mixtures. In murine models similar methods can be used with post-treatment cultures of splenic lymphocytes and measurement of cytokine responses in Balb/c mice using the epitopes described.

[00290] The number of responsive CD4 T-cells to specific antigenic epitopes can be quantitated by IFN-gamma ELISPOT assays and both CD4 and CD8 T-cell responses can be measured by intracellular cytokine staining together with T-cell subtype classification in peripheral blood lymphocyte cultures (Smaill et.al. 2013).

EXAMPLE 7

[00291] Human-derived CPP structures can include, but are not limited to, the first region, which can comprise the HOX D12 homeodomain, the HOX C12 homeodomain, or other first region structures, such as those described in Table 3 (SEQ ID Nos. 1-19). The first region can be conjugated to a second region such that the first region can be used to carry 'cargo' to intracellular and/or intranuclear targets that may be abnormally expressed in disease states. Such targets may include nucleic acid structures such as genes, mRNA and miRNA. Here DNA, RNA, LNA, PNA, γPNA, other standard or modified nucleic acid or a combination of these components can be designed to bind to these intracellular and/or intranuclear targets and reduce abnormal expression or activation characteristic of the disease state. Table 4 shows nucleotide sequences, and three examples are described below.

[00292] Table 4: Nucleotide Sequence Identities

SEQ. ID No.	Descriptor	Nucleotide Sequence
75	miRNA132 target sequence to which cargo binds	5'-UAACAGUCUACAGCCAUGGUCG-3'
76	Cargo sequence that binds to miRNA21	5'-TCAACATCAGTCTGATAAGCTA-3'
77	miRNA21 target sequence to which the cargo binds	5'- TAGCTTATCAGACTGATGTTGA-3'
78	Cargo sequence that binds to miRNA132	5'-CGACCATGGCTGTAGACTGTTA-3'

SEQ. ID No.	Descriptor	Nucleotide Sequence
79	Cargo sequence that binds to DYRK1b	5'-GTGGTGAAAGCCTATGATCAT-3'

[00293] **Affecting the Ras Pathway**: In one or more embodiments, a second region cargo binds to an miRNA132 target sequence to affect the Ras pathway.

[00294] The Ras pathway is abnormally expressed in many types of cancer and constructs that affect the pathway would reduce the growth of tumors dependent on it. Diagnostic tests would identify those patients whose tumors would be most likely to respond to this treatment. Tumor cell lines known to have an activated Ras pathway can be used to demonstrate the biological activity of Ras-inhibiting constructs described below. They will reduce the growth of these tumor cells in vitro and reduce the growth of tumors in murine xenograft models that use these cells following systemic treatment.

[00295] In one embodiment, the HOX C12 amino acid sequence first region is SEQ ID No. 1:

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNR RMKKKRLL

In another embodiment, the HOX D12 amino acid sequence first region is SEQ ID No. 2:

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNR RMKKKRVV

[00296] In another embodiment, the second region cargo may be, for example, nucleic acid SEQ ID No. 76 (Table 4) (or a variation thereof):

[00297] 5'-TCAACATCAGTCTGATAAGCTA-3'In yet another embodiment, for example, the complete conjugate comprises SEQ ID Nos. 1 and 76.

[00298] **Affecting Tumor Suppressor Pathways in Many Cancers**: The second region cargo binds to miRNA21 target sequence to affect tumor suppressor pathways found in many types of cancers. As in the Ras pathway example described above, in a variety of embodiments the first region homeodomain

sequence may be, for example, HOX C12 (SEQ ID No. 1) or HOX D12 (SEQ ID No. 2) (see Table 3). In one or more embodiments, the conjugate's second region cargo binds to an miRNA21 target sequence, which sequence includes but is not limited to SEQ. ID No. 77 (Table 4), or any variation thereof. Thus, in one embodiment the second region cargo, for example, 5'-CGACCATGGCTGTAGACTGTTA-3' (SEQ. ID No. 76), binds to the miRNA21 target sequence, for example, 5'-TAGCTTATCAGACTGATGTTGA-3' (SEQ. ID No. 77) to affect tumor suppressor pathways found in many types of cancers.

[00299] Testing these constructs in vitro and in murine xenograft tumor models can confirm their activity by measuring reduced tumor cell growth and tumor growth. Treatment of cancer patients found to have tumors that have abnormal expression of this miRNA will have clinical benefit. In one embodiment, the second region cargo sequence (SEQ. ID No. 79) binds to target sequence DYRK1b. In other embodiments variations to SEQ. ID No. 79 bind to DYRK1b, or a functional variation thereof.

[00300] **Affecting Gene Expression**: In one or more embodiments, the second region cargo binds to DYRK1b to reduce gene expression.

[00301] Over expression of this gene is present in many cancers including lung and pancreatic cancers. Mutations in DYRK1b that result in increased expression also are associated with familial inheritance of type II diabetes, central obesity and early coronary artery disease, all features of the metabolic syndrome. It is likely that this pathway is also contributing to the pathogenesis of type II diabetes and obesity in the general population. Thus systemic treatment with inhibitors of DYRK1b such as those described below will have efficacy in cancers with abnormal high expression of DYRK1b and also in patients with type II diabetes and other features of metabolic syndrome. It will be especially effective in those patients with identified mutations in DYRK1b that lead to over expression. As in the Ras and tumor suppressor pathways examples discussed above, a variety of embodiments the first region homeodomain sequence may be, for example HOX C12 (SEQ ID No. 1) or HOX D12 (SEQ ID No. 2) (see Table 3). In one embodiment, the sequence 5'-GTGGTGAAAGCCTATGATCAT-3' (SEQ. ID No. 79, see Table 4) or variation thereof, binds to DYRK1b. In other embodiments variations to SEQ. ID No. 79 bind to DYRK1b, or a functional variation thereof.

[00302] For certain comparisons between groups reported in the studies described in the examples above, significance was tested at $p < 0.05$.

[00303] From the foregoing, it will be appreciated that specific embodiments have been described herein for purposes of illustration, but that various modifications may be made without deviating from the spirit and scope of the disclosure. Accordingly, the disclosure is not limited except as by the appended claims.

CLAIMS

We claim:

1. A conjugate comprising:

a first region comprising a homeodomain structure and having a polypeptide sequence as described in any one of SEQ ID Nos. 1 to 19 or a variant thereof; and

a second region comprising a functional or regulatory polypeptide or protein not naturally associated with the first region.

2. A conjugate comprising:

a first region comprising at least one of a HOX D12 homeodomain or a HOX C12 homeodomain; and

a second region comprising a functional or regulatory polypeptide or protein not naturally associated with the first region.

3. A conjugate of claim 1 or claim 2 wherein the second region includes at least one of a NEMO binding domain, a PC1 CTT-derived fusion protein, an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies, a gene product of RPE65, a zinc finger peptide, an Ag85A 99-118 epitope peptide, or an Ag85A 70-78 epitope peptide.

4. The conjugate of claim 3 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

5. The conjugate of claim 3 wherein the PC1 CTT-derived fusion protein has a polypeptide sequence set forth in at least one of SEQ. ID Nos. 23 or 26 or a variant thereof.

6. The conjugate of claim 3 wherein the enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies has a polypeptide sequence of at least one of SEQ. ID Nos. 30, 45, or 48 or a variant thereof.

7. The conjugate of claim 3 wherein the gene product of RPE65 has a polypeptide sequence that includes at least SEQ. ID No. 33 or a variant thereof.

8. The conjugate of claim 3 wherein the zinc finger peptide sequence includes at least one of SEQ. ID Nos. 36, 29, or 42 or a variant thereof.

9. The conjugate of claim 3 wherein the Ag85A 99-118 epitope peptide has a sequence that includes at least one of SEQ. ID Nos. 51, 54, 57, or 60 or a variant thereof.

10. The conjugate of claim 3 wherein the Ag85A 70-78 epitope peptide has a sequence that includes at least one of SEQ. ID Nos. 63, 66, or 69 or a variant thereof.

11. The conjugate of claim 1 or claim 2 wherein the second region is conjugated to the C-terminus of the first region.

12. The conjugate of claim 1 or claim 2 wherein the second region is conjugated to the N-terminus of the first region.

13. The conjugate of any one of claims 1 and 2 in the form of a fusion protein.

14. The conjugate of any one of claims 1 and 2 wherein the second region interacts with an intracellular target and/or an intranuclear target.

15. The conjugate of claim 14 wherein the interaction of the second region with the intracellular target and/or intranuclear target affects one or more of the following: NF-κB activation, uveitis, PC1 transcription factor activity in renal tubular cells with polycystic kidney disease mutations, glucocerebrosidase substrate in cells with Gaucher Disease mutations of GCase, glycosaminoglycans in subjects with Hurler Syndrome or Hunter Syndrome, retinal membrane guanylyl cyclase substrate in retinal cells deficient in retinal membrane guanylyl cyclase, regulation of Huntingtin

mutant gene transcription in cells with Huntington's Disease mutations, and delivery of antigenic epitopes into cytoplasm of antigen-presenting cells for provoking an effective immune response in a vaccine embodiment such as for a tuberculosis vaccine to prevent tuberculosis in subjects exposed to the infection.

16. The conjugate of any claim 1 or claim 2 wherein the second region has a polypeptide sequence that includes SEQ. ID Nos. 23 or 26 or variant thereof, and wherein the conjugate further comprises a noncompetitive V2 receptor antagonist.

17. The conjugate of claim 1 or claim 2 wherein the second region has a polypeptide sequence selected from the group consisting of SEQ ID Nos: 20, 23, 26, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, and 72, or a variant thereof.

18. The conjugate of claim 1 or claim 2, further comprising a linker sequence between the first and second regions.

19. The conjugate of claim 1 wherein the first and second regions combined have a polypeptide sequence selected from the group consisting of SEQ ID Nos: 21, 22, 24, 25, 27, 28, 31, 32, 34, 35, 37, 38, 40, 41, 43, 44, 46, 47, 49, and 50, 52, 53, 55, 56, 58, 59, 61, 62, 64, 65, 67 , 68, 70, 71, 73 and 74, or a variant thereof.

20. The conjugate of claim 19 wherein an initiating methionine residue of the sequence is removed prior to use as an active and/or therapeutic agent.

21. The conjugate of claim 1 wherein the first and second regions combined have a sequence comprising a homeodomain structure and at least one of a NEMO binding domain, a PC1 CTT-derived fusion protein, an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies, a gene product of RPE65, a zinc finger peptide, an Ag85A 99-118 epitope peptide, and an Ag85A 70-78 epitope peptide.

22. The conjugate of claim 21 wherein the second region interacts with an intracellular target and/or an intranuclear target.

23. The conjugate of claim 22 wherein the interaction of the second region with an intracellular target and/or intranuclear target affects one or more of the following: NF-κB activation, uveitis, PC1 transcription factor activity in renal tubular cells with polycystic kidney disease mutations, glucocerebrosidase substrate in cells with Gaucher Disease mutations of GCase, glycosaminoglycans in subjects with Hurler Syndrome or Hunter Syndrome, retinal membrane guanylyl cyclase substrate in retinal cells deficient in retinal membrane guanylyl cyclase, regulation of Huntingtin mutant gene transcription in cells with Huntington's Disease mutations, and delivery of antigenic epitopes into cytoplasm of antigen-presenting cells for provoking an effective immune response as the active component of vaccines as for example in a tuberculosis vacceine to prevent tuberculosis in subjects exposed to the infection.

24. The conjugate of claim 19 or claim 21, further comprising a linker sequence between the first and second regions.

25. A composition comprising the conjugate of claim 1 and a pharmaceutically acceptable carrier.

26. A composition comprising the conjugate of claim 2 and a pharmaceutically acceptable carrier.

27. The composition of claims 25 or claim 24 wherein the composition is in the form of an inhalable composition, an eye drop formulation other ophthalmic composition for local or injectable use, an enema, a topical composition, or an injectable composition including injectable implants for sustained release.

28. The conjugate of claim 1 or claim 2 wherein, when administered to a subject in a formulation, the conjugate enters the central nervous system.

29. The conjugate of claim 1 or claim 2 wherein, when administered in a formulation to a subject, the conjugate elicits a reduced unwanted immune response in the subject compared to an unwanted immune response elicited by the second region when not conjugated to the first region.

30. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the conjugate enters the central nervous system in a subject.

31. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 21 to the subject, wherein the conjugate enters the central nervous system of the subject.

32. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the conjugate prevents or ameliorates immune responses to antigenic peptides in the subject such that there is at least one of (a) no significant reduction in peptide exposure, and (b) no significant increase in the incidence of immune-mediated adverse clinical events.

33. A method of treating a dry eye in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the first region is conjugated to a NEMO binding domain.

34. The method of claim 33 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

35. A method of treating autoimmune and inflammation-related hair loss in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

36. A method of treating autoimmune and inflammation-related hair loss in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

37. The method of claim 35 or claim 36 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

38. A method of treating an inflammatory airway disease in a subject comprising administering an inhalable formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

39. A method of treating an inflammatory airway disease in a subject comprising administering an inhalable formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

40. The method of claim 38 or claim 39 wherein the inflammatory airway disease is chronic obstructive pulmonary disease.

41. The method of claim 38, wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

42. A method of treating colo-rectal inflammatory bowel disease in a subject comprising administering an enema formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

43. A method of treating colo-rectal inflammatory bowel disease in a subject comprising administering an enema formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

44. The method of claim 42 or claim 43 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

45. A method of treating a condition in a subject comprising administering a systemic formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

46. A method of treating a condition in a subject comprising administering a systemic formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

47. The method of claim 45 or claim 46 wherein the condition is at least one of unstable angina, acute myocardial infarction and acute stroke.

48. The method of claim 45 or claim 46 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

49. A method of treating ischemia reperfusion injury following transient ischemia in a subject comprising administering a systemic formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

50. A method of treating ischemia reperfusion injury following transient ischemia in a subject comprising administering a systemic formulation comprising the conjugate of claim 2, wherein the second region is a NEMO binding domain.

51. The method of claim 49 or claim 50 wherein the ischemia reperfusion injury is in the brain, kidney or heart.

52. The method of claim 49 or claim 50 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

53. A method of treating polycystic kidney disease in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a PC1 CTT-derived fusion protein, a PC1 CTT-derived p200 peptide or a PC1 CTT-derived p21 peptide.

54. A method of treating polycystic kidney disease in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a PC1 CTT-derived fusion protein, a PC1 CTT-derived p200 peptide or a PC1 CTT-derived p21 peptide.

55. The method of claim 53 or claim 54 wherein the second region has a polypeptide sequence set forth in at least one of SEQ. ID Nos. 23 or 26 or a variant thereof.

56. The method of claim 53 or claim 54 wherein the conjugate is further conjugated to a noncompetitive V2 receptor antagonist for targeting renal cysts of collecting duct cells.

57. A method of treating a lysosomal storage disease in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region of the conjugate comprises an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies.

58. A method of treating a lysosomal storage disease in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region of the conjugate comprises an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies.

59. The method of claim 57 or claim 58 wherein the enzyme is selected from the group consisting of glucocerebrosidase, alpha-L-iduronidase, and iduronidate-2-sulfatase.

60. The method of claim 57 or claim 58 wherein the enzyme includes a polypeptide sequence as set forth in at least one of SEQ. ID Nos. 30, 45, or 48 or a variant thereof.

61. A method of treating Leber Congentital Amourosis type 1 in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region includes a gene product of RPE65.

62. A method of treating Leber Congentital Amourosis type 1 in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region includes a gene product of RPE65.

63. The method of claim 61 or claim 62 wherein the gene product of RPE65 has a polypeptide sequence as set forth in SEQ. ID No. 33 or a variant thereof.

64. A method of treating Huntington's Disease in a subject comprising administering the conjugate of claim 1 to the subject, wherein the second region includes a zinc finger peptide.

65. A method of treating Huntington's Disease in a subject comprising administering the conjugate of claim 2 to the subject, wherein the second region includes a zinc finger peptide.

66. The method of claim 64 or claim 65, wherein the zinc finger peptide sequence includes at least one of SEQ. ID Nos. 36, 29, or 42 or a variant thereof.

67. A method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region comprises an Ag85A 99-118 epitope peptide.

68. The method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region comprises an Ag85A 99-118 epitope peptide.

69. The method of claim 67 or claim 68 wherein the Ag85A 99-118 epitope peptide sequence includes at least one of SEQ. ID Nos. 51, 54, 57, or 60 or a variant thereof.

70. A method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region comprises an Ag85A 70-78 epitope peptide.

71. The method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region comprises an Ag85A 70-78 epitope peptide.

72. The method of claim 70 or claim 71 wherein the Ag85A 70-78 epitope peptide sequence includes at least one of SEQ. ID Nos. 63, 66, or 69 or a variant thereof.

73. A conjugate prepared by a method comprising the steps:

culturing a host cell transformed with an expression vector comprising a nucleic acid encoding a conjugate according to claim 1 under conditions which provide for the expression of the conjugate within the host cell; and

recovering the conjugate by affinity purification.

74. A conjugate comprising:

a first region comprising a homeodomain structure; and

a second region comprising a functional or regulatory nucleic acid or peptide nucleic acid not naturally associated with the first region.

75. The conjugate of claim 74 wherein the first region is a HOX D12 homeodomain.

76. The conjugate of claim 74 wherein the first region is a HOX C12 homeodomain.

77. A conjugate comprising:

a first region comprising a homeodomain structure having a polypeptide sequence described in any one of SEQ ID Nos. 1 to 19 or a variant thereof; and

a second region comprising a functional or regulatory nucleic acid or peptide nucleic acid not naturally associated with the first region.

78. The conjugate of any one of claims 74 to 77 wherein the second region comprises DNA, RNA, LNA, PNA, γPNA, or a combination of these components that has intracellular biological activity.

79. The conjugate of any one of claims 74 to 77 wherein the second region includes a nucleic acid that binds to miRNA21 or to DYRK1b.

80. The conjugate of any one of claims 74 to 78 wherein the second region has a nucleotide sequence as set forth in at least one of SEQ ID Nos. 76, 78, or 79 or a variant thereof.

81. The conjugate of any one of claims 74 to 79 wherein the second region is conjugated to the C-terminus of the first region.

82. The conjugate of any one of claims 74 to 79 wherein the second region is conjugated to the N-terminus of the first region.

83. The conjugate of any one of claims 74 to 79 wherein the first region is linked to the second region with a peptidic bond.

84. The conjugate of any one of claims 74 to 79 wherein the second region is conjugated to the first region via a non-peptidic bond.

85. The conjugate of any one of claims 74 to 79 wherein the second region interacts with an intracellular target and/or an intranuclear target.

86. The conjugate of claim 85 wherein the second region interacts with an intracellular target and/or an intranuclear target via interactions with an miRNA or a protein complex that binds an miRNA.

87. The conjugate of claim 86 wherein the second region is a nucleic acid or PNA that binds to miRNA132 for affecting the Ras pathway.

88. The conjugate of claim 86 wherein the second region is a nucleic acid sequence or PNA that stably hybridizes to an miRNA nucleotide sequence as set forth in SEQ ID No. 75 or a variant thereof.

89. The conjugate of claim 88 wherein the second region has a nucleotide sequence as set forth in SEQ ID No. 78 or to a portion of said nucleotide sequence or a variant thereof that stably hybridizes to miRNA132.

90. The conjugate of claim 88 wherein the second region is selected based on the specificity of an interaction with miRNA132.

91. The conjugate of claim 88 wherein the second region consists of modified nucleotides to increase stability or reduce degradation.

92. The conjugate of claim 86 wherein the second region is a nucleic acid sequence or PNA that binds to miRNA21 for affecting a tumor suppressor pathway or tumor growth.

93. The conjugate of claim 92 wherein the second region comprises an nucleotide sequence as set forth in SEQ ID No. 76 or variant thereof or can stably hybridize to a nucleotide sequence as set forth in SEQ ID No. 77 or a variant thereof.

94. The conjugate of any one of claims 74, 75 and claims 77 to 93 wherein the first region homeodomain structure has a polypeptide sequence as set forth in SEQ ID No. 2 or a variant thereof.

95. The conjugate of any one of claims 74 to 79 wherein the second region interacts with an intracellular target and/or an intranuclear target via interactions with an mRNA for affecting splicing or protein translation.

96. The conjugate of claim 95 wherein the second region binds to DYRK1b.

97. The conjugate of claim 96 in which the second region has a nucleotide sequence as set forth in SEQ ID No. 79 or a variant thereof.

98. The conjugate of claim 74 wherein the first region and the second region are attached via “click chemistry.”

99. The conjugate of claim 74 wherein the second region comprises PNA, and wherein the first region and the second region is attached via a peptide bond.

100. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 74 to the patient.

101. A method of treating cancer in a subject comprising adminsistering formulation comprising the conjugate of claim 95 to the subject.

102. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 96 to the subject.

103. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 97 to the subject.

104. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 74 to the subject.

105. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 95 to the subject.

106. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 96 to the subject.

107. A method of treating a metabolic syndrome, a lipid disorder or obesity in a subject comprising administering a formulation comprising the conjugate of claim 97 to the subject.

108. The method of treating cancer of claim 100 comprising administration of the formulation as an intravenous solution.

109. The method of treating a metabolic syndrome, a lipid disorder and/or obesity of claim 104 comprising administration of the formulation in sustained release form.

110. A method of treating tuberculosis in a subject comprising administrating a formulation comprising the conjugate of claim 1 to the subject, wherein the second region includes at least a portion of one of a Ag85A 99-118 epitope peptide or a Ag85A 70-78 epitope peptide.

111. The method of treating tuberculosis in a subject according to claim 110 wherein:

the formulation is administered to the subject at least once via an inhaled route, intranasal route and/or by intramuscular, intradermal or subcutaneous parenteral injection; and

administration of the formulation provokes a protective immune response that prevents the onset of active lung infection with Mycobacterium tuberculosis in the subject.

112. A method of treating tuberculosis in a subject comprising administrating a formulation comprising the conjugate of claim 1 to the subject, wherein the second region has a polypeptide sequence of at least one of SEQ ID Nos. 51, 54, 57, 60, 63, 66, 69 and 72 or a variant thereof.

113. A method of treating in a subject any one or more of dry eye, autoimmune-related hair loss, inflammation-related hair loss, chronic obstructive pulmonary disease, an inflammatory airway disease, unstable angina, acute myocardial infarction, acute stroke, and ischemia reperfusion injury to organs, comprising:

administering at least once a formulation comprising the conjugate of claim 1 wherein the second region comprises a NEMO binding domain, and

wherein administration of the formulation inhibits NF-κB activity, reduces blood concentration of TNF-α, IP-10, SAA, IL-6, Kc and/or GM-CSF, and/or increases Interleukin-10 levels in blood.

114. The method of claim 113 wherein the second region has a polypeptide sequence as set forth in SEQ ID No. 20 or a variant thereof.

115. The method of claim 113 wherein the first region comprises a polypeptide sequence as set forth in any of SEQ ID Nos. 1 or 2 or a variant thereof.

116. The conjugate of any one of claims 1, 2, 74 and 77 wherein the first region comprises a polypeptide sequence derived from a human gene.

117. The conjugate of claim 116 wherein the second region includes a polypeptide sequence derived from a human gene or variant thereof.

118. The conjugate of claim 116 wherein the second region includes a nucleic acid sequence derived from a human gene or variant thereof.

119. The method of any one of claims 30-72 and 100-115 wherein the subject is a human.

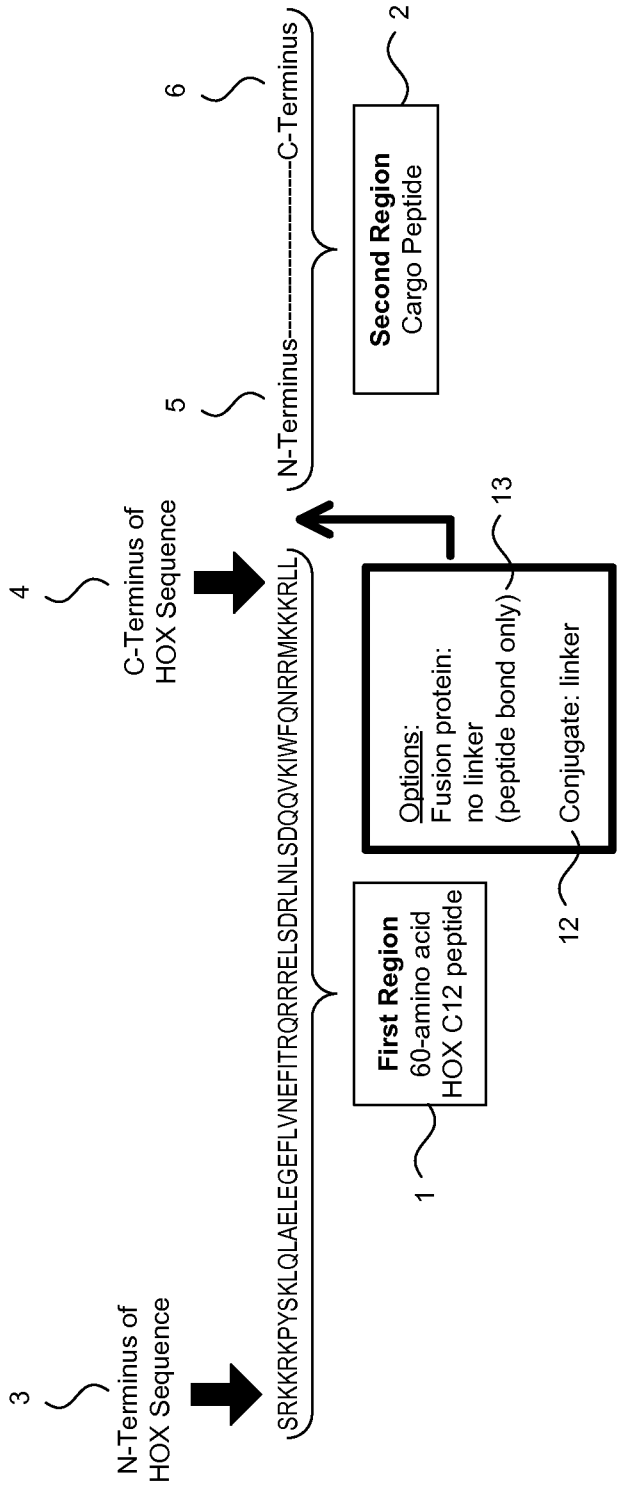

3
N-Terminus of HOX Sequence
SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL
4
C-Terminus of HOX Sequence
5
N-Terminus----------C-Terminus
6
First Region
60-amino acid
HOX C12 peptide
1
Second Region
Cargo Peptide
2
Options:
Fusion protein:
no linker
(peptide bond only)
13
Conjugate: linker
12

FIG. 1

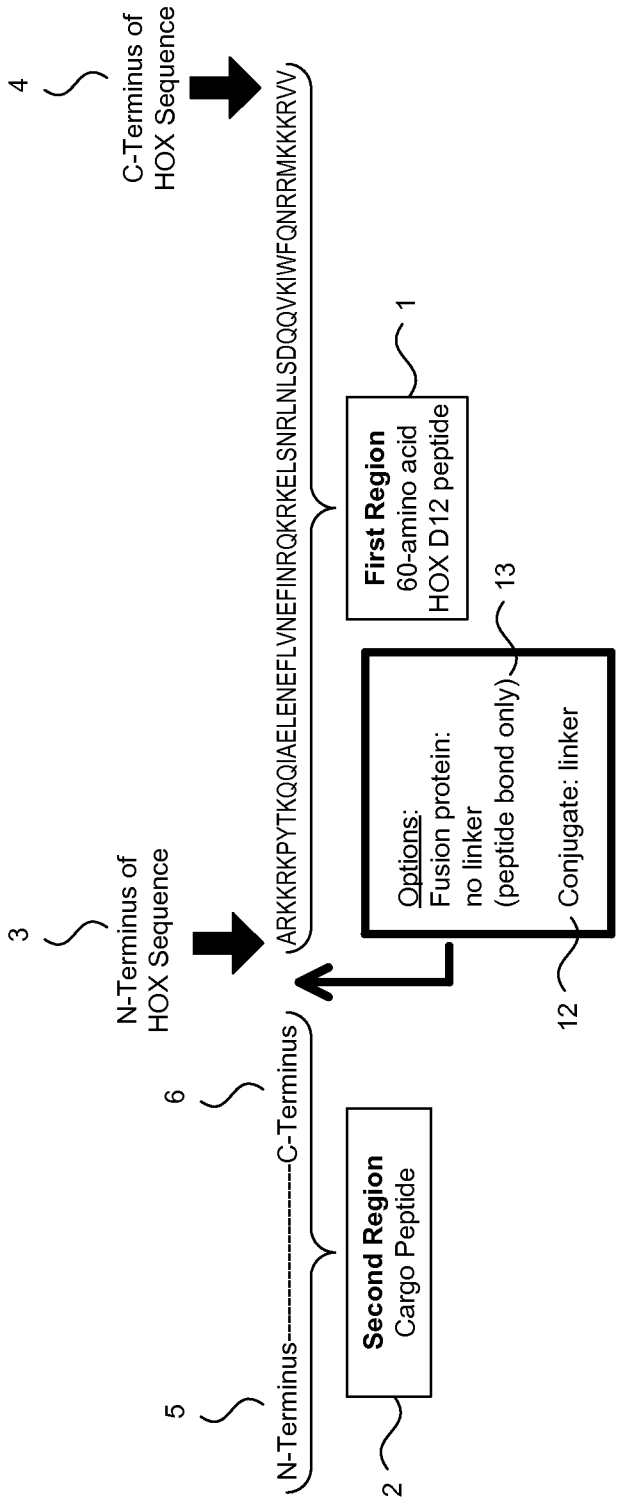

4
C-Terminus of HOX Sequence
3
N-Terminus of HOX Sequence
ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV
First Region
60-amino acid
HOX D12 peptide
1
13
Options:
Fusion protein:
no linker
(peptide bond only)
Conjugate: linker
12
6
5
N-Terminus----------C-Terminus
Second Region
Cargo Peptide
2

FIG. 2

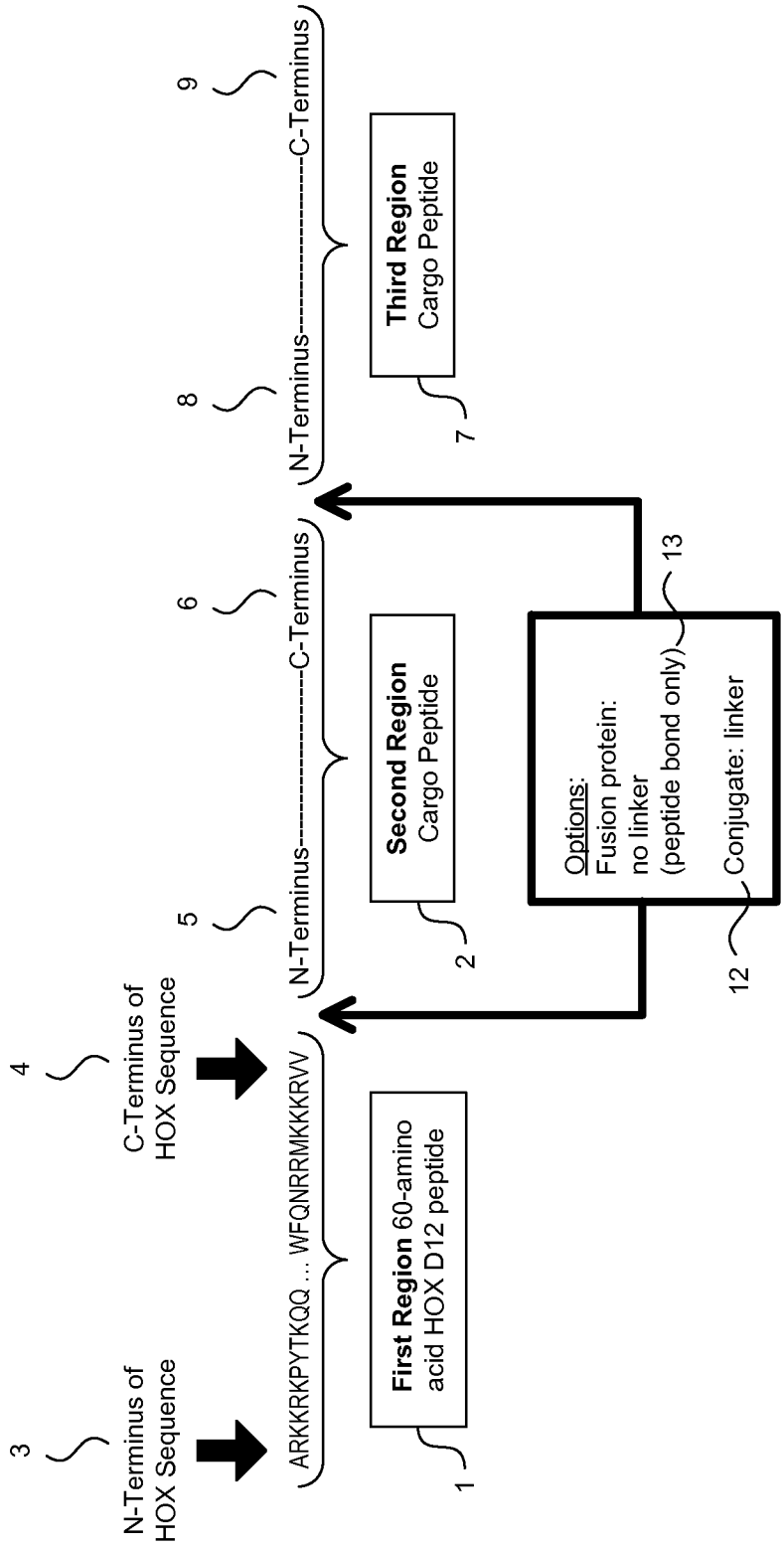

3
N-Terminus of HOX Sequence
4
C-Terminus of HOX Sequence
ARKKRKPYTKQQ ... WFQNRRMKKKRVV
First Region 60-amino acid HOX D12 peptide
1
5
N-Terminus----------C-Terminus
Second Region Cargo Peptide
2
6
N-Terminus----------C-Terminus
8
N-Terminus----------C-Terminus
Third Region Cargo Peptide
7
9
C-Terminus
Options:
Fusion protein:
no linker
(peptide bond only)
13
Conjugate: linker
12

FIG. 3A

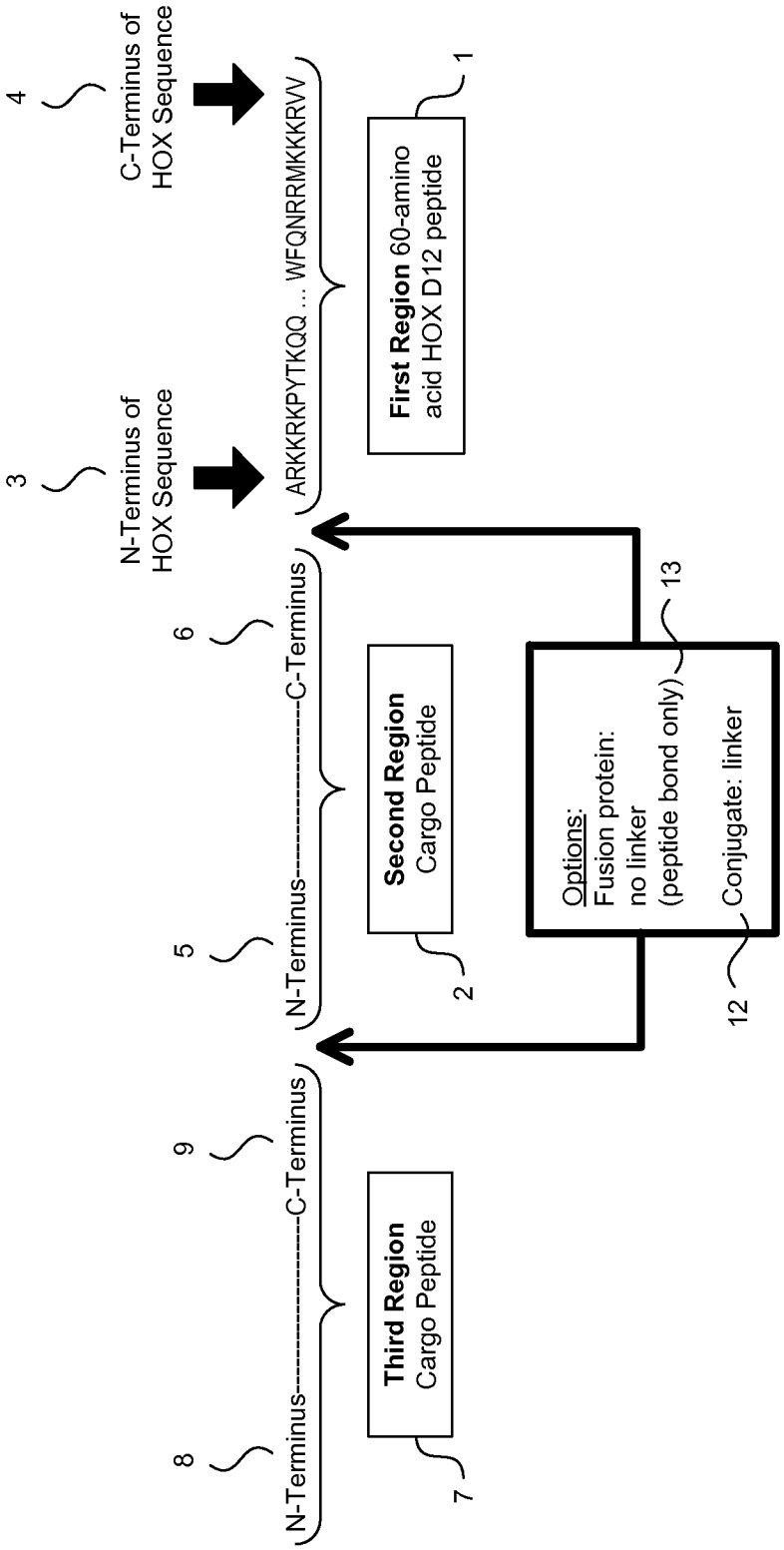

4
C-Terminus of HOX Sequence
3
N-Terminus of HOX Sequence
ARKKRKPYTKQQ ... WFQNRRMKKKRVV
1
First Region 60-amino acid HOX D12 peptide
6
C-Terminus
5
N-Terminus
Second Region Cargo Peptide
2
Options:
Fusion protein:
no linker
(peptide bond only)
13
Conjugate: linker
12
9
C-Terminus
8
N-Terminus
Third Region Cargo Peptide
7

FIG. 3B

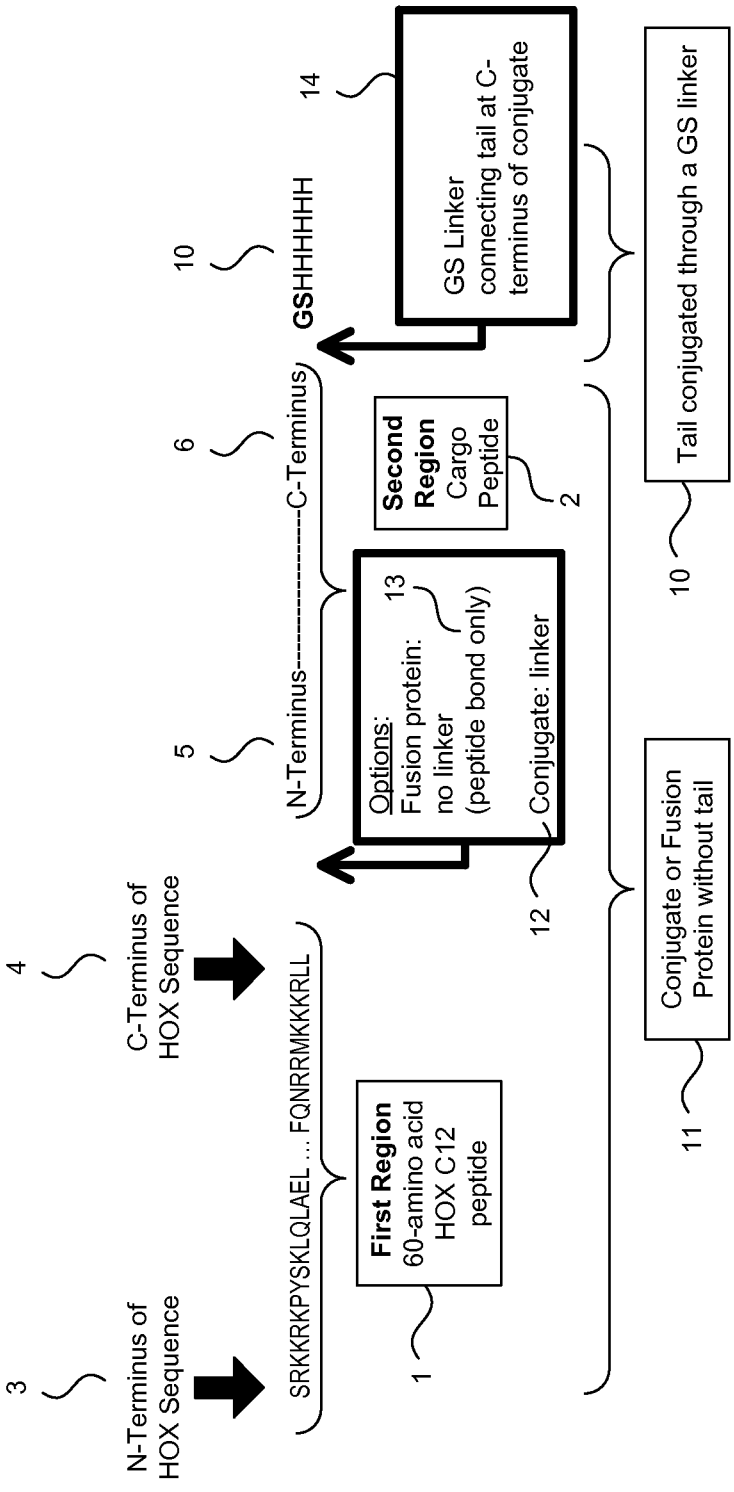

3
N-Terminus of HOX Sequence
4
C-Terminus of HOX Sequence
SRKKRKPYSKLQLAEL ... FQNRRMKKKRLL
First Region 60-amino acid HOX C12 peptide
1
5
N-Terminus
6
C-Terminus
10
GSHHHHHH
14
GS Linker connecting tail at C-terminus of conjugate
Second Region Cargo Peptide
2
Options:
Fusion protein: no linker (peptide bond only)
13
Conjugate: linker
12
Tail conjugated through a GS linker
10
Conjugate or Fusion Protein without tail
11

FIG. 4

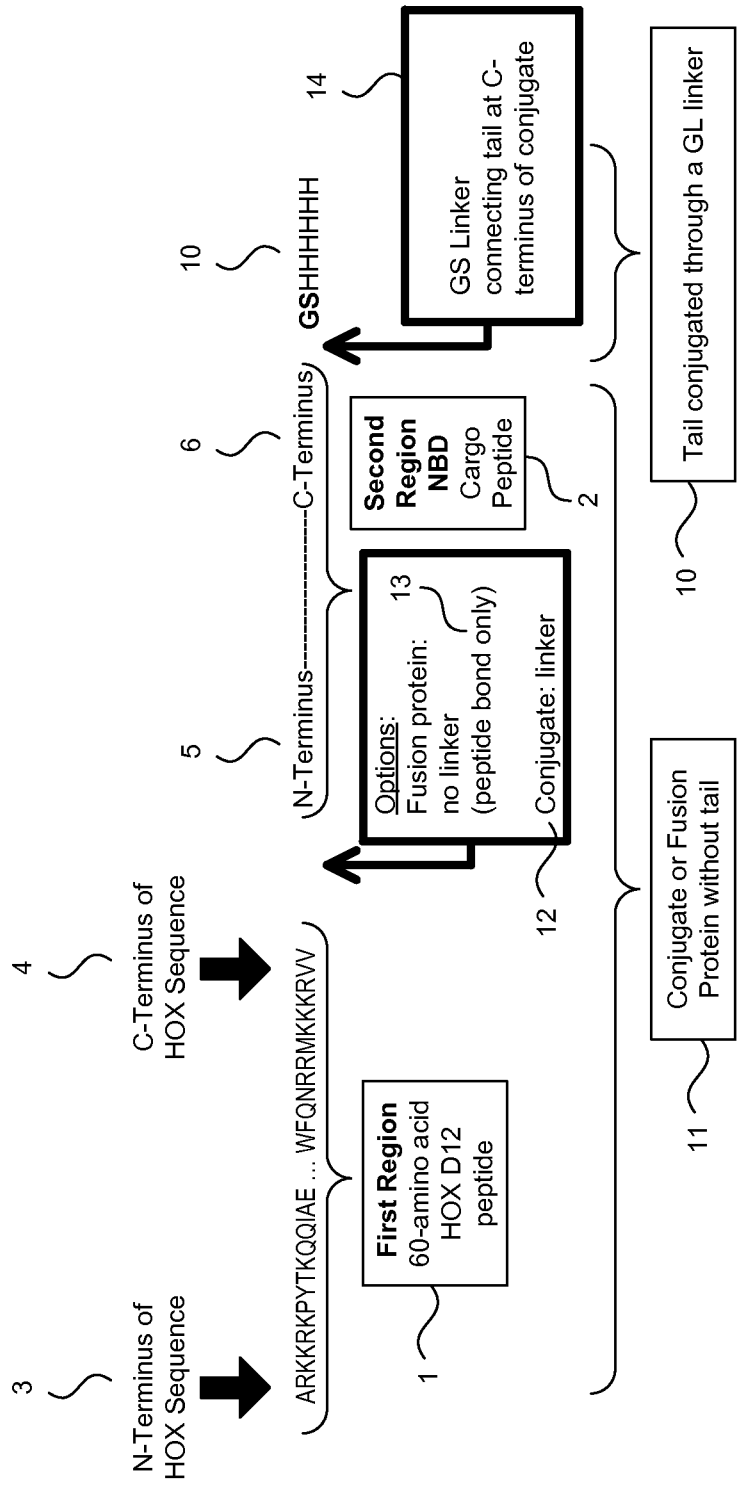

3
N-Terminus of HOX Sequence
4
C-Terminus of HOX Sequence
ARKKRKPYTKQQIAE ... WFQNRRMKKKRVV
First Region 60-amino acid HOX D12 peptide
1
5
N-Terminus
6
C-Terminus
10
GSHHHHHH
14
GS Linker connecting tail at C-terminus of conjugate
Second Region NBD Cargo Peptide
2
Options:
Fusion protein: no linker (peptide bond only)
13
Conjugate: linker
12
Conjugate or Fusion Protein without tail
11
Tail conjugated through a GL linker
10

FIG. 5

6x HIS
TEV Cleavage
Translation Map
His-TEV-PPL-002
1 ATGGCCCATCACCACCACCATCACAGCGCTGCAGAAAATCTGTACTTTCAGAGCCGTAAG
1 M G H H H H H H S A A E N L Y F Q S R K
61 AAGCGTAAACCGTATTCCAAACTGCAACTGGCGGAGTTGGAAGGTGAGTTCCTGGTTAAC
21 K R K P Y S K L 1 L A E L E G E F L V N
121 GAATTCATTACCCGTCAGCGCCGTCGCGAGCTGAGCGATCGCCTGAACCTGTCTGACCAA
41 E F I T R Q R R R E L S D R L N L S D Q
181 CAAGTGAAAATCTGGTTTCAGAATCGTCGTATGAAGAAAAAGCGCCTGCTGACCGCGTTG
61 Q V K I W F Q N R R M K K K R L L T A L
241 GACTGGAGCTGGCTGCAGACGGAG
81 D W S W L Q T E
His-TEV-PPL-002
GC Percentage: 51.568%

FIG. 6

6x HIS
TEV Cleavage
Translation Map
His-TEV-PPL-002
1 ATGGCTCATCACCACCATCACCACAGCGCAGCGGAGAACTTGTATTTTCAAGCCCGTAAG
1 M A H H H H H H S A A E N L Y F Q A R K
61 AAACGTAAACCGTACACCAAGCAGCAAATTGCGGAGCTGGAGAATGAATTCCTGGTCAAT
21 K R K P Y T K Q Q L A E L E N E F L V N
121 GAATTCATCAACCGTCAGAAGCGCAAAGAACTGAGCAATCGCCTGAACCTGTCCGACCAG
41 E F I N R Q K R K E L S D R L N L S D Q
181 CAAGTGAAAATCTGGTTTCAAAACCGTCGCATGAAGAAAACGTGTTGTTACGGCGCTG
61 Q V K I W F Q N R R M K K K R V V T A L
241 GATTGGAGCTGGCTGCAGACCGAG
81 D W S W L Q T E
His-TEV-PPL-003
GC Percentage: 48.780%

FIG. 7

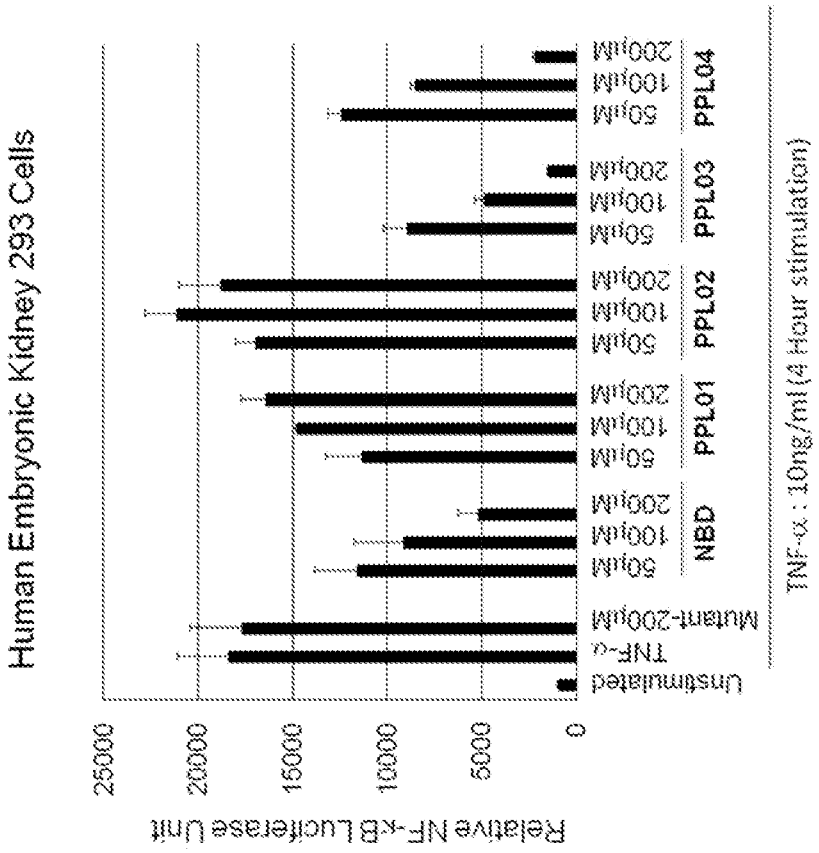

Human Embryonic Kidney 293 Cells
Relative NF-κB Luciferase Unit
25000
20000
15000
10000
5000
0
Unstimulated
TNF-α
Mutant-200μM
NBD
50μM
100μM
200μM
PPL01
50μM
100μM
200μM
PPL02
50μM
100μM
200μM
PPL03
50μM
100μM
200μM
PPL04
50μM
100μM
200μM
TNF-α : 10ng/ml (4 Hour stimulation)

FIG. 8B

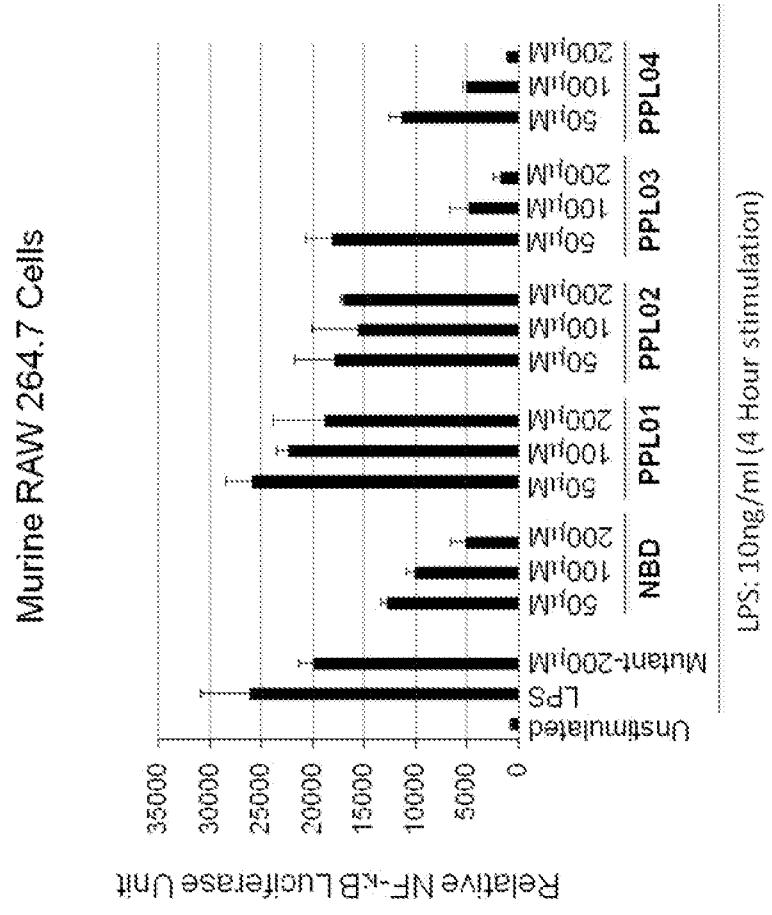

Murine RAW 264.7 Cells
Relative NF-κB Luciferase Unit
35000
30000
25000
20000
15000
10000
5000
0
Unstimulated
LPS
Mutant-200μM
NBD
50μM
100μM
200μM
PPL01
50μM
100μM
200μM
PPL02
50μM
100μM
200μM
PPL03
50μM
100μM
200μM
PPL04
50μM
100μM
200μM
LPS: 10ng/ml (4 Hour stimulation)

FIG. 8A

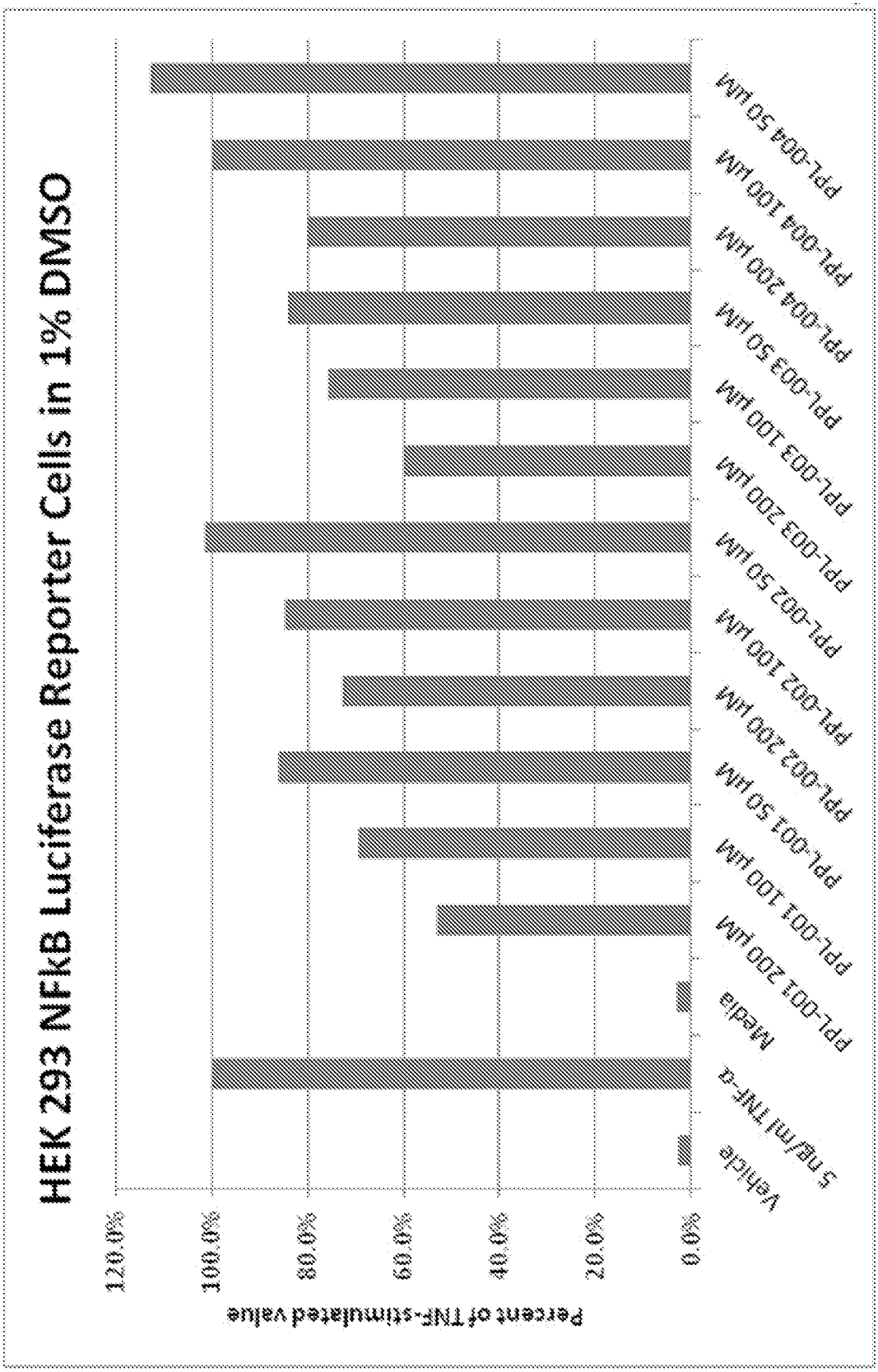

HEK 293 NFkB Luciferase Reporter Cells in 1% DMSO
Percent of TNF-stimulated value
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Vehicle
5 ng/ml TNF-α
Media
PPL-001 200 uM
PPL-001 100 uM
PPL-002 50 uM
PPL-002 200 uM
PPL-002 100 uM
PPL-002 50 uM
PPL-003 200 uM
PPL-003 100 uM
PPL-003 50 uM
PPL-004 200 uM
PPL-004 100 uM
PPL-004 50 uM

FIG. 9

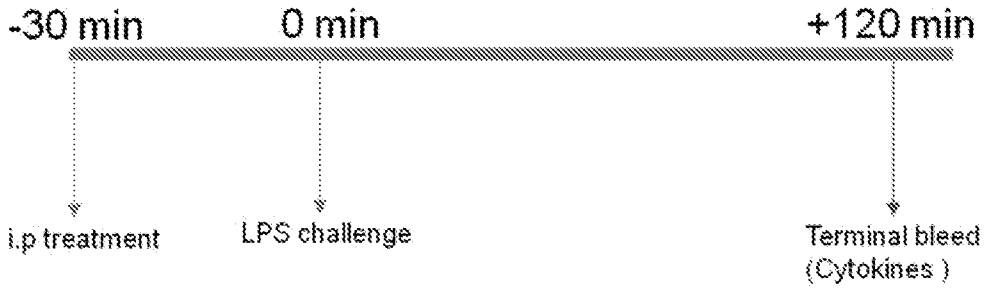

-30 min
0 min
+120 min
i.p treatment
LPS challenge
Terminal bleed
(Cytokines)

FIG. 10

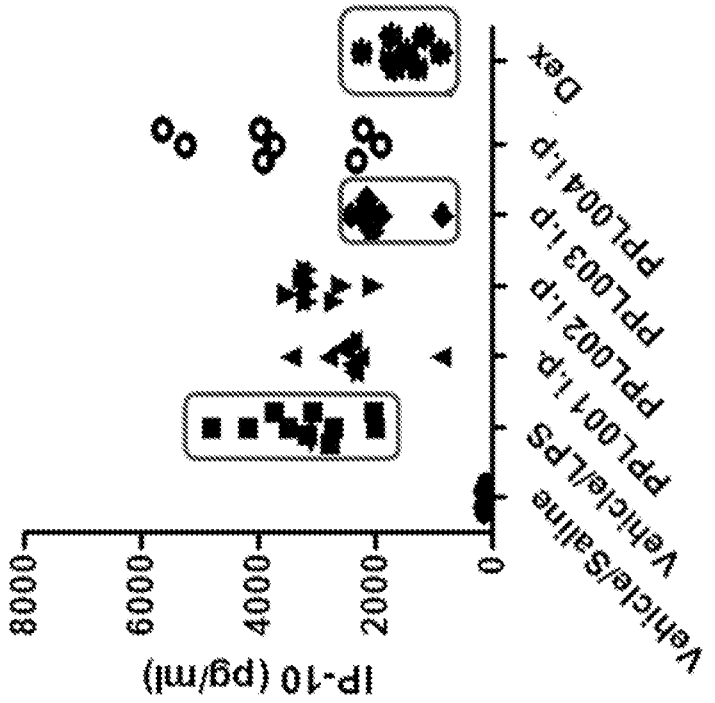


FIG. 11B

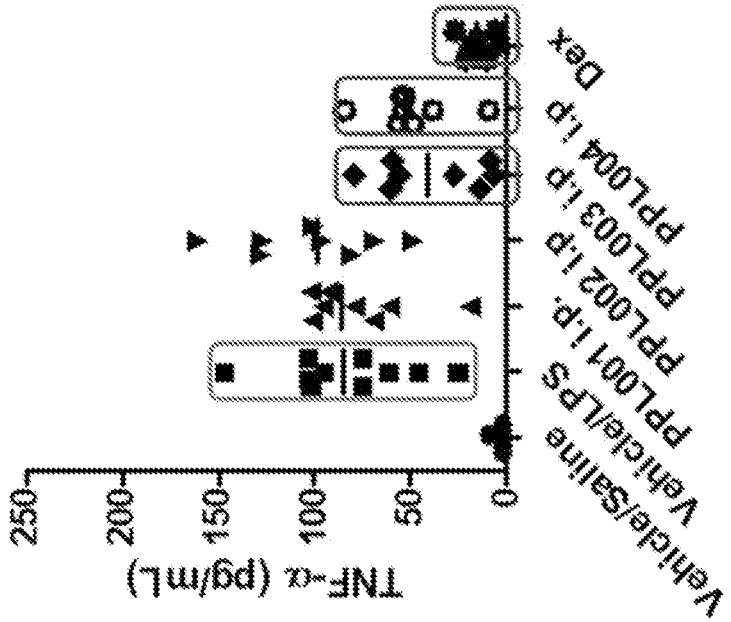


FIG. 11A

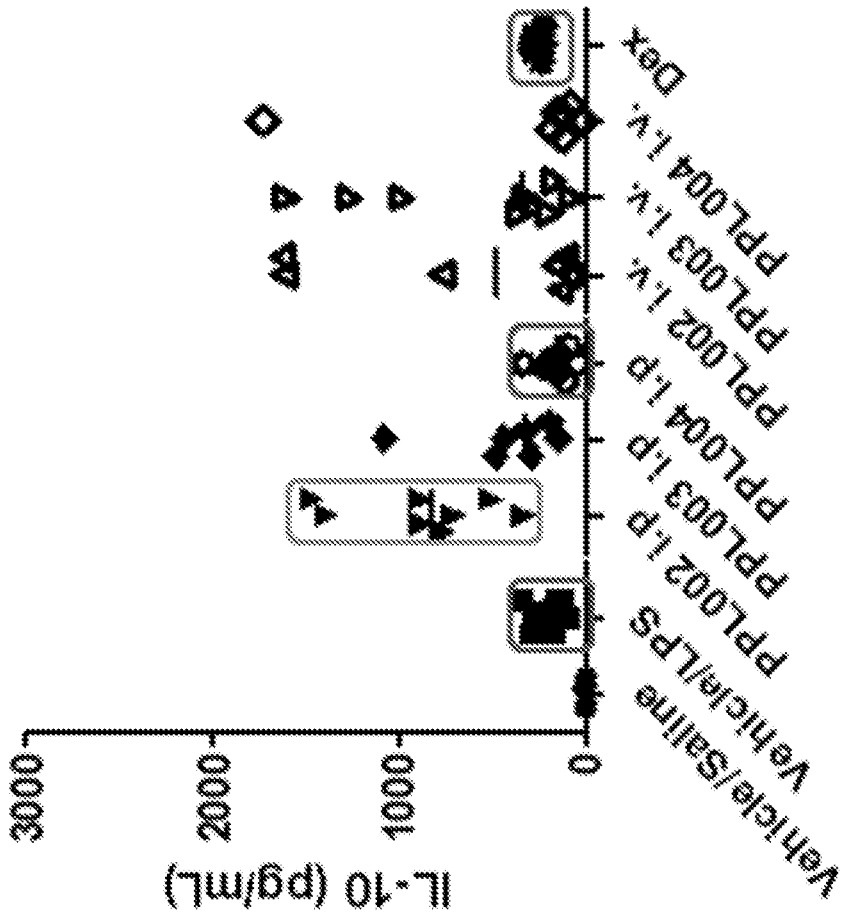

IL-10 (pg/mL)
3000
2000
1000
0
Vehicle/Saline
Vehicle/LPS
PPL002 1.p
PPL003 1.p
PPL004 1.p
PPL002 1.v
PPL003 1.v
PPL004 1.v
Dex

FIG. 12

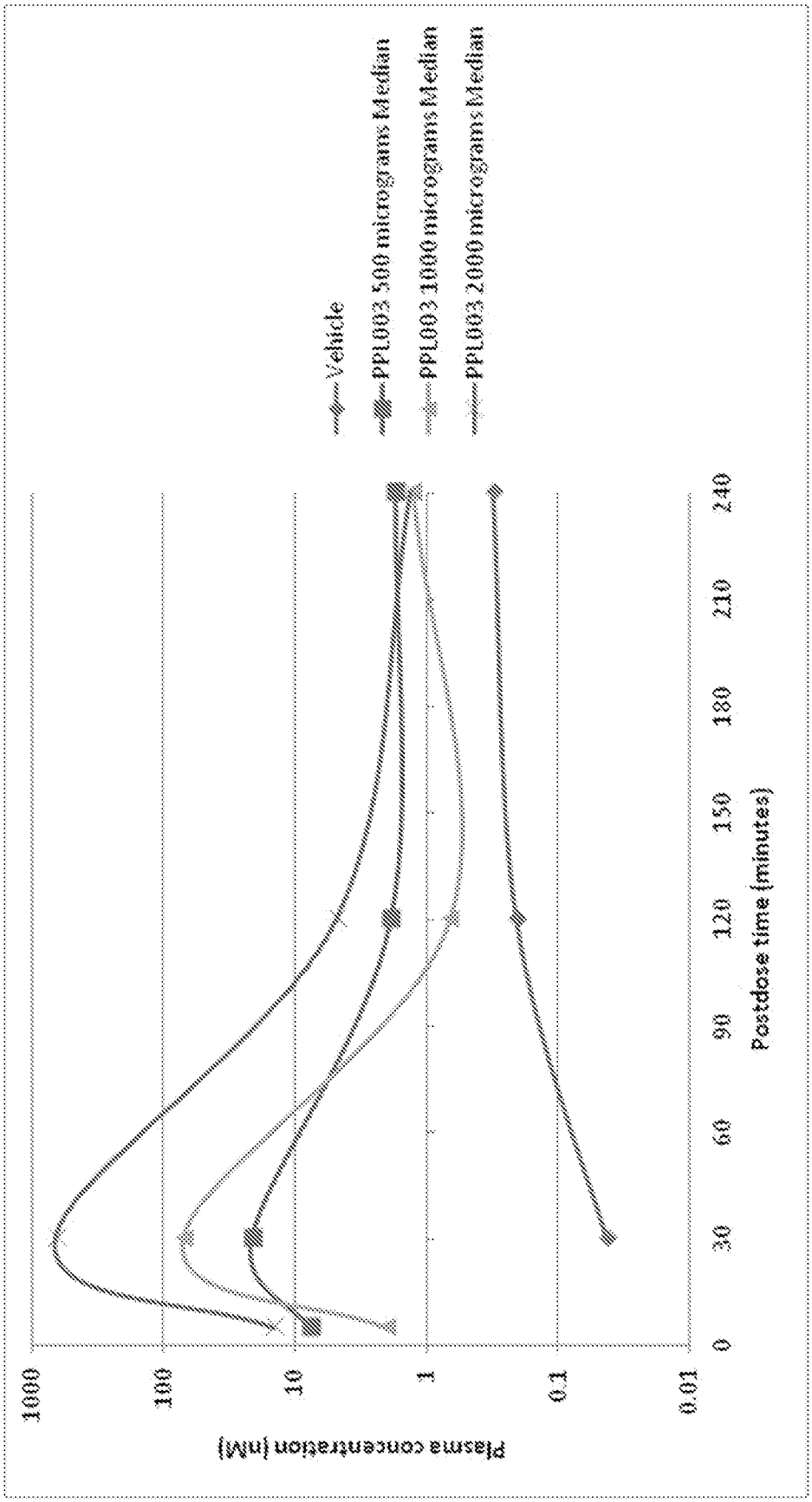

Vehicle
PPL003 500 micrograms Median
PPL003 1000 micrograms Median
PPL003 2000 micrograms Median
Plasma concentration (nM)
1000
100
10
1
0.1
0.01
0
30
60
90
120
150
180
210
240
Postdose time (minutes)

FIG. 13

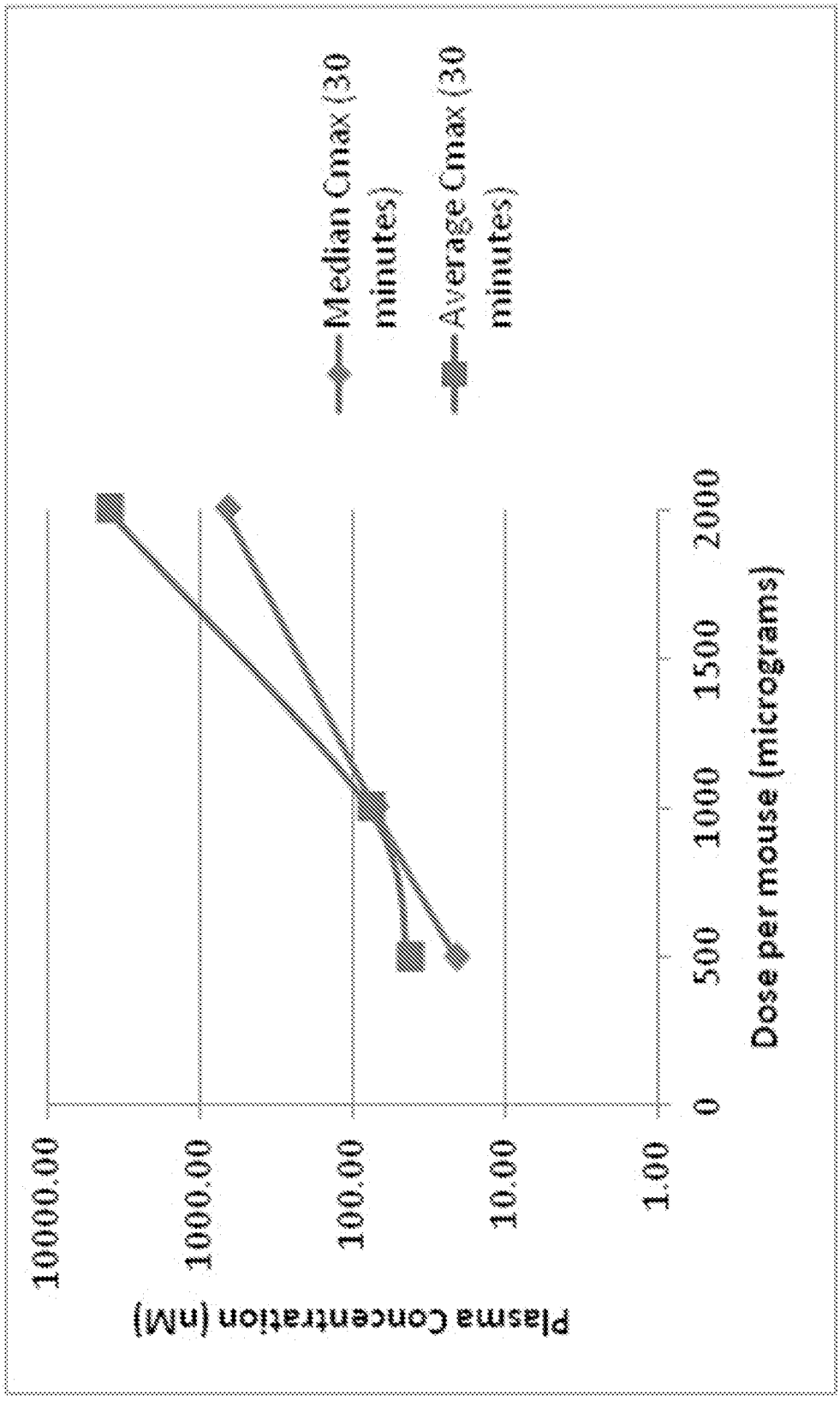

Median Cmax (30 minutes)
Average Cmax (30 minutes)
Plasma Concentration (nM)
10000.00
1000.00
100.00
10.00
1.00
0
500
1000
1500
2000
Dose per mouse (micrograms)

FIG. 14

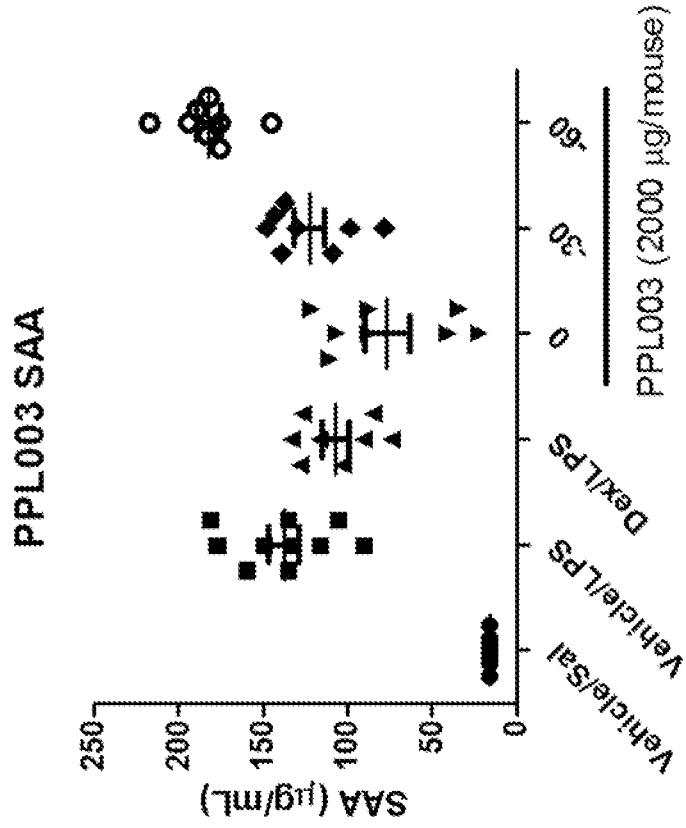


FIG. 15B

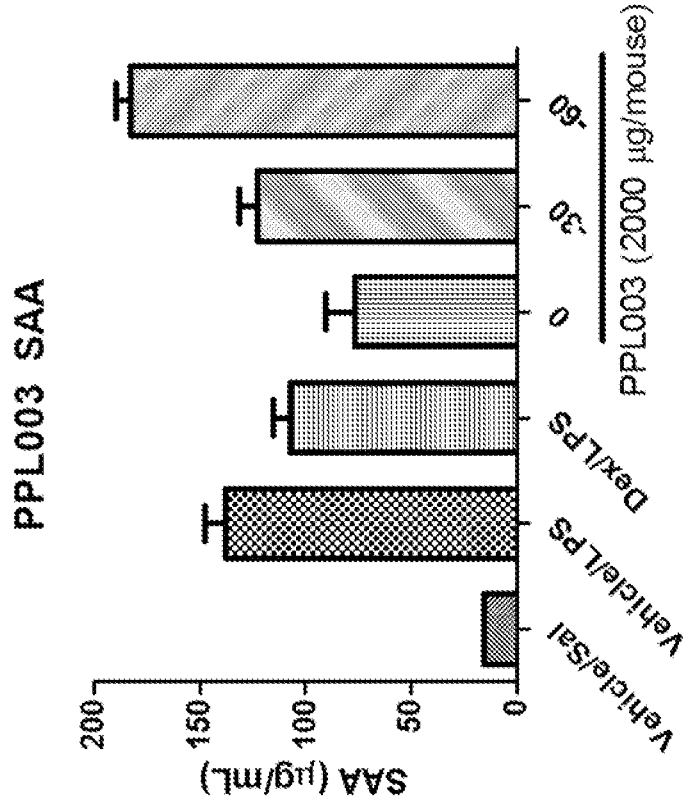


FIG. 15A

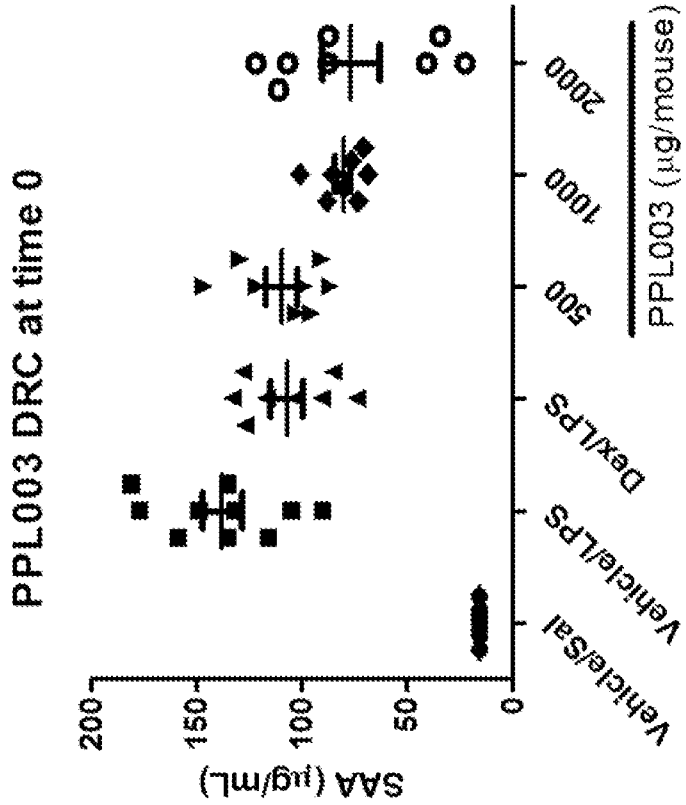


FIG. 16B

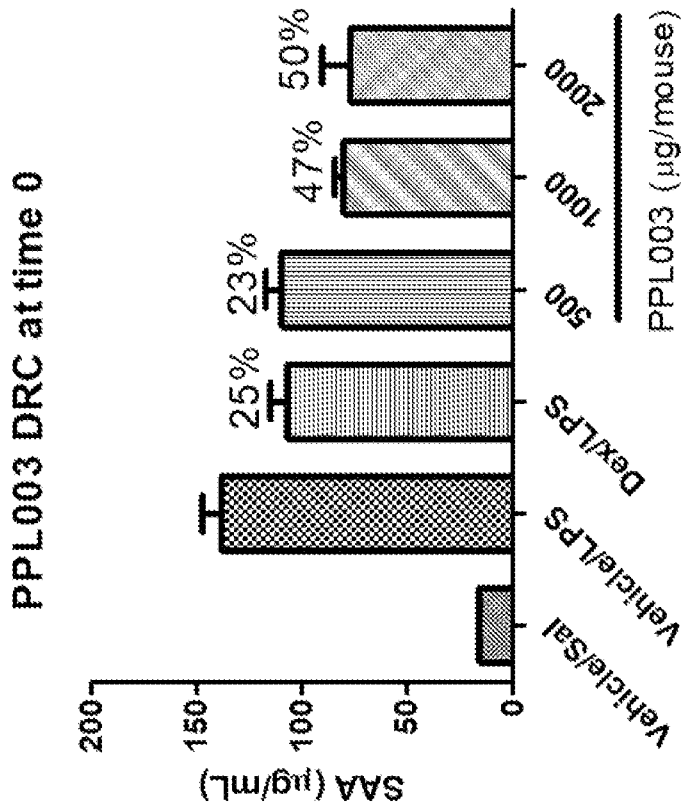


FIG. 16A

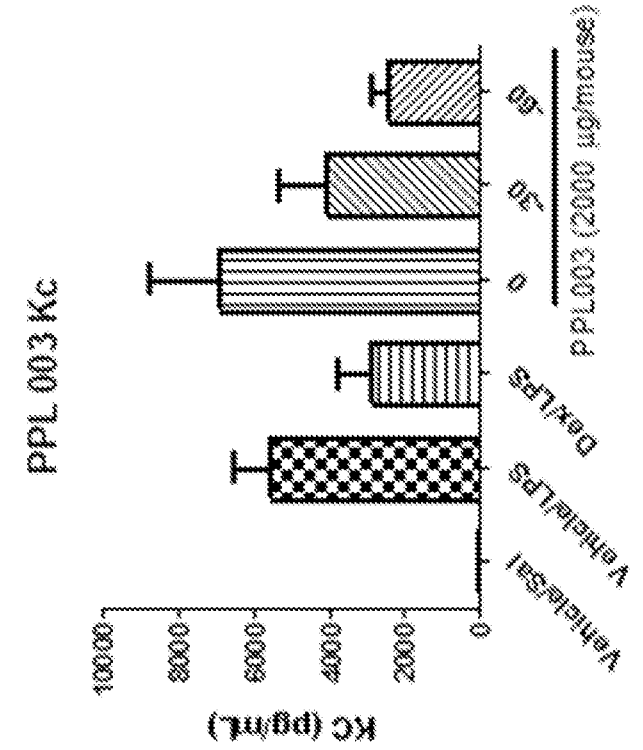


FIG. 17B

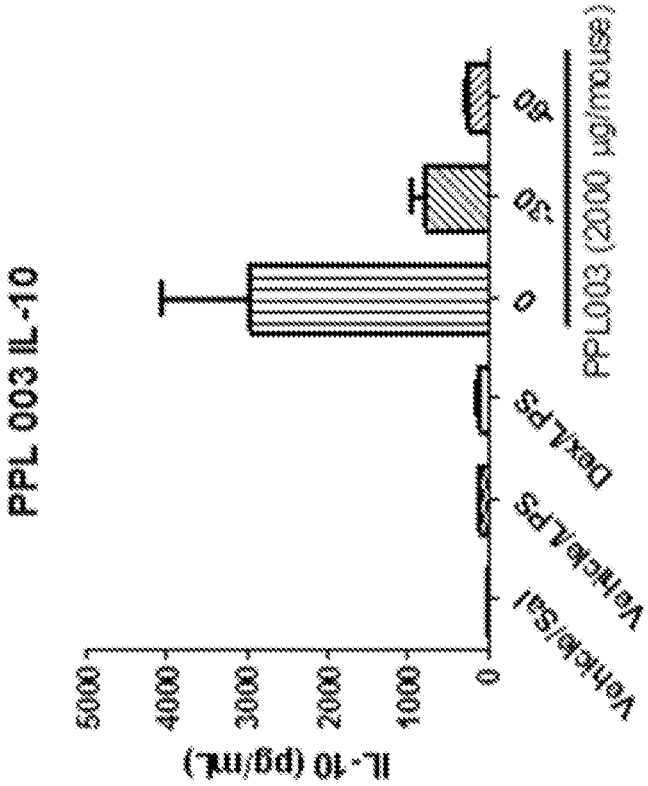


FIG. 17A

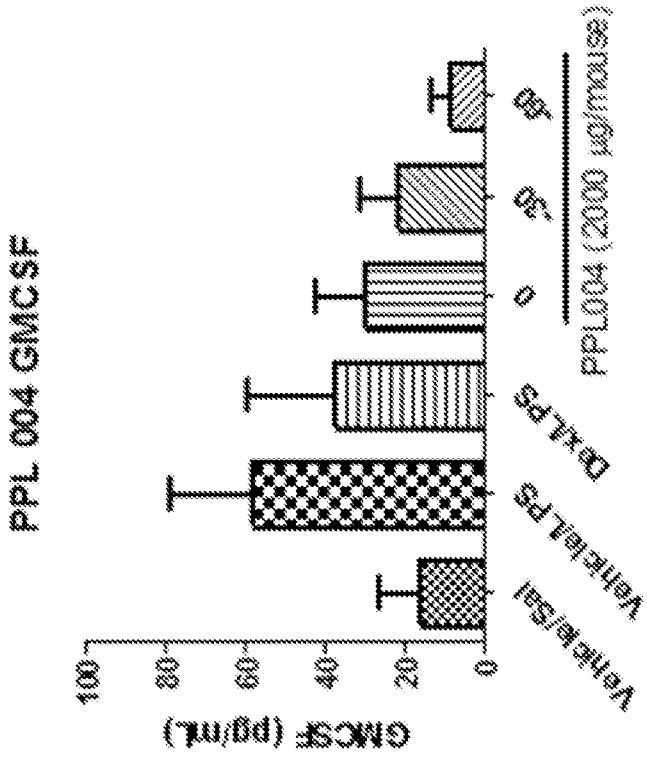


FIG. 18B

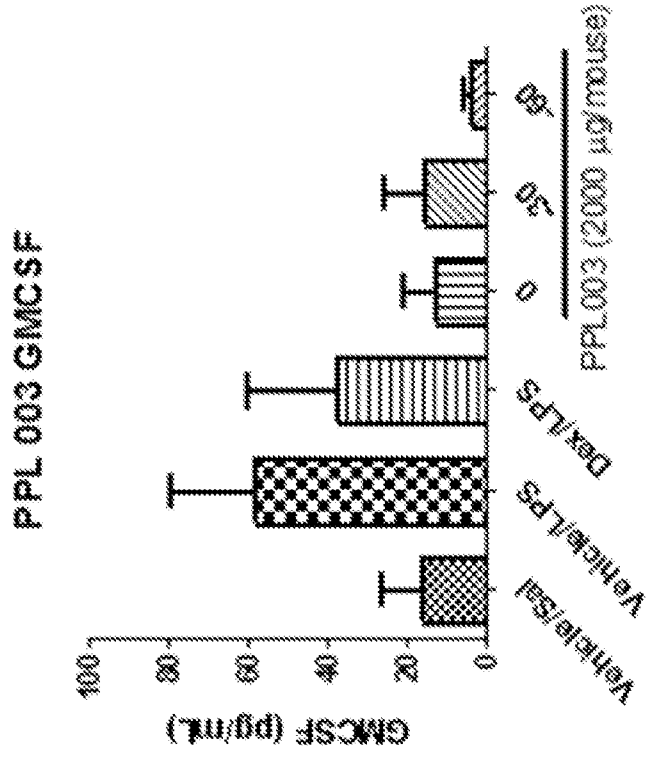


FIG. 18A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 February 2015 (26.02.2015)

WIPO | PCT

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(30) Priority Data:
61/833,819 11 June 2013 (11.06.2013) US

(71) Applicant: **PORTAGE PHARMACEUTICALS LTD.**; Viking House, Nelson Street, Douglas, Isle Of Man, IM1 2AH (VG).

(72) Inventors; and
(71) Applicants : **LITTMAN, Bruce, H.** [US/US]; 28 Prentice Williams Road, Stonington, CT 06378 (US). **THOMPSON, John, F.** [US/US]; 80 Hallmark Drive, Warwick, RI 02886 (US). **MARCOUX, Frank, W.** [US/US]; 18 Barnes Road, Stonington, CT 06378 (US).

(74) Agents: **FOURNIER, David, B.** et al.; Perkins Coie Llp, P.o.box 1247, Seattle, WA 98111-1247 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:
19 November 2015

WO 2015/025217 A3

(54) Title: STRUCTURE, MANUFACTURING AND USES OF HUMAN-DERIVED CELL-PERMEABLE PEPTIDES CONJUGATED WITH SPECIFIC BIOLOGICALLY ACTIVE CARGO PEPTIDES

(57) Abstract: Embodiments disclosed herein provide compositions for conjugates, including fusion proteins, and methods of using them to treat a variety of conditions. In some embodiments, the conjugates and/or fusion proteins incorporate a 60-amino acid human homeodomain (e.g., peptides derived from human HOX genes), to translocate functional and regulatory peptides and proteins or other biologically active molecules such as nucleic acids, which are not naturally associated with the human homeodomain, across cell and nuclear membranes to intended sites of action without provoking an unwanted immune response that may reduce exposure to the conjugate and/or result in a clinical adverse event. In further embodiments, disclosed conjugates and fusion proteins can pass through the blood-brain barrier to allow entry into the CNS. In various embodiments, the disclosed compositions are suitable for delivery into a cell (i) the expression product of a gene of interest and/or (ii) novel peptides or polynucleotides to regulate gene function.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 14/02029

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 47/48 (2015.01)
CPC - A61K 47/48238, C07K 1/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 47/48 (2015.01)
CPC - A61K 47/48238, C07K 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched (keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWEST, Google Scholar
Search terms: HOX-C12, HOX C12, HOXC12, HOX-D12, HOX D12, HOXD12, HOX, homeobox, homeodomain, conjugate, chimeric, fusion, transport, translocate, deliver, permeabilize, penetrate, cell, cytoplasm, membrane, metabolic syndrome, lipid disorder, obesity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2011/0256168 A1 (CRISANTI) 20 October 2011 (20.10.2011) para [0009]-[0014], [0018], [0062], [0073], [0075], [0092]-[0097]	74, 78/74, 100, 104, 108, 109, (116-118)/74 --- 79/74, 98-99 --- 2, 3/2, (11-15)/2, 18/2, 26, (28-30)/2, (32-33)/2, 36, 39, 40/39, 43, 46, 47/46, 50, 51/50, 54, 56/54, 58, 59/58, 62, 65, 68, 71, 75, 76, (78-79)/(75-76), (116-118)/2
Y	GRIVEAU et al., Silencing of miR-21 by locked nucleic acid-lipid nanocapsule complexes sensitize human glioblastoma cells to radiation-induced cell death. Int J Pharm, published online 31 May 2013, Vol 454, No 2, pp 765-74. Especially abstract.	79/74
Y	GOGOI et al., A versatile method for the preparation of conjugates of peptides with DNA/PNA/analog by employing chemo-selective click reaction in water. Nucleic Acids Res, 2007, Vol 35, No 21, page e139. Especially abstract	98
Y	POOGA et al., Cell penetrating PNA constructs regulate galanin receptor levels and modify pain transmission in vivo. Nat Biotechnol, September 1998, Vol 16, No 9, pp 857-61. Especially abstract.	99

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
07 July 2015 (07.07.2015)	28 JUL 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 14/02029

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0190691 A1 (EPENETOS et al.) 29 July 2010 (29.07.2010) para [0036]	2, 3/2, (11-15)/2, 18/2, 26, (28-30)/2, (32-33)/2, 36, 39, 40/39, 43, 46, 47/46, 50, 51/50, 54, 56/54, 58, 59/58, 62, 65, 68, 71, 75, 76, (78-79)/(75-76), (116-118)/2
A	CANTILE et al., The HOX genes network in uro-genital cancers: mechanisms and potential therapeutic implications. Curr Med Chem, 2011, Vol 18, No 32, pp 4872-84. Especially p 5, col 1, para 4; p 7, col 2, para 2	2, 3/2, (11-15)/2, 18/2, 26, (28-30)/2, (32-33)/2, 36, 39, 40/39, 43, 46, 47/46, 50, 51/50, 54, 56/54, 58, 59/58, 62, 65, 68, 71, 75, 76, (78-79)/(75-76), (116-118)/2
A	US 2011/0206610 A1 (ROTHBARD et al.) 25 August 2011 (25.08.2011) para [0087]	2, 3/2, (11-15)/2, 18/2, 26, (28-30)/2, (32-33)/2, 36, 39, 40/39, 43, 46, 47/46, 50, 51/50, 54, 56/54, 58, 59/58, 62, 65, 68, 71, 75, 76, (78-79)/(75-76), (116-118)/2
A	WO 2013/158819 A2 (HAECKER) 14 October 2013 (14.10.2013) para [0011]	2, 3/2, (11-15)/2, 18/2, 26, (28-30)/2, (32-33)/2, 36, 39, 40/39, 43, 46, 47/46, 50, 51/50, 54, 56/54, 58, 59/58, 62, 65, 68, 71, 75, 76, (78-79)/(75-76), (116-118)/2
L	WO 98/07859 A2 (MERBERG et al.) 26 February 1998 (26.02.1998) p 39, ln 12-20 (Document incorporated by reference by US 2011/0256168 A1 to Crisanti)	104, 109

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 14/02029

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

No electronic sequence listing was submitted in response to the PCT/ISA/225 issued on 03 April 2015. Therefore, the international search was not carried out on the basis of that non-compliant electronic sequence listing.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 14/02029

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

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

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

2. ☒ Claims Nos.: (see claims below)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 1, 3/1, 4-10, (11-15)/1, 16-17, 18/1, 19-25, 27, (28-30)/1, 31, (32-33)/1, 34-35, 37-38, 40/38, 41-42, 44-45, 47/45, 48-49, 51/49, 52-53, 55, 56/53, 57, 59/57, 60-61, 63-64, 66-67, 69-70, 72-73, 77, (78-79)/77, 80-97, 101-103, 105-107, 110-115, (116-118)/(1,77), 119 are unsearchable because the applicant failed to submit a valid CRF to the ISA/225 of 03 April 2015. The USPTO cannot supply a search for the sequences listed in this application. Accordingly, the claims requiring a sequence are held as unsearchable.

3. ☒ Claims Nos.: 27, 80-97, 101-103, 105-107, 119
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

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

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

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

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

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(54) Title: STRUCTURE, MANUFACTURING AND USES OF HUMAN-DERIVED CELL-PERMEABLE PEPTIDES CONJUGATED WITH SPECIFIC BIOLOGICALLY ACTIVE CARGO PEPTIDES

(57) Abstract: Embodiments disclosed herein provide compositions for conjugates, including fusion proteins, and methods of using them to treat a variety of conditions. In some embodiments, the conjugates and/or fusion proteins incorporate a 60-amino acid human homeodomain (e.g., peptides derived from human HOX genes), to translocate functional and regulatory peptides and proteins or other biologically active molecules such as nucleic acids, which are not naturally associated with the human homeodomain, across cell and nuclear membranes to intended sites of action without provoking an unwanted immune response that may reduce exposure to the conjugate and/or result in a clinical adverse event. In further embodiments, disclosed conjugates and fusion proteins can pass through the blood-brain barrier to allow entry into the CNS. In various embodiments, the disclosed compositions are suitable for delivery into a cell (i) the expression product of a gene of interest and/or (ii) novel peptides or polynucleotides to regulate gene function.

AMENDED CLAIMS
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1. A conjugate comprising:
a first region comprising a homeodomain structure and having a polypeptide sequence as described in any one of SEQ ID Nos. 1 to 19 or a variant thereof; and
a second region comprising a functional or regulatory polypeptide or protein not naturally associated with the first region.

2. A conjugate comprising:
a first region comprising at least one of a HOX D12 homeodomain or a HOX C12 homeodomain; and
a second region comprising a functional or regulatory polypeptide or protein not naturally associated with the first region.

3. A conjugate of claim 1 or claim 2 wherein the second region includes at least one of a NEMO binding domain, a PC1 CTT-derived fusion protein, an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies, a gene product of RPE65, a zinc finger peptide, an Ag85A 99-118 epitope peptide, or an Ag85A 70-78 epitope peptide.

4. The conjugate of claim 3 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

5. The conjugate of claim 3 wherein the PC1 CTT-derived fusion protein has a polypeptide sequence set forth in at least one of SEQ. ID Nos. 23 or 26 or a variant thereof.

6. The conjugate of claim 3 wherein the enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies has a polypeptide sequence of at least one of SEQ. ID Nos. 30, 45, or 48 or a variant thereof.

7. The conjugate of claim 3 wherein the gene product of RPE65 has a polypeptide sequence that includes at least SEQ. ID No. 33 or a variant thereof.

8. The conjugate of claim 3 wherein the zinc finger peptide sequence includes at least one of SEQ. ID Nos. 36, 29, or 42 or a variant thereof.

9. The conjugate of claim 3 wherein the Ag85A 99-118 epitope peptide has a sequence that includes at least one of SEQ. ID Nos. 51, 54, 57, or 60 or a variant thereof.

10. The conjugate of claim 3 wherein the Ag85A 70-78 epitope peptide has a sequence that includes at least one of SEQ. ID Nos. 63, 66, or 69 or a variant thereof.

11. The conjugate of claim 1 or claim 2 wherein the second region is conjugated to the C-terminus of the first region.

12. The conjugate of claim 1 or claim 2 wherein the second region is conjugated to the N-terminus of the first region.

13. The conjugate of any one of claims 1 and 2 in the form of a fusion protein.

14. The conjugate of any one of claims 1 and 2 wherein the second region interacts with an intracellular target and/or an intranuclear target.

15. The conjugate of claim 14 wherein the interaction of the second region with the intracellular target and/or intranuclear target affects one or more of the following: NF-κB activation, uveitis, PC1 transcription factor activity in renal tubular cells with polycystic kidney disease mutations, glucocerebrosidase substrate in cells with Gaucher Disease mutations of GCase, glycosaminoglycans in subjects with Hurler Syndrome or Hunter Syndrome, retinal membrane guanylyl cyclase substrate in retinal cells deficient in retinal membrane guanylyl cyclase, regulation of Huntingtin

mutant gene transcription in cells with Huntington's Disease mutations, and delivery of antigenic epitopes into cytoplasm of antigen-presenting cells for provoking an effective immune response in a vaccine embodiment such as for a tuberculosis vaccine to prevent tuberculosis in subjects exposed to the infection.

16. The conjugate of any claim 1 or claim 2 wherein the second region has a polypeptide sequence that includes SEQ. ID Nos. 23 or 26 or variant thereof, and wherein the conjugate further comprises a noncompetitive V2 receptor antagonist.

17. The conjugate of claim 1 or claim 2 wherein the second region has a polypeptide sequence selected from the group consisting of SEQ ID Nos: 20, 23, 26, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, and 72, or a variant thereof.

18. The conjugate of claim 1 or claim 2, further comprising a linker sequence between the first and second regions.

19. The conjugate of claim 1 wherein the first and second regions combined have a polypeptide sequence selected from the group consisting of SEQ ID Nos: 21, 22, 24, 25, 27, 28, 31, 32, 34, 35, 37, 38, 40, 41, 43, 44, 46, 47, 49, and 50, 52, 53, 55, 56, 58, 59, 61, 62, 64, 65, 67 , 68, 70, 71, 73 and 74, or a variant thereof.

20. The conjugate of claim 19 wherein an initiating methionine residue of the sequence is removed prior to use as an active and/or therapeutic agent.

21. The conjugate of claim 1 wherein the first and second regions combined have a sequence comprising a homeodomain structure and at least one of a NEMO binding domain, a PC1 CTT-derived fusion protein, an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies, a gene product of RPE65, a zinc finger peptide, an Ag85A 99-118 epitope peptide, and an Ag85A 70-78 epitope peptide.

22. The conjugate of claim 21 wherein the second region interacts with an intracellular target and/or an intranuclear target.

23. The conjugate of claim 22 wherein the interaction of the second region with an intracellular target and/or intranuclear target affects one or more of the following: NF-κB activation, uveitis, PC1 transcription factor activity in renal tubular cells with polycystic kidney disease mutations, glucocerebrosidase substrate in cells with Gaucher Disease mutations of GCase, glycosaminoglycans in subjects with Hurler Syndrome or Hunter Syndrome, retinal membrane guanylyl cyclase substrate in retinal cells deficient in retinal membrane guanylyl cyclase, regulation of Huntingtin mutant gene transcription in cells with Huntington's Disease mutations, and delivery of antigenic epitopes into cytoplasm of antigen-presenting cells for provoking an effective immune response as the active component of vaccines as for example in a tuberculosis vacceine to prevent tuberculosis in subjects exposed to the infection.

24. The conjugate of claim 19 or claim 21, further comprising a linker sequence between the first and second regions.

25. A composition comprising the conjugate of claim 1 and a pharmaceutically acceptable carrier.

26. A composition comprising the conjugate of claim 2 and a pharmaceutically acceptable carrier.

27. The composition of claims 25 or claim 24 wherein the composition is in the form of an inhalable composition, an eye drop formulation other ophthalmic composition for local or injectable use, an enema, a topical composition, or an injectable composition including injectable implants for sustained release.

28. The conjugate of claim 1 or claim 2 wherein, when administered to a subject in a formulation, the conjugate enters the central nervous system.

29. The conjugate of claim 1 or claim 2 wherein, when administered in a formulation to a subject, the conjugate elicits a reduced unwanted immune response in the subject compared to an unwanted immune response elicited by the second region when not conjugated to the first region.

30. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the conjugate enters the central nervous system in a subject.

31. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 21 to the subject, wherein the conjugate enters the central nervous system of the subject.

32. A method of treating a condition in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the conjugate prevents or ameliorates immune responses to antigenic peptides in the subject such that there is at least one of (a) no significant reduction in peptide exposure, and (b) no significant increase in the incidence of immune-mediated adverse clinical events.

33. A method of treating a dry eye in a subject comprising administering a formulation comprising the conjugate of claim 1 or claim 2 to the subject, wherein the first region is conjugated to a NEMO binding domain.

34. The method of claim 33 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

35. A method of treating autoimmune and inflammation-related hair loss in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

36. A method of treating autoimmune and inflammation-related hair loss in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

37. The method of claim 35 or claim 36 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

38. A method of treating an inflammatory airway disease in a subject comprising administering an inhalable formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

39. A method of treating an inflammatory airway disease in a subject comprising administering an inhalable formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

40. The method of claim 38 or claim 39 wherein the inflammatory airway disease is chronic obstructive pulmonary disease.

41. The method of claim 38, wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

42. A method of treating colo-rectal inflammatory bowel disease in a subject comprising administering an enema formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

43. A method of treating colo-rectal inflammatory bowel disease in a subject comprising administering an enema formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

44. The method of claim 42 or claim 43 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

45. A method of treating a condition in a subject comprising administering a systemic formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

46. A method of treating a condition in a subject comprising administering a systemic formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a NEMO binding domain.

47. The method of claim 45 or claim 46 wherein the condition is at least one of unstable angina, acute myocardial infarction and acute stroke.

48. The method of claim 45 or claim 46 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

49. A method of treating ischemia reperfusion injury following transient ischemia in a subject comprising administering a systemic formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a NEMO binding domain.

50. A method of treating ischemia reperfusion injury following transient ischemia in a subject comprising administering a systemic formulation comprising the conjugate of claim 2, wherein the second region is a NEMO binding domain.

51. The method of claim 49 or claim 50 wherein the ischemia reperfusion injury is in the brain, kidney or heart.

52. The method of claim 49 or claim 50 wherein the NEMO binding domain has a polypeptide sequence set forth in SEQ. ID No. 20 or a variant thereof.

53. A method of treating polycystic kidney disease in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region is a PC1 CTT-derived fusion protein, a PC1 CTT-derived p200 peptide or a PC1 CTT-derived p21 peptide.

54. A method of treating polycystic kidney disease in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region is a PC1 CTT-derived fusion protein, a PC1 CTT-derived p200 peptide or a PC1 CTT-derived p21 peptide.

55. The method of claim 53 or claim 54 wherein the second region has a polypeptide sequence set forth in at least one of SEQ. ID Nos. 23 or 26 or a variant thereof.

56. The method of claim 53 or claim 54 wherein the conjugate is further conjugated to a noncompetitive V2 receptor antagonist for targeting renal cysts of collecting duct cells.

57. A method of treating a lysosomal storage disease in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region of the conjugate comprises an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies.

58. A method of treating a lysosomal storage disease in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region of the conjugate comprises an enzyme that restores enzymatic function in subjects with inherited enzyme deficiencies.

59. The method of claim 57 or claim 58 wherein the enzyme is selected from the group consisting of glucocerebrosidase, alpha-L-iduronidase, and iduronidate-2-sulfatase.

60. The method of claim 57 or claim 58 wherein the enzyme includes a polypeptide sequence as set forth in at least one of SEQ. ID Nos. 30, 45, or 48 or a variant thereof.

61. A method of treating Leber Congentital Amourosis type 1 in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region includes a gene product of RPE65.

62. A method of treating Leber Congentital Amourosis type 1 in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region includes a gene product of RPE65.

63. The method of claim 61 or claim 62 wherein the gene product of RPE65 has a polypeptide sequence as set forth in SEQ. ID No. 33 or a variant thereof.

64. A method of treating Huntington's Disease in a subject comprising administering the conjugate of claim 1 to the subject, wherein the second region includes a zinc finger peptide.

65. A method of treating Huntington's Disease in a subject comprising administering the conjugate of claim 2 to the subject, wherein the second region includes a zinc finger peptide.

66. The method of claim 64 or claim 65, wherein the zinc finger peptide sequence includes at least one of SEQ. ID Nos. 36, 29, or 42 or a variant thereof.

67. A method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region comprises an Ag85A 99-118 epitope peptide.

68. The method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region comprises an Ag85A 99-118 epitope peptide.

69. The method of claim 67 or claim 68 wherein the Ag85A 99-118 epitope peptide sequence includes at least one of SEQ. ID Nos. 51, 54, 57, or 60 or a variant thereof.

70. A method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 1 to the subject, wherein the second region comprises an Ag85A 70-78 epitope peptide.

71. The method of treating tuberculosis in a subject comprising administering a formulation comprising the conjugate of claim 2 to the subject, wherein the second region comprises an Ag85A 70-78 epitope peptide.

72. The method of claim 70 or claim 71 wherein the Ag85A 70-78 epitope peptide sequence includes at least one of SEQ. ID Nos. 63, 66, or 69 or a variant thereof.

73. A conjugate prepared by a method comprising the steps:

culturing a host cell transformed with an expression vector comprising a nucleic acid encoding a conjugate according to claim 1 under conditions which provide for the expression of the conjugate within the host cell; and

recovering the conjugate by affinity purification.

74. A conjugate comprising:

a first region comprising a homeodomain structure that is a HOX D12 homeodomain or a HOX C12 homeodomain; and

a second region comprising a functional or regulatory nucleic acid or peptide nucleic acid not naturally associated with the first region.

75. A conjugate comprising:

a first region comprising a homeodomain structure having a polypeptide sequence described in any one of SEQ ID Nos. 1 to 19 or a variant thereof; and

a second region comprising a functional or regulatory nucleic acid or peptide nucleic acid not naturally associated with the first region.

76. The conjugate of claim 74 or claim 75 wherein the second region comprises DNA, RNA, LNA, PNA, γPNA, or a combination of these components that has intracellular biological activity.

77. The conjugate of claim 74 or claim 75 wherein the second region includes a nucleic acid that binds to miRNA21 or to DYRK1b.

78. The conjugate of any one of claims 74 to 76 wherein the second region has a nucleotide sequence as set forth in at least one of SEQ ID Nos. 76, 78, or 79 or a variant thereof.

79. The conjugate of any one of claims 74 to 77 wherein the second region is conjugated to the C-terminus of the first region.

80. The conjugate of any one of claims 74 to 77 wherein the second region is conjugated to the N-terminus of the first region.

81. The conjugate of any one of claims 74 to 77 wherein the first region is linked to the second region with a peptidic bond.

82. The conjugate of any one of claims 74 to 77 wherein the second region is conjugated to the first region via a non-peptidic bond.

83. The conjugate of any one of claims 74 to 77 wherein the second region interacts with an intracellular target and/or an intranuclear target.

84. The conjugate of claim 83 wherein the second region interacts with an intracellular target and/or an intranuclear target via interactions with an miRNA or a protein complex that binds an miRNA.

85. The conjugate of claim 84 wherein the second region is a nucleic acid or PNA that binds to miRNA132 for affecting the Ras pathway.

86. The conjugate of claim 84 wherein the second region is a nucleic acid sequence or PNA that stably hybridizes to an miRNA nucleotide sequence as set forth in SEQ ID No. 75 or a variant thereof.

87. The conjugate of claim 86 wherein the second region has a nucleotide sequence as set forth in SEQ ID No. 78 or to a portion of said nucleotide sequence or a variant thereof that stably hybridizes to miRNA132.

88. The conjugate of claim 86 wherein the second region is selected based on the specificity of an interaction with miRNA132.

89. The conjugate of claim 86 wherein the second region consists of modified nucleotides to increase stability or reduce degradation.

90. The conjugate of claim 84 wherein the second region is a nucleic acid sequence or PNA that binds to miRNA21 for affecting a tumor suppressor pathway or tumor growth.

91. The conjugate of claim 90 wherein the second region comprises an nucleotide sequence as set forth in SEQ ID No. 76 or variant thereof or can stably hybridize to a nucleotide sequence as set forth in SEQ ID No. 77 or a variant thereof.

92. The conjugate of any one of claims 74 and 75 to 91 wherein the first region homeodomain structure has a polypeptide sequence as set forth in SEQ ID No. 2 or a variant thereof.

93. The conjugate of any one of claims 74 to 77 wherein the second region interacts with an intracellular target and/or an intranuclear target via interactions with an mRNA for affecting splicing or protein translation.

94. The conjugate of claim 93 wherein the second region binds to DYRK1b.

95. The conjugate of claim 94 in which the second region has a nucleotide sequence as set forth in SEQ ID No. 79 or a variant thereof.

96. The conjugate of claim 74 wherein the first region and the second region are attached via “click chemistry.”

97. The conjugate of claim 74 wherein the second region comprises PNA, and wherein the first region and the second region is attached via a peptide bond.

98. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 74 to the patient.

99. A method of treating cancer in a subject comprising administering formulation comprising the conjugate of claim 93 to the subject.

100. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 94 to the subject.

101. A method of treating cancer in a subject comprising administering a formulation comprising the conjugate of claim 95 to the subject.

102. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 74 to the subject.

103. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 93 to the subject.

104. A method of treating a metabolic syndrome, a lipid disorder and/or obesity in a subject comprising administering a formulation comprising the conjugate of claim 94 to the subject.

105. A method of treating a metabolic syndrome, a lipid disorder or obesity in a subject comprising administering a formulation comprising the conjugate of claim 95 to the subject.

106. The method of treating cancer of claim 98 comprising administration of the formulation as an intravenous solution.

107. The method of treating a metabolic syndrome, a lipid disorder and/or obesity of claim 102 comprising administration of the formulation in sustained release form.

108. A method of treating tuberculosis in a subject comprising administrating a formulation comprising the conjugate of claim 1 to the subject, wherein the second region includes at least a portion of one of a Ag85A 99-118 epitope peptide or a Ag85A 70-78 epitope peptide.

109. The method of treating tuberculosis in a subject according to claim 108 wherein:

the formulation is administered to the subject at least once via an inhaled route, intranasal route and/or by intramuscular, intradermal or subcutaneous parenteral injection; and

administration of the formulation provokes a protective immune response that prevents the onset of active lung infection with Mycobacterium tuberculosis in the subject.

110. A method of treating tuberculosis in a subject comprising administrating a formulation comprising the conjugate of claim 1 to the subject, wherein the second region has a polypeptide sequence of at least one of SEQ ID Nos. 51, 54, 57, 60, 63, 66, 69 and 72 or a variant thereof.

111. A method of treating in a subject any one or more of dry eye, autoimmune-related hair loss, inflammation-related hair loss, chronic obstructive pulmonary disease, an inflammatory airway disease, unstable angina, acute myocardial infarction, acute stroke, and ischemia reperfusion injury to organs, comprising:

administering at least once a formulation comprising the conjugate of claim 1 wherein the second region comprises a NEMO binding domain, and

wherein administration of the formulation inhibits NF-κB activity, reduces blood concentration of TNF-α, IP-10, SAA, IL-6, Kc and/or GM-CSF, and/or increases Interleukin-10 levels in blood.

112. The method of claim 111 wherein the second region has a polypeptide sequence as set forth in SEQ ID No. 20 or a variant thereof.

113. The method of claim 111 wherein the first region comprises a polypeptide sequence as set forth in any of SEQ ID Nos. 1 or 2 or a variant thereof.

114. The conjugate of any one of claims 1, 2, 74 and 75 wherein the first region comprises a polypeptide sequence derived from a human gene.

115. The conjugate of claim 114 wherein the second region includes a polypeptide sequence derived from a human gene or variant thereof.

116. The conjugate of claim 114 wherein the second region includes a nucleic acid sequence derived from a human gene or variant thereof.

117. The method of any one of claims 30-72 and 98-113 wherein the subject is a human.

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按照条约第19条修改的权利要求书7页

(54)发明名称

与特异性生物活性货物肽偶联的源自人类的细胞渗透肽的结构、制造和用途

(57)摘要

本文所公开的实施方案提供了用于偶联物，包括融合蛋白质的组合物，和使用其治疗多种病状的方法。在一些实施方案中，偶联物和/或融合蛋白质合并有60-氨基酸人类同源域（例如，来源于人类HOX基因的肽），以使与人类同源域并不天然缔合的功能和调控肽以及蛋白质或其它生物活性分子（诸如核酸）跨越细胞和核膜易位至预期作用位点，而不会引发可能减少暴露于偶联物和/或导致临床不良事件的不当免疫反应。在其它实施方案中，所公开的偶联物和融合蛋白质可以穿过血脑屏障以允许进入CNS中。在各种实施方案中，所公开的组合物适合于向细胞中递送(i)所关注的基因的表达产物和/或(ii)用以调控基因功能的新颖肽或多核苷酸。

CN 105555317 A

1.一种偶联物,其包含:

包含同源域结构并且具有如SEQ ID No.1至19中的任一者所描述的多肽序列或其变体的第一区;以及

与所述第一区并不天然缔合的包含功能或调控多肽或蛋白质的第二区。

2.一种偶联物,其包含:

包含HOX D12同源域或HOX C12同源域中的至少一者的第一区;以及

与所述第一区并不天然缔合的包含功能或调控多肽或蛋白质的第二区。

3.如权利要求1或2所述的偶联物,其中所述第二区包括NEMO结合域、源自PC1CTT的融合蛋白质、恢复具有遗传性酶缺乏的受试者中的酶功能的酶、RPE65的基因产物、锌指肽、Ag85A 99-118表位肽、或Ag85A 70-78表位肽中的至少一者。

4.如权利要求3所述的偶联物,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

5.如权利要求3所述的偶联物,其中所述源自PC1CTT的融合蛋白质具有SEQ.ID No.23或26中的至少一者所列的多肽序列或其变体。

6.如权利要求3所述的偶联物,其中所述恢复具有遗传性酶缺乏的受试者中的酶功能的酶具有SEQ.ID No.30、45或48中的至少一者的多肽序列或其变体。

7.如权利要求3所述的偶联物,其中所述RPE65的基因产物具有包括至少SEQ.ID No.33的多肽序列或其变体。

8.如权利要求3所述的偶联物,其中所述锌指肽序列包括SEQ.ID No.36、29或42中的至少一者或其变体。

9.如权利要求3所述的偶联物,其中所述Ag85A 99-118表位肽具有包括SEQ.ID No.51、54、57或60中的至少一者的序列或其变体。

10.如权利要求3所述的偶联物,其中所述Ag85A 70-78表位肽具有包括SEQ.ID No.63、66或69中的至少一者的序列或其变体。

11.如权利要求1或2所述的偶联物,其中所述第二区偶联至所述第一区的C端。

12.如权利要求1或2所述的偶联物,其中所述第二区偶联至所述第一区的N端。

13.如权利要求1和2中任一项所述的偶联物,其呈融合蛋白质的形式。

14.如权利要求1和2中任一项所述的偶联物,其中所述第二区与细胞内标靶和/或核内标靶相互作用。

15.如权利要求14所述的偶联物,其中所述第二区与所述细胞内标靶和/或核内标靶的所述相互作用影响以下一者或多者:NF-κB活化、葡萄膜炎、具有多囊性肾病突变的肾小管细胞中的PC1转录因子活性、具有GCase的戈谢病突变的细胞中的葡糖脑苷脂酶底物、患有赫尔勒综合症或亨特综合症的受试者中的葡糖胺聚糖、缺乏视网膜鸟苷酸环化酶的视网膜细胞中的视网膜鸟苷酸环化酶底物、具有亨廷顿氏病突变的细胞中的亨廷顿蛋白突变基因转录的调控,以及抗原表位递送至抗原呈递细胞的细胞质中用于在诸如结核病疫苗的疫苗实施方案中引发有效免疫反应以预防暴露于感染的受试者的结核病。

16.如权利要求1或2所述的偶联物,其中所述第二区具有包括SEQ.ID No.23或26的多肽序列或其变体,并且其中所述偶联物进一步包含非竞争性V2受体拮抗剂。

17.如权利要求1或2所述的偶联物,其中所述第二区具有选自由SEQ ID No:20、23、26、

30、33、36、39、42、45、48、51、54、57、60、63、66、69以及72组成的群组的多肽序列或其变体。

18.如权利要求1或2所述的偶联物，其进一步包含介于所述第一与第二区之间的连接子序列。

19.如权利要求1所述的偶联物，其中所述第一和第二区组合起来具有选自由SEQ ID No:21、22、24、25、27、28、31、32、34、35、37、38、40、41、43、44、46、47、49和50、52、53、55、56、58、59、61、62、64、65、67、68、70、71、73以及74组成的群组的多肽序列或其变体。

20.如权利要求19所述的偶联物，其中在用作活性剂和/或治疗剂之前去除所述序列的起始甲硫氨酸残基。

21.如权利要求1所述的偶联物，其中所述第一和第二区组合起来具有包含同源域结构以及NEMO结合域、源自PC1CTT的融合蛋白质、恢复具有遗传性酶缺乏的受试者中的酶功能的酶、RPE65的基因产物、锌指肽、Ag85A 99-118表位肽、以及Ag85A 70-78表位肽中的至少一者的序列。

22.如权利要求21所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶相互作用。

23.如权利要求22所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶的所述相互作用影响以下一者或多者:NF-κB活化、葡萄膜炎、具有多囊性肾病突变的肾小管细胞中的PC1转录因子活性、具有GCase的戈谢病突变的细胞中的葡糖脑苷脂酶底物、患有赫尔勒综合症或亨特综合症的受试者中的葡糖胺聚糖、缺乏视网膜鸟苷酸环化酶的视网膜细胞中的视网膜鸟苷酸环化酶底物、具有亨廷顿氏病突变的细胞中的亨廷顿蛋白突变基因转录的调控，以及抗原表位递送至抗原呈递细胞的细胞质中用于引发有效免疫反应作为如结核病疫苗的疫苗的活性组分以预防暴露于感染的受试者的结核病。

24.如权利要求19或21所述的偶联物，其进一步包含介于所述第一与第二区之间的连接子序列。

25.一种组合物，其包含如权利要求1所述的偶联物和医药学上可接受的载剂。

26.一种组合物，其包含如权利要求2所述的偶联物和医药学上可接受的载剂。

27.如权利要求25和24所述的组合物，其中所述组合物呈可吸入组合物、滴眼液制剂、用于局部或可注射使用的其它眼用组合物、灌肠剂、局部组合物、或包括用于持续释放的可注射植入物的可注射组合物的形式。

28.如权利要求1或2所述的偶联物，其中当在制剂中施用于受试者时，所述偶联物进入中枢神经系统。

29.如权利要求1或2所述的偶联物，其中当在制剂中施用于受试者时，所述偶联物与当不偶联至所述第一区时由所述第二区引出的不当免疫反应相比在受试者中引出减少的不当免疫反应。

30.一种治疗受试者的病状的方法，其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者，其中所述偶联物进入受试者的中枢神经系统。

31.一种治疗受试者的病状的方法，其包括将包含如权利要求21所述的偶联物的制剂施用于所述受试者，其中所述偶联物进入所述受试者的中枢神经系统。

32.一种治疗受试者的病状的方法，其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者，其中所述偶联物预防或改善所述受试者中对抗原肽的免疫反应以使

得以下至少一者存在:(a)肽暴露没有显著减少,以及(b)免疫介导的不良临床事件的发生率没有显著增加。

33.一种治疗受试者的干眼症的方法,其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者,其中所述第一区偶联至NEMO结合域。

34.如权利要求33所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

35.一种治疗受试者的自体免疫和发炎相关的脱发的方法,其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者,其中所述第二区是NEMO结合域。

36.一种治疗受试者的自体免疫和发炎相关的脱发的方法,其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者,其中所述第二区是NEMO结合域。

37.如权利要求35或36所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

38.一种治疗受试者的发炎性气道疾病的方法,其包括将包含如权利要求1所述的偶联物的可吸入制剂施用于所述受试者,其中所述第二区是NEMO结合域。

39.一种治疗受试者的发炎性气道疾病的方法,其包括将包含如权利要求2所述的偶联物的可吸入制剂施用于所述受试者,其中所述第二区是NEMO结合域。

40.如权利要求38或39所述的方法,其中所述发炎性气道疾病是慢性阻塞性肺病。

41.如权利要求38所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

42.一种治疗受试者的结肠直肠发炎性肠病的方法,其包括将包含如权利要求1所述的偶联物的灌肠制剂施用于所述受试者,其中所述第二区是NEMO结合域。

43.一种治疗受试者的结肠直肠发炎性肠病的方法,其包括将包含如权利要求2所述的偶联物的灌肠制剂施用于所述受试者,其中所述第二区是NEMO结合域。

44.如权利要求42或43所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

45.一种治疗受试者的病状的方法,其包括将包含如权利要求1所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

46.一种治疗受试者的病状的方法,其包括将包含如权利要求2所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

47.如权利要求45或46所述的方法,其中所述病状是不稳定性心绞痛、急性心肌梗塞以及急性中风中的至少一者。

48.如权利要求45或46所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

49.一种治疗受试者在短暂缺血后的缺血再灌注损伤的方法,其包括将包含如权利要求1所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

50.一种治疗受试者在短暂缺血后的缺血再灌注损伤的方法,其包括施用包含如权利要求2所述的偶联物的全身制剂,其中所述第二区是NEMO结合域。

51.如权利要求49或50所述的方法,其中所述缺血再灌注损伤是在脑、肾脏或心脏中。

52.如权利要求49或50所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多

肽序列或其变体。

53.一种治疗受试者的多囊性肾病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区是源自PC1CTT的融合蛋白质、源自PC1CTT的p200肽、或源自PC1CTT的p21肽。

54.一种治疗受试者的多囊性肾病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区是源自PC1CTT的融合蛋白质、源自PC1CTT的p200肽、或源自PC1CTT的p21肽。

55.如权利要求53或54所述的方法，其中所述第二区具有SEQ.ID No.23或26中的至少一者所列的多肽序列或其变体。

56.如权利要求53或54所述的方法，其中所述偶联物进一步偶联至非竞争性V2受体拮抗剂用于靶向集合管细胞的肾囊肿。

57.一种治疗受试者的溶酶体贮积病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述偶联物的所述第二区包含恢复具有遗传性酶缺乏的受试者中的酶功能的酶。

58.一种治疗受试者的溶酶体贮积病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述偶联物的所述第二区包含恢复具有遗传性酶缺乏的受试者中的酶功能的酶。

59.如权利要求57或58所述的方法，其中所述酶选自由葡糖脑苷脂酶、α-L-艾杜糖苷酸酶以及艾杜糖醛酸-2-硫酸酯酶组成的群组。

60.如权利要求57或58所述的方法，其中所述酶包括如SEQ.ID No.30、45或48中的至少一者所列的多肽序列或其变体。

61.一种治疗受试者的1型莱伯先天性黑朦的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包括RPE65的基因产物。

62.一种治疗受试者的1型莱伯先天性黑朦的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包括RPE65的基因产物。

63.如权利要求61或62所述的方法，其中所述RPE65的基因产物具有如SEQ.ID No.33所列的多肽序列或其变体。

64.一种治疗受试者的亨廷顿氏病的方法，其包括将如权利要求1所述的偶联物施用于所述受试者，其中所述第二区包括锌指肽。

65.一种治疗受试者的亨廷顿氏病的方法，其包括将如权利要求2所述的偶联物施用于所述受试者，其中所述第二区包括锌指肽。

66.如权利要求64或65所述的方法，其中所述锌指肽序列包括SEQ.ID No.36、29或42中的至少一者或其变体。

67.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 99-118表位肽。

68.一种治疗受试者的结核病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 99-118表位肽。

69.如权利要求67或68所述的方法，其中所述Ag85A 99-118表位肽序列包括SEQ.ID No.51、54、57或60中的至少一者或其变体。

70.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 70-78表位肽。

71.一种治疗受试者的结核病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 70-78表位肽。

72.如权利要求70或71所述的方法，其中所述Ag85A 70-78表位肽序列包括SEQ.ID No.63、66或69中的至少一者或其变体。

73.一种偶联物，其通过包括以下步骤的方法制备：

将用包含编码如权利要求1所述的偶联物的核酸的表达载体转化的宿主细胞在提供所述偶联物在所述宿主细胞内的表达的条件下培养；以及

通过亲和纯化回收所述偶联物。

74.一种偶联物，其包含：

包含同源域结构的第一区；以及

与所述第一区并不天然缔合的包含功能或调控核酸或肽核酸的第二区。

75.如权利要求74所述的偶联物，其中所述第一区是HOX D12同源域。

76.如权利要求74所述的偶联物，其中所述第一区是HOX C12同源域。

77.一种偶联物，其包含：

包含具有SEQ ID No.1至19中的任一者所描述的多肽序列或其变体的同源域结构的第一区；以及

与所述第一区并不天然缔合的包含功能或调控核酸或肽核酸的第二区。

78.如权利要求74至77中任一项所述的偶联物，其中所述第二区包含具有细胞内生物活性的DNA、RNA、LNA、PNA、γPNA、或这些组分的组合。

79.如权利要求74至77中任一项所述的偶联物，其中所述第二区包括结合至miRNA21或DYRK1b的核酸。

80.如权利要求74至78中任一项所述的偶联物，其中所述第二区具有如SEQ ID No.76、78或79中的至少一者所列的核苷酸序列或其变体。

81.如权利要求74至79中任一项所述的偶联物，其中所述第二区偶联至所述第一区的C端。

82.如权利要求74至79中任一项所述的偶联物，其中所述第二区偶联至所述第一区的N端。

83.如权利要求74至79中任一项所述的偶联物，其中所述第一区用肽键连接至所述第二区。

84.如权利要求74至79中任一项所述的偶联物，其中所述第二区经由非肽键偶联至所述第一区。

85.如权利要求74至79中任一项所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶相互作用。

86.如权利要求85所述的偶联物，其中所述第二区经由与miRNA或结合miRNA的蛋白质复合物的相互作用而与细胞内标靶和/或核内标靶相互作用。

87.如权利要求86所述的偶联物，其中所述第二区是结合至miRNA132用于影响Ras路径的核酸或PNA。

88.如权利要求86所述的偶联物，其中所述第二区是稳定地杂交至如SEQ ID No.75所列的miRNA核苷酸序列或其变体的核酸序列或PNA。

89.如权利要求88所述的偶联物，其中所述第二区具有稳定地杂交至miRNA132的如SEQ ID No.78所列的核苷酸序列或所述核苷酸序列的一部分或其变体。

90.如权利要求88所述的偶联物，其中所述第二区基于与miRNA132的相互作用的特异性来选择。

91.如权利要求88所述的偶联物，其中所述第二区由用以增加稳定性或减少降解的修饰核苷酸组成。

92.如权利要求86所述的偶联物，其中所述第二区是结合至miRNA21用于影响肿瘤抑制路径或肿瘤生长的核酸序列或PNA。

93.如权利要求92所述的偶联物，其中所述第二区包含如SEQ ID No.76所列的核苷酸序列或其变体或可以稳定地杂交至如SEQ ID No.77所列的核苷酸序列或其变体。

94.如权利要求74、75以及77至93中任一项所述的偶联物，其中所述第一区同源域结构具有如SEQ ID No.2所列的多肽序列或其变体。

95.如权利要求74至79中任一项所述的偶联物，其中所述第二区经由与mRNA相互作用用于影响拼接或蛋白质翻译而与细胞内标靶和/或核内标靶相互作用。

96.如权利要求95所述的偶联物，其中所述第二区结合至DYRK1b。

97.如权利要求96所述的偶联物，其中所述第二区具有如SEQ ID No.79所列的核苷酸序列或其变体。

98.如权利要求74所述的偶联物，其中所述第一区和所述第二区经由“点击化学”而连接。

99.如权利要求74所述的偶联物，其中所述第二区包含PNA，并且其中所述第一区和所述第二区经由肽键连接。

100.一种治疗受试者的癌症的方法，其包括将包含如权利要求74所述的偶联物的制剂施用于所述患者。

101.一种治疗受试者的癌症的方法，其包括将包含如权利要求95所述的偶联物的制剂施用于所述受试者。

102.一种治疗受试者的癌症的方法，其包括将包含如权利要求96所述的偶联物的制剂施用于所述受试者。

103.一种治疗受试者的癌症的方法，其包括将包含如权利要求97所述的偶联物的制剂施用于所述受试者。

104.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求74所述的偶联物的制剂施用于所述受试者。

105.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求95所述的偶联物的制剂施用于所述受试者。

106.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求96所述的偶联物的制剂施用于所述受试者。

107.一种治疗受试者的代谢综合症、脂质紊乱或肥胖的方法，其包括将包含如权利要求97所述的偶联物的制剂施用于所述受试者。

108.如权利要求100所述的治疗癌症的方法，其包括施用作为静脉内溶液的所述制剂。

109.如权利要求104所述的治疗代谢综合症、脂质紊乱和/或肥胖的方法，其包括施用呈持续释放形式的所述制剂。

110.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包括Ag85A 99-118表位肽或Ag85A 70-78表位肽之一的至少一部分。

111.如权利要求110所述的治疗受试者的结核病的方法，其中：

经由吸入途径、鼻内途径和/或通过肌肉内、皮内或皮下肠道外注射将所述制剂至少一次施用于所述受试者；并且

所述制剂的施用引发防止所述受试者中结核分枝杆菌的活动性肺部感染发作的保护性免疫反应。

112.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区具有SEQ ID No.51、54、57、60、63、66、69以及72中的至少一者的多肽序列或其变体。

113.一种治疗受试者的干眼症、自体免疫相关的脱发、发炎相关的脱发、慢性阻塞性肺病、发炎性气道疾病、不稳定性心绞痛、急性心肌梗塞、急性中风以及对器官的缺血再灌注损伤中的任何一者或多者的方法，其包括：

至少一次施用包含如权利要求1所述的偶联物的制剂，其中所述第二区包含NEMO结合域，并且

其中所述制剂的施用抑制NF-κB活性，降低TNF-α、IP-10、SAA、IL-6、Kc和/或GM-CSF的血液浓度，和/或增加血液中的白介素10水平。

114.如权利要求113所述的方法，其中所述第二区具有如SEQ ID No.20所列的多肽序列或其变体。

115.如权利要求113所述的方法，其中所述第一区包含如SEQ ID No.1或2中的任一者所列的多肽序列或其变体。

116.如权利要求1、2、74以及77中任一项所述的偶联物，其中所述第一区包含来源于人类基因的多肽序列。

117.如权利要求116所述的偶联物，其中所述第二区包括来源于人类基因的多肽序列或其变体。

118.如权利要求116所述的偶联物，其中所述第二区包括来源于人类基因的核酸序列或其变体。

119.如权利要求30至72和100至115中任一项所述的方法，其中所述受试者是人类。

与特异性生物活性货物肽偶联的源自人类的细胞渗透肽的结构、制造和用途

技术领域

[0001] 本文所公开的实施方案一般涉及经由施用细胞渗透肽偶联物治疗多种病状或疾病的组合物和方法，这些偶联物特别地(但不仅限于)呈利用来源于由人类同源盒(HOX)基因和含有具有细胞渗透特性的同源域的其它人类基因编码的氨基酸同源域序列的肽的融合蛋白质或蛋白质/核酸复合物的形式。更特别地，实施方案涉及一种递送系统，其包含连接至一种或多种功能或调控肽或蛋白质的人类同源域或其变体或部分。这些肽促进进入组织、细胞以及细胞核中，由此允许含有生物活性第二区或“货物”的融合蛋白质或偶联物到达其作用位点用于治疗目的。

背景技术

[0002] 在具有因异常基因功能或缺陷功能所致的表现的疾病中，基因可以经由将诸如核酸、肽以及蛋白质的生物活性分子直接递送至其细胞内和核内作用位点以直接地或间接地经由干扰转录、翻译或转录因子产生和作用而影响基因表达来调控，并且还可以置换缺失或缺陷蛋白产物以在具有胚系或体细胞突变的个体中提供这些类型的分子。在遗传缺陷的个体中生物学上重要的蛋白质的直接置换因以下两点而受阻：(i)这些蛋白质不能到达其正常发挥功能的细胞内位点和组织位点，诸如中枢神经系统(CNS)，以及(ii)这些蛋白质的免疫原性。

发明内容

[0003] 本文所公开的实施方案连同一定范围的修改一起提供用于偶联物，包括融合蛋白质的组合物，和使用其治疗多种病状的方法。偶联物或融合蛋白质合并有源自人类HOX基因的60氨基酸序列或变体或其它人类同源域序列或其变体或部分，以使与人类同源域并不天然缔合的功能和调控分子或其变体或部分跨越细胞和核膜易位至其预期作用位点，而不会引发可能减少偶联物的暴露和/或有效性，或可能产生不良临床事件的不当免疫反应。此外，这类偶联物和融合蛋白质还通过促进穿过血脑屏障并且然后进入CNS中的细胞(诸如小胶质细胞和神经元)中而允许进入CNS中；其还允许这些功能或调控分子啮合至其细胞质内和核内标靶。向细胞中直接递送(i)所关注的基因的表达产物或(ii)能够调控基因功能的新颖分子的能力在医学领域中具有广泛应用。

[0004] 除了促进生物活性分子递送至局部(local/topical)和全身细胞内和核内标靶之外，来自人类HOX基因的肽序列还促进跨越血脑屏障的转运，由此提供CNS细胞内暴露于含有这些HOX基因序列的分子。利用跨越当前药物递送的屏障(诸如血脑屏障)递送治疗的技术应用，全身施用可以通过跨越血脑屏障递送药物来靶向中枢神经系统的癌症。这类应用的实例是源自HOX基因的肽，其与亚德里亚霉素、阿霉素或针对诸如Her2的癌细胞标靶用于治疗脑转移的Her2阳性乳癌的单克隆抗体(例如，曲妥珠单抗(trastuzumab))偶联。另外，以其它方式在CNS中并不达到治疗水平的抗细菌和抗病毒剂能够穿透血脑屏障并且成功地

治疗动物模型中的细胞内和细胞外细菌和病毒CNS感染。

[0005] 在一个实施方案中，偶联物包含具有SEQ ID No.1-19中的任一者所描述的序列或其变体或部分的第一区，偶联至与第一区并不天然缔合的第二区，其中第二区是具有SEQ ID No.20、23、26、30、33、36、39、42、45、48、51、54、57、60、63、66、69以及72中的任一者所列的序列的多肽或变体或其变体或部分。在一个实施方案中，第一区来源于人类基因。

[0006] 在一个实施方案中，偶联物包含：

[0007] (a)包含两个HOX基因HOX C12和HOX D12之一的60-氨基酸域或其变体部分的第一区；以及

[0008] (b)与第一区并不天然缔合的第二区；并且其中至少第一区是非变性的。

[0009] 根据一个实施方案，第二区可以包含核酸。这可以经由叠氮基与炔基之间的特定1,3偶极胡伊斯根环加成反应(1,3dipolar Huisgen cycloaddition reaction)，称为‘点击反应’而缔合并且用于合成肽-寡核苷酸偶联物。或者，这个实施方案可以被视作蛋白质/核酸复合物，其中第二区可以包含核酸结合域，其能够结合作为复合物的一部分的核酸。

[0010] 根据另一个实施方案，第一和第二区经由特定1,3偶极胡伊斯根环加成反应(即，‘点击反应’)而缔合，其中寡核苷酸充当miRNA或针对miRNA的拮抗剂以影响基因表达。这些寡核苷酸可以包含DNA、RNA、LNA(锁核酸)、PNA(肽核酸)或其它核酸。

[0011] 根据另一个实施方案，第一和第二区经由标准肽键连接，其中核碱基连接至肽骨架而不是磷酸二酯骨架。这些核碱基中的一些或所有能够杂交至细胞中的其它核酸并且影响基因表达。这些核碱基可以是PNA、γPNA或含有具有类似特性的其它侧链。

[0012] 在另一个实施方案中，肽或肽-核酸偶联物可以用于影响miRNA的活性，包括(但不限于)拮抗miRNA-132用于抑制Ras路径和癌症，拮抗miR-21用于抑制癌症或NF-κB路径和发炎，或影响对健康可能具有有害和/或不合需要的作用的其它miRNA和路径的功能。

[0013] 根据另一个实施方案，偶联物呈融合蛋白质的形式。在这个实施方案中，第二区是功能或调控蛋白质。

[0014] 在另一个实施方案中，偶联物可以包含：

[0015] (a)包含具有细胞渗透能力的人类同源域的第一区，其连接至

[0016] (b)包含诸如功能酶的蛋白质的与第一区并不天然缔合的第二区。

[0017] 在另一个实施方案中，组合物包含偶联物或融合蛋白质，其可以采用以下形式中的任一者：可吸入组合物；滴眼液或用于局部或可注射使用的其它眼用组合物；灌肠剂；局部组合物；或可注射组合物，包括用于持续释放的可注射植入物。

[0018] 所公开的另一个实施方案是一种方法，其包括允许含有一种或多种生物活性肽或蛋白质的偶联物或融合蛋白质易位至CNS的细胞的步骤。

[0019] 其它实施方案包含用以防止作为偶联物或融合蛋白质递送至其预期细胞溶质或核作用位点的其它方面具抗原性的蛋白质的免疫原性的方法。

[0020] 这些和其它实施方案将在下文进一步详细地公开。

附图说明

[0021] 图1示出了在货物肽的N端的例示性60-氨基酸人类HOX C12同源域(第一区)或其变体或部分。

[0022] 图2示出了在60-氨基酸人类HOX D12同源域或其变体或部分的N端的例示性货物肽(第二区)。

[0023] 图3a示出了在其C端具有货物肽(第二和第三区)的例示性60-氨基酸人类HOX D12同源域(第一区)或其变体或部分,其中第三区可以用于使整个结构靶向特定组织或细胞类型。

[0024] 图3b示出了在其N端具有货物肽(第二和第三区)的例示性60-氨基酸人类HOX D12同源域(第一区)或其变体或部分,其中第三区可以用于使整个结构靶向特定组织或细胞类型。

[0025] 图4示出了在货物肽(第二区)的N端的例示性60-氨基酸人类HOX C12同源域(第一区)或其变体或部分,以及经GS连接子偶联至C端的His尾。

[0026] 图5示出了在NBD货物肽(第二区)的N端的例示性60-氨基酸人类HOX D12同源域(第一区)或其变体或部分,以及经GS连接子偶联至C端的His尾。

[0027] 图6示出了质粒pJ411:129925的例示性序列,其显示6HIS前导子、SAA的变迁序列、继之以TEV裂解位点(ENLYFQS)以产生具有适当N端的SEQ.ID No.21(也称作PPL-002)。

[0028] 图7示出了质粒pJ411:129926的例示性序列,其显示6HIS前导子、SAA的变迁序列、继之以TEV裂解位点(ENLYFQS)以产生具有适当N端的SEQ ID No.22(也称作PPL-003)。

[0029] 图8a示出了在鼠类RAW 264.7细胞中NF-κB活化的抑制(LPS4小时刺激)。误差条代表SEM。

[0030] 图8b示出了在人胚肾(HEK)293细胞(TNF-α4小时刺激)中NF-κB活化的抑制。误差条代表SEM。

[0031] 图9示出了由含有NBD货物的细胞渗透肽对HEK 293NF-κB荧光素酶报告细胞中TNF-α刺激的NF-κB活化的抑制。图10:测定含有NBD货物的细胞渗透肽的体内活性的时间轴:将小鼠用肽预处理,通过注射脂多糖(LPS)而激发,并且在指定的时间获取血液样品用于细胞因子检验。

[0032] 图11a示出了在LPS注射和肽或地塞米松(dexamethasone,Dex)预处理之后的TNF-α反应。每个数据点代表单只小鼠的值。

[0033] 图11b示出了在LPS注射和肽或地塞米松(Dex)预处理之后的IP-10反应。每个数据点代表单只小鼠的值。

[0034] 图12示出了在LPS注射和肽或地塞米松(Dex)预处理之后的IL-10反应。每个数据点代表单只小鼠的值。

[0035] 图13示出了在以500、1000以及2000微克给药之后血浆中的PPL-003浓度。

[0036] 图14示出了按剂量的血浆中的PPL-003C_{max},其显示C_{max}的平均值和中值(30分钟)。

[0037] 图15a和15b示出了在LPS激发并且在LPS刺激之前0、30以及60分钟以2000微克PPL-003预处理之后2小时的淀粉样A蛋白(SAA)反应。图15a中的误差条代表SEM,并且图15b中的数据点是个别的小鼠,水平线是中值。

[0038] 图16a和16b示出了在PPL-003以500、1000以及2000微克的剂量预处理之后对LPS的淀粉样A蛋白(SAA)反应。图16a中的误差条代表SEM,并且图16b中的数据点是个别的小鼠,水平线是中值。百分比是相对于媒剂/LPS的抑制百分比。

[0039] 图17a和17b分别示出了在LPS刺激之前0、30以及60分钟施以2000微克PPL-003剂

量之后的IL-10和小鼠Kc反应。误差条代表SEM。

[0040] 图18a和18b分别示出了在LPS刺激之前0、30以及60分钟施以2000微克PPL-003或PPL-004剂量之后GM-CSF反应的抑制。误差条代表SEM。

具体实施方式

[0041] 人类HOX基因和其它同源域编码基因在胚胎发育中是重要的并且包括小区域，这些小区域与基因触角足(antennapedia，Antp)同源，但其中大多数氨基酸包括于促进其功能的独特人类序列中。触角足的蛋白质序列的特征为存在结合至特定DNA标靶元件的60-氨基酸基序(同源域)。Antp同源域(参看美国专利号7,968,512)和来自Antp基因的小得多的序列已经显示促进生物活性(“货物”)肽进入组织和细胞中以到达其作用位点用于治疗目的。

[0042] 虽然Antp的同源域和小区域可以用于使体外和动物模型中的蛋白质，包括功能和调控蛋白质易位，但来自人类HOX基因的肽序列和某些其它人类同源域序列也可以促进诸如肽、蛋白质以及核苷酸的生物活性分子转运至其细胞内和核内作用位点，(i)不会引发可能减少偶联物的暴露和/或有效性或可能产生不良事件的不当免疫反应，以及(ii)效率高于Antp偶联物和融合蛋白质。将包括所关注的基因的表达产物的生物活性分子直接递送至细胞中的能力具有广泛应用，特别是在医学领域中。HOX和其它人类同源域肽也能够使核酸易位。这对于利用基因调控机制的应用尤其有利。

[0043] 几个所公开的实施方案和其修改涉及新颖偶联物，其可以采用融合蛋白质的形式，各自包含来源于人类HOX基因或来源于含有具有细胞渗透特性的同源域的其它人类基因的60-氨基酸肽或其变体或部分(1，例如，如图1中所示)，连接(例如，12或13，如图1中所示)至第二区(2)：本文所公开的生物活性“货物”肽中的至少一者。图1示出了一个实施方案，其包含60-氨基酸人类HOX C12同源域(“第一区”)(1)，在其C端(4)连接至生物活性货物肽(“第二区”)(2)的N端(5)。图2示出了一个替代性实施方案，其包含60-氨基酸人类HOX D12同源域(“第一区”)(1)，在其N端(3)连接至生物活性货物肽(“第二区”)(2)的C端(6)。两个实施方案中的连接可以是简单的肽键(13)或本领域中已知的连接子序列(12)，条件是融合蛋白质或偶联物的功能不会因其添加而受损。

[0044] 术语“受损”指的是如对于本文所论述的结果中的任一者的治疗功效减小所反映的偶联物实施方案的功能降低，例如，受损的功能可以用与(1)呈融合蛋白质的形式的偶联物或(2)包含替代性连接子的偶联物所观测到的免疫原性的下降相比免疫原性的下降减弱来反映。

[0045] 货物肽包括小合成肽与较大蛋白质，诸如进入细胞中用于治疗目的的抗体或抗体的结合区。(Schutze-Redelmeier M-P等“由果蝇触角足同源域将外源性抗原引入1类MHC加工和呈递路径中在体内预激细胞毒性T细胞(Introduction of exogenous antigens into the MHC class 1 processing and presentation pathway by Drosophila antennapedia homeodomain primes cytotoxic T cells in vivo)”J.Imunol.157(2):650-55,1996年7月15日；M.J.May等“阻断NEMO与IκB激酶复合物的相互作用的肽对NF-κB活化的选择性抑制(Selective inhibition of NF-κB activation by a peptide that blocks the interaction of NEMO with the IκB kinase complex)”Science 289:1550-

54,2000年9月1日;I Strickland和S Ghosh.“细胞渗透NBD肽用于抑制发炎的用途(Use of cell permeable NBD peptides for suppression of inflammation)”Ann.Rheum.Dis.65(增刊3):iii75-iii82,2006;C.Avignolo等“经由scFv抗体的触角足蛋白质转导域向c-Myc蛋白质的内化(Internalization via Antennapedia protein transduction domain of an scFv antibody toward c-Myc protein)”FASEB J.22:1237-45,2008。)

[0046] 术语“肽”、“蛋白质”以及“多肽”同义地使用。

[0047] 术语“人类同源域”指的是(1)源自人类HOX的同源域,诸如表3中分别以SEQ ID No.1和SEQ ID No.2示出的HOX C12和HOX D12序列,或其变体或部分;以及(2)具有细胞渗透活性的任何其它人类同源域,诸如SEQ ID No.3至19,或其变体或部分。

[0048] 短语“并不天然缔合”意指偶联物或融合蛋白质的整个序列在自然界中未发现,并且整个序列并不由自然界中所发现的单个基因编码。

[0049] 短语“操作性地连接”意指第一和第二区连接起来以使得第二区能够使细胞膜易位。这种连接可以经由应用“点击”化学方法或本领域中已知的其它方法来产生或可以用区域之间的肽键合并成融合蛋白质。

[0050] 分子生物学/生物技术领域的一般技术人员应了解,表3和4中所示的序列的众多变异将处于本文所公开的实施方案内。如本文所用的同源性指的是核苷酸或氨基酸序列的一致性或接近一致性。如本领域中所了解,核苷酸错配可以在密码子中的第三或摆动碱基处发生,而不会引起翻译的多肽序列中的氨基酸取代。而且,可以耐受基因序列的某些区域中的微小核苷酸修饰(例如,取代、插入或缺失),只要这类修饰引起的氨基酸序列的变化并不改变最终基因产物的功能性。特定DNA序列的同源物可以由本领域的技术人员使用核酸的交叉杂交的测试在如本领域中充分了解的严格度条件下鉴别(如Hames等,核酸杂交(Nucleic Acid Hybridisation),(1985)IRL Press,Oxford,UK中所描述)。同源性的程度通常依据所比较的序列之间的一致性百分比来测量。

[0051] 术语“变体”指的是不同于参考多肽或蛋白质,但保留基本特性的多肽或蛋白质。多肽的典型变体的氨基酸序列不同于另一种参考多肽。一般来说,差异是有限的以使得参考多肽和变体的序列整体上密切相似(同源)并且在许多区域中是一致的。变体和参考多肽的氨基酸序列可能因一个或多个修饰(例如,取代、添加和/或缺失)而不同。取代或插入的氨基酸残基可能是或可能不是由遗传密码编码的氨基酸残基。多肽的变体可以是天然存在的,诸如等位基因变体,或其可以是据知并不天然存在的变体。

[0052] 修饰和变化可以在本公开的多肽和蛋白质的结构中进行并且仍然得到具有与多肽类似的特征的分子(例如,保守氨基酸取代)。举例来说,可以用某些氨基酸取代序列中的其它氨基酸,而活性没有明显损失。因为多肽的相互作用能力和性质定义那种多肽或蛋白质的生物功能活性,所以某些氨基酸序列取代可以在多肽或蛋白质序列中进行并且仍然获得具有类似特性的多肽或蛋白质。

[0053] 氨基酸取代一般是基于氨基酸侧链取代基的相对相似性,例如,其疏水性、亲水性、电荷、大小等等。将前述特征中的一者或多者考虑在内的例示性取代为本领域的技术人员所熟知,并且包括(但不限于)(原始残基:例示性取代):(Ala:Gly、Ser)、(Arg:Lys)、(Asn:Gln、His)、(Asp:Glu、Cys、Ser)、(Gln:Asn)、(Glu:Asp)、(Gly:Ala)、(His:Asn、Gln)、(Ile:Leu、Val)、(Leu:Ile、Val)、(Lys:Arg)、(Met:Leu、Tyr)、(Ser:Thr)、(Thr:Ser)、(Tip:

Tyr)、(Tyr:Trp、Phe)以及(Val:Ile、Leu)。因此,本公开的实施方案考虑到如上文所陈述的多肽或蛋白质的功能或生物等效物。特别地,多肽和蛋白质的实施方案可以包括与所关注的多肽和蛋白质具有约50%、60%、70%、80%、90%以及95%序列一致性的变体。

[0054] 如本领域中已知的"一致性"是两个或更多个多肽或蛋白质序列之间的关系,如通过比较这些序列所确定。在本领域中,"一致性"还指的是多肽或蛋白质之间的序列相关性的程度,如由这类序列的串之间的匹配所确定。"一致性"可以容易地通过已知的生物信息方法来计算。

[0055] 第一区:所公开的偶联物或融合蛋白质实施方案的第一区可以包含来源于HOX C12或HOX D12基因的天然或合成60-氨基酸肽或其变体或部分或具有细胞渗透活性的任何其它人类同源域,诸如SEQ ID No.3至19,或其变体或部分。

[0056] 在一个实施方案中,HOX C12(SEQ ID No.1)氨基酸序列是:

[0057] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0058] 在另一个实施方案中,HOX D12(SEQ ID No.2)氨基酸序列是:

[0059] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0060] 或者,其它实施方案将包括具有渗透特性以允许偶联物和融合蛋白质的细胞内和核内递送的17个额外人类60-氨基酸同源域序列或其变体或部分中的任一者。此外,这些额外序列各自提供以其它方式将不会穿透血脑屏障的分子的CNS穿透。在第一区中具有HOX C12和HOX D12同源域的这些偶联物和融合蛋白质的氨基酸序列的实例示于表3中(SEQ ID No.21-22、24-25、27-28、31-32、34-35、37-38、40-41、43-44、46-47、49-50、52-53、55-56、58-59、61-62、64-65、67-68、70-71以及73-74,或其变体或部分),并且其它人类同源域或其变体或部分(SEQ ID No.3-19)中的任一者以及这些序列的变异可以取代HOX C12和D12同源域中的任一者。人类同源域序列中的每一者与60-氨基酸Antp序列的一致性小于50%,并且因此在结构上是不同的并且具有独特的人类特性。另外,因为表中所示的所有同源域序列具有人类起源,所以其将在人类中没有抗原性。人类同源域序列中的每一者连同其所来源的人类基因的名称的缩写(SEQ ID No.1至19)一起在下文列于表3中。货物序列可能具有或可能不具有人类起源,这取决于其预期功能。

[0061] 另外,可以使用合成变体,条件是其保留使膜易位的能力。合成变体将一般因取代、特别是保守取代而不同于天然存在的蛋白质。短语"保守氨基酸变化"在本文中意指将来自氨基酸群组(即,疏水、极性、酸性或碱性)之一的氨基酸用相同群组内的氨基酸置换。这类变化的一个实例是缬氨酸被甲硫氨酸置换,反之亦然。保守取代的其它实例通过参考下表1可见:

[0062] 表1:保守氨基酸取代

[0063]

脂肪族	非极性	GAP	ILV
	极性-不带电	CSTM	NQ
	极性-带电	DE	RK
芳香族			HFWY
其它			N

[0064] 这类变体可以直接地合成或使用标准重组DNA技术,诸如定点诱变来制备。在将要进行插入的情况下,编码插入连同5'和3'侧接区的合成DNA对应于插入位点任一侧的天然

存在的序列。侧接区将含有对应于天然存在的序列中的位点的适宜限制位点以使得可以用适当的酶切割序列并且将合成DNA接合至切点中。然后表达DNA以制得编码的蛋白质。这些方法仅仅说明本领域中已知用于操纵DNA序列的众多标准技术，并且还可以使用其它已知技术。与来源于HOX-C12和HOX-D12的所要求的60-氨基酸序列或其变体或部分保留至少50％序列一致性的变体将可能维持其细胞渗透性特征并且保留其人类特征，从而产生低免疫原性潜力。天然存在或合成的HOX序列使膜易位的能力可以通过本领域中已知的常规方法来测试。编码SEQ ID No.1-19(表3)的氨基酸或其变体或部分的任何多核苷酸可以用于本文的融合蛋白质或偶联物中。

[0065] 第二区肽或蛋白质:所公开的偶联物或融合蛋白质实施方案的第二区可以包含与第一区并不天然缔合的任何肽或蛋白质序列。编码第一区的基因可能也编码或可能不编码第二区。第二区可能也来自或可能不来自与第一区相同的种类，但第一和第二区将以不同于天然情形的方式存在于偶联物或融合蛋白质实施方案中。

[0066] 融合蛋白质或偶联物实施方案的第二区可以是任何长度的肽或蛋白质，只要其当包括于融合蛋白质或偶联物中时对其标靶具生物活性。

[0067] 第二区核酸:第二区可以包括可以具治疗活性的任何核酸。在一个实施方案中，核酸可以是DNA或RNA。在另一个实施方案中，核酸是寡核苷酸或PNA。

[0068] 在一个实施方案中，第二区可以包括核碱基置换标准氨基酸的任何肽核酸，诸如PNA，并且第二区可以具治疗活性，包括经由拮抗或活化miRNA起作用，以影响基因表达或其它生物过程。

[0069] 在另一个实施方案中，第二区可以包括任何肽核酸，诸如PNA，并且其中第二区可以具治疗活性，包括经由拮抗或活化mRNA起作用，这是通过活化或抑制拼接或翻译以影响基因表达或其它生物过程。

[0070] 在包含蛋白质/核酸复合物的某些实施方案中，复合物可以进一步包含核酸或PNA作为第二区的一部分，其当在细胞内部时结合至RISC复合物或miRNA。举例来说，结合至miR132miRNA可以抑制癌症或NF-κB。这样结合至miRNA分子可以形成稳定的复合物，其中miRNA分子可以变得不能影响基因的表达，在未结合的miRNA的情况下通常将会影响。

[0071] 在包含蛋白质/核酸复合物的一些实施方案中，复合物可以进一步包含核酸或PNA作为第二区的一部分，其当在细胞内部时结合至基因组DNA。举例来说，在转录因子结合位点处结合至DNA可以防止转录因子结合和/或活性并且因此影响基因转录。

[0072] 在包含蛋白质/核酸复合物的其它实施方案中，复合物可以进一步包含核酸、LNA或PNA作为第二区的一部分，其当在细胞内部时结合至mRNA。举例来说，结合至DYRK1b mRNA可以下调DYRK1b表达和/或翻译。

[0073] 在包含蛋白质/核酸复合物的额外实施方案中，复合物可以进一步包含核酸(或DNA)结合域作为第二区的一部分。在一个实施方案中，核酸结合域可以用以介导蛋白质组分与核酸或PNA组分的特定的高亲和力和非共价相互作用。

[0074] 核酸结合域可以是RNA或DNA结合域，例如，转录因子、特别是酵母或人类转录因子的DNA结合域。举例来说，GAL4可导域介导蛋白质与DNA序列CGGAGGACAGTCCTCCG的选择性结合(Cavey等 J Mol Biol 209:423, 1989)。此外，DNA结合域由GAL4氨基酸2至147组成。DNA结合域可以结合至第二域上的单股或双股DNA。

[0075] 所公开的偶联物或融合蛋白质实施方案的其它应用包括抗细菌和抗病毒措施的开发。举例来说，源自HOX基因或源自其它人类同源域的细胞渗透肽可以用于在受感染细胞的细胞质中转运重组抗体、天然存在的先天免疫效应分子或DNA结合分子并且其破坏细菌、寄生虫或干扰细菌或病毒复制的关键步骤。

[0076] 适合的肽和蛋白质包括具有治疗和/或诊断应用的那些，诸如(但不限于)：细胞因子、趋化因子、激素、抗体、工程改造的免疫球蛋白样分子、单链抗体、偶联物、酶、免疫共刺激分子、免疫调节分子、反义RNA、靶蛋白的反式显性阴性突变体、毒素(诸如内毒素A、大肠杆菌素A、d-内毒素、白喉毒素、炭疽杆菌毒素、霍乱毒素、百日咳毒素、大肠杆菌毒素、志贺毒素或志贺样毒素、条件毒素)、抗原、肿瘤抑制蛋白和生长因子、膜蛋白、血管活性蛋白和肽、抗病毒蛋白和核糖酶，以及衍生物。当包括时，这类编码序列通常可以操作性地连接至适合的启动子，其可以是驱动核糖酶表达的启动子，或不同的启动子。

[0077] 一个或多个实施方案可以提供一种通过动物或人类中的基因疗法治疗个体的医药组合物，其中这种组合物包含治疗有效量的偶联物或融合蛋白质。通常，医师应确定将最适合于个别受试者的实际剂量并且其将随着特定个体的年龄、体重以及反应而变化。

[0078] 递送诸如基因或蛋白质的一种或多种治疗分子的融合蛋白质或偶联物实施方案可以单独或与其它治疗或治疗组分组合使用。可以治疗的疾病包括(但不限于)：癌症、神经疾病、遗传疾病、心脏病、中风、关节炎、病毒和细菌感染，以及免疫系统的疾病。适合的治疗基因包括编码以下的那些：肿瘤抑制蛋白、酶、前药活化酶、免疫调节分子、抗体、工程改造的免疫球蛋白样分子、偶联物、激素、膜蛋白、血管活性蛋白或肽、细胞因子、趋化因子、抗病毒蛋白、反义RNA以及核糖酶。

[0079] 偶联物还可以含有一种或多种细胞因子编码核酸或细胞因子。适合的细胞因子和生长因子包括(但不限于)：ApoE、Apo-SAA、BDNF、心肌营养素1、EGF、ENA-78、嗜酸性粒细胞趋化因子(Eotaxin)、嗜酸性粒细胞趋化因子2、依杜斯2(Exodus-2)、酸性FGF、碱性FGF、成纤维细胞生长因子10(Marshall 1998Nature Biotechnology 16:129)、FLT3配体(Kimura等(1997))、不规则趋化因子(Fractalkine)(CX3C)、GDNF、G-CSF、GM-CSF、GF-[β]1、胰岛素、IFN-[γ]、IGF-I、IGF-II、IL-1[α]、IL-1[β]、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8(72a.a.)、IL-8(77a.a.)、IL-9、IL-10、IL-11、IL-12、IL-13、IL-15、IL-16、IL-17、IL-18(IGIF)、抑制素[α]、抑制素[β]、IP-10、角化细胞生长因子2(KGF-2)、KGF、瘦素(Leptin)、LIF、淋巴细胞趋化因子(Lymphotactin)、苗勒管抑制物质(Mullerian inhibitory substance)、单核细胞集落抑制因子、单核细胞趋化蛋白(Marshall1998，同上)、M-CSF、MDC(67a.a.)、MDC(69a.a.)、MCP-1(MCAF)、MCP-2、MCP-3、MCP4、MDC(67a.a)、MDC(69a.a.)、MIG、MIP-1[α]、MIP-1[β]、MIP-3[α]、MIP-3[β]、MIP4、骨髓祖细胞抑制因子1(MPIF-1)、NAP-2、神经营养因子(Neurturin)、神经生长因子、[β]-NGF、NT-3、NT-4、制瘤素M(Oncostatin M)、PDGF-AA、PDGF-AB、PDGF-BB、PF-4、RANTES、SDF1[α]、SDF1[β]、SCF、SCGF、干细胞因子(SCF)、TARC、TGF-[α]、TGF-[β]、TGF-[β]2、TGF-[β]3、肿瘤坏死因子(TNF)、TNF-[α]、TNF-[β]、TNF-1、TPO、VEGF、GCP-2GRO/IMGSA、GRO-[β]、GRO-[γ]、HCC1以及1-309。还预期，同源细胞因子和生长因子可以用作适合的动物系统中的货物用于研究或兽医学应用。

[0080] 所公开的融合蛋白质或偶联物实施方案可以包含本领域的技术人员已知的其它适合的域。举例来说，内质网滞留信号发挥功能以影响内化的偶联物或蛋白质/核酸复合物

的细胞内途径。适合的内质网滞留信号可以是哺乳动物的内质网滞留信号。

[0081] 还可以存在用以增强核酸或蛋白质脱离细胞囊泡系统并且由此加强由这个途径转移核酸的易位域。这个域可以用以减少或避免在蛋白质/核酸内化至靶细胞中之后的溶酶体降解。适合的易位域可来源于毒素，特别是细菌毒素，诸如外毒素A、大肠杆菌素A、d-内毒素、白喉毒素、炭疽杆菌毒素、霍乱毒素、百日咳毒素、大肠杆菌毒素、志贺毒素或志贺样毒素。

[0082] 可以修饰第一结合域以靶向除了核以外的细胞位点。

[0083] 另外或替代地，识别细胞表面结构的靶细胞特异性结合域可能存在，诸如靶细胞上的受体蛋白或表面抗原。

[0084] 术语“偶联物”在本文中包含一种类别的结构，包括融合蛋白质，其中第一区60-氨基酸人类同源域序列或其变体或部分经由肽键或其它类型的键(包括共价键与非共价键)直接偶联。偶联物可以包括使同源域序列连接至与第一区并不天然缔合的第二区功能或调控肽或蛋白质(“货物”肽)的连接子区。可以利用本领域中已知的多种连接子(短的连接序列)中的任一者以形成偶联物，条件是偶联物的功能不会因其添加而受损。因此，第二区能够穿过细胞或核膜易位。举例来说，对于本领域中已知的多种连接子，参看Chen等“融合蛋白质连接子:特性、设计以及功能性(Fusion protein linkers:property,design and functionality)”Advanced Drug Delivery Reviews.http://dx.doi.org/10.1016/J.addr.2012.09.039。在替代性实施方案中，术语“融合蛋白质”用于指特定子类的偶联物，其在没有使用这类连接子形成偶联物并且这些域完全由肽键连接时存在。

[0085] 第一(HOX或其它人类同源域)区和第二(货物)区可以由可裂解连接子区连接，这个区可以是适合用于这个目的的任何区，条件是偶联物的功能不会因其添加而受损。可裂解连接子区是蛋白酶可裂解连接子，不过可以使用例如可由小分子裂解的其它连接子。这些包括可由溴化氰裂解的Met-X位点、可由羟胺裂解的Asn-Gly、可由弱酸裂解的Asp-Pro、以及尤其可由NBS-粪臭素裂解的Trp-X。蛋白酶裂解位点需要较温和的裂解条件并且在例如因子Xa、凝血酶以及胶原酶中发现。可以使用这些当中的任一者。精确序列在本领域中是可得的并且技术人员将不难选择适合的裂解位点。举例来说，由因子Xa靶向的蛋白酶裂解区是I E G R。由肠激酶靶向的蛋白酶裂解区是D D D D K。由凝血酶靶向的蛋白酶裂解区是L V P R G。可裂解连接子区可以是由细胞内蛋白酶靶向的可裂解连接子区。连接子可能并不是功能所必需的，但连接子可以包括于第一与第二区之间以允许在不使功能受损的情况下第二区的靶向释放或增强具有连接子连接的第二区的生物活性。

[0086] 表2-示出本领域中已知的连接子的部分清单，修改自Chen等,2012

[0087]

$(GGGGS)_n$，其中n＝1、2、3或4
$(Gly)_n$，其中n＝6或8
$(EAAAK)_n$，其中n＝1-3
$A(EAAAK)_4ALEA(EAAAK)_4A$
PAPAP
AEAAAKEAAAKA
$(Ala\text{-}Pro)_n$，其中n＝10-34 aa
VSQTSKLTR↓AETVFPDV
PLG↓LWAc
RVL↓AEA；EDVVCC↓SMSY；GGIEGR↓GS
TRHRQPR↓GWE；AGNRVRR↓SVG；RRRRRRR↓R↓R
GFLG↓
$A(EAAAK)_4ALEA(EAAAK)_4A$
↓＝在这个位置处可裂解

[0088] 所公开的实施方案允许强效治疗作用，包括有效易位至许多所鉴别的肽中的任一者的细胞内和核内作用位点中用于指定的治疗性治疗，其中(i)CNS是靶组织；并且(ii)与靶组织无关，这种易位不会引发可能减少偶联物的暴露和/或有效性或产生不良或不合需要的临床事件的不当免疫反应。“第二区”的生物活性肽与人类同源域序列或其变体或部分(“第一区”)在连接之前并不天然缔合。

[0089] 此外，在多种实施方案中，偶联物或融合蛋白质可以包含也包含货物肽的第三区。图3a和3b示出了包含60-氨基酸人类同源域(此处缩写示为“第一区”)和可以使融合蛋白质或偶联物靶向其细胞作用位点的额外货物蛋白的替代性实施方案。在图3a中，左侧的人类HOX D12同源域(1)在其C端(4)连接至所关注的生物活性货物肽(“第二区”)(2)的N端(5)，而所关注的第三货物肽(“第三区”)(7)在其N端(8)连接至“第二区”(2)的C端(6)。这个第三区可以保持经由肽键连接或融合至整个融合蛋白质或可以经过设计用于在靶向的组织中吸收或结合至特异性位点之后去除。

[0090] 图3b示出了一个替代性实施方案，其中右侧的人类HOX D12同源域(1)经其N端(3)连接至第二区(2)的C端(6)，而第三区货物肽(7)经第二区(2)的N端(5)连接。这些连接可以是简单的肽键(13)或本领域中已知的连接子序列(12)，或两者的组合，条件是偶联物的功能不会因连接子序列的添加而受损。第二货物肽(“第三区”)(7)可以使偶联物或融合蛋白质靶向其细胞作用位点。参看实施例2。

[0091] 本文中的偶联物和融合蛋白质实施方案可以根据文献中所描述的标准分子生物学技术中的任一者产生。参看例如Ausubel等(2002)分子生物学中的短方案：来自分子生物学通用方案的方法概要(Short Protocols in Molecular Biology:A Compendium of Methods from Current Protocols in Molecular Biology)，第5版.John Wiley&Sons。可以使用的包括纯化方法的制造方法也在美国专利号7,968,512中公开，这个专利的全部内容以引用的方式并入本文中。

[0092] “表达载体”或“质粒”(在本文中可互换使用)可以用于产生偶联物或其组分以将异源性DNA引入宿主细胞中，用于表达或复制。技术人员对适当载体的选择将取决于其预期用途(即，DNA扩增或DNA表达)、将要插入载体中的DNA的大小、以及将要用载体转化的宿主

细胞。每个载体取决于其预期用途而含有各种组分，其包含以下一者或多者：复制起点、一个或多个标志基因、增强子元件、启动子、转录终止序列以及信号序列。

[0093] 核酸的来源可以通过参考所公布的文献或由诸如NCBI或EMBL的机构所提供的数据库来确定。所关注的序列的鉴别可以通过使用BLAST、BLAT或其它同源性搜索算法来实现。此外，编码所需的第一或第二区的核酸可以获自学术或商业来源，其中这类来源愿意提供这种材料，或在只有序列数据可用的情况下通过合成或克隆适当序列而获得。一般来说，这可以通过参考描述所讨论的基因的克隆的文献来源而完成。或者，在有限的序列数据可用的情况下或在需要表达与已知核酸同源或以其它方式相关的核酸的情况下，例示性核酸可以用杂交至本领域中已知的核酸序列的那些核苷酸序列表征。

[0094] 短语“杂交严格度”指的是多核酸杂交体稳定的条件。这类条件为本领域的一般技术人员显而易见。而且，如本领域的技术人员所了解，杂交体的稳定性反映在序列同源性每降低1％，杂交体的熔融温度(T_m)就下降约1至1.5℃。一般来说，杂交体的稳定性随着钠离子浓度和温度而变。杂交反应通常是在较高严格度的条件下进行，继之以不同严格度下的洗涤。

[0095] 如本文所用的短语“高严格度”指的是允许只有那些在65-68℃下在1M Na+中形成稳定杂交体的核酸序列杂交的条件。高严格度条件可以例如通过在含有6×SSC、5×登哈特氏溶液(Denhardt's)、1％SDS(十二烷基硫酸钠)、0.1焦磷酸钠以及0.1mg/ml变性鲑鱼精DNA作为非特异性竞争剂的水溶液中杂交来提供。在杂交之后，高严格度洗涤可以在几个步骤中完成，其中最后洗涤(约30分钟)是在杂交温度下在0.2-0.1×SSC、0.1％SDS中进行。

[0096] 短语“中等严格度”指的是除了温度为约60-62℃之外，等效于在上述溶液中杂交的条件。在那种情况下，最后洗涤是在杂交温度下在1×SSC、0.1％SDS中进行。

[0097] 低严格度指的是等效于在约50-52℃下在上述溶液中杂交的条件。在那种情况下，最后洗涤是在杂交温度下在2×SSC、0.1％SDS中进行。

[0098] 应了解，可以使用多种缓冲液(例如，基于甲酰胺的缓冲液)和温度来修改和重复这些条件。登哈特氏溶液和SSC为本领域的技术人员所熟知，其它适合的杂交缓冲液也是如此(参看例如Sambrook等编(1989)分子克隆：实验室手册(Molecular Cloning:A Laboratory Manual),Cold Spring Harbor Laboratory Press,New York或Ausubel等编(1990)分子生物学通用方案(Current Protocols in Molecular Biology),John Wiley& Sons,Inc.)。最佳杂交条件必须按经验确定，因为探针的长度和GC含量也起作用。

[0099] 在一个实施方案中，偶联物或融合蛋白质可以经由使用包含核酸序列和启动子的表达载体来产生，这种表达载体用于在例如植物细胞(包括海藻)中、在诸如大肠杆菌的细菌中、或在诸如中国仓鼠卵巢(CHO)细胞或酵母细胞的真核细胞中重组合成。

[0100] 在另一个实施方案中，用表达载体转化宿主细胞。

[0101] 在另一个实施方案中，核酸序列编码偶联物或融合蛋白质用于通过重组技术合成和制造的目的。

[0102] 在一些实施方案中，偶联物或融合蛋白质是非变性的，意指其可以按其原生状态存在，这种形式的蛋白质在完整细胞中以其三维结构出现。

[0103] 术语“非变性”可能也暗示，但未必暗示特定的非变性步骤。变性改变蛋白质分子的三维形状而不破坏其肽键；如果这类过程也通过其整体三维结构的变化来实现，那么可

能破坏二硫键，或可能对蛋白质中的某些基团进行化学改性。

[0104] 在其它实施方案中，使偶联物或融合蛋白质复性，通过这个过程使变性蛋白质返回其变性之前的原始构象。对于肽，一般用二硫化物还原剂和脲引起可逆变性，并且对于核酸，用热和盐引起可逆变性。

[0105] 在一个实施方案中，第一区在第二区的N端。在另一个实施方案中，第一区在第二区的C端。

[0106] 本领域中已知的纯化方法可以用于制备根据所公开的实施方案的偶联物和融合蛋白质的过程中，例如，如Zachariou,M.(2010)亲和色谱法:方法和方案(Affinity Chromatography:Methods and Protocols)，第2版Totoua,NJ:Humana Press中所描述。偶联物或融合蛋白质可以获自细菌或真核细胞溶解产物，因为变性试剂、pH的小变化以及摩尔渗透压浓度的差异可能对肽的易位特性有影响。获得和纯化人类同源域肽，包括源自HOX的肽的条件在本文中公开。

[0107] HOX肽偶联物跨越细胞表面膜易位的能力可以取决于重组蛋白质的构象。举例来说，通过使用暴露于少量清洁剂(离子和非离子)或变性剂(脲或胍盐)的细菌细胞提取物或纯化蛋白质的多肽易位可以防止或抑制易位。这种构象依赖性特性可以通过在原生条件下纯化HOX肽偶联物或其它人类同源域偶联物而保留。

[0108] 在一个实施方案中，从细菌溶解产物中纯化第一(人类同源域或其变体或部分)与第二(生物活性肽或蛋白质)区。在其它实施方案中，从植物细胞溶解产物中纯化偶联物或融合蛋白质。在另一个实施方案中，从真核细胞溶解产物、培养基或发酵液中纯化偶联物或融合蛋白质。

[0109] 一个实施方案是一种制备偶联物或融合蛋白质的方法，其包括：

[0110] (a)在提供来自表达载体的偶联物在宿主细胞内表达的条件下培养宿主细胞；以及

[0111] (b)回收偶联物，这种回收包括

[0112] (i)使氨基酸尾或其它特异性配体融合至偶联物，这个尾能够结合至至少一个底物并且不结合至另一个底物，并且其中促使偶联物或融合蛋白质经由这个尾结合至至少一个底物，并且其中宿主细胞的组分不结合至这个底物；以及

[0113] (ii)使偶联物和宿主细胞的其余组分与其它底物接触以使得偶联物不会结合并且宿主细胞的其余组分结合至其它底物。

[0114] 另一个实施方案是一种制备偶联物或融合蛋白质的方法，其包括：

[0115] (i)在提供来自表达载体的融合蛋白质在宿主细胞内表达的条件下培养宿主细胞，这个宿主细胞用包含操作性地连接至启动子并编码融合蛋白质的核酸的表达载体转化，其中

[0116] (a)第一区包含HOX肽或其它人类同源域肽；并且

[0117] (b)与第一区并不天然缔合的第二区包含肽或蛋白质；以及

[0118] (ii)回收融合蛋白质，这种方法包括使氨基酸尾或其它配体融合至偶联物，这个尾能够结合至至少一个底物并且不结合至另一个底物，并且其中促使偶联物经由这个尾结合至至少一个底物以使得宿主细胞的组分不结合至这个底物；并且使偶联物与其它底物接触以使得偶联物不会结合并且宿主细胞的其余组分结合至其它底物。

[0119] 在另一个实施方案中，作为亲和纯化过程的一部分，实施方案包括使用连接至偶联物或融合蛋白质的尾或配体；这允许正向与负向纯化步骤。

[0120] 对于所有组合的第一和第二区序列，可以将额外的氨基酸序列添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(诸如HHHHHH、HHHHHHGS，后者利用GS连接子，参看图4和5)，以及本领域的技术人员已知的其它类似标签。另外，可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端起始甲硫氨酸的序列，如果添加N端纯化域，那么甲硫氨酸将在纯化域的N端而不是人类同源域肽第一区的N端。对于任何标签，可以使用本领域中已知的肽连接子并且在用特异性蛋白酶纯化之后去除。

[0121] 在一个实施方案中，使氨基酸尾或配体融合至偶联物的C端。举例来说，图4示出了包含生物活性货物肽(2)的一个实施方案，这种生物活性货物肽(2)在其C端(6)经肽键(13)或本领域中已知的连接子序列(12)连接至人类同源域(此处缩写示为“第一区”)(1)的N端(3)。所示的GS连接子(14)使His标签(“尾”)连接至货物肽(2)的C端(6)。可以使用任何连接子，条件是其不使偶联物的功能受损。

[0122] 在一个实施方案中，固定底物是镍或钴柱、亲和素柱、或对氨基酸尾具有亲和力的抗体柱。在另一个实施方案中，使偶联物的氨基酸尾与至少两个与尾具有亲和力的固定底物连续地接触，在这种情况下镍或钴柱和/或亲和素和/或抗体可以按任何次序使用。

[0123] 在另一个实施方案中，纯化偶联物的方法包括使氨基酸尾或配体融合至偶联物或融合蛋白质，这个尾能够结合至至少一个底物，而杂质仅结合至第二底物；使偶联物与其它底物接触以使得偶联物不会结合并且其余杂质结合至其它底物。

[0124] 根据另一个实施方案，产生和纯化包含60-氨基酸人类同源域或其变体或部分和生物活性肽或蛋白质的偶联物或融合蛋白质的方法包括培养宿主细胞用于表达来自表达载体的偶联物或融合蛋白质以及随后使用本领域中已知的亲和纯化技术回收偶联物或融合蛋白质。

[0125] 当第二区是DNA结合域时，可以通过混合所形成的偶联物与核酸而形成具有核酸的复合物。

[0126] 其它实施方案包括本文所公开的实施方案的包含偶联物或融合蛋白质的医药组合物，和使用偶联物或融合蛋白质制备用于治疗疾病的药剂的方法。

[0127] 在一个实施方案中，偶联物或融合蛋白质包含连接至人类同源域或其变体或部分的生物活性肽或蛋白质，这种生物活性肽或蛋白质是功能酶。

[0128] 其它实施方案包含用于治疗所鉴别的病状或疾病的多种制剂中的任一者。

[0129] SEQ ID No.21和SEQ ID No.22的上游产生.针对能够在T7载体系统中调控表达具有TEV可裂解N端His标签的靶蛋白的两个同源盒候选蛋白质PPL-002(SEQ ID No.21，表3)和PPL-003(SEQ ID No.22，表3)产生细菌表达构建体。T7表达系统适合于产生用于动物功效研究的材料；然而，也涵盖其它表达载体/系统并且为分子生物学/生物技术领域中的一般技术人员所知。

[0130] 将以合成方式创造的密码子优化的基因序列克隆至载体中用于在卡那霉素选择下所选蛋白质的可诱导表达。同源盒蛋白质PPL-002(例如，质粒pJ411:129925)和PPL-003(例如，pJ411:129926)的DNA和对应的氨基酸序列分别示于图6和图7中。每个表达的蛋白质

含有6HIS前导子、SAA的变迁序列、继之以TEV裂解位点(ENLYFQS)以产生具有适当N端的PPL-002或PPL-003序列。

[0131] 在转化至DH5α中之后,通过限制性消化测试每个构建体的六个克隆体的插入物的存在。对所合成的DNA序列进行测序用于确认。使用每个蛋白质的验证过的表达构建体来转化细菌菌株大肠杆菌BL21(DE3),一种从Invitrogen(www.lifetechnologies.com/us/en/home/brands/invitrogen)可商购的菌株,并且制备每个最佳表达克隆体的PD甘油细胞储备液。

[0132] 在摇瓶培养物中进行初步表达优化研究。在移至小规模发酵罐之后,进行细胞生长的进一步优化。早期氧气补充和约7.1的pH设定点显示是最佳的。将诱导温度优化为约18℃。

[0133] 本文所公开的组合物实施方案可以包含医药学上可接受的载剂、稀释剂或赋形剂。术语"医药学上可接受的载剂、稀释剂或赋形剂"指的是如下任何物质:本身不是治疗剂,用作载剂或媒剂以将治疗剂递送至受试者或添加至医药组合物中以改善其处理或储存特性或允许或促进形成组合物的单位剂量,并且不会产生不可接受的毒性或与组合物中的其它组分产生不可接受的相互作用。

[0134] 医药学上可接受的载剂、赋形剂或稀释剂的选择可以基于制剂和预期施用途径以及标准医药实践来选择。这类组合物可以包含可以辅助、调控、释放或增加进入身体区室、组织、细胞内或核内标靶位点中的任何试剂,诸如粘合剂、润滑剂、悬浮剂、包衣剂、增溶剂或其它试剂。用于蛋白质的持续释放的可注射植入物还可以用于获得延长的暴露和作用。术语"持续释放"指的是偶联物以缓慢的速率从制剂中释放,从而允许在活性浓度下较长时段的暴露。

[0135] 本文所公开的包含一种或多种偶联物或融合蛋白质的组合物可以取决于所治疗的病状或其它考虑因素以许多方式施用,例如(但不限于)以下任何一者或多者:(1)吸入;(2)呈栓剂或子宫托的形式;(3)呈局部洗液、溶液、乳膏、软膏或撒布粉的形式;(4)通过使用皮肤贴片;(5)经口,呈含有诸如淀粉或乳糖的赋形剂的片剂形式,或于单独或与赋形剂混合的胶囊或胚珠中,或呈含有调味剂或着色剂的酏剂、溶液或悬浮液的形式;(6)肠道外注射,例如海绵体内、静脉内、肌肉内或皮下;(7)对于眼科疾病,其可以被配制成滴眼液或用于眼内注射;(8)对于肠道外施用,其可以呈无菌水溶液或可注射植入物的形式,这种溶液或植入物可以含有其它物质,例如,具有足够的盐或单糖含量以使溶液与血液或允许缓慢释放的物质等张;以及(9)对于经颊或舌下施用,组合物可以呈片剂或糖锭的形式施用,这些片剂或糖锭可以按常规方式配制。

[0136] 在一个实施方案中,偶联物包含:

[0137] 包含来源于以下的结构的第一区:(1)人类HOX基因,或(2)具有SEQ ID No.1-19中的任一者所描述的序列的其它人类基因,其中1-20、1-15或1-10个氨基酸残基被取代、缺失、添加和/或插入;以及

[0138] 第二区是与第一区并不天然缔合的功能或调控多肽或蛋白质。在各种实施方案中,第二区选自由以下组成的群组:NBD肽、源自PC1CTT的p200、源自PC1CTT的p21、葡糖脑苷脂酶、α-L-艾杜糖苷酸酶、艾杜糖醛酸-2-硫酸酯酶、视网膜鸟苷酸环化酶、锌指肽构建体ZF6xHunt-Kox-1、锌指肽构建体ZF12xHunt-Kox-1以及锌指肽构建体ZF18xHunt-Kox-1。

[0139] 在一个实施方案中，偶联物包含：

[0140] 包含来源于以下的结构的第一区：(1)人类HOX基因，或(2)具有SEQ ID No.1-19中的任一者所描述的序列的其它人类基因，其中1-20、1-15或1-10个氨基酸残基被取代、缺失、添加和/或插入，并且其中所述氨基酸(i)具有人类同源域活性；以及(ii)维持人类HOX基因或含有具有细胞渗透特性的同源域的其它人类基因的螺旋区的细胞渗透活性；以及

[0141] 第二区是与第一区并不天然缔合的功能或调控多肽或蛋白质。在各种实施方案中，第二区选自由以下组成的群组：NBD肽、源自PC1CTT的p200、源自PC1CTT的p21、葡糖脑苷脂酶、α-L-艾杜糖苷酸酶、艾杜糖醛酸-2-硫酸酯酶、视网膜鸟苷酸环化酶、锌指肽构建体ZF6xHunt-Kox-1、锌指肽构建体ZF12xHunt-Kox-1以及锌指肽构建体ZF18xHunt-Kox-1。

[0142] 在一个实施方案中，细胞培养物中融合蛋白质或偶联物的活性浓度小于约115μM、小于约100μM、小于约90μM、小于约80μM、小于约70μM、小于约65μM、小于约60μM、小于约55μM、小于约50μM、小于约45μM、小于约40μM、小于约35μM、小于约30μM、小于约25μM、小于约20μM、小于约15μM、小于约10μM、小于约5μM、或小于约1μM，例如，小于约1μM至约3μM、小于约1μM至约6μM、小于约1μM至约8μM、小于约1μM至约15μM、小于约1μM至约25μM、小于约1μM至约50μM、小于约1μM至约70μM、小于约1μM至约85μM、小于约1μM至约110μM、小于约10μM至约110μM、小于约15μM至约70μM、小于约15μM至约60μM、小于约20μM至约55μM、或小于约25μM至约45μM。

[0143] 在其它实施方案中，在小鼠模型(每20g小鼠)中经(1)静脉内(i.v.)或腹膜内(i.p.)注射、(2)局部制剂或(3)吸入制剂所递送(每天或根据需要)的剂量为至少500μg、至少450μg、至少400μg、至少350μg、至少300μg、至少250μg、至少200μg、至少150μg、至少100μg、至少80μg、至少70μg、至少60μg、至少50μg、至少40μg、至少30μg、至少20μg、至少10μg、或至少1μg，例如，约1μg至约500μg、约10μg至约450μg、约20μg至约400μg、约30μg至约350μg、约30μg至约200μg、约30μg至约100μg、约40μg至约300μg、约40μg至约200μg、约50μg至约100μg、约50μg至约90μg、约55μg至约85μg、约60μg至约80μg、约60μg至约100μg、约1μg至约200μg、约1μg至约100μg、约1μg至约90μg、约1μg至约80μg、约1μg至约70μg、约1至约60μg、约1至约50μg、约1至约40μg、约1至约30μg、约1至约20μg、约1至约15μg、约1至约12μg、约1至约10μg、约1至约8μg、约1至约6μg、约1至约4μg、或约1至约3μg。

[0144] 在其它实施方案中，在小鼠模型中经静脉内(i.v.)或腹膜内(i.p.)注射所递送(每天或根据需要)的剂量为至少100mg/kg、小于约80mg/kg、小于约45mg/kg、小于约40mg/kg、小于约30mg/kg、小于约25mg/kg、小于约20mg/kg、小于约15mg/kg、小于约12mg/kg、小于约10mg/kg、小于约8mg/kg、小于约4mg/kg、小于约2mg/kg、或小于约1mg/kg，例如，小于约1mg/kg至约50mg/kg、约5mg/kg至约40mg/kg、约8mg/kg至约30mg/kg、约10mg/kg至约20mg/kg、或约12mg/kg至约15mg/kg、约8mg/kg至约12mg/kg、约5mg/kg至约9mg/kg、约3mg/kg至约6mg/kg、约2mg/kg至约5mg/kg、约2mg/kg至约4mg/kg、约1mg/kg至约3mg/kg、约1mg/kg至约2.0mg/kg。

[0145] 在其它实施方案中，在人类(70kg体重)中经吸入制剂所递送(每天或根据需要)的剂量为至少约600mg、至少约500mg、至少约450mg、至少约400mg、至少约350mg、至少约300mg、至少约250mg、至少约200mg、至少约150mg、至少约125mg、至少约100mg、至少约75mg、至少约50mg、至少约25mg、至少约20mg、至少约15mg、至少约10mg、至少约5mg、至少约1mg、至

少约500μg、至少约450μg、至少约400μg、至少约350μg、至少约300μg、至少约250μg、至少约200μg、至少约150μg、至少约100μg、至少约80μg、至少约70μg、至少约60μg、至少约50μg、至少约40μg、至少约30μg、至少约20μg、至少约10μg、或至少约1μg，例如，介于约1μg至约750μg、约1μg至约500μg、约10μg至约450μg、约20μg至约400μg、约30μg至约350μg、约30μg至约200μg、约30μg至约100μg、约40μg至约300μg、约40μg至约200μg、约50μg至约100μg、约50μg至约90μg、约55μg至约85μg、约60μg至约80μg、约60μg至约100μg、约1μg至约200μg、约1μg至约100μg、约1μg至约90μg、约1μg至约80μg、约1μg至约70μg、约1至约60μg、约1至约50μg、约1至约40μg、约1至约30μg、约1至约20μg、约1至约15μg、约1至约12μg、约1至约10μg、约1至约8μg、约1至约6μg、约1至约4μg、约1μg至约3μg、约1μg至约1mg、约1μg至约2mg、约1μg至约5mg、约1μg至约10mg、约1mg至约10mg、约1mg至约15mg、约2mg至约20mg、约3mg至约30mg、约4mg至约40mg、约5mg至约50mg、约5mg至约80mg、约5mg至约110mg、约10mg至约150mg、约10mg至约80mg、约20mg至约70mg、约20mg至约60mg、约30mg至约60mg、约120mg至约190mg、约130mg至约180mg、约130mg至约200mg、约140mg至约250mg、约180mg至约300mg、约190mg至约350mg、约220mg至约400mg、约250mg至约425mg、约280mg至约460mg、约300mg至约480mg、约350mg至约490mg、约380mg至约550mg、约400mg至约580mg、约480mg至约590mg、或约520mg至约600mg之间。

[0146] 在其它实施方案中，在人类中和小鼠模型中经局部制剂所递送(每天或根据需要)的剂量小于约5%wt/vol、小于约4.5%wt/vol、小于约3.5%wt/vol、小于约2.5%wt/vol、小于约1.5%wt/vol、小于约0.5%wt/vol、小于约0.4%wt/vol、小于约0.3%wt/vol、小于约0.2%、小于约0.1%wt/vol、小于约0.09%wt/vol、小于约0.08%wt/vol、小于约0.07%wt/vol、小于约0.06%wt/vol、小于约0.05%wt/vol、小于约0.04%wt/vol、小于约0.03%wt/vol、小于约0.02%wt/vol、小于约0.01%wt/vol、小于约0.008%wt/vol、小于约0.006%wt/vol、小于约0.004%wt/vol、或小于约0.002%wt/vol，例如，介于约0.002%wt/vol与约5%wt/vol、约0.01%wt/vol与约4%wt/vol、约0.05%wt/vol与约3%wt/vol、约0.02%wt/vol与约2.5%wt/vol、约0.03%wt/vol与约2%wt/vol、约0.05%wt/vol与约1%wt/vol、约0.06%wt/vol与约0.9%wt/vol、约0.07%wt/vol与约0.6%wt/vol、约0.08%wt/vol与约0.4%wt/vol、约0.09%wt/vol与约0.2%wt/vol、或约0.09wt/vol与约0.1%wt/vol之间。

[0147] 在其它实施方案中，在人类(70kg体重)中经局部制剂所递送(每天或根据需要)的剂量小于约70μg、小于约50μg、小于约45μg、小于约40μg、小于约30μg、小于约25μg、小于约20μg、小于约15μg、小于约12μg、小于约10μg、小于约8μg、小于约4μg、小于约2μg、或小于约1μg，例如，约1μg至约50μg、约5μg至约40μg、约8μg至约30μg、约10μg至约20μg、或约12μg至约15μg、约8μg至约12μg、约5μg至约9μg、约3μg至约6μg、约2μg至约5μg、或小于约1μg至约3μg。

[0148] 在另一个实施方案中，在人类中经持续释放制剂的静脉内、皮下或肌肉内注射或可注射植入物所递送(每天或根据需要)的全身剂量小于约100mg/kg、小于约80mg/kg、小于约45mg/kg、小于约40mg/kg、小于约30mg/kg、小于约25mg/kg、小于约20mg/kg、小于约15mg/kg、小于约12mg/kg、小于约10mg/kg、小于约8mg/kg、小于约4mg/kg、小于约2mg/kg、小于约1mg/kg、小于约0.1mg/kg、或小于约0.01mg/kg，例如，小于约0.01mg/kg至约50mg/kg、小于约5mg/kg至约40mg/kg、小于约8mg/kg至约30mg/kg、小于约10mg/kg至约20mg/kg、或小于约

12mg/kg至约15mg/kg、小于约8mg/kg至约12mg/kg、小于约5mg/kg至约9mg/kg、小于约3mg/kg至约6mg/kg、小于约2mg/kg至约5mg/kg、小于约2mg/kg至约4mg/kg、小于约1mg/kg至约3mg/kg、小于约0.2mg/kg至约2.0mg/kg、小于约0.1mg/kg至约1.5mg/kg、或小于约0.01mg/kg至约2.00mg/kg。

[0149] 在其它实施方案中，经除了持续释放、吸入或局部制剂的静脉内、皮下或肌肉内注射或可注射植入物之外的任何制剂递送(每天或根据需要)至人类(基于70kg体重)的剂量为至少约600mg、至少约500mg、至少约450mg、至少约400mg、至少约350mg、至少约300mg、至少约250mg、至少约200mg、至少约150mg、至少约125mg、至少约100mg、至少约75mg、至少约50mg、至少约25mg、至少约20mg、至少约15mg、至少约10mg、至少约5mg、至少约1mg、至少约500μg、至少约450μg、至少约400μg、至少约350μg、至少约300μg、至少约250μg、至少约200μg、至少约150μg、至少约100μg、至少约80μg、至少约70μg、至少约60μg、至少约50μg、至少约40μg、至少约30μg、至少约20μg、至少约10μg、或至少约1μg，例如，介于约1μg至约750μg、约1μg至约500μg、约10μg至约450μg、约20μg至约400μg、约30μg至约350μg、约30μg至约200μg、约30μg至约100μg、约40μg至约300μg、约40μg至约200μg、约50μg至约100μg、约50μg至约90μg、约55μg至约85μg、约60μg至约80μg、约60μg至约100μg、约1μg至约200μg、约1μg至约100μg、约1μg至约90μg、约1μg至约80μg、约1μg至约70μg、约1至约60μg、约1至约50μg、约1至约40μg、约1至约30μg、约1至约20μg、约1至约15μg、约1至约12μg、约1至约10μg、约1至约8μg、约1至约6μg、约1至约4μg、约1μg至约3μg、约1μg至约1mg、约1μg至约2mg、约1μg至约5mg、约1μg至约10mg、约1mg至约10mg、约1mg至约15mg、约2mg至约20mg、约3mg至约30mg、约4mg至约40mg、约5mg至约50mg、约5mg至约80mg、约5mg至约110mg、约10mg至约150mg、约10mg至约80mg、约20mg至约70mg、约20mg至约60mg、约30mg至约60mg、约120mg至约190mg、约130mg至约180mg、约130mg至约200mg、约140mg至约250mg、约180mg至约300mg、约190mg至约350mg、约220mg至约400mg、约250mg至约425mg、约280mg至约460mg、约300mg至约480mg、约350mg至约490mg、约380mg至约550mg、约400mg至约580mg、约480mg至约590mg、或约520mg至约600mg之间。

[0150] 在一个实施方案中，制剂以6小时、12小时、每天或每隔一天的时间间隔或以每周或每月为基础全身或局部(例如，眼内注射、灌肠制剂、吸入)施用以引出所需效益或以其它方式提供治疗作用。在另一个实施方案中，制剂根据需要施用以引出所需效益或以其它方式提供治疗作用。

[0151] 在另一个实施方案中，心肌梗塞(MI)后心输出量的改善将通过超声波评估。在另一个实施方案中，中风后神经功能的改善将通过测定在呈递后一个月具有小于17的NIHSS的患者百分比来评估。在另一个实施方案中，人类同源域或其变体或部分的偶联物或融合蛋白质的免疫原性将通过测定淋巴细胞对3H-胸苷的并入来评估。在另一个实施方案中，人类同源域或其变体或部分的偶联物或融合蛋白质的免疫原性将通过测定由接受体动物或人类产生的特异性抗体的水平来评估。在另一个实施方案中，缺血再灌注损伤的程度将使用已知的组织病理学技术以检查中风模型中坏死组织的面积来评估。在另一个实施方案中，GAG积聚将使用已知的组织病理学技术来评估。在另一个实施方案中，在亨廷顿氏病(Huntington's Disease)的小鼠模型中的行为和运动缺陷将使用评价前爪的握力和在旋转平衡杆上的平衡能力的已公布方法来评估。在其它实施方案中，每个结果将通过已知方

法来评估。

[0152] 在一个实施方案中,在用所公开的融合蛋白质或偶联物实施方案中的任一者处理一个或多个人类或动物受试者之后,受试者将展现以下结果中的一者或多者:

[0153] (a)呼吸短促或呼吸困难减少;

[0154] (b)细胞因子产生减少;

[0155] (c)血液中的TNF-α浓度降低;

[0156] (d)血液中的IL-1浓度降低;

[0157] (e)血液中的IL-6浓度降低;

[0158] (f)支气管肺泡灌洗液(BALF)发炎性细胞数目减少;

[0159] (g)BALF细胞因子水平(例如,TNF-α、IL-1、IL-6、IL-8)下降;

[0160] (h)肺功能(例如,FEV1)改善;

[0161] (i)功能(包括运动)能力改善;

[0162] (j)需要紧急护理或住院的COPD恶化减轻;

[0163] (k)泪液分泌朝着正常水平恢复;

[0164] (l)脱发减少;

[0165] (m)毛发生长增加;

[0166] (n)通过MRI的坏死脑组织的面积减小;

[0167] (o)C反应蛋白(CRP)减少;

[0168] (p)急性死亡率(即,在一个月时)下降;

[0169] (q)如通过超声波测量的心肌梗塞(MI)后心输出量改善;

[0170] (r)如通过测定在呈递后一个月具有小于17的NIHSS的患者百分比测量的中风后神经功能改善;

[0171] (s)抑制肾生长;

[0172] (t)与使用单独的货物第二区的先前处理相比对60-氨基酸人类同源域(或其变体或部分)偶联物或融合蛋白质的过敏反应和输液反应减少;

[0173] (u)抗货物肽特异性抗体水平下降;

[0174] (v)GCase底物葡糖神经酰胺的积聚减少;

[0175] (w)硫酸皮肤素的尿排泄量减少;

[0176] (x)硫酸乙酰肝素的尿排泄量减少;

[0177] (y)网膜电图(ERG)功能恢复;

[0178] (z)如经由标准临床方法测定的视敏度恢复;

[0179] (aa)如使用统一亨廷顿氏病评定量表(UHDRS)总运动评分和/或其它临床验证的评分方法评估的运动缺陷正常化或改善;

[0180] (bb)如通过标准生长和发育儿科技术评估的骨骼发育正常化;

[0181] (cc)如通过标准儿科技术评估的脑发育正常化;

[0182] (dd)通过MRI评估的肾囊肿大小和数目的进展减缓;

[0183] (ee)血液中的抗炎性白介素10(IL-10)水平增加;

[0184] (ff)BALF中的抗炎性白介素10(IL-10)水平增加;

[0185] (gg)血液中的IP-10水平下降;

[0186] (hh)BALF中的IP-10水平下降;和/或

[0187] (ii)血液中的淀粉样A蛋白(SAA)减少;

[0188] (jj)血液或BALF中的小鼠Kc或人类IL-8减少;

[0189] (kk)血液或BALF中的GM-CSF减少;

[0190] (ll)防止分级玻璃体混浊和/或分级前房细胞增加;

[0191] (mm)防止视敏度退化;

[0192] (nn)肿瘤细胞生长下降和/或肿瘤生长下降;

[0193] (oo)DYRK1b的表达减少;和/或

[0194] (pp)NF-κB调控基因的活化减少。

[0195] 在另一个实施方案中,患者将经过例如约1天至患者一生的时段,经过约1天至约200周、约1天至约100周、约1天至约80周、约1天至约50周、约1天至约40周、约1天至约20周、约1天至约15周、约1天至约12周、约1天至约10周、约1天至约5周、约1周至约4周、约2周至约3周、约1天至约2周、约1周、约1至5天、约1至3天、或约1至2天的时段来治疗。

[0196] 在包含在所提供的实施例中、在其它研究和处理(包括用于人类和动物应用的动物研究)以及兽医处理中所提议的研究中的任一者中所利用的融合蛋白质或偶联物制剂的另一个实施方案中,除非另有指示,否则处理组成员或处理组各自与基线或对照相比将展现以下结果中的一者或多者:

[0197] (a)抑制NF-κB转录活性;

[0198] (b)细胞因子产生减少;

[0199] (c)抑制血液中的TNF-α浓度;

[0200] (d)抑制血液中的IL-1浓度;

[0201] (e)抑制血液中的IL-6浓度;

[0202] (f)支气管肺泡灌洗液(BALF)发炎性细胞数目减少;

[0203] (g)支气管肺泡灌洗液(BALF)细胞因子水平(例如,TNF-α、IL-1、IL-6、IL-8)下降;

[0204] (h)上皮角膜中的杯状细胞浸润减少;

[0205] (i)结膜中的杯状细胞损失减弱;

[0206] (j)淋巴管生成减少;

[0207] (k)泪液分泌朝着正常水平恢复;

[0208] (l)脱发减少或毛发生长增加;

[0209] (m)如使用MRI评估的坏死脑组织的面积减小;

[0210] (n)C反应蛋白(CRP)减少;

[0211] (o)急性死亡率(即,在一个月时)下降;

[0212] (p)如通过超声波测量的心肌梗塞(MI)后心输出量改善;

[0213] (q)如通过在呈递后一个月具有小于17的NIHSS的患者百分比测量的中风后神经功能改善;

[0214] (r)抑制肾生长;

[0215] (s)与通过用人类同源域-p200融合蛋白质或偶联物,例如HOX C12-p200或HOX D12-p200处理所引出的变化相比血液中骨钙素浓度的变化减弱;

[0216] (t)如经由淋巴细胞对3H-胸苷的并入所评估的对60-氨基酸人类同源域(或其变

体或部分)偶联物或融合蛋白质的过敏反应和输液反应减少;

[0217] (u)抗货物肽特异性抗体水平下降;

[0218] (v)GCase底物葡糖神经酰胺的积聚减少;

[0219] (w)脑中α-突触核蛋白/泛素聚集体的积聚减少;

[0220] (x)硫酸皮肤素的尿排泄量减少;

[0221] (y)硫酸乙酰肝素的尿排泄量减少;

[0222] (z)如经由组织病理学测定的硫酸皮肤素减少;

[0223] (aa)如经由组织病理学测定的硫酸乙酰肝素减少;

[0224] (bb)网膜电图(ERG)功能恢复;

[0225] (cc)如经由标准临床方法测定的视敏度恢复;

[0226] (dd)HD鼠类模型中的突变体亨廷顿蛋白(Huntingtin)基因表达减少或抑制;

[0227] (ee)如使用统一亨廷顿氏病评定量表(UHDRS)总运动评分评估的患者中的运动缺陷正常化或改善;

[0228] (ff)如使用评价前爪的握力和在旋转平衡杆上的平衡能力的已公布方法评估的小鼠中的运动缺陷正常化或改善;

[0229] (gg)如在HD鼠类模型中通过分子方法和组织学分析评估的神经元损失减少和/或纹状体细胞体积减小;

[0230] (hh)血液中的抗炎性白介素10(IL-10)水平增加;

[0231] (ii)BALF中的抗炎性白介素10(IL-10)水平增加;

[0232] (jj)血液中的IP-10水平下降;

[0233] (kk)BALF中的IP-10水平下降;

[0234] (ll)血液中的血清淀粉样A蛋白(SAA)减少;

[0235] (mm)血液或BALF中的人类IL-8减少;

[0236] (nn)血液或BALF中的GM-CSF减少;

[0237] (oo)防止分级玻璃体混浊和/或分级前房细胞增加;

[0238] (pp)防止视敏度退化;

[0239] (qq)肿瘤细胞生长减少和/或肿瘤生长减少;

[0240] (rr)DYRK1b的表达减少;和/或

[0241] (ss)NF-κB调控基因的活化减少。

[0242] 在另一个实施方案中,使用包含临床研究中所公开的融合蛋白质和/或偶联物实施方案的制剂处理将延续例如约1天至约52周、约1天至约26周、约1天至约16周、约1天至约12周、约1天至约10周、约1天至约5周、约1周至约4周、约2周至约3周、约1天至约2周、约1周、约1至6天、约1至4天、或约1至2天的时段。

[0243] 在另一个实施方案中,在使用包含所公开的融合蛋白质和/或偶联物实施方案的制剂处理之后,如实施例中的研究中所公开的(1)患者或(2)处理组,包括动物模型中的实验动物(诸如小鼠),与对照相比展现以下结果中的一者或多者:

[0244] (a)NF-κB转录活性抑制至少约99%、至少约95%、至少约90%、至少约80%、至少约70%、至少约60%、至少约50%、至少约40%、至少约35%、至少约30%、至少约20%、至少约15%、至少约10%、或至少约5%,例如,约30%至约99%、约80%至约90%、约70%至约

90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0245] (b)血液和/或BALF中的细胞因子产生和/或浓度降低至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0246] (c)血液和/或BALF中的TNF-α浓度降低至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0247] (d)血液和/或BALF中的IL-1浓度降低至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0248] (e)血液和/或BALF中的IL-6浓度降低至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0249] (f)支气管肺泡灌洗液(BALF)发炎性细胞数目减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0250] (g)支气管肺泡灌洗液(BALF)细胞因子(例如，TNF-α、IL-1、IL-6、IL-8)减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0251] (h)上皮角膜中的杯状细胞浸润减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0252] (i)结膜中的杯状细胞损失减弱至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0253] (j)淋巴管生成减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0254] (k)如通过希尔默测试(Schirmer test)所测量的泪液分泌朝着正常水平恢复，其中希尔默湿润性增加大于20mm、大于15mm、大于10mm、大于5mm，例如，介于5mm与20mm、10mm与20mm、5mm与15mm、5mm与10mm、或15mm至20mm之间(与基线或对照相比的实际变化％或中值变化％)；

[0255] (l)脱发减少或毛发生长增加至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0256] (m)坏死脑组织的面积减小至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0257] (n)血清C反应蛋白(CRP)减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变

化％或中值变化％)；

[0258] (o)急性死亡率(即,在一个月时)下降至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0259] (p)如通过超声波测量的心肌梗塞(MI)后心输出量改善至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)

[0260] (q)如通过在呈递后一个月具有小于17的NIHSS的患者百分比测量的中风后神经功能改善至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0261] (r)肾生长抑制至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0262] (s)血液中使用包含具有细胞膜渗透特性的人类同源域的HOX C12-p21、HOX D12-p21或其它含p21的融合蛋白质或偶联物的骨钙素浓度的变化减弱至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与通过使用例如包含具有细胞膜渗透特性的人类同源域的HOX C12-p200融合蛋白质或偶联物或其它含p200的融合蛋白质或偶联物处理所引出的变化相比的实际变化％或中值变化％)；

[0263] (t)如经由淋巴细胞对3H-胸苷的并入所评估的60-氨基酸人类同源域(或其变体或部分)偶联物或融合蛋白质与其单独的货物蛋白相比的过敏反应和输液反应减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％,例如,约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约

40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(从基线或对照的实际变化％或中值变化％)；

[0264] (u)在用60-氨基酸人类同源域(或其变体或部分)偶联物或融合蛋白质处理之后与用其单独的货物蛋白处理相比抗货物肽特异性抗体水平下降至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(从基线或对照的实际变化％或中值变化％)；

[0265] (v)GCase底物葡糖神经酰胺的积聚减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0266] (w)脑中α-突触核蛋白/泛素聚集体的积聚减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0267] (x)硫酸皮肤素的尿排泄量减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0268] (y)硫酸乙酰肝素的尿排泄量减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0269] (z)如经由组织病理学测定的硫酸皮肤素减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0270] (aa)如经由组织病理学测定的硫酸乙酰肝素减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0271] (bb)网膜电图(ERG)功能恢复至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0272] (cc)如经由标准临床方法测定的视敏度恢复至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0273] (dd)HD鼠类模型中的突变体亨廷顿蛋白基因表达减少或抑制至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0274] (ee)如使用统一亨廷顿氏病评定量表(UHDRS)总运动评分以绝对量评估的患者中的运动缺陷正常化或改善至少约4个点、至少约3个点、至少约2个点、至少约1.5个点、至少约1个点、至少约0.5个点、至少约0.4个点、至少约0.3个点、或至少约0.2个点，例如，约0.5至约2个点、约1至约2个点、或约1.5至约2.5个点，和/或总运动评分改善至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、约10％至约80％、约10％至约30％、约10％至约20％、约20％至约40％、约30％至约50％(与基线和未处理的对照组相比)；

[0275] (ff)如使用评价前爪的握力和在旋转平衡杆上的平衡能力的已公布方法评估的小鼠中的运动缺陷正常化或改善至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约

90％、约25％至约90％、约5％至约85％、约10％至约80％、约10％至约30％、约10％至约20％、约20％至约40％、约30％至约50％(与基线和未处理的对照组相比)；

[0276] (gg)如在HD鼠类模型中通过分子方法和组织学分析评估的神经元损失减少和/或纹状体细胞体积减小至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0277] (hh)血浆(血液)和/或BALF中的IL-10增加至少约3,000％、至少约2,000％、至少约1,000％、至少约500％、至少约380％、至少约360％、至少约340％、至少约320％、至少约300％、至少约280％、至少约260％、至少约240％、至少约220％、至少约200％、至少约180％、至少约160％、至少约140％、至少约120％、至少约110％、至少约100％、至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约400％至约500％、约450％至约500％、约420％至约480％、约300％至约450％、约350％至约430％、约320％至约380％、约200％至约350％、约250％至约330％、约220％至约280％、约100％至约250％、约150％至约230％、约120％至约180％、约50％至约150％、约60％至约130％、约80％至约110％、约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0278] (ii)血液或BALF中的IL-10增至至少约300pg/mL、至少约400pg/mL、至少约500pg/mL、至少约600pg/mL、至少约800pg/mL、至少约1000pg/mL、至少约1200pg/mL、至少约1400pg/mL、至少约1600pg/mL、至少约1800pg/mL、至少约2000pg/mL、至少约2200pg/mL、至少约2400pg/mL、至少约2600pg/mL、至少约2800pg/mL、至少约3000pg/mL、至少约4000pg/ml，例如，约300mg/mL至约3900pg/mL、约380pg/mL至约2900pg/mL、约400pg/mL至约2800pg/mL、约450pg/mL至约2500pg/mL、约500pg/mL至约2400pg/mL、约800pg/mL至约3000pg/mL、约900pg/mL至约2900pg/mL、约1200pg/mL至约2900pg/mL、约1400pg/mL至约2900pg/mL、约1800pg/mL至约2900pg/mL、约2000pg/mL至约2900pg/mL、约2200pg/mL至约2900pg/mL、约2200pg/mL至约3000pg/mL、约2500pg/mL至约3000pg/mL、或约2500pg/mL至约3000pg/mL；

[0279] (jj)血液和BALF中的IP-10水平下降至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0280] (kk)血液(血浆)中的血清淀粉样A蛋白(SAA)减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少

约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0281] (ll)血液和/或BALF中的小鼠Kc或人类IL-8减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0282] (mm)血液或BALF中的GM-CSF减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；

[0283] (nn)基于本领域中的那些专家所知的普遍认可的临床准则，防止分级玻璃体混浊和/或分级前房细胞有临床上显著的增加；

[0284] (oo)基于本领域中的那些专家所知的普遍使用和认可的准则，防止视敏度退化有临床上显著的变化；

[0285] (pp)肿瘤细胞生长减少和/或肿瘤生长减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；和/或

[0286] (rr)DYRK1b的表达减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)；和/或

[0287] (ss)NF-κB调控基因的活化减少至少约99％、至少约95％、至少约90％、至少约80％、至少约70％、至少约60％、至少约50％、至少约40％、至少约35％、至少约30％、至少约20％、至少约15％、至少约10％、或至少约5％，例如，约30％至约99％、约80％至约90％、约70％至约90％、约60％至约90％、约50％至约90％、约40％至约90％、约35％至约90％、约30％至约90％、约25％至约90％、约5％至约85％、或约10％至约80％(与基线或对照相比的实际变化％或中值变化％)。

[0288] 根据所公开的实施方案和其修改，偶联物或融合蛋白质可以单独或与其它治疗或

其它治疗的组分组合使用。可以治疗的疾病和病症或病状包括(但不限于):癌症、炎症或发炎性疾病、皮肤病症、发烧、心血管效应、出血、凝固和急性期反应、恶病质、厌食、急性感染、HIV感染、休克状态、移植物抗宿主反应、自体免疫疾病、再灌注损伤、脑膜炎、偏头痛以及阿司匹林依赖性抗血栓形成;肿瘤生长、侵入和扩散、血管生成、转移、恶性腹水以及恶性胸腔积液;脑缺血、缺血性心脏病、骨关节炎、类风湿性关节炎、骨质疏松症、哮喘、多发性硬化、神经退化、阿尔茨海默氏病(Alzheimer's disease)、动脉粥样硬化、中风、血管炎、克罗恩氏病(Crohn's disease)以及溃疡性结肠炎;牙周炎、牙龈炎;牛皮癣、异位性皮炎、慢性溃疡、大疱性表皮松解症;角膜溃疡、视网膜病以及手术创口愈合;鼻炎、过敏性结膜炎、湿疹、过敏症;再狭窄、充血性心脏衰竭、子宫内膜异位、动脉粥样硬化或内膜硬化、细胞因子和细胞增殖/分化活性;免疫抑制剂或免疫刺激剂活性(例如,用于治疗免疫缺陷,包括人类免疫缺陷病毒感染;调控淋巴细胞生长;治疗癌症和许多自体免疫疾病,以及预防移植排斥反应或诱导肿瘤免疫性);调控造血,例如治疗骨髓或淋巴疾病;促进骨骼、软骨、肌腱、韧带以及神经组织的生长,例如用于愈合创口、治疗灼伤、溃疡以及牙周病和神经退化;抑制或活化促卵泡激素(调节生育力);趋化/趋化因子活性(例如,用于动员特定细胞类型至损伤或感染部位);止血和溶栓活性(例如,用于治疗血友病和中风);抗炎活性(用于治疗例如败血性休克或克罗恩氏病);作为抗微生物剂;例如代谢或行为的调节剂;作为止痛剂;治疗特异性缺陷病症;在人类或兽医学中治疗例如牛皮癣;巨噬细胞抑制和/或T细胞抑制活性并且因此抗炎活性;抗免疫活性,即,对细胞和/或体液免疫反应的抑制作用,包括与发炎不相关的反应;抑制巨噬细胞和T细胞附着至细胞外基质组分和纤连蛋白的能力,以及上调T细胞中的fas受体表达;抑制不当免疫反应和发炎,包括关节炎(包括类风湿性关节炎)、与超敏相关的发炎、过敏反应、哮喘、全身红斑狼疮、胶原病和其它自体免疫疾病、与动脉粥样硬化相关的发炎、动脉硬化、动脉粥样硬化性心脏病、再灌注损伤、心脏骤停、心肌梗塞、血管发炎性病症、呼吸窘迫综合症或其它心肺疾病、与消化性溃疡相关的发炎、溃疡性结肠炎和其它胃肠道疾病、肝纤维化、肝硬化或其它肝病、甲状腺炎或其它腺病、肾小球肾炎或其它肾和泌尿系疾病、耳炎或其它耳鼻喉病、皮炎或其它皮肤病、牙周病或其它牙病、睾丸炎或附睾睾丸炎、不育、睾丸创伤或其它免疫相关的睾丸疾病、胎盘功能不良、胎盘功能不全、习惯性流产、子痫、先兆子痫和其它免疫和/或发炎相关的妇科疾病、后葡萄膜炎、中间葡萄膜炎、前葡萄膜炎、结膜炎、脉络膜视网膜炎、葡萄膜视网膜炎、视神经炎、眼内炎症(例如,视网膜炎或囊样黄斑水肿)、交感性眼炎、巩膜炎、色素性视网膜炎、退化性眼底疾病的免疫和发炎组分、眼部创伤的发炎组分、由感染引起的眼炎、增生性玻璃体视网膜病变、急性缺血性视神经病变、过多疤痕(例如,在青光眼滤过手术之后)、对眼部植入物的免疫和/或发炎反应以及其它免疫和发炎相关的眼科疾病、与自体免疫疾病或病状或病症相关的发炎(其中在中枢神经系统(CNS)中或任何其它器官中免疫和/或发炎抑制都将是有益的)、帕金森氏病(Parkinson's disease)、帕金森氏病治疗所致的并发症和/或副作用、AIDS相关的痴呆复合症、HIV相关的脑病、德维克氏病(Devic's disease)、西登哈姆舞蹈病(Sydenham chorea)、阿尔茨海默氏病和其它退化性疾病、CNS病状或病症、斯托克斯(stokes)的发炎组分、小儿麻痹后综合症、精神病症的免疫和发炎组分、脊髓炎、脑炎、亚急性硬化性全脑炎、脑脊髓炎、急性神经病、亚急性神经病、慢性神经病、格巴二氏综合症(Guillaim-Barre syndrome)、西登哈姆舞蹈病、重症肌无力、假性脑瘤、唐氏综合症(Down's Syndrome)、亨廷

顿氏病、肌萎缩侧索硬化、CNS压迫或CNS创伤或CNS感染的发炎组分、肌肉萎缩和营养不良的发炎组分、以及免疫和发炎相关的疾病、中枢和周围神经系统的病状或病症、创伤后炎症、败血性休克、传染病、手术的发炎性并发症或副作用、骨髓移植或其它移植并发症和/或副作用、基因疗法的发炎和/或免疫并发症和副作用(例如,因病毒载体感染),或与AIDS相关的炎症,以遏制或抑制体液和/或细胞免疫反应,以通过减少单核细胞或淋巴细胞的量治疗或改善单核细胞或白细胞增殖疾病(例如白血病),在天然或人工细胞、组织以及器官(诸如角膜、骨髓、器官、晶状体、起博器、天然或人工皮肤组织)移植的情况下用于预防和/或治疗移植排斥反应。

[0289] 表3:肽序列本体

[0290]

序列 ID 编号	序列所来源的描述符或基因	序列
1	HOX C12	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLL
2	HOX D12	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVV
3	OTP	QKRHRTRFTPAQLNELERSFAKTHYPDIFMREELALRIGLTE SRVQVWFQNRRAKWKKRK
4	TLX1	KKKPRTSFTRLQICELEKRFHRQKYLASAERAALAKALKM TDAQVKTWFQNRRTKWRRQT
5	TLX3	RKKPRTSFSRVQICELEKRFHRQKYLASAERAALAKSLKMT DAQVKTWFQNRRTKWRRQT
6	TLX2	RKKPRTSFSRSQVLELERRFLRQKYLASAERAALAKALRM TDAQVKTWFQNRRTKWRRQT
7	NKX6-1	RKHTRPTFSGQQIFALEKTFEQTKYLAGPERARLAYSLGMT ESQVKVWFQNRRTKWRKKH
8	NKX6-2	KKHSRPTFSGQQIFALEKTFEQTKYLAGPERARLAYSLGMT ESQVKVWFQNRRTKWRKRH
9	NKX6-3	KKHTRPTFTGHQIFALEKTFEQTKYLAGPERARLAYSLGMT ESQVKVWFQNRRTKWRKKS

[0291]

序列 ID 编号	序列所来源的描述符或基因	序列
10	NKX3-2	KKRSRAAFSHAQVFELERRFNHQRYLSGPERADLAASLKLT ETQVKIWFQNRRYKTKRRQ
11	BARHL1	PRKARTAFTDHQLAQLERSFERQKYLSVQDRMELAASLNL TDTQVKTWYQNRRTKWKRQT
12	BARHL2	PRKARTAFSDHQLNQLERSFERQKYLSVQDRMDLAAALNL TDTQVKTWYQNRRTKWKRQT
13	BARX1	GRRSRTVFTELQLMGLEKRFEKQKYLSTPDRIDLAESLGLS QLQVKTWYQNRRMKWKKIV
14	BARX2	PRRSRTIFTELQLMGLEKKFQKQKYLSTPDRLDLAQSLGLT QLQVKTWYQNRRMKWKKMV
15	BSX	RRKARTVFSDSQLSGLEKRFEIQRYLSTPERVELATALSLSET QVKTWFQNRRMKHKKQL
16	DLX2	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAASLGLT QTQVKIWFQNRRSKFKKMW
17	DLX3	VRKPRTIYSSYQLAALQRRFQKAQYLALPERAELAAQLGLT QTQVKIWFQNRRSKFKKLY
18	DBX1	GMLRRAVFSDVQRKALEKMFQKQKYISKPDRKKLAAKLG LKDSQVKIWFQNRRMKWRNSK
19	DBX2	GILRRAVFSEDQRKALEKMFQKQKYISKTDRKKLAINLGLK ESQVKIWFQNRRMKWRNSK
20	NEMO 结合域（“NBD”）（仅第二区肽）	TALDWSWLQTE

[0292]

序列 ID 编号	序列所来源的描述符或基因	序列
21	**HOX C12-NBD**（整个序列：组合的 SEQ ID No. 1 和 20）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLTALDWSWLQTE
22	**HOX D12-NBD**（整个序列：组合的 SEQ ID No. 2 和 20）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVTALDWSWLQTE
23	**来源于 PC1 CTT 的 p200 肽**（仅第二区肽）	VILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGL SKVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDA SHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQAVFEALLTQF DRLNQATEDVYQLEQQLHSLQGRRSSRAPAGSSRGPSPGLR PALPSRLARASRGVDLATGPSRTPLRAKNKVHPSST
24	**HOX C12-p200**（整个序列：组合的 SEQ ID No. 1 和 23，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLVILRWRYHALRGELYRPAWE PQDYEMVELFLRRLRLWMGLSKVKEFRHKVRFEGMEPLPS RSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGRLG TRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHSL QGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPS RTPLRAKNKVHPSST
25	**HOX D12-p200**（整个序列：组合的 SEQ ID No. 2 和 23，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVVILRWRYHALRGELYRPAW EPQDYEMVELFLRRLRLWMGLSKVKEFRHKVRFEGMEPLP SRSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGRL GTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHS LQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGP SRTPLRAKNKVHPSST
26	**p21 肽**（PKD 结构的替代性第二区肽）	IRRIRLWMGLSKVKEFRHKVR
27	**HOX C12-p21**（整个序列：组合的 SEQ ID No. 1 和 26，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLIRRIRLWMGLSKVKEFRHKV R

[0293]

序列 ID 编号	序列所来源的描述符或基因	序列
28	**HOX D12-p21**（整个序列：组合的 SEQ ID No. 2 和 26，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVIRRIRLWMGLSKVKEFRHK VR
29	**VRQ397-**（非竞争性 V2 受体拮抗剂，其可以连接至用于靶向含 p21 和 p200 的 HOX 或人类同源域融合肽的蛋白质结构）	CRAVKY
30	**葡糖脑苷脂酶氨基酸序列（"GCase"）**（仅第二区肽）	ARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTFSRYES TRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFG GAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPMA SCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQL AQRPVSLLASPWTSPTWLKTNGAVNGKGSLKGQPGDIYHQ TWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQ CLGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLL LPHWAKVVLTDPEAAKYVHGIAVHWYLDFLAPAKATLGET HRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSI ITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPIIVDIT KDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVA LMHPDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIH TYLWRRQ

[0294]

序列 ID 编号	序列所来源的描述符或基因	序列
31	**HOX C12-GCase**（整个序列：组合的 SEQ ID No. 1 和 30，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLARPCIPKSFGYSSVVCVCNA TYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHT GTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQ NLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQL HNFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPWTSPTWLK TNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKL QFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFIARDLGP TLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYV HGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSK FWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLAL NPEGGPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKF IPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLNRSSK DVPLTIKDPAVGFLETISPGYSIHTYLWRRQ
32	**HOX D12-GCase**（整个序列：组合的 SEQ ID No. 2 和 30，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVARPCIPKSFGYSSVVCVCNA TYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHT GTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQ NLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQL HNFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPWTSPTWLK TNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKL QFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFIARDLGP TLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYV HGIAVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSK FWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLAL NPEGGPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKF IPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLNRSSK DVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

[0295]

序列 ID 编号	序列所来源的描述符或基因	序列
33	**RetGC-1**（仅第二区肽）	AVFTVGVLGPWACDPIFSRARPDLAARLAAARLNRDPGLA GGPRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPAAC RPAELLAEEAGIALVPWGCPWTQAEGTTAPAVTPAADALYA LLRAFGWARVALVTAPQDLWVEAGRSLSTALRARGLPVAS VTSMEPLDLSGAREALRKVRDGPRVTAVIMVMHSVLLGGE EQRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALA NSSQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSD LNLQQVSPLFGTIYDAVFLLARGVAEARAAAGGRWVSGAA VARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAAGDRLFATY MLDPARGSFLSAGTRMHFPRGGSAPGPDPSCWFDPNNICG GGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMV SGPNKIILTVDDITFLHPHGGTSRKVAQGSRSSLGARSMSDI RSGPSQHLDSPNIGVYEGDRVWLKKFPGDQHIAIRPATKTAF SKLQELRHENVALYLGLFLARGAEGPAALWEGNLAVVSEH CTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHHRGV AHGRLKSRNCIVDGRFVLKITDHGHGRLLEAQKVLPEPPRA EDQLWTAPELLRDPALERRGTLAGDVFSLAIIMQEVVCRSA PYAMLELTPEEVVQRVRSPPPLCRPLVSMDQAPVECILLMK QCWAEQPELRPSMDHTFDLFKNINKGRKTNIIDSMLRMLE QYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVAEALKT GTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYT LFDAIIGSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIAN MSLDILSAVGTFRMRHMPEVPVRIRIGLHSGPCVAGVVGLT MPRYCLFGDTVNTASRMESTGLPYRIHVNLSTVGILRALDS GYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPKPPDLQ PGSSNHGISLQEIPPERRRKLEKARPGQFS

[0296]

序列 ID 编号	序列所来源的描述符或基因	序列
34	**HOX C12-RetGC-1**（整个序列：组合的 SEQ ID No. 1 和 33，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLAVFTVGVLGPWACDPIFSRA RPDLAARLAAARLNRDPGLAGGPRFEVALLPEPCRTPGSLG AVSSALARVSGLVGPVNPAACRPAELLAEEAGIALVPWGCP WTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDL WVEAGRSLSTALRARGLPVASVTSMEPLDLSGAREALRKV RDGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTDGSLV FLPFDTIHYALSPGPEALAALANSSQLRRAHDAVLTLTRHCP SEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLA RGVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGD EEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPR GGSAPGPDPSCWFDPNNICGGGLEPGLVFLGFLLVVGMGL AGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPHGG TSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIGVYEGDR VWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFL ARGAEGPAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDW MFKSSLLLDLIKGIRYLHHRGVAHGRLKSRNCIVDGRFVLKI TDHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPALERR GTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRSP PPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFD LFKNINKGRKTNIIDSMLRMLEQYSSNLEDLIRERTEELELE KQKTDRLLTQMLPPSVAEALKTGTPVEPEYFEQVTLYFSDI VGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDVYKVETIG DAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHM PEVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRM ESTGLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKGA EDTFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQEIPPERRR KLEKARPGQFS

[0297]

序列 ID 编号	序列所来源的描述符或基因	序列
35	**HOX D12-RetGC-1**（整个序列：组合的 SEQ ID No. 2 和 33，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVAVFTVGVLGPWACDPIFSRA RPDLAARLAAARLNRDPGLAGGPRFEVALLPEPCRTPGSLG AVSSALARVSGLVGPVNPAACRPAELLAEEAGIALVPWGCP WTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDL WVEAGRSLSTALRARGLPVASVTSMEPLDLSGAREALRKV RDGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTDGSLV FLPFDTIHYALSPGPEALAALANSSQLRRAHDAVLTLTRHCP SEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLA RGVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGD EEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPR GGSAPGPDPSCWFDPNNICGGGLEPGLVFLGFLLVVGMGL AGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPHGG TSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIGVYEGDR VWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFL ARGAEGPAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDW MFKSSLLLDLIKGIRYLHHRGVAHGRLKSRNCIVDGRFVLKI TDHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPALERR GTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRVRSP PPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFD LFKNINKGRKTNIIDSMLRMLEQYSSNLEDLIRERTEELELE KQKTDRLLTQMLPPSVAEALKTGTPVEPEYFEQVTLYFSDI VGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDVYKVETIG DAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHM PEVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRM ESTGLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKGA EDTFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQEIPPERRR KLEKARPGQFS
36	**ZF6xHunt-Kox-1**（仅第二区肽），示出 6 个锌指重复	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR KFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIR THTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRIC MRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH TKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGI IKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTA QQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWL VEREIHQETHPDSETAFEIKSSV

[0298]

序列 ID 编号	序列所来源的描述符或基因	序列
37	**HOX C12-ZF6xHunt-Kox-1**（整个序列：组合的 SEQ ID No. 1 和 36，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGS GGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTL VTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL GYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKS SV
38	**HOX D12-ZF6xHunt-Kox-1**（整个序列：组合的 SEQ ID No. 2 和 36，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGS GGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTL VTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSL GYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKS SV
39	**ZF12xHunt-Kox-1**（仅第二区肽），示出 12 个锌指重复	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGR KFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIR THTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRIC MRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH TKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICM RNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHT KIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDI CGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQ RHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGG GGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAK SLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVML ENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHP DSETAFEIKSSV

[0299]

序列 ID 编号	序列所来源的描述符或基因	序列
40	**HOX C12-ZF12xHunt-Kox-1**（整个序列，组合的 SEQ ID No. 1 和 39，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGS GGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEK PFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQ RATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSP QHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTRE EWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRL EKGEEPWLVEREIHQETHPDSETAFEIKSSV
41	**HOX D12-ZF12xHunt-Kox-1**（整个序列，组合的 SEQ ID No. 2 和 39，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGS GGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEK PFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQ RATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSP QHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTRE EWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRL EKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0300]

序列 ID 编号	序列所来源的描述符或基因	序列
42	**ZF18xHunt-Kox-1**（仅第二区肽），示出 18 个锌指重复	TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV
43	**HOX C12-ZF18xHunt-Kox-1**（整个序列，组合的 SEQ ID No. 1 和 42，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0301]

序列 ID 编号	序列所来源的描述符或基因	序列
44	**HOX D12-ZF12xHunt-Kox-1**（整个序列，组合的 SEQ ID No. 2 和 42，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNFSQRA TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGS GGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEK PFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQ RATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGS ERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFA QRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAER PFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQR ATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGE KPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFS QRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLR QKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKE GMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVY RNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIH QETHPDSETAFEIKSSV
45	**α-L-艾杜糖苷酸酶**（第二区 653 氨基酸第二区肽，包括 27 氨基酸信号序列）	MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARAL WPLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGA VPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFTHLDGYLD LLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKDLVSSL ARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVSMTMQGF LNYYDACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLR HCHDGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVA QQIRQLFPKFADTPIYNDEADPLVGWSLPQPWRADVTYAA MVVKVIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHPFA QRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQL WAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIY ASDDTRAHPNRSVAVTLRLRGVPPGPGLVYVTRYLDNGLCS PDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGG RLTLRPALRLPSLLLVHVCARPEKPPGQVTRLRALPLTQGQL VLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLF VFSPDTGAVSGSYRVRALDYWARPGPFSDPVPYLEVPVPRG PPSPGNP

[0302]

序列 ID 编号	序列所来源的描述符或基因	序列
46	**HOX C12-α-L-艾杜糖苷酸酶**（整个序列，组合的 SEQ ID No. 1 和 45，还示出起始甲硫氨酸残基）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLMRPLRPRAALLALLASLLA APPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHSQ ADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWLLELVTT RGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGH FTDFEDKQQVFEWKDLVSSLARRYIGRYGLAHVSKWNFET WNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRL GGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGEAGVRLDYI SLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEAD PLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSA FPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQL LRKPVLTAMGLLALLDEEQLWAEVSQAGTVLDSNHTVGVL ASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLR GVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQF RRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCA RPEKPPGQVTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEI QFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALD YWARPGPFSDPVPYLEVPVPRGPPSPGNP
47	**HOX D12-α-L-艾杜糖苷酸酶**（整个序列，组合的 SEQ ID No. 2 和 45，还示出起始甲硫氨酸残基）	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVMRPLRPRAALLALLASLLA APPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHSQ ADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWLLELVTT RGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGH FTDFEDKQQVFEWKDLVSSLARRYIGRYGLAHVSKWNFET WNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRL GGPGDSFHTPPRSPLSWGLLRHCHDGTNFFTGEAGVRLDYI SLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEAD PLVGWSLPQPWRADVTYAAMVVKVIAQHQNLLLANTTSA FPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQL LRKPVLTAMGLLALLDEEQLWAEVSQAGTVLDSNHTVGVL ASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLR GVPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQF RRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCA RPEKPPGQVTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEI QFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALD YWARPGPFSDPVPYLEVPVPRGPPSPGNP

[0303]

序列 ID 编号	序列所来源的描述符或基因	序列
48	艾杜糖醛酸-2-硫酸酯酶（第二区 550 氨基酸序列，包括 25 氨基酸信号序列）	MPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLL IIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAV CAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFK ENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKY ENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAI QLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENIT LAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIP VDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAF TSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPE AGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGL AGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLP GNPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDY RYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYN DSQGGDLFQLLMP
49	HOX C12-艾杜糖醛酸-2-硫酸酯酶（整个序列，组合的 SEQ ID No. 1 和 48，还示出起始甲硫氨酸）	MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNL SDQQVKIWFQNRRMKKKRLLMPPPRTGRGLLWLGLVLSSV CVALGSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRS PNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLY DFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISS NHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLL CPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVG YHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNP WMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYL DTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAK YSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQ LMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVE LCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDI PQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLAN FSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

[0304]

序列 ID 编号	序列所来源的描述符或基因	序列
50	**HOX D12-艾杜糖醛酸-2-硫酸酯酶**(整个序列，组合的 SEQ ID No. 2 和 48，还示出起始甲硫氨酸)	MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNL SDQQVKIWFQNRRMKKKRVVMPPPRTGRGLLWLGLVLSS VCVALGSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVR SPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRL YDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGIS SNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLL CPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVG YHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNP WMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYL DTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAK YSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQ LMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVE LCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDI PQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLAN FSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP
51	**跨越 Ag85A 99-118 表位的四个中的第一个肽**(第二区 15 氨基酸序列，TB 疫苗)	ETFLTSELPGWLQAN
52	**HOX C12-Ag85A 99-118 表位，四个中的第一个肽**(整个序列，组合的 SEQ ID No. 1 和 51，TB 疫苗)	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLETFLTSELPGWLQAN
53	**HOX D12-Ag85A 99-118 表位，四个中的第一个肽**(整个序列，组合的 SEQ ID No. 2 和 51，TB 疫苗)	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVETFLTSELPGWLQAN

[0305]

序列 ID 编号	序列所来源的描述符或基因	序列
54	**跨越 Ag85A 99-118 表位的四个中的第二个肽**（第二区 15 氨基酸序列，TB 疫苗）	SELPGWLQANRHVKP
55	**HOX C12-Ag85A 99-118 表位，四个中的第二个肽**（整个序列，组合的 SEQ ID No. 1 和 54，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLSELPGWLQANRHVKP
56	**HOX D12-Ag85A 99-118 表位，四个中的第二个肽**（整个序列，组合的 SEQ ID No. 2 和 54，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVSELPGWLQANRHVKP
57	**跨越 Ag85A 99-118 表位的四个中的第三个肽**（第二区 15 氨基酸序列，TB 疫苗）	WLQANRHVKPTGSAV
58	**HOX C12-Ag85A 99-118 表位，四个中的第三个肽**（整个序列，组合的 SEQ ID No. 1 和 57，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLWLQANRHVKPTGSAV

[0306]

序列 ID 编号	序列所来源的描述符或基因	序列
59	**HOX D12-Ag85A 99-118 表位，四个中的第三个肽**（整个序列，组合的 SEQ ID No. 2 和 57，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVWLQANRHVKPTGSAV
60	**跨越 Ag85A 99-118 表位的四个中的第四个肽**（第二区 15 氨基酸序列，TB 疫苗）	RHVKPTGSAVVGLSM
61	**HOX C12-Ag85A 99-118 表位，四个中的第四个肽**（整个序列，组合的 SEQ ID No. 1 和 60，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLRHVKPTGSAVVGLSM
62	**HOX D12-Ag85A 99-118 表位，四个中的第四个肽**（整个序列，组合的 SEQ ID No. 2 和 60，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVRHVKPTGSAVVGLSM
63	**跨越 Ag85A 70-78 表位的三个中的第一个肽**（第二区 15 氨基酸序列，TB 疫苗）	QSGLSVVMPVGGQSS

[0307]

序列 ID 编号	序列所来源的描述符或基因	序列
64	**HOX C12-Ag85A 70-78 表位，三个中的第一个肽**（整个序列，组合的 SEQ ID No. 1 和 63，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLQSGLSVVMPVGGQSS
65	**HOX D12-Ag85A 70-78 表位，三个中的第一个肽**（整个序列，组合的 SEQ ID No. 2 和 63，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVQSGLSVVMPVGGQSS
66	**跨越 Ag85A 70-78 表位的三个中的第二个肽**（第二区 15 氨基酸序列，TB 疫苗）	VVMPVGGQSSFYSDW
67	**HOX C12-Ag85A 70-78 表位，三个中的第二个肽**（整个序列，组合的 SEQ ID No. 1 和 66，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLVVMPVGGQSSFYSDW
68	**HOX D12-Ag85A 70-78 表位，三个中的第二个肽**（整个序列，组合的 SEQ ID No. 2 和 66，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVVVMPVGGQSSFYSDW

[0308]

序列 ID 编号	序列所来源的描述符或基因	序列
69	**跨越 Ag85A 70-78 表位的三个中的第三个肽**（第二区 15 氨基酸序列，TB 疫苗）	GGQSSFYSDWYQPAC
70	**HOX C12-Ag85A 70-78 表位，三个中的第三个肽**（整个序列，组合的 SEQ ID No. 1 和 69，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLLGGQSSFYSDWYQPAC
71	**HOX D12-Ag85A 70-78 表位，三个中的第三个肽**（整个序列，组合的 SEQ ID No. 2 和 69，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVVGGQSSFYSDWYQPAC
72	**Ag85A 表位**（第二区货物）	AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA

[0309]

序列 ID 编号	序列所来源的描述符或基因	序列
73	**HOX C12-Ag85A**（整个序列，组合的 SEQ ID No. 1 和 72，TB 疫苗）	SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLS DQQVKIWFQNRRMKKKRLL AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA
74	**HOX D12-Ag85A**（整个序列，组合的 SEQ ID No. 2 和 72，TB 疫苗）	ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLS DQQVKIWFQNRRMKKKRVV AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA

[0310] 各种特征和实施方案以及所选修改现在将经由非限制性实施例来描述。在起始甲硫氨酸包括于完整序列中以允许重组合成的所有实施例中，起始甲硫氨酸可以在纯化期间被去除以得到具有所指示的人类同源域序列的活性产物。在描述完整偶联物的用途的段落中提及完整偶联物的序列号指的是在去除起始甲硫氨酸之后的偶联物。

[0311] 实施例1

[0312] 由60-氨基酸人类同源域和抑制NF-κB转录因子产生的货物NBD肽组成的偶联物或融合肽或蛋白质是经由干扰蛋白质-蛋白质相互作用来调控基因表达的实施方案的实例。

[0313] 在一个实施方案中，HOX C12氨基酸序列第一区是SEQ ID No.1：

[0314] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0315] 在另一个实施方案中，HOX D12氨基酸序列第一区是SEQ ID No.2：

[0316] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0317] 在这两个实施方案中，这个第一区经由简单的肽键或者本领域中已知的连接子(参看表2)连接至NEMO结合域“货物”肽的NF-κB必需修饰子(NEMO)，缩写为NBD。可以使用任何连接子，条件是其添加不使偶联物的功能受损。NBD氨基酸序列是SEQ ID No.20，第二区：

[0318] TALDWSWLQTE

[0319] 并且合并有HOX C12第一区(SEQ ID No.1)并且仅含有连接两个区的肽键的整个

融合蛋白质序列实施方案是：

[0320] SEQ ID No.21，

[0321]

SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLTALDWSWLQTE

[0322] 合并有HOX D12第一区(SEQ ID No.2)并且仅含有连接两个区的肽键的整个融合蛋白质序列实施方案是：

[0323] SEQ ID No.22：

[0324]

ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVTALDWSWLQTE

[0325] SEQ ID No 21和22中的每一者的大小允许以合成方式产生整个融合蛋白质。如果其经由重组合成而产生，那么SEQ ID No.21和22或其变体或部分可以包括N端甲硫氨酸。连接子不是功能所必需的，但在替代性实施方案中连接子可以包括于第一与第二区之间，条件是其不使偶联物的功能受损。为了那个目的，可以使用本领域中已知的任何连接子(参看例如表2)来产生这个偶联物的实施方案，并且将至少部分改变SEQ ID No.21和22，达到连接子序列将桥连这些第一和第二区的程度。

[0326] 在其它实施方案中，可以将额外的氨基酸序列(“尾”)添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(HHHHHH)，以及本领域的技术人员已知的其它类似标签。这类标签可能包括或可能不包括连接子。另外，可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端起始甲硫氨酸的序列，如果添加N端纯化域，那么甲硫氨酸将在纯化域的N端而不是人类同源域肽第一区的N端。图5示出了HOX D12同源域偶联至NBD货物肽并且经GS连接子进一步偶联至His标签的一个实施方案。

[0327] 在各种实施方案中，SEQ ID No.21和22或其一部分可以经过配制用于全身或局部递送以治疗发炎性疾病。局部递送的实施方案包括以下实例：诸如吸入液体或干粉制剂，用于慢性阻塞性肺病(COPD)；使用结肠和/或直肠软膏的结肠灌肠制剂，用于溃疡性结肠炎或克罗恩氏病(发炎性肠病)；滴眼液制剂，用于发炎性眼病，诸如干眼症(干燥性角膜结膜炎)、葡萄膜炎以及巩膜炎；以及局部软膏和乳膏制剂，用于发炎性和自体免疫皮肤病，诸如牛皮癣和斑秃。全身制剂包括静脉内、皮下和肌肉内注射以及可注射植入物制剂以供持续释放用于治疗慢性发炎性疾病，诸如类风湿性关节炎、溃疡性结肠炎、克罗恩氏病、牛皮癣等，以及急性发炎性病状，诸如发炎性动脉粥样硬化斑块不稳定性，用于预防急性心肌梗塞(冠状动脉血栓形成)和中风，预防中风和心肌梗塞中(梗塞尺寸减小和功能改善)和器官移植患者中(器官功能改善)的缺血再灌注损伤。在NF-κB发炎路径的活化涉及于某些癌症的发病和进展中的程度上，如文献中广泛报道，例如在结肠、肺以及一些皮肤癌中，全身施用包括癌症治疗。

[0328] 以化学方式合成SEQ ID No.21(在所描述的研究中标记为PPL-002)和SEQ ID No.22(在所描述的研究中标记为PPL-003)，并且表征其相应的物理和生物特性并与先前已经描述的具有相同的11-氨基酸NEMO结合域(NBD)货物(SEQ ID No.20)的两个基于触角足的细胞渗透膜构建体相比较(M.J.May等“阻断NEMO与IκB激酶复合物的相互作用的肽对NF-κB活化的选择性抑制(Selective inhibition of NF-κB activation by a peptide that

blocks the interaction of NEMO with the IκB kinase complex)"Science 289:1550-54,2000年9月1日;I Strickland和S Ghosh."细胞渗透NBD肽用于抑制发炎的用途(Use of cell permeable NBD peptides for suppression of inflammation)"Ann.Rheum.Dis.65(增刊3):iii75-iii82,2006)。这些融合肽之一利用来自触角足同源域的N端17-氨基酸序列和在其C末端的11-氨基酸NBD肽并且在这些研究中标记为PPL-004。其它基于触角足的构建体是组合触角足的60氨基酸同源域与相同的11-氨基酸NBD货物的融合肽并且在这些研究中标记为PPL-001。

[0329] 物理特征:SEQ ID No.22(PPL-003)与SEQ ID No.21(PPL-002)相比在其溶解度和盐相互作用方面不同,使得其更适于医药开发。其与已公布的基于触角足的NBD肽(PPL-004)和其它基于触角足的NBD肽PPL-001相比在这些特征方面也不同。

[0330] PPL-001、PPL-002以及PPL-004肽可溶于水;然而,其在含盐缓冲液,诸如通常用作医药媒剂或制剂的那些中并不展示溶解度。在医药施用中常规使用的10mM Tris缓冲盐水(pH 7.4)中,PPL-003甚至在所测试的最高浓度5mg/mL(0.566mM)下仍可溶为澄清溶液,而PPL-002(1mg/mL)形成混浊溶液,这可能是归因于盐与肽上的带电基团的相互作用。基于触角足的NBD肽(PPL-001和PPL-004)也不溶于这种缓冲盐水溶液。使用1mM精氨酸、0.01%和0.1%Tween 20或添加组氨酸至Tris缓冲盐水中以及使用柠檬酸盐缓冲溶液并不改善PPL-001、PPL-002或PPL-004的溶解度特征。以结构差异为基础并未预测PPL-003与PPL-002和基于触角足的肽相比不同的溶解度特征。另外,只有PPL-003可以在5mg/mL下完全溶解于磷酸盐缓冲生理盐水溶液(pH 7.4)中。

[0331] 我们使用本领域中已知的技术测试所公开的人类HOX C12或HOX D12同源域-NBD渗透肽(融合蛋白质)的某些实施方案当与用内毒素刺激之前的细胞一起预孵育时抑制NF-κB活化和诸如肿瘤坏死因子α(TNF-α)的细胞因子形成的能力。我们利用包含SEQ ID No.21和/或No.22或其一部分的实施方案进行体外研究,其中测量TNF-α刺激的NF-κB转录并且研究内毒素刺激的细胞因子产生。

[0332] 偶联至人类HOX C12或HOX D12同源域(SEQ ID No.21和/或22或其变体或部分)的NBD货物肽的实施方案在介于约50μM至约200μM之间的各种浓度下在这两个体外检验中都具活性,并且通过已知的方法测量TNF-α刺激的NF-κB转录活性和/或细胞因子产生并且与对照组相比较。

[0333] 使用NF-κB荧光素酶报告细胞系的体外活性:利用TNF-α刺激的人胚肾细胞系(HEK 293)和内毒素(LPS)刺激的鼠类巨噬细胞样细胞系(RAW 264.7)(两者都具有NF-κB荧光素酶报告基因)测量NF-κB活化的抑制的体外检验用于测量具有NBD货物的细胞渗透肽的实施方案的生物活性(Park等磷酸肌醇依赖性激酶1整合T细胞受体和CD28辅受体信号传导以实现NF-κB诱导和T细胞活化(Phosphoinositide-dependent kinase 1integrates T cell receptor and CD28co-receptor signaling to effect NF-κB induction and T cell activation).Nat Immunol.10(2):158-166,2009年2月)。(图8a和8b)

[0334] 图8a、8b和9的关键点:

[0335]

未受刺激或媒剂	未受内毒素（LPS）或 TNF-α 刺激的细胞
LPS	在与对照缓冲液一起孵育 3 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞
突变体：200 微摩尔浓度	在与 NBD 突变体非活性肽一起孵育 3 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞
NBD	在与具有基于触角足的 17-氨基酸同源域序列和 C 端 11-氨基酸 NBD 序列（SEQ ID No. 020 是 NBD 部分）的商购肽一起孵育 30 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞
PPL-001	在与具有基于触角足的 60-氨基酸同源域序列和 C 端 11-氨基酸 NBD 序列（SEQ ID No. 020）的 Portage Pharmaceuticals 生产的肽一起孵育 3 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞
PPL-002（SEQ ID No. 21）	在与具有 60-氨基酸人类 HOX-C12 同源域序列（SEQ ID No. 001）和 C 端 11-氨基酸 NBD 序列（SEQ ID No. 020）的 Portage Pharmaceuticals 生产的肽一起孵育 3 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞
PPL-003（SEQ ID No. 22）	在与具有 60-氨基酸人类 HOX-D12 同源域序列（SEQ ID 002）和 C 端 11-氨基酸 NBD 序列（SEQ ID 020）的 Portage Pharmaceuticals 生产的肽一起孵育 3 小时后用 10 ng/mL 内毒素（LPS）或 10 ng/mL 人类 TNF-α 刺激 4 小时的细胞

[0336] 在初始实验中，首先将这些肽溶解于DMSO中，然后在磷酸盐缓冲盐水中稀释，随后添加至含有NF-κB荧光素酶报告细胞系的完全组织培养基中。在这些研究中，发现PPL-003具有与PPL-004和具有一致结构的PPL-004的可商购型式类似的活性。在一个测试中，NBD序列有变化，致使不能结合至NBD靶序列的对照突变体肽是非活性的，从而展示这个测试对用作货物的NBD序列的特异性。

[0337] 当对于所有肽将DMSO的最终浓度调整至1％并且使用HEK293细胞测量其抑制人类TNF-α刺激(5ng/ml)的NF-κB活化的能力时，所有肽都存在明显的剂量反应。然而，PPL-003在相同和最低的50微摩尔浓度下似乎比其它更强效。图9报告了以未经肽预处理的TNF-α刺激的细胞百分比表示的荧光素酶信号。

[0338] 在啮齿动物全身静脉内内毒素激发模型中，使用如下文所描述包含偶联至人类HOX C12或HOX D12同源域(SEQ ID No.21和/或22)的NBD货物肽的实施方案制剂的全身静脉内(i.v.)或腹膜内(i.p.)施用并且在未来的研究中以每20g小鼠1μg至至少2,000μg或更大的剂量对小鼠进行预处理。在血液中测量多个发炎性和抗炎性细胞因子以及急性期蛋白血清淀粉样蛋白A(SAA)，并且将结果与媒剂阴性对照组和地塞米松预处理的阳性对照组相比较。

[0339] 含NBD货物的细胞渗透肽的体内活性：在通过注射50微克/公斤剂量的内毒素(LPS)激发之前通过腹膜内注射每只小鼠500微克(μg)(10mg/kg)剂量的肽或地塞米松(3mg/kg)30分钟预处理的小鼠中进行初始研究。在LPS注射之后2小时测定肽预处理抑制发炎性细胞因子反应或刺激对LPS的抗炎性细胞因子反应的能力(图10)。

[0340] 在LPS注射之后两小时小鼠血浆中的细胞因子水平在用含NBD肽处理的小鼠中下降(图11a和11b)。举例来说，TNF-α和IP-10(CXCL10)的水平通过用PPL-003预处理而有力地下降，其中一些小鼠类似于地塞米松处理组展示这些细胞因子的水平下降。令人感兴趣的

是,PPL-003关于其减少IP-10反应的能力与PPL-004不同。虽然IP-10水平通过用PPL-003进行腹膜内预处理而降至与在地塞米松处理后所测量的那些类似的水平,但PPL-004腹膜内预处理对IP-10血浆水平没有影响。对特异性细胞因子的这些选择性影响对于PPL-003作为用于发炎性疾病的治疗剂具有重要意义。

[0341] 用PPL-002和PPL-003实施方案预处理还使得抗炎性细胞因子白介素10(IL-10)的水平增加。在通过注射50微克/公斤剂量的内毒素(LPS)激发之前30分钟以每只小鼠500微克(10mg/kg)腹膜内与静脉内给予肽。以3mg/kg腹膜内给予的地塞米松对IL-10水平没有影响,而PPL-002(SEQ ID No.21)的腹膜内预处理使得IL-10显著增加($p<0.01$)。PPL-002(SEQ ID No.21)和PPL-003(SEQ ID No.22)实施方案的静脉内预处理也增加IL-10水平。令人感兴趣的是,在LPS激发之后增加IL-10水平的这种能力在用PPL-004预处理的小鼠中没有观察到,PPL-004是具有来自触角足同源域的N端17-氨基酸序列和在其C末端的11-氨基酸NBD肽的已公布的NBD细胞渗透肽(图12),其中在PPL-004与媒剂预处理组之间不存在可检测的差异。

[0342] 源自大肠杆菌的重组PPL-003的活性和药物动力学.通过在10mM Tris缓冲生理盐水溶液(pH 7.4)中腹膜内注射至小鼠来施用如上文所描述(参看SEQ ID No.21和SEQ ID No.22的上游产生)在大肠杆菌中产生并纯化的重组PPL-003(SEQ ID No.22)实施方案,并且通过利用针对SEQ ID No.20NBD货物的亲和纯化的兔抗体的ELISA检验测量PPL-003的血浆浓度。用500μg[微克]、1,000μg或2,000μg PPL-003注射小鼠,并且在所指示的时间点对血液进行取样(图13)。用EDTA对血液进行抗凝处理并且通过离心分离血浆。通过ELISA测量PPL-003水平,并且使用化学合成的纯PPL-003的标准曲线来计算。血浆PPL-003在给药后5分钟是可测量的,并且Cmax是在约30分钟时,在给药后约240分钟达到检测下限(图13-14)。

[0343] 如前述,在用重组PPL-003给药之后用LPS激发小鼠,并且在LPS激发之后2小时测量对LPS注射的多个细胞因子反应。用PPL-003给药与LPS激发同时、在LPS激发之前30分钟或在LPS激发之前60分钟。为作比较,在LPS激发之前30分钟注射地塞米松。

[0344] 淀粉样A蛋白(SAA)、TNFα、IP-10、GM-CSF、IL-6、KC(人类IL-8的等效物,CXCL1)以及MCP-1的LPS刺激的血浆水平降至低于地塞米松预处理所达成的水平,而抗炎性细胞因子IL-10水平显著升高。一般来说,最大抑制是在LPS激发之前30分钟给药下,不过SAA抑制在同时注射PPL-003和LPS下是最大的(图15a、15b、16a以及16b)。SAA浓度的平均百分比降低在图16a中指示高于条杆。IL-10增加和Kc减少在LPS注射之前60分钟给药下是最大的。(图17a和17b)

[0345] 图18a和18b示出了当注射(i.p.)PPL-003时在小鼠中对静脉内LPS(内毒素)激发的GM-CSF反应的抑制。在LPS之前60和30分钟施用,并且与LPS同时施用(0分钟)。GM-CSF降低大于在用仅提供适度抑制的地塞米松预处理之后所观察到的。PPL-003在相同的微克剂量下比PPL-004更强效,不过PPL-004的摩尔剂量比等效微克剂量的PPL-003约高三倍。

[0346] 局部递送(local delivery/topical delivery)将防止不当的全身活性,这种全身活性在含NBD肽的融合蛋白质的情况下可能导致易染上感染。作为用于治疗诸如慢性阻塞性肺病(COPD)的发炎性气道疾病的吸入制剂,将评价所公开的人类HOX C12或HOX D12同源域-NBD偶联物或融合蛋白质实施方案(SEQ ID No.21或22或其一部分)中的一者或多者在充分描述的啮齿动物(小鼠和大鼠)模型中在吸入内毒素之前通过吸入这些化合物预处

理之后抑制气道发炎反应的能力。具体地说，我们将在例如介于约1μM至约115μM之间的各种浓度下利用一个或多个人类同源域-NBD融合蛋白质或偶联物实施方案进行研究，并且评价在BALF中内毒素刺激的细胞和细胞因子反应的对应的最大抑制。另外，在用小剂量的吸入内毒素激发的人类中对研究进行规划以评价(i)由喷雾器递送的液体制剂实施方案；以及(ii)干粉制剂实施方案，每个这样的制剂包含SEQ ID No.21和/或22或其一部分。这些制剂和递送系统实施方案将在患有发炎性气道疾病(诸如COPD)的患者中具有功效。

[0347] 在吸入内毒素激发的啮齿动物中，经由吸入制剂以每只小鼠至少1μg至至少约200μg或更大的剂量向小鼠施用包含偶联至人类HOX C12或HOX D12同源域(例如，SEQ ID No.21和/或22或其一部分)的NBD货物肽的实施方案。将通过已知的方法测量支气管肺泡灌洗液(BALF)中的TNF-α和IL-6浓度，并且将结果与对照组相比较。

[0348] 而且，在吸入内毒素激发的啮齿动物中，经由吸入制剂以每只小鼠至少1μg至至少约200μg或更大的剂量向小鼠施用包含偶联至人类HOX C12或HOX D12同源域或其变体或部分(例如，SEQ ID No.21和/或22或其一部分)的NBD货物肽的另一个实施方案。将通过已知的方法测量支气管肺泡灌洗液(BALF)发炎性细胞数目，并且将结果与对照组相比较。

[0349] 在吸入内毒素激发的人类中，以每70kg人至少1μg至至少约600mg或更大的剂量将包含偶联至人类同源域或其变体或部分(例如，SEQ ID No.21和/或22或其一部分)的NBD货物肽的吸入制剂实施方案施用于健康志愿者。将通过已知的方法测量支气管肺泡灌洗液(BALF)中的TNF-α、IL-6和IL-1浓度以及发炎性细胞数目，并且将结果与对照组相比较。

[0350] 干眼症(干燥性角膜结膜炎)是局部发炎导致泪液产生减少的医学病状。在其极端情况下，发生角膜损伤。在干空气或有风状况下诸如灼烧、疼痛、过度流泪反射的干眼症症状是很常见的，并且用“人工泪液”处理轻度情况。然而，对于中度至重度情况需要局部免疫抑制疗法，例如，由Allergan制造的环孢素滴眼液(Restasis®)。最近，已经牵涉Toll样受体(TLR)信号传导的重要性，并且已经开发了鼠类模型(Redfern等“患有实验性干眼症的小鼠中的Toll样受体表达和活化(Toll-Like Receptor Expression and Activation in Mice with Experimental Dry Eye)”发表于Invest.Ophthalmol.Vis.Sci.中54(2):1554-63,2013年2月28日)。最初，我们将在鼠类实验性干眼症(EDE)模型中进行研究，其中可以评价发炎性生物标志物和病理变化。举例来说，我们将评价在局部制剂中施用的含NBD肽的融合蛋白质或偶联物的一个或多个实施方案对以下的影响：(1)上皮角膜中的杯状细胞浸润和结膜中的损失；(2)泪液分泌；以及(3)淋巴管生成；和/或(4)泪腺组织中的发炎性细胞积聚。

[0351] 在EDE啮齿动物模型中，如果溶解度允许的话，那么以每只小鼠至少1μg至至少约500μg或更大的剂量，每天、每隔一天或每周长期施用局部制剂，包含偶联至人类HOX C12或HOX D12同源域或其变体或部分(SEQ ID No.21和/或22或其一部分)的NBD货物肽的另一个实施方案。将通过已知的方法评估泪液产生、上皮角膜中的杯状细胞浸润以及结膜中的杯状细胞损失，并且将结果与对照相比较。

[0352] 在更严重的干眼症啮齿动物模型中，如果溶解度允许的话，那么以每20g小鼠至少1μg至至少约500μg或更大的剂量，每天、每隔一天或每周施用局部制剂，包含偶联至人类HOX C12或HOX D2同源域或其变体或部分(例如，SEQ ID No.21和/或22或其一部分)的NBD货物肽的另一个实施方案。将通过已知的方法评估淋巴管生成和角膜病理，并且将结果与

对照相比较。

[0353] 在人类中，以每70kg人小于1μg至约100μg的剂量，每天、每隔一天或每周长期施用局部制剂，包含偶联至人类HOX C12或HOX D12同源域或其变体或部分(例如，SEQ ID No.21和/或22或其一部分)的NBD货物肽的另一个实施方案，将通过希尔默测试来测量与接受安慰剂处理的对照受试者相比泪液分泌朝着正常水平的变化。

[0354] 斑秃是常见的自体免疫皮肤病，导致头皮和身体上的其它地方的脱发。其通常始于头皮上的一个或多个小的、圆的、平滑的斑点，并且可以进展至全头皮脱发(全头秃)或全身脱发(普秃)。斑秃影响总人口的约2％，单独在美国包括五百万人以上。这种常见的皮肤病是高度不可预测的和周期性的。毛发可以在任何时间长回或再次脱落，并且病程对于每个人是不同的。

[0355] 在适当的洗发精或局部制剂中配制用于施加至头皮和其它涉及区域，我们将评价人类HOX C12或HOX D12同源域-NBD融合蛋白质实施方案或替代性的含NBD蛋白质的偶联物实施方案以影响(1)通过皮肤活组织检查对源自T细胞的细胞因子的下游发炎反应，(2)脱发，以及(3)毛发再生。在斑秃患者中的这个双盲研究中，在脱发发展的相同阶段的成对信号病灶将用安慰剂(单独的局部制剂)和含人类HOX C12或HOX D12同源域-NBD肽的制剂处理。将在两个病灶中测量毛发生长和毛囊发炎并且在未处理的病灶中作比较。在其它平行组研究中，将测量信号病灶的脱发和生长以及新病灶的数目并且在处理组之间作比较。

[0356] 在啮齿动物斑秃模型，例如邓迪实验秃毛大鼠(Dundee Experimental Bald Rat，DEBR)模型中，以及在斑秃患者中，以小于约0.01％wt/vol至小于约5％wt/vol的剂量，每天、每隔一天或每周长期施用局部制剂，包含偶联至人类HOX C12或HOX D12同源域或其变体或部分(SEQ ID No.21和/或22或其一部分)的NBD货物肽的实施方案。将通过已知的方法在处理对比未处理的病灶中评估脱发和毛发生长。

[0357] 作为静脉内或腹膜内制剂施用，我们将评价所描述的源自人类基因的渗透NBD融合蛋白质或偶联物(SEQ ID No.21和/或22或其一部分)的实施方案对啮齿动物进行的预处理对充分描述的动物模型(包括中风模型)中的缺血再灌注损伤的影响。具体地说，我们将通过已知的组织病理学技术在这个中风模型中检查坏死组织的面积，缺血再灌注损伤的指标。

[0358] 在啮齿动物中风模型中，在缺血事件1小时内开始亚急性施用，并且以诸如每6小时、12小时或每天的时间间隔持续一至三天，以小于约0.1mg/kg至小于约100mg/kg的剂量继续施用静脉内或腹膜内制剂，包含偶联至人类HOX C12或HOX D12同源域或其变体或部分(SEQ ID No.21和/或22或其一部分)的NBD货物肽的另一个实施方案。将使用已知的组织病理学方法评估处理组中坏死脑组织的面积。

[0359] 葡萄膜炎是眼睛的常见发炎性病状。葡萄膜炎的许多型式可能是威胁视力的眼部发炎性疾病。实验性自体免疫葡萄膜视网膜炎(EAU)被认为是人类葡萄膜炎的动物模型。用于治疗人类葡萄膜炎的免疫抑制剂在这样的动物模型中是有效的。此外，NF-κB发炎路径，HOX C12和HOX D12同源域-NBD融合蛋白质的标靶，据报道涉及于EAU发病中。另外，存在特定形式的家族性肉芽肿性葡萄膜炎，布劳综合症(Blau syndrome)，其可能由人类NOD2基因的特异性突变引起并且与NF-κB发炎直接相关。

[0360] 将评价配制成局部施用于眼睛的人类HOX D12同源域-NBD融合蛋白质实施方案减

轻动物葡萄膜炎模型的发炎的能力。向缺乏NOD2的小鼠给予胞壁酰二肽(MDP)的玻璃体内注射,并且可以在虹膜内测量血管内反应并且可以通过体内显微术和组织学来评估细胞侵润。或者,作为局部功效的初步测试,可以评价融合蛋白质在小鼠中减轻施用LPS内毒素所引起的眼部发炎的能力。预期,在指示动物葡萄膜炎模型中的有效性之后,可以在葡萄膜炎的更常见类型中考虑人类试验。用于葡萄膜炎临床试验的临床终点包括在16周疗法之后分级玻璃体混浊从基线的平均变化和分级前房细胞从基线的平均变化。防止退化通过未观测到玻璃体混浊增加大于或等于2个等级所示,通过防止前房细胞增加大于或等于2个等级或防止视敏度从基线退化大于或等于0.3logMAR所示。

[0361] 在一个实施方案中,可以关于导致血管内血栓形成和不稳定性心绞痛、急性心肌梗塞以及急性中风的动脉粥样硬化斑块不稳定性的临床结果来评价用所描述的源自人类的渗透NBD偶联物(SEQ ID No.21和/或22或其一部分)的静脉内制剂处理人类受试者的作用。在具有不稳定性心绞痛、急性MI和/或急性中风的症状的人类受试者中,潜在的病理与发炎性动脉粥样硬化斑块不稳定性相关,其中在动脉粥样硬化病灶内由巨噬细胞产生促血栓形成因子,这导致血栓形成和部分或完全血管闭塞。具体地说,评价可以在呈递至急性护理设施时开始。进一步评价可以包括在呈递后24小时观测/测量急性和亚急性处理一至五天对发炎的生物标志物,诸如C反应蛋白(CRP)的影响。可以在呈递后约一个月评估临床结果,诸如死亡率、心脏功能(在急性MI的情况下的射血分数)以及用国家卫生研究院中风量表(National Institutes of Health Stroke Scale,NIHSS)测量的神经功能(在急性中风的情况下)。NIHSS是提供中风相关的神经缺陷的定量量度的系统评估工具。举例来说,具有低于12-14的NIHSS值的中风患者将具有80%的机会获得良好或极佳的结果,而高于20-26的NIHSS值具有小于20%的机会获得良好或极佳的结果。因此,在这些研究中,在一个月时具有低于17的NIHSS评分的人类受试者的百分比可以用作神经功能的终点。

[0362] 在另一个实施方案中,可以评价在约0.1mg/kg至小于约100mg/kg的日剂量下源自人类基因的细胞渗透NBD偶联物(SEQ ID No.21和/或22或其一部分)的每日静脉内疗法。将通过已知的方法在血清中评估在24小时下的CRP浓度。另外,我们将在呈递后一个月评估:(1)如通过具有小于17的NIHSS的患者百分比测量的中风后神经功能;(2)如通过超声波测量的MI后心输出量;以及(3)急性死亡率水平。将结果与对照相比较。

[0363] 包含偶联至NBD肽的SEQ ID No.1至19中的任一者或其变体或部分的替代性实施方案包括多种包含本领域中已知的任何一个或多个连接子的融合蛋白质或偶联物,条件是融合蛋白质或偶联物的功能不会因其添加而受损。

[0364] 实施例2

[0365] 用于多囊性肾病的PC1细胞质C端尾肽

[0366] 抑制蛋白质-蛋白质相互作用的人类HOX C12或HOX D12同源域(或其变体或部分)融合蛋白质的另一个实施方案是第二区由200氨基酸肽组成的融合蛋白质,这个肽的序列来源于PC1蛋白质的细胞质C端尾区(PC1CTT),也称作p200(Lal等"多囊蛋白1C端尾与β-连环蛋白相关联并且抑制典型的Wnt信号传导(Polycystin-1C-terminal tail associates withβ-catenin and inhibits canonical Wnt Signaling)"Hum.Mol.Genet.17(20):3105-17,2008年10月15日,包括补充材料)。PC1基因和/或PC2基因通常在多囊性肾病(PKD)中发生突变。这些突变是遗传的或由体细胞突变或这些事件的组合产生并且使得从PC1蛋

白质的c端细胞质尾区裂解的转录因子对基因的活化失调。p200蛋白质阻断这个转录因子的作用，并且如果其可以有效地递送至其在肾小管细胞内的作用位点就可能能够恢复PKD患者中的正常肾小管发育。

[0367] 在一个实施方案中，第一区人类HOX C12同源域60-氨基酸序列是：

[0368] SEQ ID No.1(表3)：

[0369] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0370] 在另一个实施方案中，第一区人类HOX D12同源域60-氨基酸序列是：

[0371] SEQ ID No.2(表3)：

[0372] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0373] 并且所连接的第二区是来源于PC1CTT序列的p200肽；这个第二部分的氨基酸序列是：

[0374] SEQ ID No.23(表3)：

[0375]

VILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGLSKVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRAKNKVHPSST

[0376] 在其它实施方案中，第二区是此处所示的货物序列上的变异。在添加起始甲硫氨酸下，含有HOX C12的整个融合蛋白质序列实施方案如SEQ ID No.24(表3)所示：

[0377]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLVILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGLSKVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRAKNKVHPSST

[0378] 并且在添加起始甲硫氨酸下，含有HOX D12的整个融合蛋白质序列实施方案如SEQ ID No.25(表3)所示：

[0379]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVVILRWRYHALRGELYRPAWEPQDYEMVELFLRRLRLWMGLSKVKEFRHKVRFEGMEPLPSRSSRGSKVSPDVPPPSAGSDASHPSTSSSQLDGLSVSLGRLGTRCEPEPSRLQAVFEALLTQFDRLNQATEDVYQLEQQLHSLQGRRSSRAPAGSSRGPSPGLRPALPSRLARASRGVDLATGPSRTPLRAKNKVHPSST

[0380] 连接子不是功能所必需的，但连接子可以包括于SEQ ID No.1与23或其一部分之间或SEQ ID No.2与23或其一部分之间，而不会使功能受损。可以使用本领域中已知的连接子，并且将至少部分改变SEQ ID No.24和25的实施方案，达到连接子序列将桥连每个偶联物的第一和第二区的程度。

[0381] 在其它实施方案中，p200肽可以偶联至SEQ ID No.3至19中的任一者。

[0382] 在其它实施方案中，可以将额外的氨基酸序列添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(HHHHHH)，以及本领域的技术人员已知的其它类似标签。这类标签可能包括或可能不包括连接子。另外，可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端

起始甲硫氨酸的序列，如果添加N端纯化域，那么甲硫氨酸将在纯化域的N端而不是人类HOX C12或HOX D12同源域的N端。

[0383] 包括60-氨基酸人类HOX C12或HOX D12同源域或其变体或部分与第二区中的PC1CTT p200肽的融合蛋白质实施方案将包括于全身制剂中，包括用于持续释放的静脉内、皮下、肌肉内以及可注射植入物制剂。这些融合蛋白质还可以经过配制以包括靶向其的特异性结构用于在其被肾小球滤过并且进入肾单位之后由肾小管细胞再吸收或经由特异性受体再吸收，诸如可以经由血液靶向肾囊肿细胞的加压素2受体(V2R，参看下文)。参看例如图3，其示出了代表这样的结构的第三区。如所提及，融合蛋白质的生物活性可以在体外在囊肿样结构形成受抑制的具有PKD突变的肾小管细胞培养物中，以及在囊肿形成和肾生长的进展受抑制的啮齿动物PKD模型中展示(Lal等2008)。我们将评价融合蛋白质实施方案(没有起始甲硫氨酸的SEQ ID No.24或25或其一部分，或替代性的含PC1 CTT p200肽的偶联物实施方案)在体外在这些肾小管细胞培养物中以及在动物PKD模型(例如，Pkd1转基因小鼠(Pkd2W525/-))中对囊肿形成的影响。在这个PKD-1转基因小鼠模型中，我们将通过肾大小增长的超声波抑制(即，肾生长的抑制)来测量。在PKD患者中，使用所公开的在第二区中包含PC1 CTT p200肽的融合蛋白质和/或偶联物实施方案中的一者或多者的医药制剂，我们将通过MRI总肾体积以及囊肿大小和数目的进展来评价。

[0384] 在用于治疗PKD的含PC1CTT肽的融合蛋白质或偶联物的另一个实施方案中，蛋白质的第二区是p21，保留p200的一些而不是所有生物活性的p200分子的一部分。参看表3，SEQ ID No.26。p21肽当在上文所描述的PKD肾小管细胞培养物中遗传表达时，以类似于在这些细胞中表达整个p200肽的作用的方式使细胞恢复正常小管样表型。从p200构建体中去除p21氨基酸还消除了p200表达校正培养物中这些细胞的囊肿样表型的能力。不同于p200，p21并不活化Wnt信号传导路径并且因此可能不会诱导成骨活性。这些差异可以在体内使用小鼠PKD模型中的成骨性骨形成生物标志物(如骨钙素)来测量，其中还可以使用超声波测量对肾囊肿形成和肾大小的进展的有益作用。

[0385] 60-氨基酸人类HOX C12同源域第一区(SEQ ID No.1)的氨基酸序列示于上文。这个第二区p21(SEQ ID No.26)的氨基酸序列是：

[0386] IRRIRLWMGLSKVKEFRHKVR。

[0387] 在其它实施方案中，第二区是此处所示的货物序列上的变异。

[0388] 某些实施方案，诸如各自具有起始甲硫氨酸的含有HOX C12和HOX D12的融合蛋白质，在下文和表3中分别以SEQ ID No.27和28示出。

[0389] SEQ ID No.27：

[0390]
MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLIRRIRLWMGLSKVKEF
RHKVR

[0391] SEQ ID No.28：

[0392]
MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVIRRIRLWMGLSKVKEF
RHKVR

[0393] 连接子不是功能所必需的，但连接子可以包括于例如SEQ ID No.1与27或28之间

和SEQ ID No.2与27或28之间，而不会使功能受损。可以使用本领域中已知的任何连接子，并且将至少部分改变SEQ ID No.27和28的实施方案，达到连接子序列将桥连这些第一和第二区的程度。

[0394] 在其它实施方案中，p21肽可以偶联至SEQ ID No.3至19中的每一者。

[0395] 在PKD-1转基因小鼠模型中，我们将使用超声波评价长期每天腹膜内或静脉内注射包括(1)我们的人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物实施方案和(2)我们的人类HOX C12或HOX D12同源域-p21融合蛋白质或偶联物实施方案的制剂对肾大小的影响。

[0396] 在PKD-1转基因小鼠模型中，包含偶联至人类HOX C12或HOX D12同源域(在p21的情况下为SEQ ID No.27和28，并且在p200的情况下为SEQ ID No.24和25)的PC1CTT货物肽(p200(SEQ ID No.23)或p21(SEQ ID No.26))的实施方案，或因连接子(如果有的话)的类型而变化的替代性的含PC1CTT肽的实施方案，如果溶解度允许的话，那么以每只小鼠至少约1μg至至少500μg或更大的剂量。将通过超声波评估肾大小和生长速率，并且将来自接受人类HOX C12或HOX D12同源域-p21处理制剂的组的结果与对照组和接受人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物处理制剂的组的那些结果相比较。

[0397] 在PKD-1转基因小鼠模型中，我们将进一步评价包含人类HOX C12或HOX D12同源域-p21和人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物的另一个实施方案与对照组相比减少肾生长的能力，并且比较人类同源域-p21与人类同源域-p200的变化相比之下对血液中的成骨性骨生物标志物(诸如骨钙素)的影响。如果溶解度允许的话，那么将以每只小鼠至少约1μg至至少500μg或更大的剂量在制剂中施用人类HOX C12或HOX D12同源域-p21和人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物实施方案。将通过已知的方法评估血液中的骨钙素浓度，并且将来自接受诸如人类HOX C12或HOX D12同源域-p21处理制剂的实施方案的组的结果与对照组和接受诸如人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物处理制剂的实施方案的组的那些结果相比较。

[0398] 在通过长期每天给予上文所描述的制剂全身处理的PKD患者中，将以小于约0.01mg/kg至小于约100mg/kg的剂量施用包含经肽键偶联至人类HOX C12或HOX D12同源域或其变体或部分(例如，在p21的情况下为SEQ ID No.27-28的全序列实施方案或其变体或部分，并且在p200的情况下为SEQ ID No.24-25的全序列实施方案或其变体或部分)的PC1CTT货物肽(p200(SEQ ID No.23)或p21(SEQ ID No.26)或其一部分)的实施方案，或因连接子(如果有的话)的类型而变化的替代性的含PC1CTT肽的偶联物实施方案。将通过已知的方法评估肾生长速率，并且将来自接受人类HOX C12或HOX D12同源域-p21处理制剂实施方案的组的结果与对照组和接受人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物处理制剂实施方案的组的那些结果相比较。

[0399] 靶向肾囊肿：肾集合管被认为是PKD中的一些或大多数囊肿的起源。集合管细胞表达加压素2受体(V2R)。加压素2受体可能通过在一些实施方案中包括连接的肽V2R拮抗剂或通过添加肽V2R拮抗剂作为融合蛋白质的额外区段而使具有第一区中的人类HOX C12或HOX D12同源域肽或其变体或部分和第二区中的p200或p21的融合蛋白质和偶联物实施方案靶向集合管起源的肾囊肿。在一些实施方案中，可以使用可去除的连接子，条件是偶联物的功能不会因其添加而受损。非竞争性肽V2R拮抗剂的一个实例由Rihakova等，“VRQ397

(CRAVKY):新颖的非竞争性V2受体拮抗剂(VRQ397(CRAVKY):a novel noncompetitive V2receptor antagonist)"Am.J.Physiol.Integr.Comp.Physiol.297:R1009-R1018,2009报道。我们意图测试非竞争性肽V2R拮抗剂VRQ397(CRAVKY)(SEQ ID No.29,示于表3中)经连接子添加至人类HOX C12或HOX D12同源域-p200和/或人类HOX C12或HOX D12同源域-p21融合(偶联物)蛋白质实施方案的N端或C端以评价功效(诸如肾大小的变化)所需的剂量以及递送至作用位点的效率,这个连接子被设计成一旦所暴露的人类同源域区已经促进细胞进入细胞质中就解除连接或溶解。此外,我们将检查肽V2R拮抗剂是否经由不同机制影响融合蛋白质或偶联物实施方案的功效。

[0400] SEQ ID No.29(表3):肽V2R六氨基酸拮抗剂:CRAVKY。

[0401] 在各种实施方案中,用于治疗PKD的靶向肽可以是以下任一者:CRAVKY-(连接子)-p200-人类同源域;CRAVKY-(连接子)-p21-人类同源域;人类同源域-p200-(连接子)-CRAVKY;人类同源域-p21-(连接子)-CRAVKY,其中CRAVKY序列在前两个实例中的蛋白质的N端和后两个实例中的蛋白质的C端。

[0402] 可以在体外在如上文所描述的PKD囊肿细胞培养物中评价包含例如连接有和未连接CRAVKY肽的p21和p200肽的实施方案的相对活性,条件是这些细胞被工程改造成在其表面上也表达V2R。可以将对例如上文所描述的各种含CRAVKY的融合蛋白质或偶联物实施方案的体外剂量反应与不含CRAVKY的相同融合蛋白质或偶联物实施方案针对使细胞培养物的体外表型从囊肿样至小管样变化的能力进行比较。另外,还可以在上文所描述的鼠类PKD模型中测试这些构建体减少肾大小的生长的相对能力。

[0403] 如果(1)观测到人类HOX C12或HOX D12同源域-p21和/或人类HOX C12或HOX D12同源域-p200融合蛋白质或偶联物制剂实施方案在PKD囊肿细胞培养物中的功效,以及(2)当使用CRAVKY肽靶向时观测到上文所描述的PDK-1转基因小鼠模型中的更高效力(即,剂量反应曲线向左偏移),那么将在PDK-1转基因小鼠模型中以及受影响患者中测试包含SEQ ID No.29的上文所描述的序列实施方案中的至少一者而不是SEQ ID No.24、25、27和/或28(或其变体或部分),或其偶联配对物。将通过已知的方法评估肾生长速率。

[0404] 替代性实施方案,包括包含偶联至(1)p200肽或(2)p21肽的SEQ ID No.1至19中的任一者或其变体或部分的那些,包括多种包含本领域中已知的任何一个或多个连接子的融合蛋白质或偶联物,条件是功能不受损,并且可以进一步包含CRAVKY肽。

[0405] 实施例3

[0406] 用于溶酶体贮积病的细胞渗透肽酶替代疗法

[0407] 强有力的证据表明细胞渗透肽能够使大的"货物"肽或蛋白质(诸如抗体)进入细胞。包含源自HOX的肽或其它人类同源域细胞渗透肽,诸如类似公开的那些的偶联物或融合蛋白质的实施方案可以伴随较大分子跨越细胞膜至细胞内作用位点。其还减少其它方面具抗原性的货物肽和蛋白质的免疫原性,并且允许其穿过血脑屏障并且进入CNS。所公开的实施方案和其修改因此还包括偶联物或融合蛋白质的第一区是60-氨基酸人类同源域或其变体或部分并且第二区是活性酶的实施方案。这些化合物将恢复遗传性酶缺乏患者中,诸如溶酶体贮积病中的酶功能。我们包括溶酶体贮积病和相关的"货物"结构的三个实例((1)对于戈谢病(Gaucher Disease),葡糖脑苷脂酶(GCase),例如,如SEQ ID No.30所示;(2)对于赫尔勒综合症(Hurler Syndrome),α-L-艾杜糖苷酸酶,例如,如SEQ ID No.45所示;以及

(3)对于亨特综合症(Hunter Syndrome),艾杜糖醛酸-2-硫酸酯酶,例如,如SEQ ID No.48所示),相同的原则适用于具有在其它溶酶体贮积病中缺乏或有缺陷的其它“货物”酶的人类同源域融合蛋白质或偶联物。

[0408] 溶酶体贮积病一般由所涉及的主要贮积物质的性质来分类,并且可以宽泛地分为以下类:(在可用时提供ICD-10编码)。

[0409] •(E75)脂质贮积病症,主要是神经鞘脂贮积病(包括戈谢氏病和尼曼-匹克病(Niemann-Pick disease)(E75.0-E75.1))、神经节苷脂贮积病(包括泰-萨二氏病(Tay-Sachs disease)(E75.2))、脑白质营养不良

[0410] •(E76.0)粘多糖贮积病(包括亨特综合症和赫尔勒病)

[0411] •(E77)糖蛋白贮积病症

[0412] •(E77.0-E77.1)粘脂贮积病

[0413] 而且,II型糖原贮积病(旁波症(Pompe disease))也是溶酶体代谢中的缺陷,不过其以其它方式归类为ICD-10中的E74.0。

[0414] 戈谢病

[0415] 戈谢病是由基因GBA 1的不存在或突变引起的遗传性溶酶体贮积病症,其继而导致GBA1产物葡糖脑苷脂酶(GCase)的缺乏。静脉内施用GCase减轻患者的戈谢病的一些症状。GCase缺乏还促成一些遗传形式的帕金森氏病。

[0416] 在一个实施方案中,第一区人类HOX C12同源域60-氨基酸序列是:

[0417] SEQ ID No.1(表3):

[0418] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0419] 在另一个实施方案中,第一区人类HOX D12同源域60-氨基酸序列是:

[0420] SEQ ID No.2(表3):

[0421] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0422] 并且在一些实施方案中,所连接的第二部分是GCase氨基酸序列:

[0423] SEQ ID No.30(表3):

[0424]

ARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQK
VKGFGGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDT
KLKIPLIHRALQLAQRPVSLLASPWTSPTWLKTNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVT
AENEPSAGLLSGYPFQCLGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYVHGI
AVHWYLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLAL
NPEGGPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLN
RSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

[0425] 在其它实施方案中,第二区是此处所示的货物序列上的变异。在另一个实施方案中,在添加起始甲硫氨酸下,具有HOX C12的融合蛋白质的整个肽序列是

[0426] SEQ ID No.31(表3):

[0427]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLARPCIPKSFGYSSVVC
VCNATYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNI

LALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQR
PVSLLASPWTSPTWLKTNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQ
CLGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAVHWYLDFLAPAKATL
GETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSP
IIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLNRSSKDVPLTIKDPAVG
FLETISPGYSIHTYLWRRQ

[0428] 在另一个实施方案中,在添加起始甲硫氨酸下,具有HOX D12的融合蛋白质的整个肽序列是

[0429] SEQ ID No.32(表3):

[0430]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVARPCIPKSFGYSSVVC
VCNATYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNI
LALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQR
PVSLLASPWTSPTWLKTNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQ
CLGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAVHWYLDFLAPAKATL
GETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSP
IIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLNRSSKDVPLTIKDPAVG
FLETISPGYSIHTYLWRRQ

[0431] 连接子可能不是功能所必需的,但在某些实施方案中连接子可以包括于例如SEQ ID No.1与30之间或SEQ ID No.2与30之间,而不会使功能受损。可以使用本领域中已知的任何连接子,条件是偶联物的功能不会因其添加而受损。将至少部分改变SEQ ID No.31和32的实施方案或其变体或部分,达到连接子序列将桥连每一者中的第一和第二区的程度。

[0432] 同样可以在最终结构中改变蛋白质糖基化。在几个糖基化位点处甘露糖的暴露可以增强包括例如SEQ ID No.31和/或32或其变体或部分的实施方案的溶酶体靶向。

[0433] 在其它实施方案中,可以将额外的氨基酸序列添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(HHHHHH),以及本领域的技术人员已知的其它类似标签。这类标签可能包括或可能不包括连接子。另外,可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端起始甲硫氨酸的序列,如果添加N端纯化域,那么甲硫氨酸将在纯化域的N端而不是人类同源域或HOX肽第一区的N端。

[0434] 包括人类同源域或其变体或部分和例如第二区中的GCase序列的偶联物或融合蛋白质实施方案将包括于全身制剂中,包括用于持续释放的静脉内、皮下、肌肉内以及可注射植入物制剂。在人类突变并入小鼠GCase序列中的鼠类戈谢病模型中,这些融合蛋白质防止各种组织中的底物积聚并且防止戈谢病的神经表现。

[0435] 我们将使用本领域中熟知的淋巴细胞增殖检验来检查60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质实施方案的免疫原性。为了研究例如60-氨基酸人类同源域或其变体或部分消除其它方面具免疫原性的蛋白质的免疫原性的一般能力,将对破伤风类毒素免疫的从受试者分离的人类单核细胞在体外与破伤风类毒素或所测定的在约1μM至约115μM范围内的各种浓度的人类同源域(或其变体或部分)肽-破伤

风类毒素偶联物一起孵育三天。然后将测定淋巴细胞对3H-胸苷的并入。

[0436] 另外，我们将评价体外酶融合蛋白质或偶联物实施方案的免疫原性。具体地说，将从对酶具有已知的输液反应和抗体形成的患者中分离单核白细胞。使用本领域中熟知的技术，将这些细胞在体外与单独的培养基和各种浓度的标准酶制剂以及各种浓度(例如，介于1μM与约115μM之间)的人类HOX C12或HOX D12同源域肽融合蛋白质和/或偶联物实施方案，诸如GCase序列或其变体或部分一起孵育。将测定淋巴细胞对3H-胸苷的并入作为淋巴细胞免疫反应的量度。

[0437] 举例来说，将评价包含偶联至60-氨基酸人类HOX C12或HOX D12同源域或其变体或部分(例如，包含SEQ ID No.24、25、27和/或28或其变体或部分的实施方案)的GCase货物肽的融合蛋白质实施方案的体外测试。在一个实施方案中，我们将使用从当用单独的酶处理时具有已知的免疫介导的输液反应的患者中分离的人类单核白细胞的淋巴细胞增殖检验来比较含有GCase肽的60-氨基酸人类同源域偶联物或融合蛋白质的免疫原性与单独的酶的免疫原性。在所测定的最佳微摩尔浓度下体外孵育三至五天之后。将通过检查淋巴细胞对3H-胸苷的并入来评估包含60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质的实施方案的免疫原性，并且将结果与对照相比较。

[0438] 稍后在临床测试中，将以在不存在临床上显著的输液反应或功能上重要的抗GCase抗体产生的情况下恢复功能的能力为基础来评价包含包括GCase序列的人类HOX C12或HOX D12同源域肽融合蛋白质或偶联物的各种实施方案。

[0439] 存在细胞检验的两个实例，其可以用于确定人类同源域-GCase偶联物实施方案是有效的，并且在何种浓度下。在正常细胞，例如，培养物中的成纤维细胞中，添加GCase的底物使得酶活化和底物(在戈谢病中积聚)减少。在GBA 1基因有缺陷或缺乏的成纤维细胞中，添加GCase的底物并不导致底物周转。然而，在具有这些细胞的培养物中，添加渐增量的具有暴露的甘露糖残基或人类同源域-GCase偶联物的人类同源域-GCase递送更多的酶至细胞并且减少例如放射性标记的底物。或者，在从戈谢病患者获取的细胞，例如白细胞中，荧光β-葡糖苷酶检验测定相对GCase活性作为疾病的诊断，并且也可用作人类细胞检验以展示诸如人类同源域-GCase融合蛋白质或偶联物的实施方案在恢复酶活性中的有效性。

[0440] 在具有Gba1基因突变的成纤维细胞培养物中，作为融合蛋白质偶联至人类HOX C12或HOX D12同源域(例如，SEQ ID No.1和/或2中的任一者或其一部分)的GCase货物肽(例如，SEQ ID No.30)将在这个体外检验中在介于约1μM至约115μM之间的各种浓度下具活性。我们将测量GCase底物葡糖神经酰胺的积聚，与对照培养物中的积聚相比较。

[0441] 小鼠的GBA1基因的点突变已经产生可靠的戈谢病模型。我们将评价每天静脉内或腹膜内施用包含人类同源域-GCase融合蛋白质或偶联物的实施方案持续至多两个月以减少这个鼠类模型中的葡糖神经酰胺(GCase底物)积聚。将以每只小鼠介于至少约1μg至至少约500μg或更大的剂量施用作为融合蛋白质偶联至人类HOX C12或HOX D12同源域(例如，SEQ ID No.1和/或2中的任一者或其一部分)的GCase货物肽(例如，SEQ ID No.30)。我们将再次评估GCase底物葡糖神经酰胺的积聚，与对照组中的积聚相比较。

[0442] 在涉及中枢神经系统的另一个小鼠戈谢病模型中，α-突触核蛋白和τ包涵体的积聚在脑区中发生并且是神经退化性疾病，诸如帕金森氏病的标志。举例来说，鼠类Gba1基因座(Gba1D409V/D409V)中的单点突变引起α-突触核蛋白/泛素聚集体在CNS中的积聚和可测

量的海马体记忆缺失。在这个小鼠模型中，我们将评价包含人类HOX C12或HOX D12同源域-GCase融合蛋白质或偶联物的实施方案穿过血脑屏障并且递送能够防止帕金森氏病的结构关联的酶的能力，目的在于与全身施用纯酶相比减少α-突触核蛋白/泛素聚集体在脑中的积聚。在这个小鼠戈谢病模型中，将以每只小鼠介于至少约1μg至至少约500μg之间的剂量静脉内或腹膜内施用作为融合蛋白质偶联至人类同源域(例如，SEQ ID No.1至19中的任一者或其一部分)的GCase货物肽(例如，SEQ ID No.30)持续至多两个月。我们将通过已知的方法评估α-突触核蛋白/泛素聚集体在脑中的积聚，与对照组中的积聚相比较。

[0443] 替代性实施方案，诸如偶联至GCase肽的SEQ ID No.1至19中的任一者或其变体或部分，包括多种包含本领域中已知的一个或多个连接子的融合蛋白质或偶联物，条件是偶联物的功能不受损。

[0444] 赫尔勒综合症

[0445] 另一种溶酶体贮积病，赫尔勒综合症，粘多糖贮积症I(MPS I)，是粘多糖贮积病之一，粘多糖贮积病是一组因缺乏葡糖胺聚糖(GAG)或粘多糖降解中所涉及的特异性溶酶体酶而引起的遗传性病症。部分降解的GAG的积聚导致干扰细胞、组织以及器官功能。赫尔勒综合症是归因于α-L-艾杜糖苷酸酶因异常IDUA基因结构或IDUA基因调控所致的遗传性缺乏并且可以产生广泛范围的具有3种主要识别的临床实体的表型累及：赫尔勒(Hurler)(MPS IH)、施艾(Scheie)(MPS IS;607016)以及赫尔勒-施艾(MPS IH/S)综合症。赫尔勒和施艾综合症分别代表MPS I临床谱系的重度和轻度端点的表型，并且赫尔勒-施艾综合症处于表型表达的中间。

[0446] 粘多糖贮积症1H(MPS1H)是1型粘多糖贮积症的重度形式，其特征为硫酸皮肤素和硫酸乙酰肝素的尿排泄量的进行性物理退化。MPS1H患者通常在生命的第一年内呈现肝脾肿大、骨骼变形、角膜混浊以及重度智力迟滞的组合。阻塞性气道疾病、呼吸道感染以及心脏并发症通常导致在10岁之前死亡。

[0447] 粘多糖贮积症1H/S(MPS1H/S)是1型粘多糖贮积症的一种形式，其特征为硫酸皮肤素和硫酸乙酰肝素的尿排泄量的进行性物理退化。MPS1H/S代表MPS1临床谱系的中间表型。其特征为相对很少的神经累及，但针对重度MPS1所描述的大多数躯体症状在青少年早期至中期发展，导致运动性的显著损失。

[0448] 粘多糖贮积症1S(MPS1S)是1型粘多糖贮积症的轻度形式，其特征为硫酸皮肤素和硫酸乙酰肝素的尿排泄量的进行性物理退化。MPS1S患者可能具有很少或没有神经累及、正常身材以及寿命期限，但呈现关节僵硬、轻度肝脾肿大、主动脉瓣膜病以及角膜混浊的发展。

[0449] 此处，我们将所有这些临床表型称作赫尔勒综合症或MPS I。其比MPS II(亨特综合症)更加常见，没有角膜混浊并且遵循更缓慢的进程(参看下文)。

[0450] 在一个实施方案中，HOX C12氨基酸序列第一区是SEQ ID No.1：

[0451] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0452] 在另一个实施方案中，HOX D12氨基酸序列第一区是SEQ ID No.2：

[0453] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0454] 第二区可以是例如SEQ ID No.45(表3)，653氨基酸α-L-艾杜糖苷酸酶序列(包括27氨基酸信号肽)：

[0455]

MRPLRPRAALLALLASLLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYV
GAVPHRGIKQVRTHWLLELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKD
LVSSLARRYIGRYGLAHVSKWNFETWNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRS
PLSWGLLRHCHDGTNFFTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEADPLVGWSL
PQPWRADVTYAAMVVKVIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKP
VLTAMGLLALLDEEQLWAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRG
VPPGPGLVYVTRYLDNGLCSPDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVH
VCARPEKPPGQVTRLRALPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSG
SYRVRALDYWARPGPFSDPVPYLEVPVPRGPPSPGNP

[0456] 在其它实施方案中,第二区是此处所示的货物序列上的变异。并且,包括具有起始甲硫氨酸的HOX C12同源域的这个实施方案的完整序列可以是例如SEQ ID No.46(表3):

[0457]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLMRPLRPRAALLALLAS
LLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWL
LELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKDLVSSLARRYIGRYGLA
HVSKWNFETWNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCHDGTNF
FTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEADPLVGWSLPQPWRADVTYAAMVVK
VIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQL
WAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRGVPPGPGLVYVTRYLDN
GLCSPDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLR
ALPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALDYWARPGPF
SDPVPYLEVPVPRGPPSPGNP

[0458] 包括具有起始甲硫氨酸的HOX D12同源域的另一个实施方案的完整序列可以是例如SEQ ID No.47(表3):

[0459]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVMRPLRPRAALLALLAS
LLAAPPVAPAEAPHLVHVDAARALWPLRRFWRSTGFCPPLPHSQADQYVLSWDQQLNLAYVGAVPHRGIKQVRTHWL
LELVTTRGSTGRGLSYNFTHLDGYLDLLRENQLLPGFELMGSASGHFTDFEDKQQVFEWKDLVSSLARRYIGRYGLA
HVSKWNFETWNEPDHHDFDNVSMTMQGFLNYYDACSEGLRAASPALRLGGPGDSFHTPPRSPLSWGLLRHCHDGTNF
FTGEAGVRLDYISLHRKGARSSISILEQEKVVAQQIRQLFPKFADTPIYNDEADPLVGWSLPQPWRADVTYAAMVVK
VIAQHQNLLLANTTSAFPYALLSNDNAFLSYHPHPFAQRTLTARFQVNNTRPPHVQLLRKPVLTAMGLLALLDEEQL
WAEVSQAGTVLDSNHTVGVLASAHRPQGPADAWRAAVLIYASDDTRAHPNRSVAVTLRLRGVPPGPGLVYVTRYLDN
GLCSPDGEWRRLGRPVFPTAEQFRRMRAAEDPVAAAPRPLPAGGRLTLRPALRLPSLLLVHVCARPEKPPGQVTRLR
ALPLTQGQLVLVWSDEHVGSKCLWTYEIQFSQDGKAYTPVSRKPSTFNLFVFSPDTGAVSGSYRVRALDYWARPGPF
SDPVPYLEVPVPRGPPSPGNP

[0460] 在一些实施方案中,同样可以在最终结构中改变蛋白质糖基化。在几个糖基化位点处甘露糖的暴露可以增强含α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案的溶酶体靶向。

[0461] 将在小鼠模型中评价由60-氨基酸人类同源域或其变体或部分和活性α-L-艾杜糖苷酸酶组成的融合蛋白质和偶联物实施方案或具有一个或多个连接子的替代性的含α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案到达(1)在(1)脑组织中积聚GAG的位点、(2)关节软骨内的软骨细胞以及(3)长骨的生长板软骨的能力。

[0462] 赫尔勒综合症的MPS 1-H敲入小鼠模型携带与赫尔勒综合症患者中所发现的突变(IDUA-W402X)类似的突变;MPS 1-H小鼠展现生物化学、代谢以及形态异常的表型,这些异常与先前所利用的赫尔勒综合症动物模型中的异常相关联,并且与这些患者中α-L-艾杜糖苷酸酶缺乏的最重度形式中的异常相关联。Wang等"携带与人类IDUA-W402X突变类似的无义突变的MPS 1-K敲入小鼠的表征(Characterization of an MPS 1-K knock-in mouse that carries a nonsense mutation analogous to the human IDUA-W402X mutation)" Molecular Genetics and Metabolism 99:62-71,2010。

[0463] 在这个模型中,如果溶解度允许的话,那么以每只小鼠1μg至至少500μg的剂量,我们将施用包含偶联至人类同源域或其变体或部分的α-L-艾杜糖苷酸酶的制剂实施方案,或具有一个或多个连接子的替代性的含α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案的静脉内或腹膜内注射。我们将使用组织病理学技术评估GAG(硫酸乙酰肝素和硫酸皮肤素)的积聚,并且将这些结果与对照组的结果相比较。

[0464] 在患有赫尔勒综合症的患者中,我们将通过测量包括硫酸乙酰肝素和硫酸皮肤素的GAG的尿排泄量的减少来评价包含活性α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案对GAG积聚的影响。具体地说,以小于约0.01mg/kg至小于约100mg/kg的剂量,将通过长期每天给予包含偶联至60-氨基酸人类HOX C12或HOX D12同源域或其变体或部分的α-L-艾杜糖苷酸酶的制剂实施方案,或具有一个或多个连接子的替代性的含α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案来全身处理患有赫尔勒综合症的患者。将通过已知的方法评估尿中的GAG排泄量(硫酸乙酰肝素和硫酸皮肤素),并且将结果与接受包含并不呈融合蛋白质或偶联物形式的α-L-艾杜糖苷酸酶的制剂实施方案的患者相比较。此外,我们将通过标准生长和发育儿科技术来评价骨骼和脑发育。

[0465] 将在体外在成纤维细胞细胞系的培养物中测试包含人类HOX C12或HOX D12同源域-α-L-艾杜糖苷酸酶融合蛋白质或偶联物的各种实施方案,这些细胞系具有如在天生患有赫尔勒综合症的患者中发生的IDUA基因的纯合突变。在这些培养物中,将测量在存在和不存在融合蛋白质或偶联物实施方案的情况下的GAG周转以确认活性酶以及硫酸皮肤素和硫酸乙酰肝素的减少的溶酶体积聚。

[0466] 在具有IDUA基因的纯合突变的成纤维细胞培养物中,包含人类HOX C12或HOX D12同源域-α-L-艾杜糖苷酸酶融合蛋白质的一个实施方案,或包含一个或多个连接子的替代性的含α-L-艾杜糖苷酸酶的融合蛋白质或偶联物实施方案将在这个体外检验中在介于约1μM至约115μM之间的最佳浓度下具活性。我们将再次测量GAG底物硫酸皮肤素和硫酸肝素的积聚,并且将我们的结果与对照培养物相比较。

[0467] 我们将使用来自具有文献记载的输液反应同时接受替代酶疗法的MPS I患者的外周血单核细胞进一步评价淋巴细胞培养物中人类HOX C12或HOX D12同源域-α-L-艾杜糖苷酸酶融合蛋白质或偶联物实施方案的免疫原性。

[0468] 在一个实施方案中,我们将使用从当接受标准α-L-艾杜糖苷酸酶替代疗法时具有

已知的过敏(输液)反应的患者中分离的人类单核白细胞中的淋巴细胞增殖检验来比较含有α-L-艾杜糖苷酸酶肽的60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质的免疫原性与单独的酶的免疫原性。在介于约1μM至约115μM之间的最佳酶浓度下体外孵育三至五天之后测量3H-胸苷并入。将通过检查淋巴细胞对3H-胸苷的并入来评估包含60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质的实施方案的免疫原性,并且将结果与仅接受酶的处理组的那些结果相比较。

[0469] 通过比较过敏输液反应的发生和IgG抗酶抗体的产生,将临床中的人类HOX C12或HOX D12同源域-酶融合蛋白质和/或偶联物实施方案的免疫原性与标准酶替代蛋白质的免疫原性相比较。

[0470] 包含例如偶联至α-L-艾杜糖苷酸酶肽的SEQ ID No.1至19中的任一者或其变体或部分的替代性实施方案包括多种融合蛋白质或偶联物实施方案,并且可以进一步包含本领域中已知的任何一个或多个连接子,条件是偶联物的功能不会因一个或多个连接子的添加而受损。

[0471] 亨特综合症

[0472] 亨特综合症或粘多糖贮积症II(MPS II)是由溶酶体酶艾杜糖醛酸-2-硫酸酯酶缺乏,导致几乎所有细胞类型、组织以及器官中的葡糖胺聚糖的进行性积聚而引起的罕见X连锁隐性病症。MPS II患者在尿中排泄过量的硫酸软骨素B(硫酸皮肤素)和硫酸类肝素(硫酸乙酰肝素)。MPS II是多系统病症。患有MPS2的大多数儿童具有重度形式伴有早期躯体异常,包括骨骼变形、肝脾肿大以及进行性心肺退化。突出特征是神经损伤,其呈现为发育迟缓和活动过度,但进展至智力迟滞和痴呆。通常因阻塞性气道疾病或心脏衰竭的结果而在15岁之前死亡。相比之下,患有轻度形式的MPS2的那些人可能存活至成年,躯体并发症减弱并且常常没有智力迟滞(Wraith,J.E.等"II型粘多糖贮积症(亨特综合症):在酶替代疗法的时代对于治疗的临床回顾和建议(Mucopolysaccharidosis type II(Hunter syndrome):a clinical review and recommendations for treatment in the era of enzyme replacement therapy)"Europ.J.Pediat.167:267-277,2008)。

[0473] 在一个实施方案中,第一区人类HOX C12同源域60-氨基酸序列是:

[0474] SEQ ID No.1(表3):

[0475] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0476] 在另一个实施方案中,第一区人类HOX D12同源域60-氨基酸序列是:

[0477] SEQ ID No.2(表3):

[0478] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0479] 并且,第二区可以是例如SEQ ID No.48,艾杜糖醛酸-2-硫酸酯酶(包含25氨基酸信号序列的550氨基酸):

[0480]

MPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNA
FAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWS
FPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIP
FRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLD
TQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFD

SASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIA
YSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDS
QGGDLFQLLMP

[0481] 在其它实施方案中,第二区是此处所示的货物序列上的变异。具有起始甲硫氨酸的组合的第一(HOX C12)和第二区的完整氨基酸序列实施方案是例如SEQ ID No.49:

[0482]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLMPPPRTGRGLLWLGLV
LSSVCVALGSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLT
GRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKT
CRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENI
TLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLA
NSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLV
ELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSD
KPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

[0483] 具有起始甲硫氨酸的组合的第一(HOX D12)和第二区的完整氨基酸序列实施方案是例如SEQ ID No.50:

[0484]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVMPPPRTGRGLLWLGLV
LSSVCVALGSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLT
GRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKT
CRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENI
TLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLA
NSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLV
ELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSD
KPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP

[0485] 在一些实施方案中,同样可以在最终结构中改变蛋白质糖基化。在几个糖基化位点处甘露糖的暴露可以增强人类HOX C12或HOX D12同源域-艾杜糖醛酸-2-硫酸酯酶融合蛋白质或偶联物实施方案的溶酶体靶向。

[0486] 在具有IDS基因的纯合突变的成纤维细胞培养物中,诸如人类HOX C12或HOX D12同源域-艾杜糖醛酸-2-硫酸酯酶融合蛋白质的实施方案,或包含一个或多个连接子的替代性的含艾杜糖醛酸-2-硫酸酯酶的融合蛋白质或偶联物实施方案将在这个体外检验中在介于约1μM至约115μM之间的各种浓度下具活性。我们将再次测量GAG底物的积聚,并且将我们的结果与对照培养物相比较。

[0487] 在也已经用于评价实验性基因治疗方法的MPSII(亨特综合症)的鼠类模型(idsy/-)中,大体形态表型的开始出现在3-4月龄时表现出,并且进行性地变得更加严重直至在60至70周龄时死亡。这个表型包括增重减少、颅面和其它骨骼异常,伴有短头盖骨和秃头症以及指趾增厚。此外,这些小鼠在开放场测试中显示不规则步态、异常步行模式以及较差移动和探究能力。当与这种酶在野生型组织中的活性相比时,这个小鼠模型中的IDS活性在肝、脾、肺、心、肾、骨骼肌、脑以及眼中是不可检测的。然而,IDS起始皮肤素和硫酸乙酰肝

素GAG的分解代谢。IDS活性的损失在生活仅几天之后引起所有组织内的GAG积聚，在成年生活阶段期间观察到显著增加；其导致进行性细胞液泡化和随之发生的细胞死亡。3和9月龄的MPSII小鼠显示细胞内高水平的GAG储存，并且如射线照相检验中所示，进行性GAG积聚也导致骨骼变形，其影响移动测试中的性能。(Polito,VA等“由AAV2/8介导的基因递送校正MPSII小鼠模型的亨特综合症(Correction of Hunter syndrome in the MPSII mouse model by AAV2/8-mediated gene delivery)”Hum.Mol.Genet.15(7):1225-36,2006年4月1日)。

[0488] 我们将通过使用荧光底物4-甲基伞形酮基-α-艾杜糖醛酸-2-硫酸酯目测和定量从这些MPSII小鼠的组织匀浆制备的蛋白质提取物中的GAG积聚来检查IDS活性。可以通过这些小鼠中的肝、脾、肺、心、肾以及骨骼肌的石蜡包埋切片的阿尔新蓝(Alcian-blue)染色在组织中目测GAG。另外，将使用蛋白质的二甲基-亚甲基蓝检验来测定GAG浓度。

[0489] 如果溶解度允许的话，那么以每只小鼠1μg至至少500μg或更大的剂量，我们将向雄性idsy/-小鼠施用包含偶联至人类HOX C12或HOX D12同源域(或其变体或部分)的艾杜糖醛酸-2-硫酸酯酶肽的制剂实施方案的静脉内或腹膜内注射。我们将使用组织病理学技术评估GAG(硫酸乙酰肝素和硫酸皮肤素)的积聚，并且将这些结果与对照组的那些结果相比较。

[0490] 在患有亨特综合症的患者中，我们意图评价使用包含人类HOX C12或HOX D12同源域-艾杜糖醛酸-2-硫酸酯酶融合蛋白质或偶联物的制剂实施方案处理相对于正常发育对疾病的全身表现(包括CNS中、骨中以及关节中的那些)的影响。将通过以小于约0.01mg/kg至小于约100mg/kg的剂量长期每天给予包含例如偶联至人类HOX C12或HOX D12同源域或其变体或部分的艾杜糖醛酸-2-硫酸酯酶的制剂实施方案，或替代性的包含艾杜糖醛酸-2-硫酸酯酶序列和一个或多个连接子的融合蛋白质或偶联物实施方案来全身处理患有亨特综合症的患者。将通过已知的方法评估尿中的GAG排泄量(硫酸乙酰肝素和硫酸皮肤素)，并且将结果与接受包含并不呈融合蛋白质或偶联物形式的艾杜糖醛酸-2-硫酸酯酶的制剂的患者相比较。此外，我们将评价通过标准生长和发育儿科技术评估的骨骼和脑发育。

[0491] 我们将通过比较处理对比对照(安慰剂)组中的增殖反应来进一步评价人类HOX C12或HOX D12同源域-艾杜糖醛酸-2-硫酸酯酶融合蛋白质或偶联物实施方案的体外免疫原性。将使用来自已经存在文献记载的输液反应同时接受替代酶疗法的MPS II患者的外周血单核细胞制备淋巴细胞培养物。将通过在培养三至五天之后测量这些细胞中3H-胸苷的并入来测定增殖反应。在一个实施方案中，我们将使用在所测定的在约1μM至约115μM范围内的最佳浓度下体外孵育三至五天之后分离的人类单核白细胞中的淋巴细胞增殖检验来比较含有人类同源域-艾杜糖醛酸-2-硫酸酯酶肽的60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质的免疫原性与单独的酶的免疫原性。将通过检查淋巴细胞对3H-胸苷的并入来评估包含60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)偶联物或融合蛋白质的实施方案的免疫原性。

[0492] 包含例如偶联至艾杜糖醛酸-2-硫酸酯酶肽的SEQ ID No.1至19中的任一者或其变体或部分的替代性实施方案包括多种融合蛋白质或偶联物，并且可以包含本领域中已知的任何一个或多个连接子，条件是偶联物的功能不会因一个或多个连接子的添加而受损。

[0493] 实施例4

[0494] 先天性失明中的视网膜鸟苷酸环化酶(RetGC-1)替代疗法

[0495] 1型莱伯先天性黑朦(Leber Congenital Amourosis type 1,LCA1):人类RetGC-1是1103氨基酸蛋白质,包括编码不含信号肽的单独的酶的氨基酸52-1103。RetGC-1是RPE65的基因产物,当其不存在或发生突变时导致失明。在这种疾病中,残留视锥光受体视力与突变体的生物化学特性相关联,暗示如果可以恢复视网膜中的RetGC-1酶活性,那么就可以达成视力恢复。本文所公开的实施方案和其修改因此包括包含60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)细胞渗透肽和RetGC-1酶作为货物肽的融合蛋白质或偶联物,并且可以在用于周期性眼内注射或局部施用的许多制剂实施方案中施用。

[0496] 在一个实施方案中,第一区人类HOX C12同源域60-氨基酸序列是:

[0497] SEQ ID No.1(表3):

[0498] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0499] 在另一个实施方案中,第一区人类HOX D12同源域60-氨基酸序列是:

[0500] SEQ ID No.2(表3):

[0501] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0502] 并且所连接的第二区是例如RetGC-1酶序列,诸如:

[0503] SEQ ID No.33(表3):

[0504]

AVFTVGVLGPWACDPIFSRARPDLAARLAAARLNRDPGLAGGPRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPV
NPAACRPAELLAEEAGIALVPWGCPWTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDLWVEAGRSLSTAL
RARGLPVASVTSMEPLDLSGAREALRKVRDGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTDGSLVFLPFDTIH
YALSPGPEALAALANSSQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLAR
GVAEARAAAGGRWVSGAAVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHF
PRGGSAPGPDPSCWFDPNNICGGGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITFL
HPHGGTSRKVAQGSRSSLGARSMSDIRSGPSQHLDSPNIGVYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQELRHE
NVALYLGLFLARGAEGPAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHHRGVAHGRL
KSRNCIVDGRFVLKITDHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPALERRGTLAGDVFSLAIIMQEVVCRSA
PYAMLELTPEEVVQRVRSPPPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNINKGRKTNIIDSML
RMLEQYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVAEALKTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEP
IEVVDLLNDLYTLFDAIIGSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRI
RIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRMESTGLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKGAED
TFWLVGRRGFNKPIPKPPDLQPGSSNHGISLQEIPPERRRKLEKARPGQFS。

[0505] 在其它实施方案中,第二区是此处所示的货物序列上的变异。并且,在另一个实施方案中,在添加起始甲硫氨酸下,包括HOX C12的融合蛋白质的整个肽序列是例如:

[0506] SEQ ID No.34(表3):

[0507]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLAVFTVGVLGPWACDPI
FSRARPDLAARLAAARLNRDPGLAGGPRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPAACRPAELLAEEAG
IALVPWGCPWTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDLWVEAGRSLSTALRARGLPVASVTSMEPL
DLSGAREALRKVRDGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALANS

SQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLARGVAEARAAAGGRWVSG
AAVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPDPSCWFD
PNNICGGGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPHGGTSRKVAQGSRS
SLGARSMSDIRSGPSQHLDSPNIGVYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFLARGAEG
PAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHHRGVAHGRLKSRNCIVDGRFVLKIT
DHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPALERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRV
RSPPPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNINKGRKTNIIDSMLRMLEQYSSNLEDLIRE
RTEELELEKQKTDRLLTQMLPPSVAEALKTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDA
IIGSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGLHSGPCVAGVVGL
TMPRYCLFGDTVNTASRMESTGLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPK
PPDLQPGSSNHGISLQEIPPERRRKLEKARPGQFS

[0508] 并且,在另一个实施方案中,在添加起始甲硫氨酸下,包括HOX D12的融合蛋白质的整个肽序列是例如:

[0509] SEQ ID No.35(表3):

[0510]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVAVFTVGVLGPWACDPI
FSRARPDLAARLAAARLNRDPGLAGGPRFEVALLPEPCRTPGSLGAVSSALARVSGLVGPVNPAACRPAELLAEEAG
IALVPWGCPWTQAEGTTAPAVTPAADALYALLRAFGWARVALVTAPQDLWVEAGRSLSTALRARGLPVASVTSMEPL
DLSGAREALRKVRDGPRVTAVIMVMHSVLLGGEEQRYLLEAAEELGLTDGSLVFLPFDTIHYALSPGPEALAALANS
SQLRRAHDAVLTLTRHCPSEGSVLDSLRRAQERRELPSDLNLQQVSPLFGTIYDAVFLLARGVAEARAAAGGRWVSG
AAVARHIRDAQVPGFCGDLGGDEEPPFVLLDTDAAGDRLFATYMLDPARGSFLSAGTRMHFPRGGSAPGPDPSCWFD
PNNICGGGLEPGLVFLGFLLVVGMGLAGAFLAHYVRHRLLHMQMVSGPNKIILTVDDITFLHPHGGTSRKVAQGSRS
SLGARSMSDIRSGPSQHLDSPNIGVYEGDRVWLKKFPGDQHIAIRPATKTAFSKLQELRHENVALYLGLFLARGAEG
PAALWEGNLAVVSEHCTRGSLQDLLAQREIKLDWMFKSSLLLDLIKGIRYLHHRGVAHGRLKSRNCIVDGRFVLKIT
DHGHGRLLEAQKVLPEPPRAEDQLWTAPELLRDPALERRGTLAGDVFSLAIIMQEVVCRSAPYAMLELTPEEVVQRV
RSPPPLCRPLVSMDQAPVECILLMKQCWAEQPELRPSMDHTFDLFKNINKGRKTNIIDSMLRMLEQYSSNLEDLIRE
RTEELELEKQKTDRLLTQMLPPSVAEALKTGTPVEPEYFEQVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDA
IIGSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIANMSLDILSAVGTFRMRHMPEVPVRIRIGLHSGPCVAGVVGL
TMPRYCLFGDTVNTASRMESTGLPYRIHVNLSTVGILRALDSGYQVELRGRTELKGKGAEDTFWLVGRRGFNKPIPK
PPDLQPGSSNHGISLQEIPPERRRKLEKARPGQFS

[0511] 连接子可能不是功能所必需的,但连接子可以在多种实施方案中包括于例如SEQ ID No.1与33之间或SEQ ID No.2与33或其变体或部分之间,而不会使功能受损。可以使用本领域中已知的任何连接子,条件是偶联物的功能不会因其添加而受损。将至少部分改变诸如SEQ ID No.34和/或35或其变体或部分的实施方案,达到连接子序列将桥连这些第一和第二区的程度。

[0512] 在其它实施方案中,可以将额外的氨基酸序列添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(HHHHHH),以及本领域的技术人员已知的其它类似标签。这类标签可能包括或可能不包括连接子。另外,可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端

起始甲硫氨酸的序列，如果添加N端纯化域，那么甲硫氨酸将在纯化域的N端而不是人类同源域或HOX肽第一区的N端。

[0513] 酶至通常有很少或不存在这种酶的细胞中的成功的功能性易位，例如HEK293细胞中的酶易位可以通过提供酶的底物并且通过标准方法测量鸟苷酸环化酶活性来测定。鸟苷酸环化酶活性增加的证据将被视为诸如具有细胞渗透能力的人类HOX C12或HOX D12同源域的实施方案可以递送功能酶至人类细胞的实验证据。

[0514] 我们将评价小鼠敲除模型中包含例如人类HOX C12或HOX D12同源域-RetGC-1酶融合蛋白质或偶联物的实施方案。最佳近似于LCA1的动物模型是编码RetGC-1的基因Gucy2e的小鼠敲除。这种小鼠展现无反应的视锥光受体并且展示LCA1表型。Jacobson等“在治疗途中测定人类莱伯先天性黑朦中视网膜鸟苷酸环化酶(RetGC1)缺乏的结果:残留视锥光受体视力与突变体的生物化学特性相关联(Determining consequences of retinal membrane guanylyl cyclase(RetGC1)deficiency in human Leber congenital amaurosis en route to therapy:residual cone-photoreceptor vision correlates with biochemical properties of the mutants).”Human Molecular Genetics 22(1):168-83,2013。

[0515] 我们将通过视网膜(眼球后)注射或通过滴眼液(即，局部)将人类同源域-RetGC-1偶联物或融合蛋白质的实施方案施用于敲除小鼠，并且评价恢复的网膜电图(ERG)功能作为回复的视力的量度。将结果与施用不含人类HOX C12或HOX D12同源域-RetGC-1的缓冲液的对照组中的那些结果相比较。在一个实施方案中，以每20g小鼠至少约1μg至至少约500μg的剂量，将通过视网膜(眼球后)注射或通过滴眼液向小鼠施用蛋白质，即，包含偶联至人类HOX C12或HOX D12同源域或其变体或部分的RetGC-1肽的制剂，或包含一个或多个连接子的替代性的含RetGC-1的融合蛋白质或偶联物实施方案。我们将检查ERG功能的恢复，与未处理的对照组相比较。

[0516] 在患有1型莱伯先天性黑朦(LCA1)的儿童中，将在以小于约0.01mg/kg至小于约100mg/kg的剂量使用包含偶联至人类HOX C12或HOX D12同源域或其变体或部分的RetGC-1肽的眼用制剂实施方案，或包含RetGC-1序列和一个或多个连接子的替代性融合蛋白质或偶联物实施方案长期(每天、每隔一天、每周两次、每周或以更低频率)处理之后评估视敏度和网膜电图功能的变化。在诸如1天、3天、1周、2周、3周或4周或更长的处理持续时间之后，将接受融合蛋白质或偶联物处理的患者与其预处理值相比较以使用标准临床方法评价视觉功能的临床上有意义的改善来评估视敏度，包括(但不限于)ERG功能。

[0517] 包含偶联至RetGC-1肽的SEQ ID No.1至19中的任一者或其变体或部分的替代性实施方案包括多种包含本领域中已知的任何一个或多个连接子的融合蛋白质或偶联物，条件是偶联物的功能不会因添加而受损。

[0518] 实施例5

[0519] 用于亨廷顿氏病(HD)的调控肽

[0520] 亨廷顿氏病(HD)，一种影响肌肉协调并导致认知衰退的神经退化性遗传病症，是由突变体亨廷顿蛋白基因(HTT)的表达而引起。其常常在30年龄段中期至40年龄段早期表现出，但可能更早开始伴有身体和心智能力随着时间而衰退;逐渐地协调运动变得很困难。在临床上，还存在HD患者中所观察到的许多细微缺陷，包括眼球运动、言语以及认知问题。

突变体HTT表达对纹状体，控制运动的脑的一部分具特异性，并且神经元损失和/或发炎在此处被认为是构成HD的临床征象的基础。

[0521] ZF6xHunt-Kox-1是特定设计的锌指蛋白，其结合更长的CAG重复并且抑制突变体HTT基因表达。其它长度的锌指蛋白也是可能的，包括(但不限于)ZF12xHunt-Kox-1和ZF18xHunt-Kox-1。具有人类HOX C12或HOX D12同源域细胞渗透肽的融合蛋白质或偶联物实施方案将使ZF6xHunt-Kox-1、ZF12xHunt-Kox-1、ZF18xHunt-Kox-1以及相关蛋白质能够进入细胞和核以有效地并且选择性地下调突变体HTT基因表达。我们将评价多种包含60-氨基酸人类HOX C12或HOX D12同源域-锌指融合蛋白质或偶联物的实施方案对神经病理的影响。为此目的，我们将包含人类同源域-锌指实施方案的静脉内制剂施用于亨廷顿氏病患者。具体地说，我们将评价人类HOX C12或HOX D12同源域肽穿过血脑屏障并且进入中枢神经系统的能力。

[0522] 在一个实施方案中，第一区人类HOX C12同源域60-氨基酸序列是：

[0523] SEQ ID No.1(表3)：

[0524] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0525] 在另一个实施方案中，第一区人类HOX D12同源域60-氨基酸序列是：

[0526] SEQ ID No.2(表3)：

[0527] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0528] 并且所连接的第二区是例如ZF6xHunt-Kox-1序列：

[0529] SEQ ID No.36(表3)：

[0530]

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0531] 在其它实施方案中，第二区是此处所示的货物序列上的变异。并且，在另一个实施方案中，在添加起始甲硫氨酸下，包括HOX C12肽的融合蛋白质的整个肽序列是例如：

[0532] SEQ ID No.37(表3)：

[0533]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0534] 并且在另一个实施方案中，在添加起始甲硫氨酸下，包括HOX D12肽的融合蛋白质的整个肽序列是例如：

[0535] SEQ ID No.38(表3)：

[0536]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNF

SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI
HLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDT
AQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0537] 在另一个实施方案或其它实施方案中,可以包括额外数目的锌指重复,如分别具有12和18个重复的第二组分的这些实例中所示:

[0538] ZF12xHunt-Kox-1,例如:

[0539] SEQ ID No.39(表3):

[0540]

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRA
TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDIC
GRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIH
TGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGS
QLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLG
YQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0541] ZF18xHunt-Kox-1,例如:

[0542] SEQ ID No.42(表3):

[0543]

TGAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRA
TLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDIC
GRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIH
TGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGS
QLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFS
QRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFAC
DICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLV
TFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAF
EIKSSV

[0544] 在其它实施方案中,示出了包括有人类同源域肽的具有额外数目的锌指重复的这些序列,例如,在包含具有起始甲硫氨酸残基的序列的实施方案中:

[0545] 人类HOX C12-ZF12xHunt-Kox-1,例如:

[0546] SEQ ID No.40(表3):

[0547]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI
HLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNF

SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQG
IIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGE
EPWLVEREIHQETHPDSETAFEIKSSV

[0548] 人类HOX D12-ZF12xHunt-Kox-1,例如:

[0549] SEQ ID No.41(表3):

[0550]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI
HLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQG
IIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGE
EPWLVEREIHQETHPDSETAFEIKSSV

[0551] 人类HOX C12-ZF18xHunt-Kox-1,例如:

[0552] SEQ ID No.43(表3):

[0553]

MSRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLTGAERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI
HLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICM
RNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEK
PFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKL
LDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0554] 人类HOX D12-ZF18xHunt-Kox-1,例如:

[0555] SEQ ID No.44(表3):

[0556]

MARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVTGAERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFA
CDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKI
HLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNF
SQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHLRQKDGGGGSGGGGSGGGGSQLVGTAERPFQCRICM
RNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEK
PFACDICGRKFAQRATLQRHTKIHTGSERPFQCRICMRNFSQRATLQRHIRTHTGEKPFACDICGRKFAQRATLQRH
TKIHLRQKDGGGGSGGGGSGGGGSQLVSSLSPQHSAVTQGIIKNKEGMDAKSLTAWSRTLVTFKDVFVDFTREEWKL

LDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKSSV

[0557] 在其它实施方案中，第二区可以是上文所示的货物序列中的任一者上的变异。

[0558] 连接子可能不是功能所必需的，但连接子可以在多种实施方案中包括于例如SEQ ID No.1与36、39或42之间或SEQ ID No.2与36、39或42或其一部分之间，而不会使功能受损。可以使用本领域中已知的任何连接子，条件是偶联物的功能不会因其添加而受损；参看例如表2。将至少部分改变诸如SEQ ID No.37、38、40、41、43和/或44的实施方案，达到连接子序列将桥连这些第一和第二区的程度。

[0559] 在其它实施方案中，可以将额外的氨基酸序列添加至氨基或羧基端以促进纯化。这类序列可以包括FLAG标签(DYKDDDDK)、myc标签(EQKLISEEDL)、His标签(HHHHHH)，以及本领域的技术人员已知的其它类似标签。另外，可以使用诸如生物素受体蛋白质(GLNDIFEAQKIEWHE)的配体连同活性BirA蛋白质。对于包括N端起始甲硫氨酸的序列，如果添加N端纯化域，那么甲硫氨酸将在纯化域的N端而不是人类同源域或HOX肽第一区的N端。将在适当细胞系中测试人类HOX C12或HOX D12同源域-锌指融合蛋白质或偶联物的实施方案以确定构建体在功能上易位并且突变体HTT基因受抑制。将使用小鼠HTT基因的第一外显子已经被具有111CAG重复(STHdhQ111/HdhQ111或STHdhQ7/HdhQ111)的人类外显子置换的敲入STHdh细胞。

[0560] 在一个实施方案中，我们将使用在所测定的可能介于约1μM至约115μM之间的最佳微摩尔浓度下在培养物中孵育敲入STHdh细胞持续诸如1小时、2小时、3小时、4小时、6小时或24小时的各种时间段之后所存在的含60-氨基酸人类HOX C12或HOX D12同源域(或其变体或部分)的融合蛋白质或偶联物检查锌指货物蛋白的易位。我们将通过已知的方法评价突变体HTT基因的表达，并且将结果与对照培养物相比较。

[0561] R6/2小鼠是最广泛使用的动物HD模型。这种敲入小鼠HD模型在脑中在已知在人类患者中受影响的位置纹状体处过度表达人类HTT基因。R6/2小鼠在表型上展示类似于HD患者中的征象和症状的运动缺陷，例如，R6/2小鼠缺乏用其前爪抓握的能力。

[0562] 在一个实施方案中，在三或四周龄时开始，我们将评价R6/2小鼠的运动技能；一半将被处理并且其它将充当对照组。我们将经由静脉内或腹膜内注射向小鼠每天、每隔一天或每三天施用制剂，包含例如SEQ ID No.37、38、40、41、42或43中的任一者或其任何部分的人类HOX C12或HOX D12同源域-锌指融合蛋白质或偶联物实施方案，持续一至两周。在四至六周龄时，我们将在即将处死以供组织学分析之前再评价运动技能。组织学分析将分为两个部分。首先，使用rtPCR，将在小鼠的处理组和对照组的每个纹状体中评价突变体HTT表达。其次，将在经过处理对比未处理的对照小鼠的纹状体中评价纹状体神经元损失的证据。将使用神经组织学实验室中熟知的技术来评估神经元损失和/或纹状体细胞体积的减少，并且将以在常用放大率下每个标准显微镜视场的神经元密度来报道。因此，我们将通过已知的方法评价突变体HTT基因的表达与脑部纹状体中的神经元损失的证据，并且在评估运动缺陷之后，将经过处理的R6/2小鼠的结果与未处理的R6/2对照的那些结果相比较；我们将进一步比较每个组的处理后运动缺陷评分与其自身的基线评分。

[0563] 在亨廷顿氏病患者的临床试验中，将通过使用统一亨廷顿氏病评定量表(UHDRS)总运动评分，一种用于评价HD疗法试验中的患者的临床工具来评价运动缺陷。其提供了HD中的治疗效益的定量评估，从而提供基于许多运动缺陷的复合评分。亨廷顿研究小组作者，

“统一亨廷顿氏病评定量表:可靠性和一致性(Unified Huntington’s Disease Rating Scale:Reliability and Consistency)”Movement Disorders 11(2):136-142,1996。一个点或更多点的处理前至处理后总运动评分的变化被视为临床上显著的。

[0564] 我们将评价在施用包含人类HOX C12或HOX D12同源域-锌指融合蛋白质或偶联物的制剂实施方案之后如HD患者中的运动技能的变化所反映的疾病进展。举例来说,SEQ ID No.37、38、40、41、42或43中的任一者或其一部分。首先,我们将评估这些患者的这个处理组中的UHDRS评分,并且然后经过六至八周的时程,以所测定的在至少0.01mg/kg至至少约500mg/kg体重的范围内的最佳剂量,每天通过静脉内或皮下注射施用融合蛋白质或偶联物制剂实施方案。将在处理后基于统一亨廷顿氏病评定量表(UHDRS)总运动评分来评价运动功能和其它HD评分缺陷;将结果与基线评分相比较。我们将相对于持续相同时段所追踪的未处理对照或历史对照来评估UHDRS的改善百分比。

[0565] 包含偶联至上文所论述的锌指肽中的任一者的SEQ ID No.1至19中的任一者或其变体或部分的替代性实施方案包括多种包含本领域中已知的任何一个或多个连接子的融合蛋白质或偶联物,条件是偶联物的功能不会因添加而受损。

[0566] 实施例6

[0567] 细胞穿透肽和疫苗应用.已经显示细胞穿透肽促进抗原表位递送至抗原呈递细胞(诸如树突状细胞)的细胞质中,从而能够分别以I类或II类主要组织相容性复合物(MHC)依赖性方式进行抗原加工并呈递至CD8和CD4T细胞并且引发有效免疫反应(Brooks等,细胞穿透肽:在疫苗递送中的应用(Cell-penetrating peptides:Application in vaccine delivery),Biochimica et Biophysica Acta 1805:25-34,2010)。已经显示利用弗林蛋白酶(furin)敏感性连接子用于与抗原表位偶联的CPP当与含有CpG基序的寡脱氧核苷酸一起施用时有效引发抗原特异性细胞溶解CD8T细胞反应(Lu等特洛伊抗原对CTL表位的TAP非依赖性呈递(TAP-independent presentation of CTL epitopes by Trojan antigens),J.Immunol.166:7063-7071,2001)。允许在抗原呈递细胞中释放抗原货物的其它偶联方法包括使用由细胞质谷胱甘肽裂解的二硫键联(Kim等通过偶联至HIV tat肽将可溶性蛋白质引入I类MHC路径中(Introduction of soluble proteins into the MHC class I pathway by conjugation to an HIV tat peptide),J.Immunol.159:1666-68,1997)以及使用经过磺基琥珀酰亚胺基4-马来酰亚胺基甲基环己烷甲酸酯(SMCC)处理的抗原肽、继而与C端半胱氨酸修饰的CPP反应(Apostolopoulos等使用穿透素将肿瘤相关抗原递送至抗原呈递细胞诱导强效免疫反应(Delivery of tumor associated antigens to antigen presenting cells using penetratin induces potent immune responses),Vaccine 24:3191-202,2006)。不含连接子的CPP融合蛋白质也已经成功地用于产生对肿瘤抗原的特异性T细胞反应(Batchu等用于骨髓瘤的基于NY-ESO-1的免疫疗法的树突状细胞的蛋白质转导(Protein transduction of dendritic cells for NY-ESO-1-based immunotherapy of myeloma),Cancer Res.65:10041-49,2005;Scheller等人类巨细胞病毒蛋白pp65:进入人类树突状细胞中的有效蛋白质载体系统(Human cytomegalovirus protein pp65:an efficient protein carrier system into human dendritic cells),Gene Ther.15:318-325,2008;Viehl等Tat乳腺珠蛋白融合蛋白质转导的树突状细胞刺激乳腺珠蛋白特异性CD4和CD8T细胞(Tat mammaglobin fusion protein transduced dendritic cells

stimulate mammaglobin-specific CD4and CD8T cells),Breast Cancer Res.Treat.91:271-78,2005)。

[0568] 传统上,疫苗已经依赖于通常为toll样受体(TLR)的配体的佐剂以使抗原靶向树突状细胞用于增强免疫反应。这种方法在疫苗的情况下并不很好地工作以预防或改善结核病中的结果。一个原因可能是II类MHC依赖性抗原呈递的TLR2依赖性抑制,也已经报道这是允许巨噬细胞中的分枝杆菌的持续感染的重要机制(Harding,C.V和Boom,W.H.,结核分枝杆菌对抗原呈递的调控:Toll样受体的卷形结构(Regulation of antigen presentation by Mycobacterim tuberculosis:a roll for Toll-like receptors).Nat Rev Microbiol.8(4):296-307,2010)。因此,CPP的使用可以促进有效的树突状细胞抗原加工和呈递并且避免TLR2促效剂的这种不合需要的作用,而许多当前的结核病疫苗候选物,诸如依赖于腺病毒载体(Appledorn等腺病毒载体诱导的先天发炎性介体、MAPK信号传导以及适应性免疫反应取决于体内TLR2与TLR9(Adenovirus Vector-Induced Innate Inflammatory Mediators,MAPK Signaling,As Well As Adaptive Immune Responses Are Dependent upon Both TLR2and TLR9In Vivo).J Immunol 181:2134-44,2008)和佐剂的那些并不如此(Frick,M.结核病疫苗管线(The Tuberculosis Vaccines Pipeline),2013年6月http://www.pipelinereport.org/2013/tb-vaccine以及Rowland,R.和McShane.Expert Rev Vaccines 10(5):645-58,2011)。

[0569] 在一个疫苗实施方案中,偶联物包含第一区,具有例如SEQ ID No.1-19中的任一者或其变体或部分所描述的序列的人类同源域或其变体或部分,偶联至与第一区并不天然缔合的第二区,其中第二区是肽抗原或作为抗原表位的抗原部分。在多种实施方案中,偶联可以呈融合蛋白质形式(肽键联)或可以利用连接子,诸如弗林蛋白酶敏感性连接子(RVKR)、二硫键联、或本领域中已知的其它类型的蛋白质-蛋白质偶联。

[0570] 结核病疫苗抗原85A和其表位.抗原85复合物是分枝杆菌中编码几种密切相关的主要分泌蛋白质的多基因家族。信号肽在成熟Ag85蛋白质中裂解。Ag85A是具有35.7kDa的分子质量和6.5的预测pI的338氨基酸肽。其也被称为细胞外α-抗原并且具有在细胞壁合成中重要的分枝菌酸转移酶活性。在使用重组腺病毒载体,重组Ag85A本身或作为一组重叠15聚体肽(单一Balb/c H-2D限制表位)对小鼠进行鼻内和全身免疫之后,Balb/c小鼠中较小肽表位的免疫原性和保护活性的分析(Tchilian等使用结核分枝杆菌抗原85A的不同制剂免疫诱导具有不同特异性和保护功效的免疫反应(Immunization with different formulations of Mycobacterium tuberculosis antigen 85A induces immune responses with different specificity and protective efficacy).Vaccine 31:4624-31,2013)展示在鼻内免疫之后对结核分枝杆菌(Mtb)激发的极佳保护。CD485A99-118和CD885A70-78反应是保护性的,而对CD885A145-152的反应没有保护性。

[0571] 使用腺病毒Ag85A疫苗以增强BCG疫苗在婴儿中的有效性的人类疫苗试验仅诱导适度的免疫反应并且不具有任何临床功效(Tameris等新结核病疫苗MVA85A在先前用BCG接种疫苗的婴儿中的安全性和功效:随机化安慰剂对照2b期试验(Safety and efficacy of MVA85A,a new tuberculosis vaccine,in infants previously vaccinated with BCG:a randomised,placebo-controlled phase 2b trial).The Lancet 381:1021-1028,2013)。这可以归因于所示的腺病毒组分的TLR2促效剂特性对引发下调免疫反应的IL-10反应的抑

制作用或归因于对一些Ag85A表位的非保护反应。

[0572] 结核病疫苗.保护性人类结核病疫苗的本发明实施方案是通过吸入、鼻内或肠道外注射途径施用的偶联物或偶联物混合物。在一个实施方案中,吸入途径在肺中提供局部免疫性。

[0573] 在Balb/c小鼠中,将测试偶联物或偶联物混合物由以下组成的概念:第一区,包括(但不限于)SEQ ID No.1-19中的任一者或其变体或部分所描述的序列的人类同源域或其变体或部分,偶联至与第一区并不天然缔合的第二区。在某些实施方案中,第二区是跨越Ag85A 99-118表位的四个肽中的一者或多者(参看表3):

[0574] Ag85A 99-118表位的四个中的第一个肽是SEQ ID No.51:ETFLTSELPGWLQAN;

[0575] Ag85A 99-118表位的四个中的第二个肽是SEQ ID No.54:SELPGWLQANRHVKP;

[0576] Ag85A 99-118表位的四个中的第三个肽是SEQ ID No.57:WLQANRHVKPTGSA;并且

[0577] Ag85A 99-118表位的四个中的第四个肽是SEQ ID No.60:RHVKPTGSAVVGLSM。

[0578] 在其它实施方案中,第二区是这四个肽中的一者或多者上的变异。这些偶联物中的几个的序列ID编号示于表3中。举例来说,在一个实施方案中,Ag85A 99-118表位的组合的第一(HOX C12)和第二区SEQ ID No.51的完整氨基酸序列是SEQ ID No.52:SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLETFLTSELPGWLQAN。同样地,在另一个实施方案中,Ag85A 99-118表位的组合的第一(HOX D12)和第二区SEQ ID No.51的完整氨基酸序列是SEQ ID No.53:ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVETFLTSELPGWLQAN。

[0579] 用于在Balb/c小鼠中测试的第二偶联物或偶联物混合物由以下组成:第一区,具有SEQ ID No.1-19中的任一者或其变体或部分所描述的序列的人类同源域或其变体或部分,偶联至与第一区并不天然缔合的第二区。在某些实施方案中,第二区是跨越Ag85A 70-78表位的三个肽中的一者或多者(参看表3):

[0580] Ag85A 70-78表位的三个中的第一个肽是SEQ ID No.63:QSGLSVVMPVGGQSS;

[0581] Ag85A 70-78表位的三个中的第二个肽是SEQ ID No.66:VVMPVGGQSSFYSDW;并且

[0582] Ag85A 70-78表位的三个中的第三个肽是SEQ ID No.69:GGQSSFYSDWYQPAC。

[0583] 在其它实施方案中,第二区是这三个肽中的一者或多者上的变异。这些偶联物中的几个的序列ID编号也示于表3中。举例来说,在一个实施方案中,Ag85A 70-78表位的组合的第一(HOX C12)和第二区SEQ ID No.63的完整氨基酸序列是SEQ ID No.64:SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLLQSGLSVVMPVGGQSS。同样地,在另一个实施方案中,Ag85A 70-78表位的组合的第一(HOX D12)和第二区SEQ ID No.63的完整氨基酸序列是SEQ ID No.65:ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVVQSGLSVVMPVGGQSS。

[0584] 因此,将要在小鼠中并且稍后在人类中测试的整个疫苗由偶联物的混合物组成,其中每个偶联物在多种实施方案中具有这些较小肽或其变异中的一者或多者作为偶联物的货物,并且这些偶联物一起提供对Ag85A 99-118的稳固CD4反应和对Ag85A 70-78的稳固CD8反应。

[0585] 在一个人类疫苗实施方案中,疫苗将由以下组成:第一区,包括(但不限于)SEQ ID No.1-19中的任一者或其变体或部分所描述的序列的人类同源域或其变体或部分,偶联至

与第一区并不天然缔合的第二区，其中在一个实施方案中，第二区是整个Ag85A 338-氨基酸肽或Ag85A的HLA限制表位，诸如显示在适当人类受试者中引发免疫反应的Ag85A的一个或多个特异性HLA限制表位(参看表3)。Ag85A肽或其人类表位可以连接至如上文所描述的第一区。在另一个实施方案中，整个Ag85A序列连接至第一区以提供单一疫苗构建体用于具有不同I类和II类HLA基因型的受试者。Ag85A序列是：MQLVDRVRGA VTGMSRRLVV GAVGAALVSG LVGAVGGTAT AGAFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA。

[0586] 此处，在上文呈粗体和加下划线字体的较长序列区是当产生成熟Ag85A序列时裂解的信号肽(剩余的296氨基酸肽)。纤连蛋白结合域紧接在其下面以粗体和斜体字体示出，并且下面呈粗体、加下划线以及斜体字体的三个氨基酸在催化域中。

[0587] 在一个实施方案中，Ag85A(不含信号肽)的第二区氨基酸序列将为：AFSRPGLP VEYLQVPSPS MGRDIKVQFQ SGGANSPALY LLDGLRAQDD FSGWDINTPA FEWYDQSGLS VVMPVGGQSS FYSDWYQPAC GKAGCQTYKW ETFLTSELPG WLQANRHVKP TGSAVVGLSM AASSALTLAI YHPQQFVYAG AMSGLLDPSQ AMGPTLIGLA MGDAGGYKAS DMWGPKEDPA WQRNDPLLNV GKLIANNTRV WVYCGNGKPS DLGGNNLPAK FLEGFVRTSN IKFQDAYNAG GGHNGVFDFP DSGTHSWEYW GAQLNAMKPD LQRALGATPN TGPAPQGA。这个Ag85A货物肽的序列ID编号(SEQ.ID No.72)连同许多可能实施方案中的两者一起示于表3中，在这些实施方案中Ag85A货物肽或变异包含偶联物或融合蛋白质的第二区：含有第一区HOX C12(SEQ.ID No.73)或HOX D12(SEQ.ID No.74)序列的那些。分子生物学/生物技术领域中的一般技术人员应了解，表3中所示的这些和其它序列中的任一者上的众多变异将处于本文所公开的实施方案内。

[0588] 疫苗诱导的免疫反应的测量.使用特异性抗原的体外全血培养和I型细胞因子反应的测量成功地用于在I期临床试验中测量对腺病毒载体Ag85A疫苗的免疫反应(Smaill等基于人类5型腺病毒的结核病疫苗尽管预先存在抗腺病毒免疫性仍诱导人类中稳固的T细胞反应(A Human Type 5Adenovirus-Based Tuberculosis Vaccine Induces Robust T Cell Responses in Humans Despite Preexisting Anti-Adenovirus Immunity).Sci.Transl.Med.5,205:1-11,2013)。这种技术可以用于测量人类中对基于CPP的Ag85A表位疫苗混合物的免疫反应。在鼠类模型中，类似方法可以使用所描述的表位与脾淋巴细胞的处理后培养和Balb/c小鼠中细胞因子反应的测量一起使用。

[0589] 可以通过IFN-γELISPOT检验来定量对特异性抗原表位有反应的CD4T细胞的数目，并且可以通过细胞内细胞因子染色以及外周血淋巴细胞培养物中的T细胞亚型分类来测量CD4与CD8T细胞反应(Smaill等2013)。

[0590] 实施例7

[0591] 源自人类的CPP结构可以包括(但不限于)第一区，其可以包含HOX D12同源域、HOX C12同源域，或其它第一区结构，诸如表3中所描述的那些(SEQ ID No.1-19)。第一区可以偶联至第二区以使得第一区可以用于携带‘货物’至可能在疾病病况中异常表达的细胞内和/或核内标靶。这类标靶可以包括核酸结构，诸如基因、mRNA以及miRNA。此处，DNA、RNA、LNA、

PNA、γPNA、其它标准或修饰的核酸或者这些组分的组合可以经过设计以结合至这些细胞内和/或核内标靶并且减少疾病病况的异常表达或活化特征。表4示出了核苷酸序列，并且下文描述了三个实施例。

[0592] 表4:核苷酸序列本体

[0593]

SEQ. ID No.	描述符	核苷酸序列
75	货物所结合的miRNA132靶序列	5'-UAACAGUCUACAGCCAUGGUCG-3'
76	结合至miRNA21的货物序列	5'-TCAACATCAGTCTGATAAGCTA-3'
77	货物所结合的miRNA21靶序列	5'-TAGCTTATCAGACTGATGTTGA-3'
78	结合至miRNA132的货物序列	5'-CGACCATGGCTGTAGACTGTTA-3'
79	结合至DYRK1b的货物序列	5'-GTGGTGAAAGCCTATGATCAT-3'

[0594] 影响Ras路径:在一个或多个实施方案中，第二区货物结合至miRNA132靶序列以影响Ras路径。

[0595] Ras路径在许多类型的癌症中异常表达并且影响这个路径的构建体将减少依赖于其的肿瘤的生长。诊断测试将鉴别肿瘤将最有可能对这种处理有反应的那些患者。已知具有活化的Ras路径的肿瘤细胞系可以用于展示下文所描述的Ras抑制构建体的生物活性。其将减少体外这些肿瘤细胞的生长并且在全身处理之后减少使用这些细胞的鼠类异种移植模型中肿瘤的生长。

[0596] 在一个实施方案中，HOX C12氨基酸序列第一区是SEQ ID No.1：

[0597] SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL

[0598] 在另一个实施方案中，HOX D12氨基酸序列第一区是SEQ ID No.2：

[0599] ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV

[0600] 在另一个实施方案中，第二区货物可以是例如核酸SEQ ID No.76(表4)(或其变异)：

[0601] 5'-TCAACATCAGTCTGATAAGCTA-3'。在另一个实施方案中，例如，完整偶联物包含SEQ ID No.1和76。

[0602] 影响许多癌症中的肿瘤抑制路径:第二区货物结合至miRNA21靶序列以影响许多类型的癌症中所发现的肿瘤抑制路径。如在上文所描述的Ras路径实施例中，在多种实施方案中，第一区同源域序列可以是例如HOX C12(SEQ ID No.1)或HOX D12(SEQ ID No.2)(参看表3)。在一个或多个实施方案中，偶联物的第二区货物结合至miRNA21靶序列，这个序列包括(但不限于)SEQ.ID No.77(表4)或其任何变异。因此，在一个实施方案中，第二区货物，例如5'-CGACCATGGCTGTAGACTGTTA-3'(SEQ.ID No.76)，结合至miRNA21靶序列，例如5'-TAGCTTATCAGACTGATGTTGA-3'(SEQ.ID No.77)，以影响许多类型的癌症中所发现的肿瘤抑制路径。

[0603] 在体外和鼠类异种移植肿瘤模型中测试这些构建体可以通过测量减少的肿瘤细胞生长和肿瘤生长来确认其活性。发现有具有这种miRNA的异常表达的肿瘤的癌症患者的

处理将具有临床效益。在一个实施方案中，第二区货物序列(SEQ.ID No.79)结合至靶序列DYRK1b。在其它实施方案中，针对SEQ.ID No.79的变异结合至DYRK1b或其功能变异。

[0604] 影响基因表达：在一个或多个实施方案中，第二区货物结合至DYRK1b以减少基因表达。

[0605] 这种基因的过度表达存在于许多癌症中，包括肺癌和胰腺癌。使得表达增加的DYRK1b中的突变也与II型糖尿病、中心性肥胖以及早期冠状动脉疾病的家族遗传、代谢综合症的所有特征相关联。这个路径可能还促成一般群体中II型糖尿病和肥胖的发病。因此，使用DYRK1b的抑制剂，诸如下文所描述的那些进行全身处理将在具有异常高的DYRK1b表达的癌症中以及具有II型糖尿病和代谢综合症的其它特征的患者中具有功效。其将在具有导致过度表达的DYRK1b中的所鉴别的突变的那些患者中尤其有效。如在Ras和肿瘤抑制路径、上文所论述的实施例、多种实施方案中，第一区同源域序列可以是例如HOX C12(SEQ ID No.1)或HOX D12(SEQ ID No.2)(参看表3)。在一个实施方案中，序列5'-GTGGTGAAAGCCTATGATCAT-3'(SEQ.ID No.79，参看表4)或其变异结合至DYRK1b。在其它实施方案中，针对SEQ.ID No.79的变异结合至DYRK1b或其功能变异。

[0606] 对于上述实施例中所描述的研究中报道的组之间的某些比较，在$p<0.05$下测试显著性。

[0607] 从前述内容应了解，出于说明的目的在本文中已经描述了特定实施方案，但在不脱离本公开的精神和范围的情况下可以作出各种修改。因此，本公开除了受随附权利要求书限制以外不受限制。

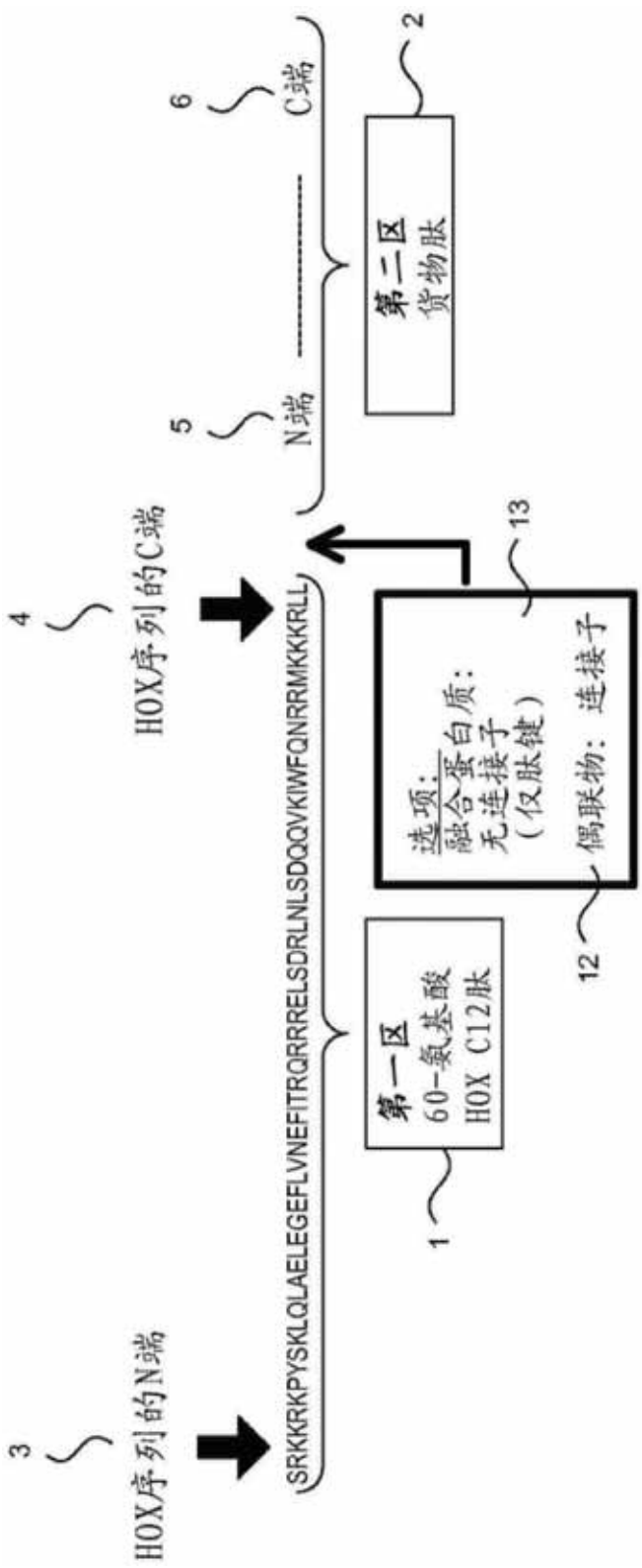

3
HOX序列的N端
SRKKRKPYSKLQLAELEGEFLVNEFITRQRRRELSDRLNLSDQQVKIWFQNRRMKKKRLL
4
HOX序列的C端
5
N端
6
C端
第一区
60-氨基酸
HOX C12肽
1
第二区
货物肽
2
选项：
融合蛋白质：
无连接子
（仅肽键）
偶联物：连接子
12
13

图1

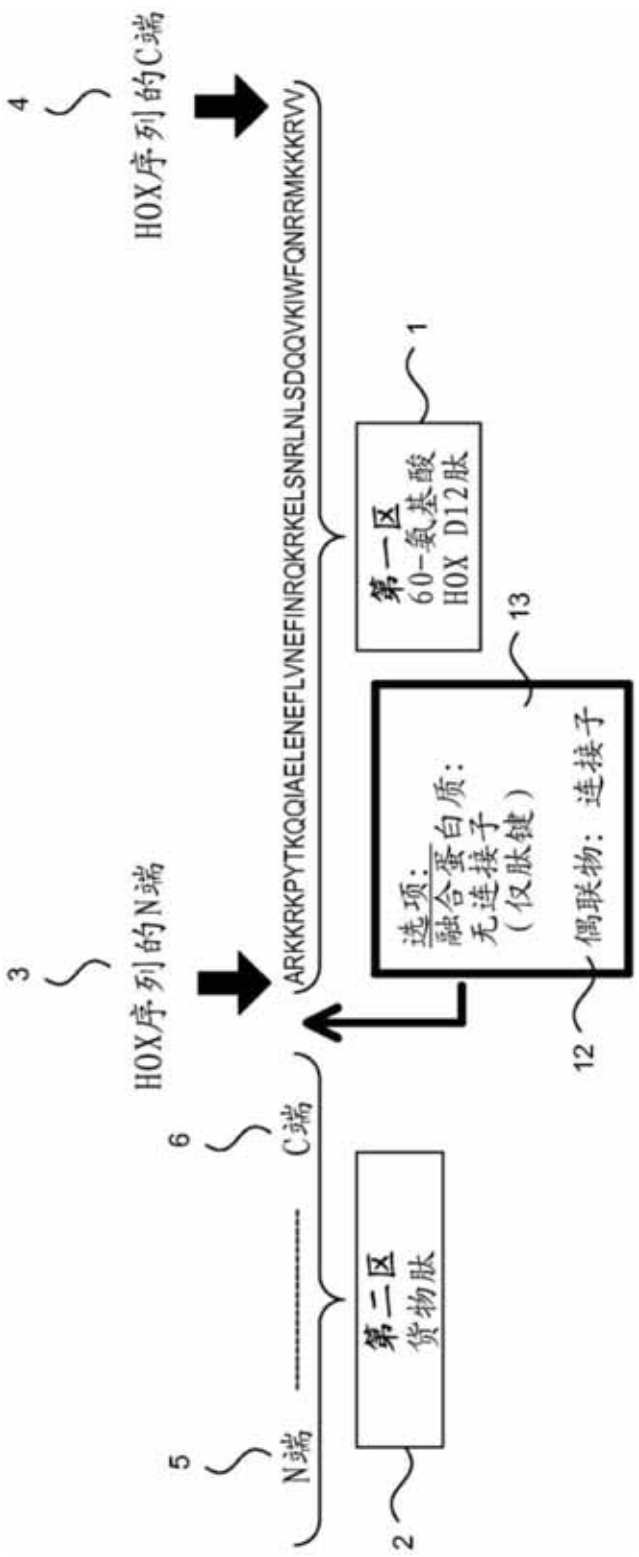

4
HOX序列的C端
3
HOX序列的N端
ARKKRKPYTKQQIAELENEFLVNEFINRQKRKELSNRLNLSDQQVKIWFQNRRMKKKRVV
1
第一区
60-氨基酸
HOX D12肽
13
选项:
融合蛋白质:
无连接子
(仅肽键)
偶联物: 连接子
12
6
C端
5
N端
2
第二区
货物肽

图2

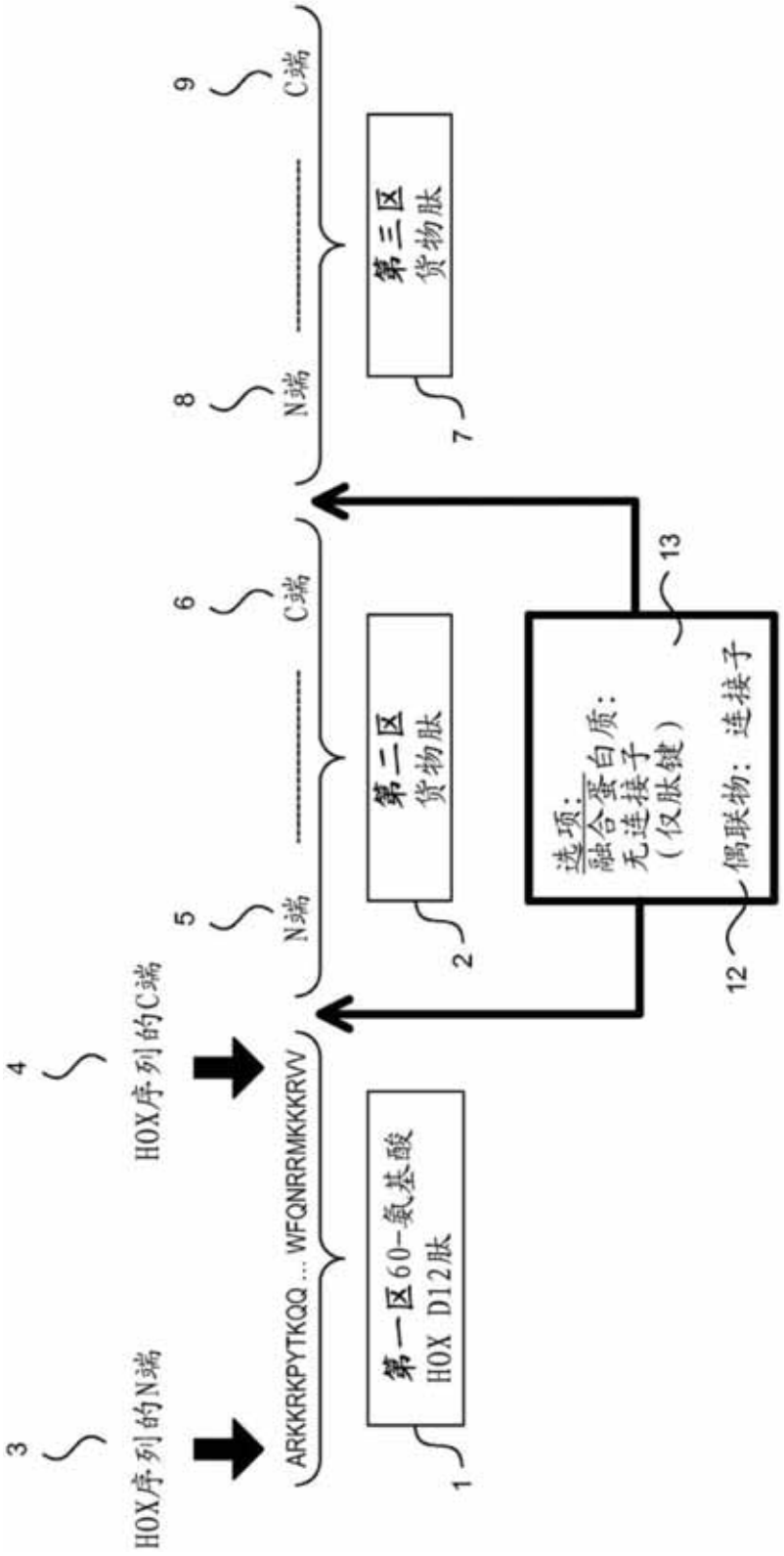

3
HOX序列的N端
4
HOX序列的C端
ARKKRKPYTKQQ ... WFQNRRMKKKRVV
第一区60-氨基酸
HOX D12肽
1
5
N端
6
C端
第二区
货物肽
2
8
N端
9
C端
第三区
货物肽
7
选项:
融合蛋白质:
无连接子
(仅肽键)
13
偶联物: 连接子
12

图3A

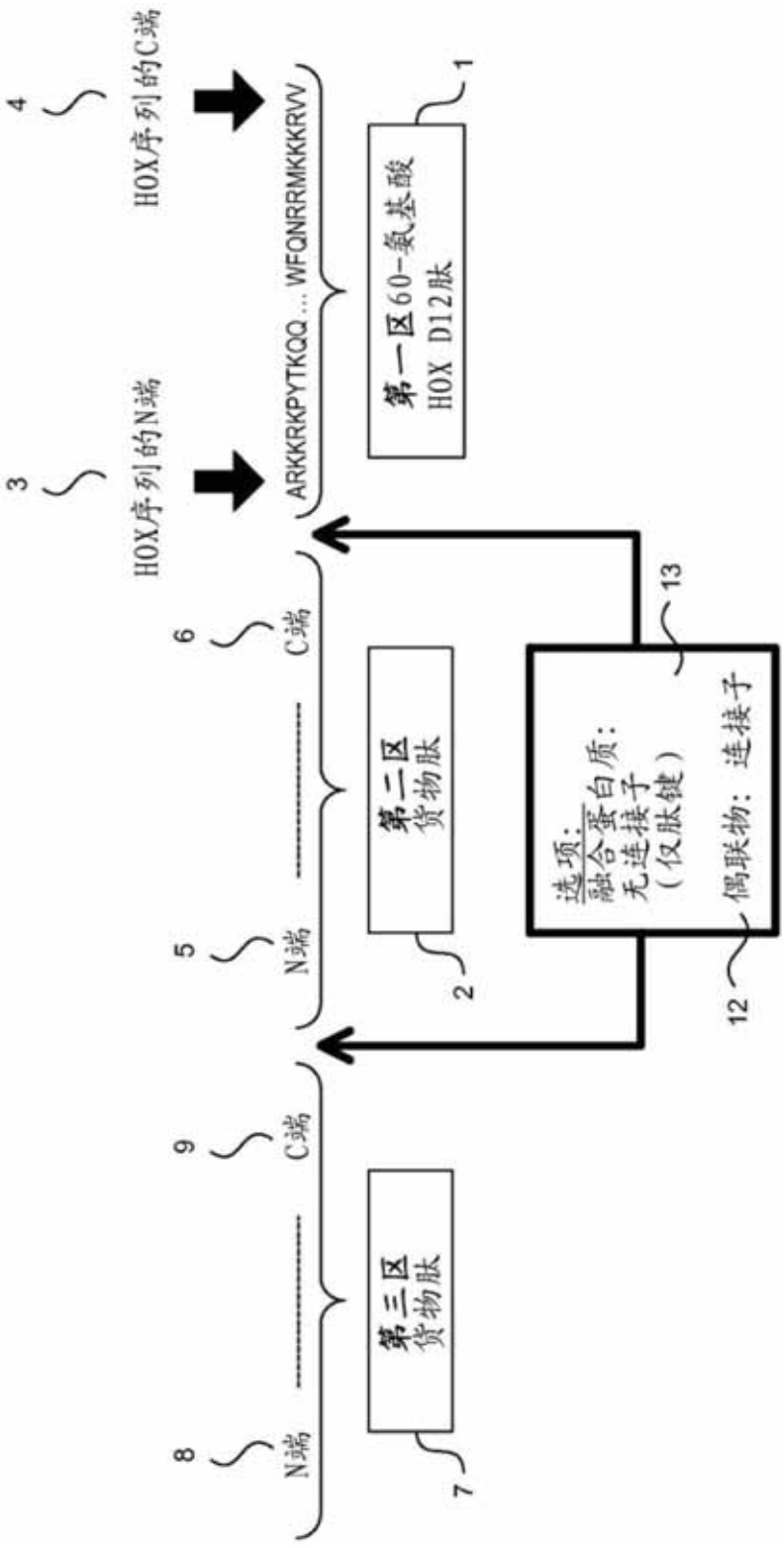

4
HOX序列的C端
3
HOX序列的N端
ARKKRKPYTKQQ ... WFQNRRMKKKRVV
1
第一区60-氨基酸
HOX D12肽
6
C端
5
N端
第二区
货物肽
2
选项:
融合蛋白质:
无连接子
(仅肽键)
13
偶联物:连接子
12
9
C端
8
N端
第三区
货物肽
7

图3B

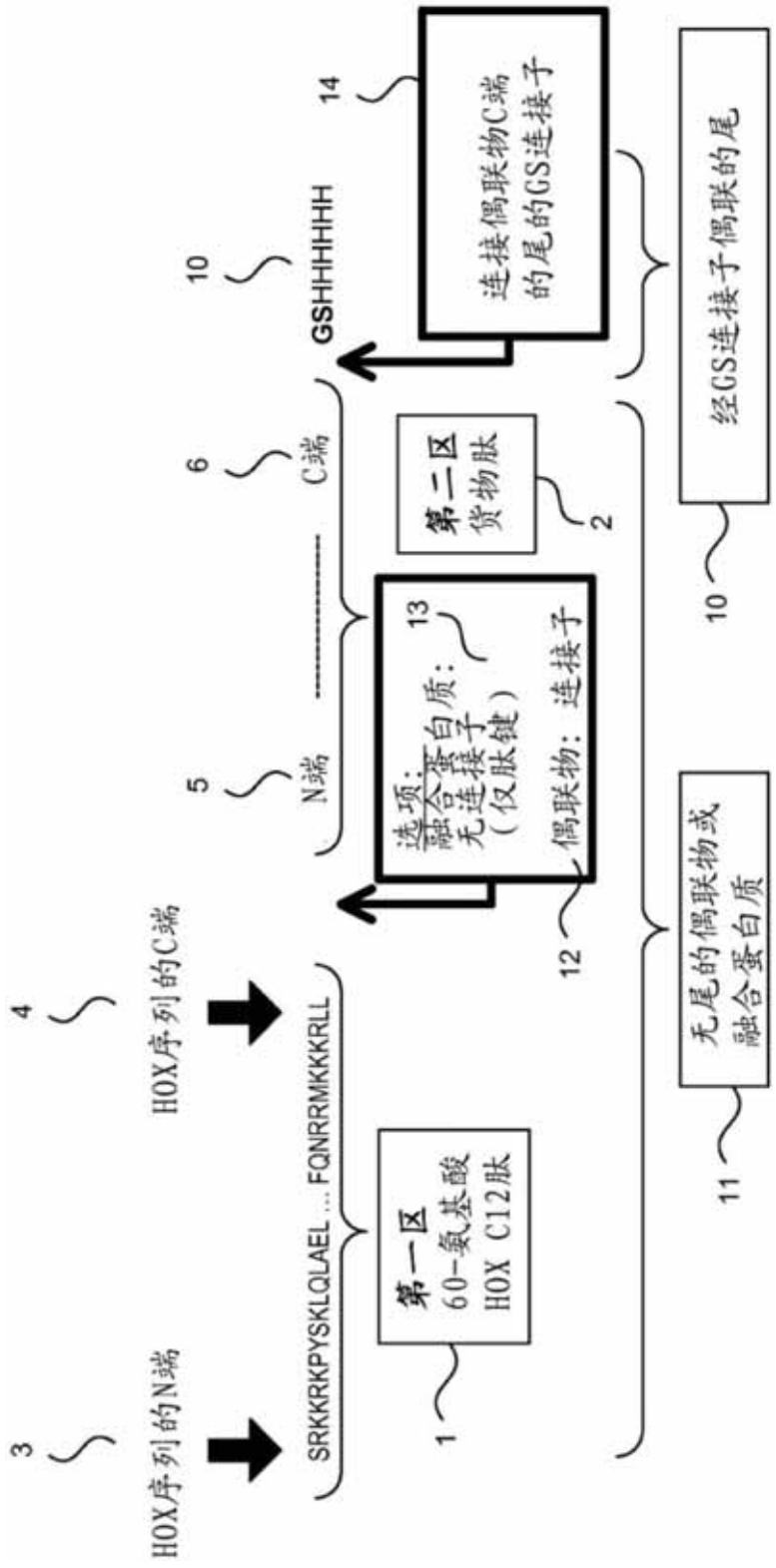

3
HOX序列的N端
4
HOX序列的C端
SRKKRKPYSKLQLAEL ... FQNRRMKKKRLL
5
N端
6
C端
10
GSHHHHHH
14
连接偶联物C端的尾的GS连接子
第一区
60-氨基酸
HOX C12肽
1
选项：融合蛋白质：无连接子（仅肽键）
13
偶联物：连接子
12
第二区
货物肽
2
经GS连接子偶联的尾
10
无尾的偶联物或融合蛋白质
11

图4

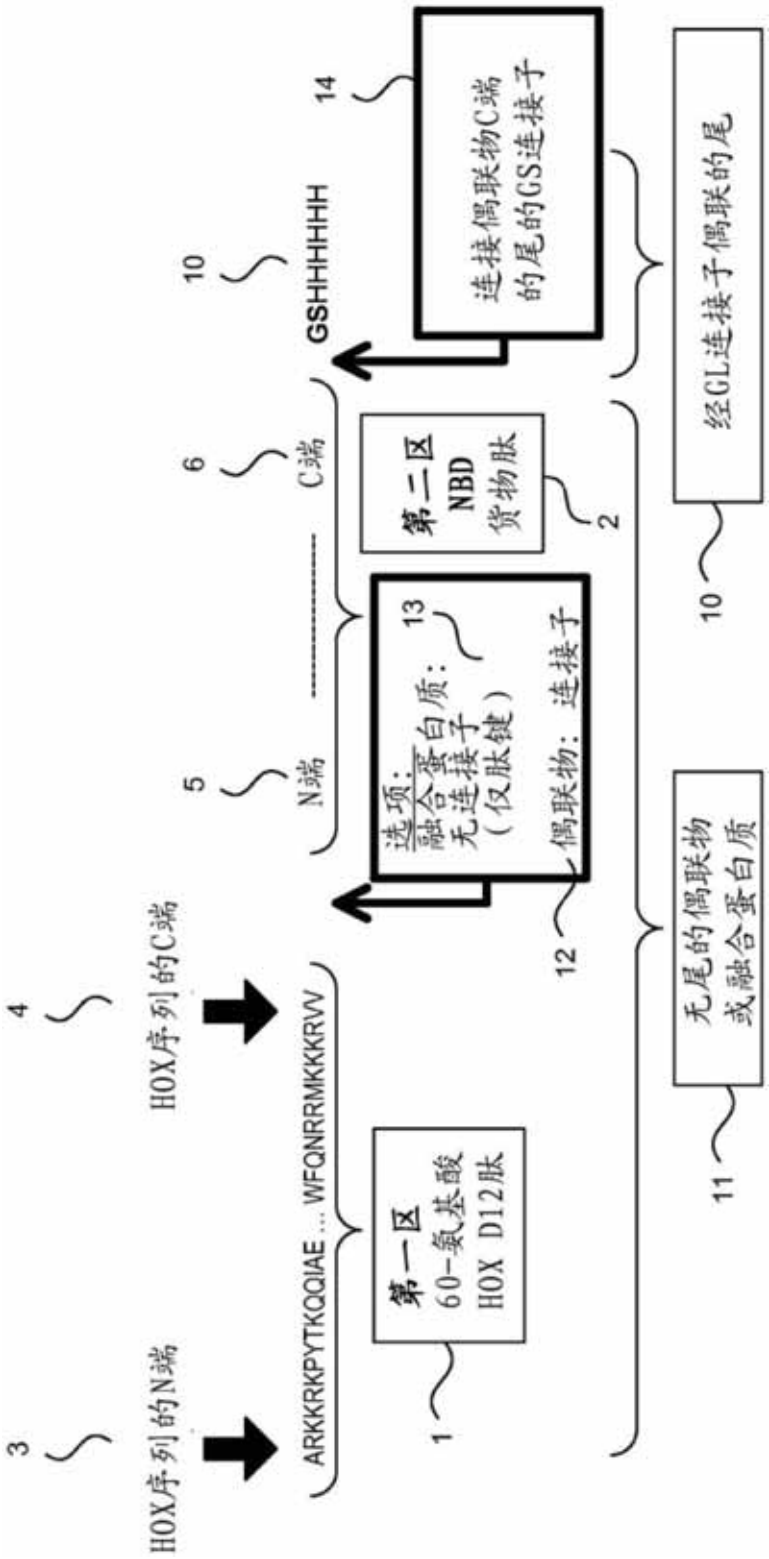

3
HOX序列的N端
4
HOX序列的C端
ARKKRKPYTKQQIAE ... WFQNRRMKKKRVV
1
第一区
60-氨基酸
HOX D12肽
5
N端
6
C端
10
GSHHHHHH
14
连接偶联物C端
的尾的GS连接子
第二区
NBD
货物肽
2
选项:
融合蛋白质:
无连接子
(仅肽键)
13
偶联物: 连接子
12
经GL连接子偶联的尾
10
无尾的偶联物
或融合蛋白质
11

图5

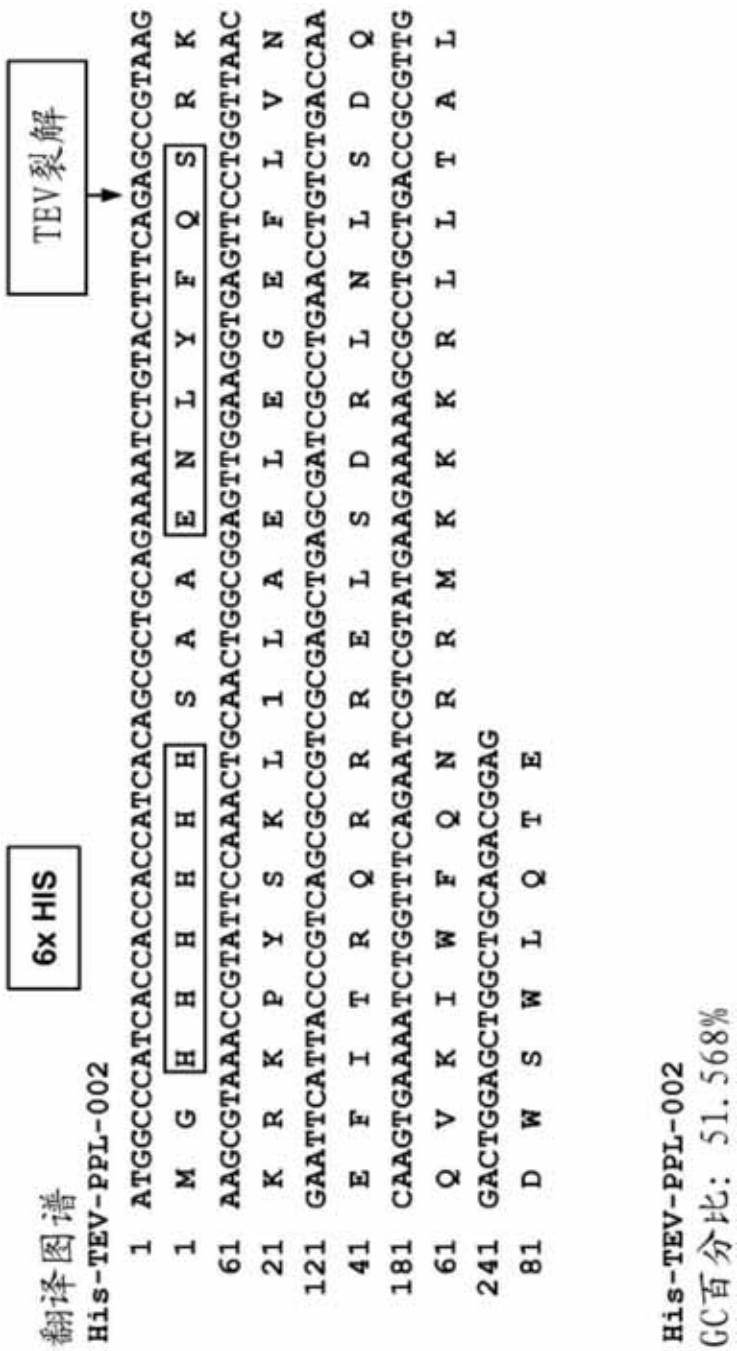

翻译图谱
His-TEV-PPL-002
6x HIS
TEV裂解
1 ATGGCCCATCACCACCACCATCACAGCGCTGCAGAAAATCTGTACTTTCAGAGCCGTAAG
1 M G H H H H H H S A A E N L Y F Q S R K
61 AAGCGTAAACCGTATTCCAAACTGCAACTGGCGGAGTTGGAAGGTGAGTTCCTGGTTAAC
21 K R K P Y S K L 1 L A E L E G E F L V N
121 GAATTCATTACCCGTCAGCGCCGTCGCGAGCTGAGCGATCGCCTGAACCTGTCTGACCAA
41 E F I T R Q R R R E L S D R L N L S D Q
181 CAAGTGAAAATCTGGTTTCAGAATCGTCGTATGAAGAAAAAGCGCCTGCTGACCGCGTTG
61 Q V K I W F Q N R R M K K K R L L T A L
241 GACTGGAGCTGGCTGCAGACGGAG
81 D W S W L Q T E
His-TEV-PPL-002
GC百分比: 51.568%

图6

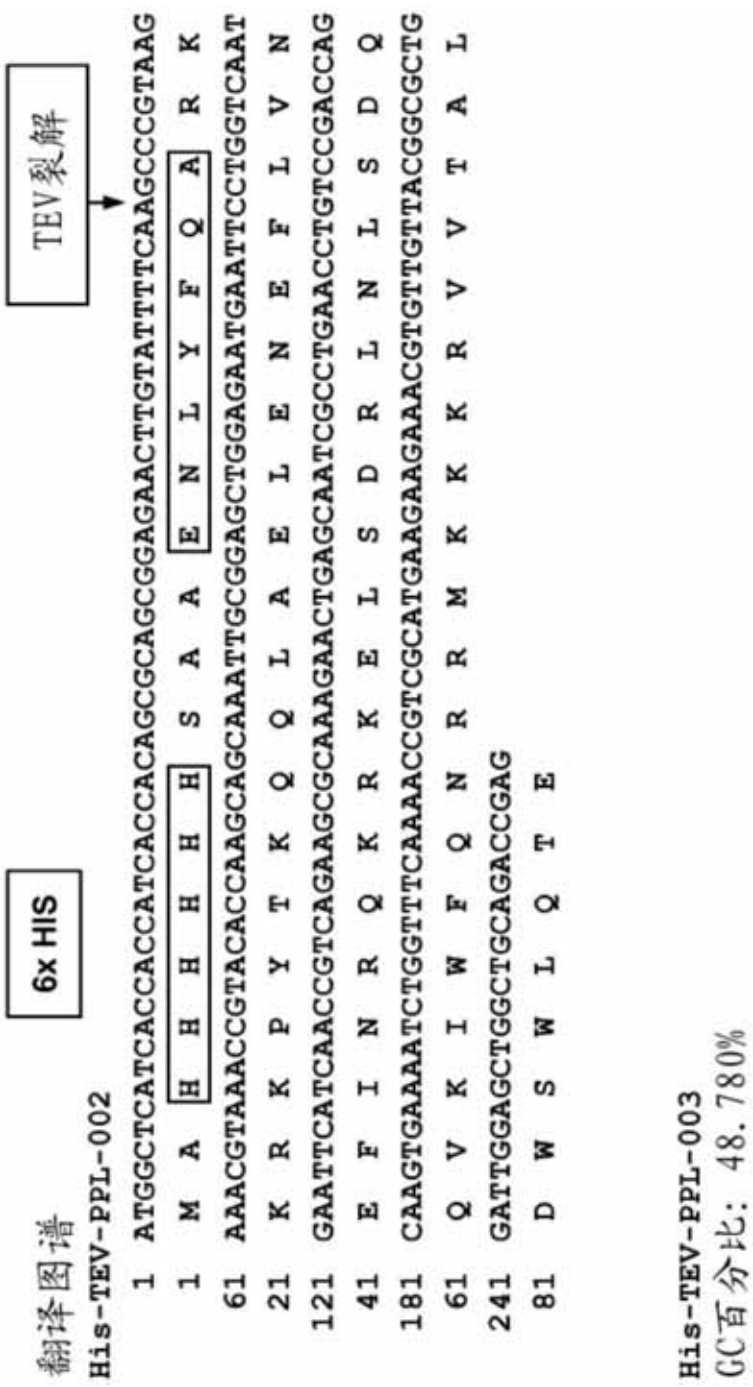

翻译图谱
6x HIS
TEV裂解
His-TEV-PPL-002
1 ATGGCTCATCACCACCATCACCACAGCGCAGCGGAGAACTTGTATTTTCAAGCCCGTAAG
1 M A H H H H H H S A A E N L Y F Q A R K
61 AAACGTAAACCGTACACCAAGCAGCAAATTGCGGAGCTGGAGAATGAATTCCTGGTCAAT
21 K R K P Y T K Q Q L A E L E N E F L V N
121 GAATTCATCAACCGTCAGAAGCGCAAAGAACTGAGCAATCGCCTGAACCTGTCCGACCAG
41 E F I N R Q K R K E L S D R L N L S D Q
181 CAAGTGAAAATCTGGTTTCAAAACCGTCGCATGAAGAAACGTGTTGTTACGGCGCTG
61 Q V K I W F Q N R R M K K K R V V T A L
241 GATTGGAGCTGGCTGCAGACCGAG
81 D W S W L Q T E
His-TEV-PPL-003
GC百分比: 48.780%

图7

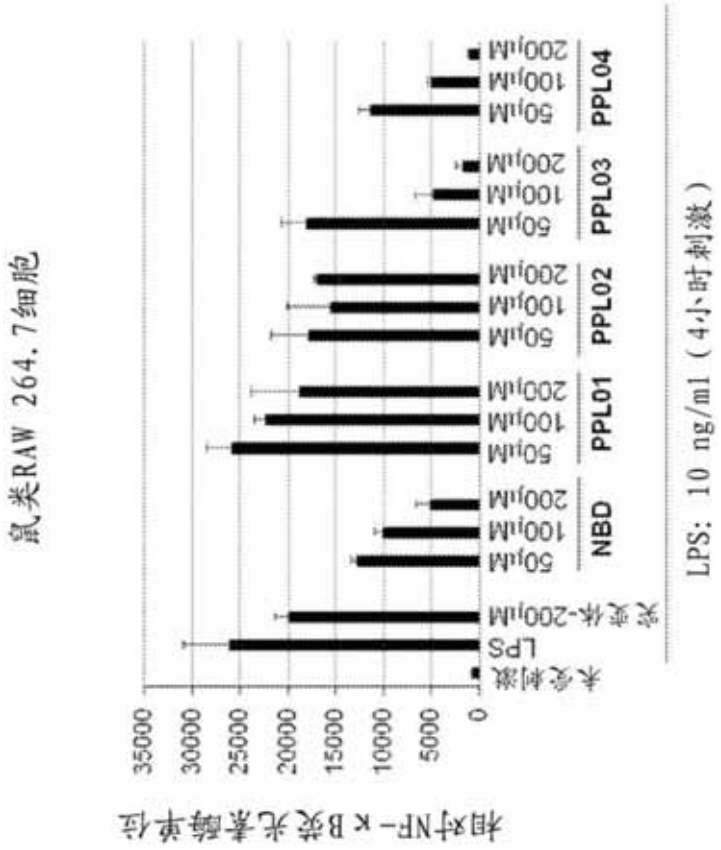

鼠类RAW 264.7细胞
相对NF-κB荧光素酶单位
35000
30000
25000
20000
15000
10000
5000
0
未受刺激
LPS
荧光体-200μM
NBD
50μM
100μM
200μM
PPL01
50μM
100μM
200μM
PPL02
50μM
100μM
200μM
PPL03
50μM
100μM
200μM
PPL04
50μM
100μM
200μM
LPS: 10 ng/ml (4小时刺激)

图8A

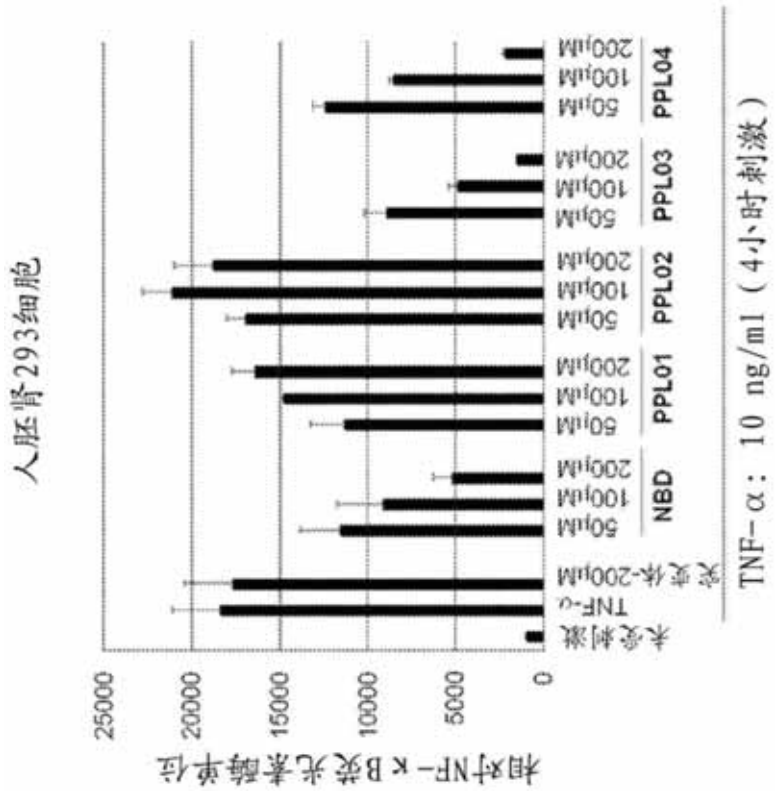

人胚肾293细胞
相对NF-κB荧光素酶单位
25000
20000
15000
10000
5000
0
未受刺激
TNF-α
受体体-200μM
NBD
50μM
100μM
200μM
PPL01
50μM
100μM
200μM
PPL02
50μM
100μM
200μM
PPL03
50μM
100μM
200μM
PPL04
50μM
100μM
200μM
TNF-α：10 ng/ml（4小时刺激）

图8B

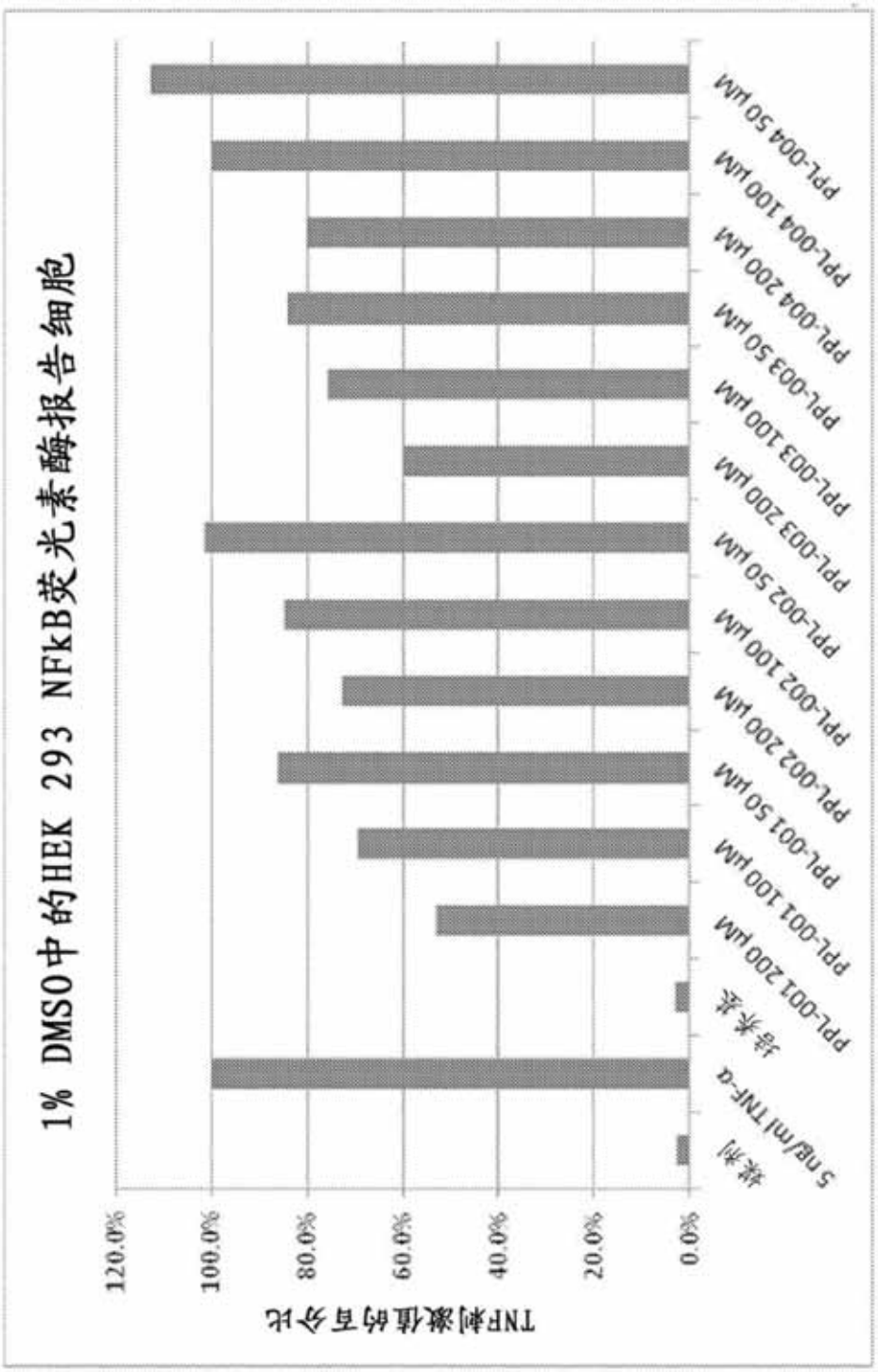

1% DMSO中的HEK 293 NFkB荧光素酶报告细胞
TNF刺激值的百分比
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
媒剂
5 ng/ml TNF-α
培养基
PPL-001 200 μM
PPL-001 100 μM
PPL-001 50 μM
PPL-002 200 μM
PPL-002 100 μM
PPL-002 50 μM
PPL-003 200 μM
PPL-003 100 μM
PPL-003 50 μM
PPL-004 200 μM
PPL-004 100 μM
PPL-004 50 μM

图9


-30 min
0 min
+120 min
腹膜内处理
LPS激发
末端放血
（细胞因子）

图10

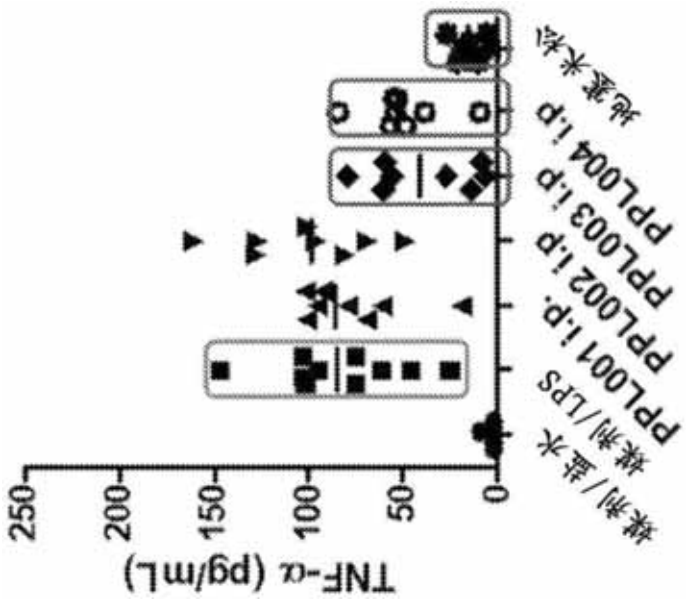

载体未处理
PPL004 i.p.
PPL003 i.p.
PPL002 i.p.
PPL001 i.p.
媒剂/LPS
媒剂/盐水
250
200
150
100
50
0
TNF-α (pg/mL)

图11A

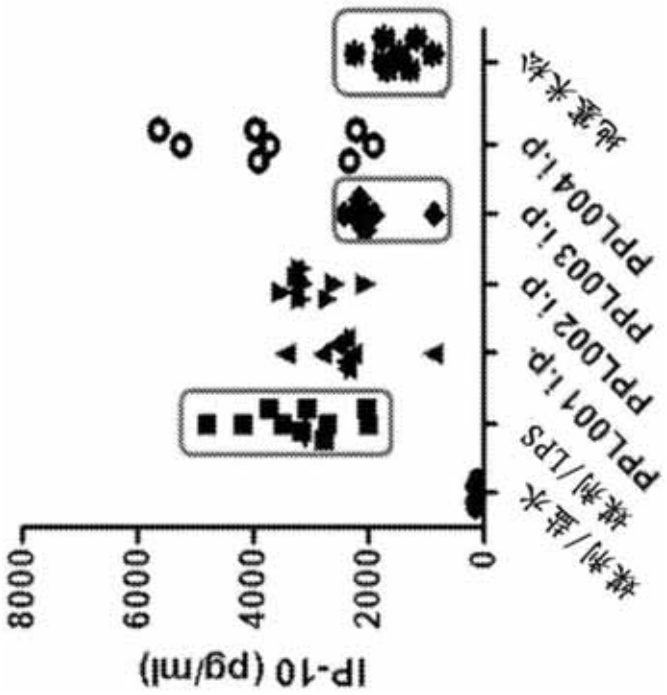

IP-10 (pg/ml)
8000
6000
4000
2000
0
媒剂/盐水
媒剂/LPS
PPL001 i.p
PPL002 i.p
PPL003 i.p
PPL004 i.p
地塞米松

图11B

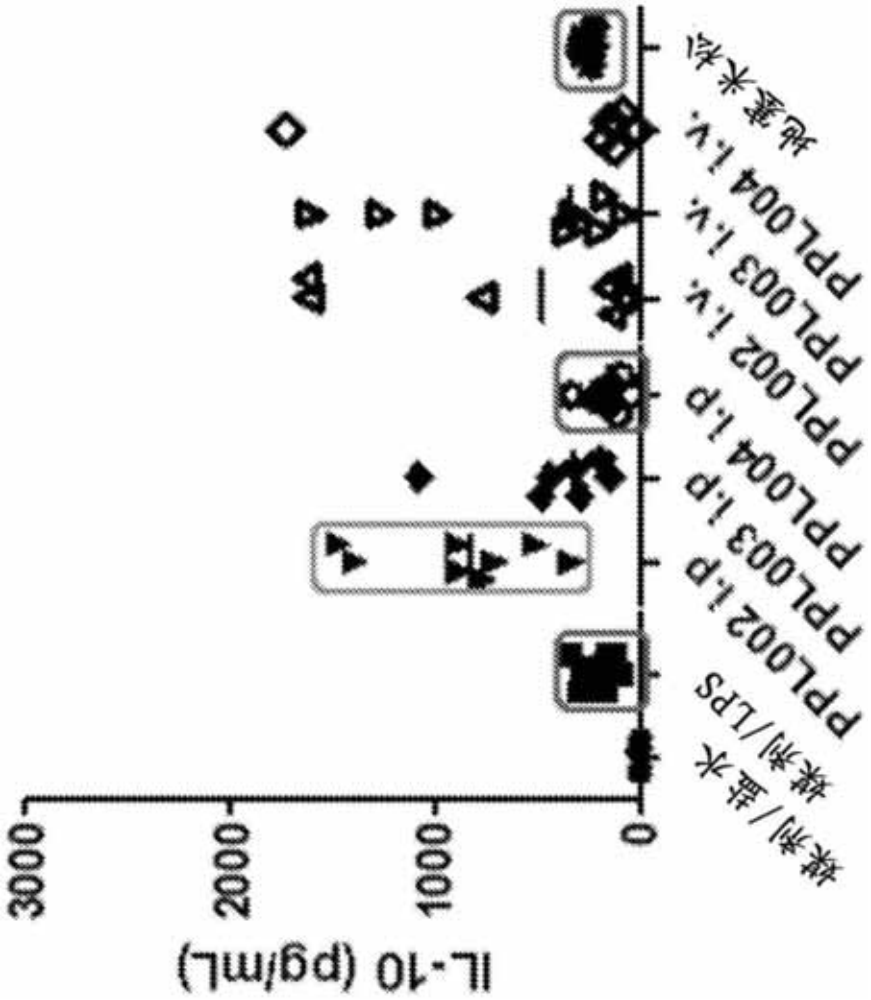

IL-10 (pg/mL)
3000
2000
1000
0
媒剂/盐水
媒剂/LPS
PPL002 i.p
PPL003 i.p
PPL004 i.p
PPL002 i.v.
PPL003 i.v.
PPL004 i.v.
地塞米松

图12

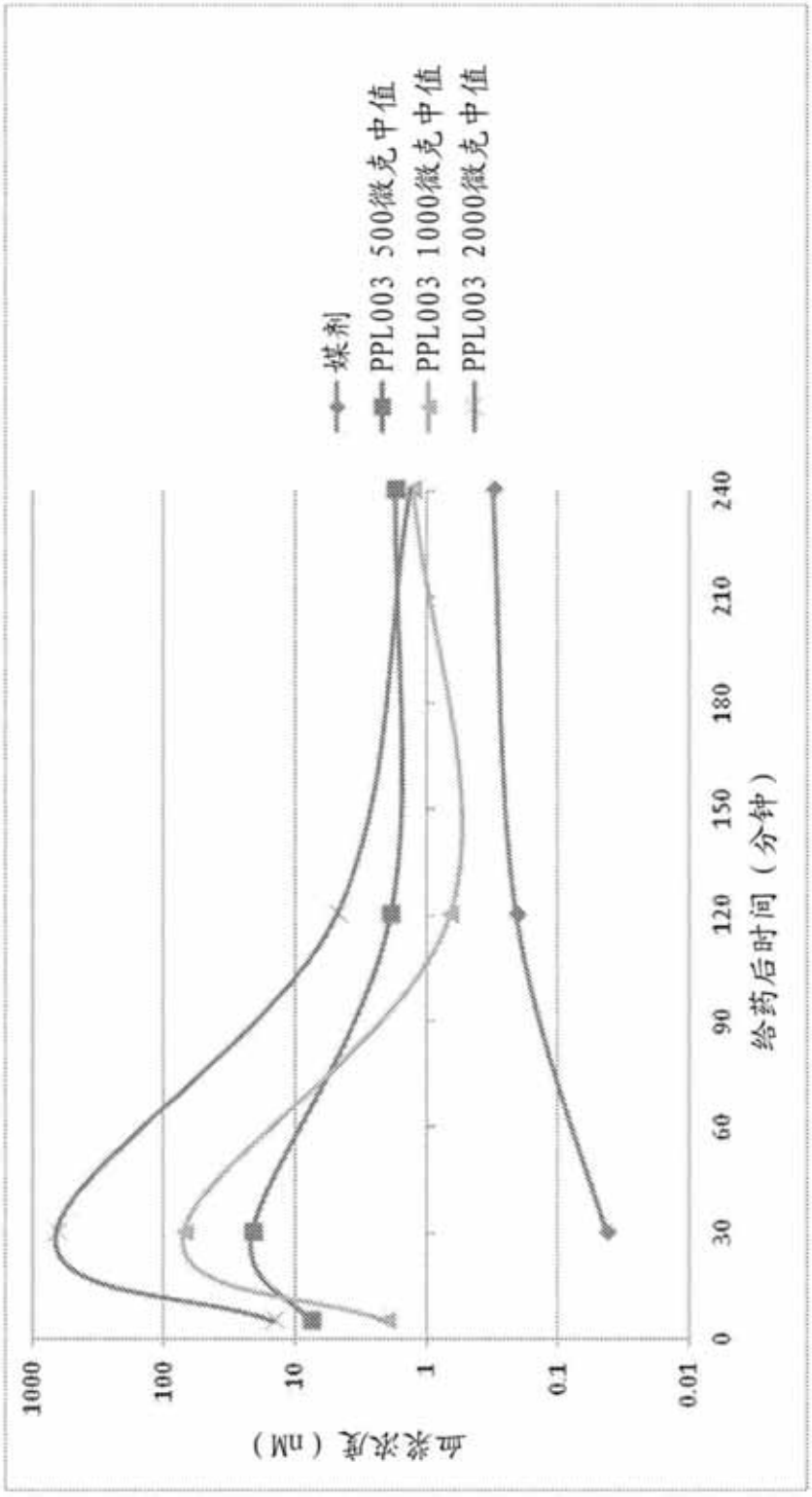

媒剂
PPL003 500微克中值
PPL003 1000微克中值
PPL003 2000微克中值
血浆浓度（nM）
1000
100
10
1
0.1
0.01
0
30
60
90
120
150
180
210
240
给药后时间（分钟）

图13

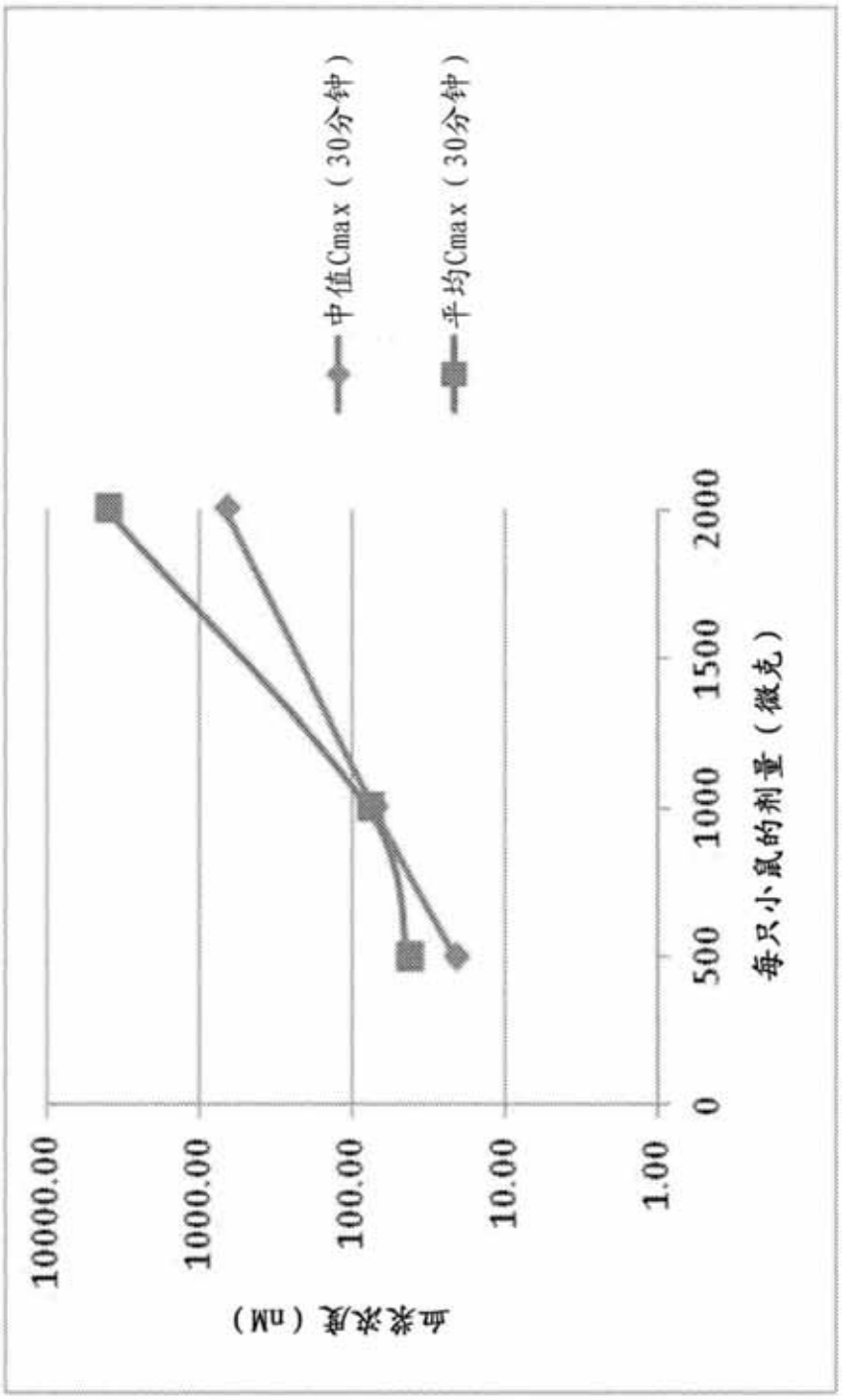

中值Cmax（30分钟）
平均Cmax（30分钟）
血浆浓度（nM）
10000.00
1000.00
100.00
10.00
1.00
0
500
1000
1500
2000
每只小鼠的剂量（微克）

图14

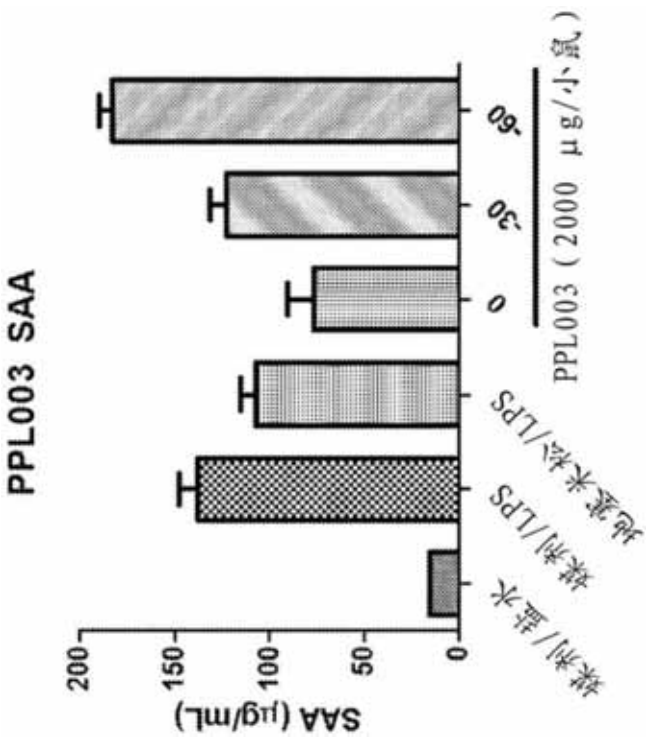


图15A

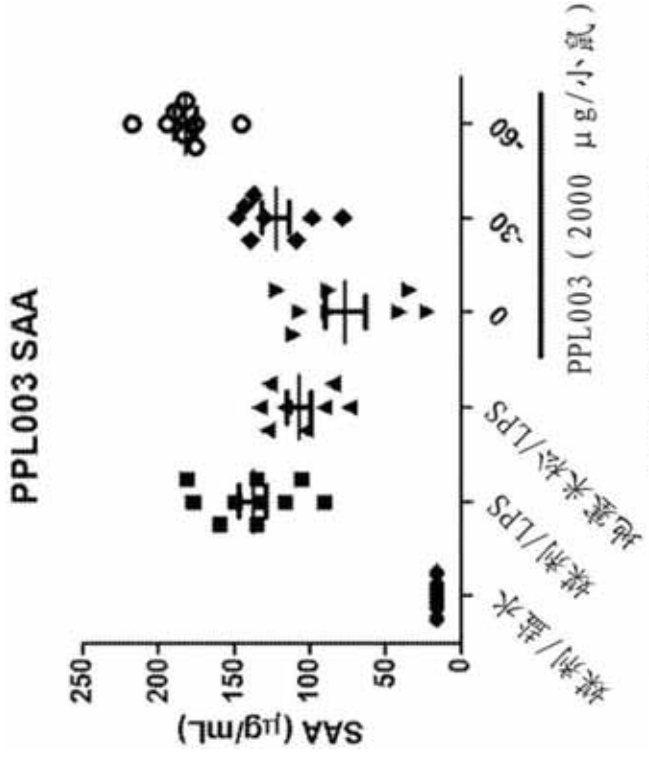


图15B

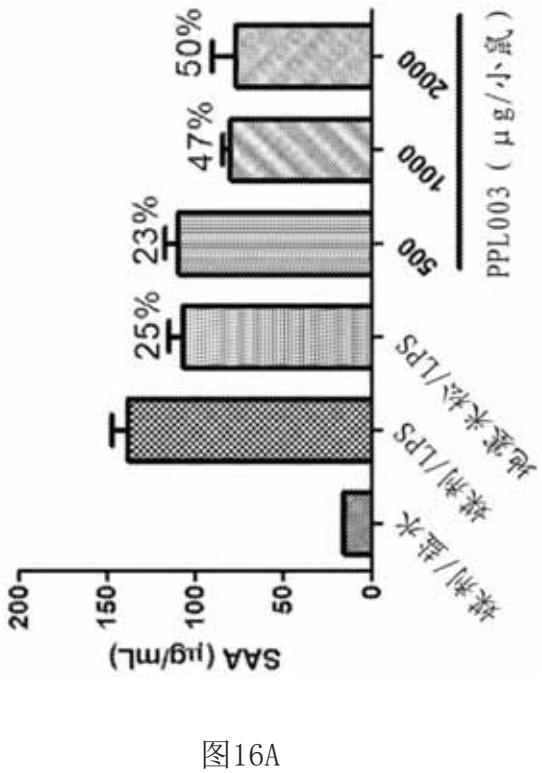


图16A

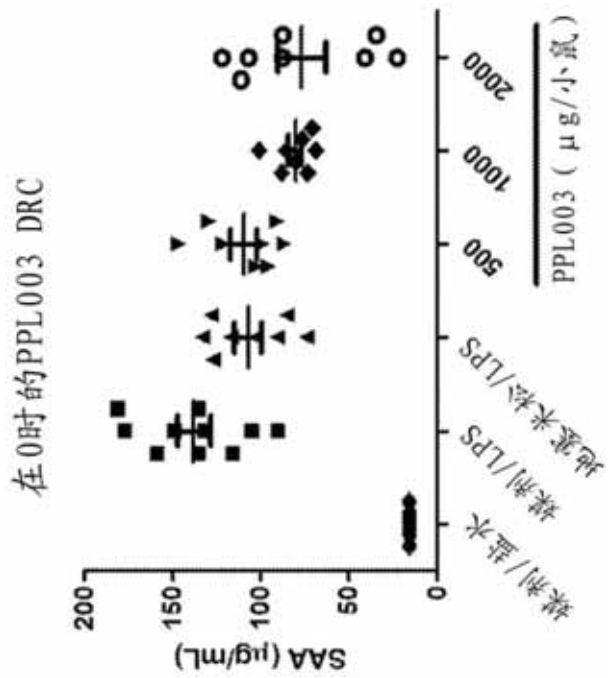

在0时的PPL003 DRC
SAA (μg/mL)
200
150
100
50
0
媒剂/盐水
媒剂/LPS
地塞米松/LPS
500
1000
2000
PPL003 (μg/小鼠)

图16B

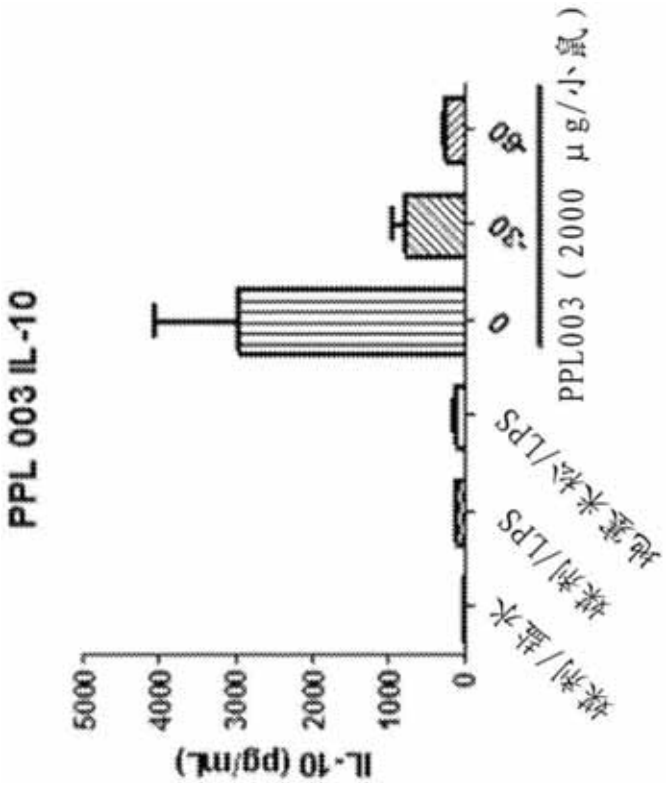

PPL 003 IL-10
IL-10 (pg/mL)
5000
4000
3000
2000
1000
0
媒剂/盐水
媒剂/LPS
地塞米松/LPS
0
30
60
PPL003 (2000 μg/小鼠)

图17A

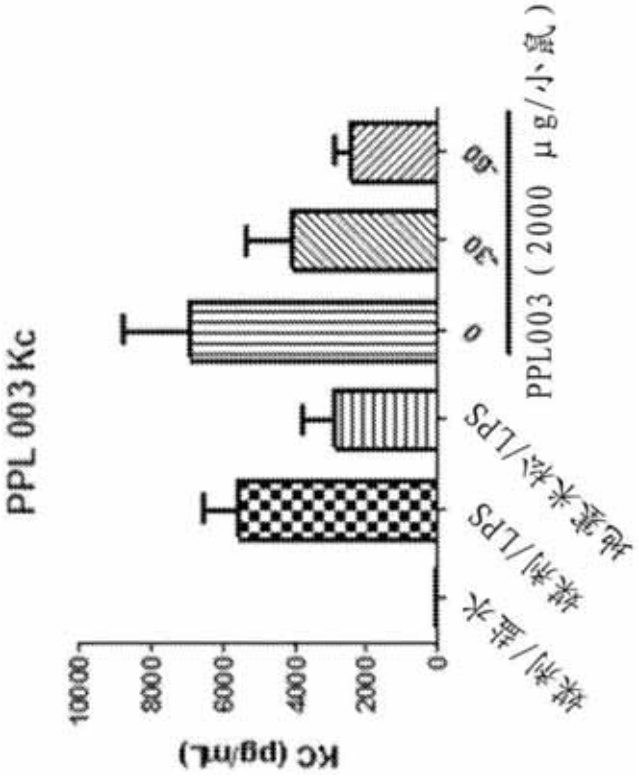

PPL 003 Kc
KC (pg/mL)
10000
8000
6000
4000
2000
0
媒剂/盐水
媒剂/LPS
地塞米松/LPS
PPL003（2000 μg/小鼠）
0
30
60

图17B

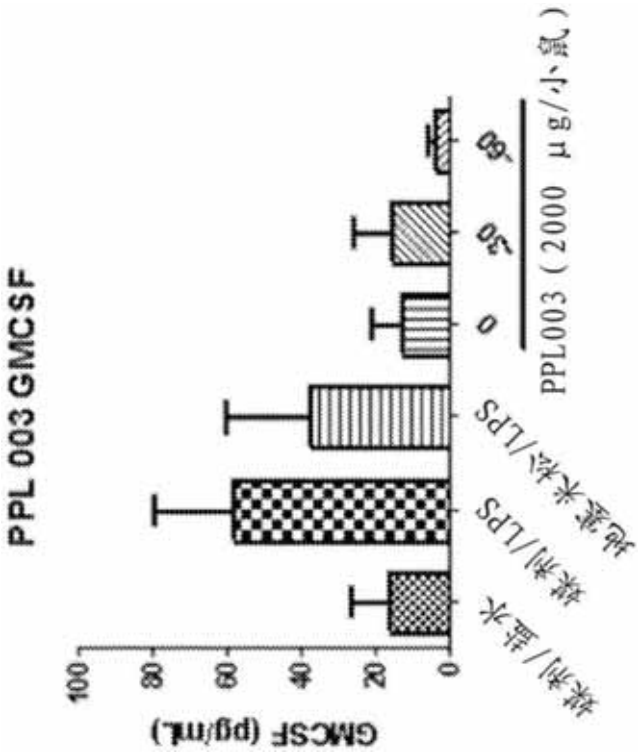

PPL 003 GMCSF
GMCSF (pg/mL)
100
80
60
40
20
0
媒剂/盐水
媒剂/LPS
地塞米松/LPS
PPL003（2000 μg/小鼠）
0
30
60

图18A

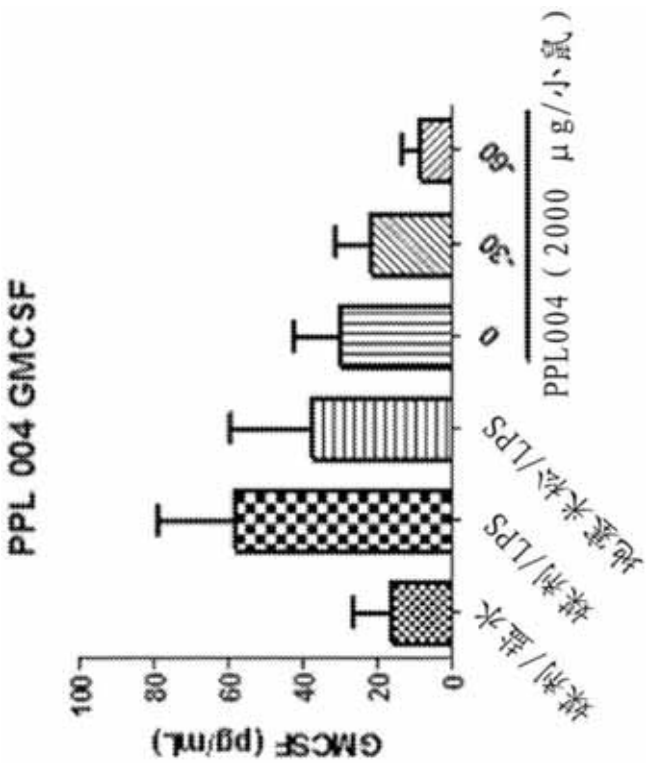

PPL 004 GMCSF
GMCSF (pg/mL)
100
80
60
40
20
0
媒剂/盐水
媒剂/LPS
地塞米松/LPS
PPL004（2000 μg/小鼠）
0
30
60

图18B

1.一种偶联物,其包含:

包含同源域结构并且具有如SEQ ID No.1至19中的任一者所描述的多肽序列或其变体的第一区;以及

与所述第一区并不天然缔合的包含功能或调控多肽或蛋白质的第二区。

2.一种偶联物,其包含:

包含HOX D12同源域或HOX C12同源域中的至少一者的第一区;以及

与所述第一区并不天然缔合的包含功能或调控多肽或蛋白质的第二区。

3.如权利要求1或2所述的偶联物,其中所述第二区包括NEMO结合域、源自PC1CTT的融合蛋白质、恢复具有遗传性酶缺乏的受试者中的酶功能的酶、RPE65的基因产物、锌指肽、Ag85A 99-118表位肽、或Ag85A 70-78表位肽中的至少一者。

4.如权利要求3所述的偶联物,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

5.如权利要求3所述的偶联物,其中所述源自PC1CTT的融合蛋白质具有SEQ.ID No.23或26中的至少一者所列的多肽序列或其变体。

6.如权利要求3所述的偶联物,其中所述恢复具有遗传性酶缺乏的受试者中的酶功能的酶具有SEQ.ID No.30、45或48中的至少一者的多肽序列或其变体。

7.如权利要求3所述的偶联物,其中所述RPE65的基因产物具有包括至少SEQ.ID No.33的多肽序列或其变体。

8.如权利要求3所述的偶联物,其中所述锌指肽序列包括SEQ.ID No.36、29或42中的至少一者或其变体。

9.如权利要求3所述的偶联物,其中所述Ag85A 99-118表位肽具有包括SEQ.ID No.51、54、57或60中的至少一者的序列或其变体。

10.如权利要求3所述的偶联物,其中所述Ag85A 70-78表位肽具有包括SEQ.ID No.63、66或69中的至少一者的序列或其变体。

11.如权利要求1或2所述的偶联物,其中所述第二区偶联至所述第一区的C端。

12.如权利要求1或2所述的偶联物,其中所述第二区偶联至所述第一区的N端。

13.如权利要求1和2中任一项所述的偶联物,其呈融合蛋白质的形式。

14.如权利要求1和2中任一项所述的偶联物,其中所述第二区与细胞内标靶和/或核内标靶相互作用。

15.如权利要求14所述的偶联物,其中所述第二区与所述细胞内标靶和/或核内标靶的所述相互作用影响以下一者或多者:NF-κB活化、葡萄膜炎、具有多囊性肾病突变的肾小管细胞中的PC1转录因子活性、具有GCase的戈谢病突变的细胞中的葡糖脑苷脂酶底物、患有赫尔勒综合症或亨特综合症的受试者中的葡糖胺聚糖、缺乏视网膜鸟苷酸环化酶的视网膜细胞中的视网膜鸟苷酸环化酶底物、具有亨廷顿氏病突变的细胞中的亨廷顿蛋白突变基因转录的调控,以及抗原表位递送至抗原呈递细胞的细胞质中用于在诸如结核病疫苗的疫苗实施方案中引发有效免疫反应以预防暴露于感染的受试者的结核病。

16.如权利要求1或2所述的偶联物,其中所述第二区具有包括SEQ.ID No.23或26的多肽序列或其变体,并且其中所述偶联物进一步包含非竞争性V2受体拮抗剂。

17.如权利要求1或2所述的偶联物,其中所述第二区具有选自由SEQ ID No:20、23、26、

30、33、36、39、42、45、48、51、54、57、60、63、66、69以及72组成的群组的多肽序列或其变体。

18.如权利要求1或2所述的偶联物，其进一步包含介于所述第一与第二区之间的连接子序列。

19.如权利要求1所述的偶联物，其中所述第一和第二区组合起来具有选自由SEQ ID No:21、22、24、25、27、28、31、32、34、35、37、38、40、41、43、44、46、47、49和50、52、53、55、56、58、59、61、62、64、65、67、68、70、71、73以及74组成的群组的多肽序列或其变体。

20.如权利要求19所述的偶联物，其中在用作活性剂和/或治疗剂之前去除所述序列的起始甲硫氨酸残基。

21.如权利要求1所述的偶联物，其中所述第一和第二区组合起来具有包含同源域结构以及NEMO结合域、源自PC1CTT的融合蛋白质、恢复具有遗传性酶缺乏的受试者中的酶功能的酶、RPE65的基因产物、锌指肽、Ag85A 99-118表位肽、以及Ag85A 70-78表位肽中的至少一者的序列。

22.如权利要求21所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶相互作用。

23.如权利要求22所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶的所述相互作用影响以下一者或多者:NF-κB活化、葡萄膜炎、具有多囊性肾病突变的肾小管细胞中的PC1转录因子活性、具有GCase的戈谢病突变的细胞中的葡糖脑苷脂酶底物、患有赫尔勒综合症或亨特综合症的受试者中的葡糖胺聚糖、缺乏视网膜鸟苷酸环化酶的视网膜细胞中的视网膜鸟苷酸环化酶底物、具有亨廷顿氏病突变的细胞中的亨廷顿蛋白突变基因转录的调控，以及抗原表位递送至抗原呈递细胞的细胞质中用于引发有效免疫反应作为如结核病疫苗的疫苗的活性组分以预防暴露于感染的受试者的结核病。

24.如权利要求19或21所述的偶联物，其进一步包含介于所述第一与第二区之间的连接子序列。

25.一种组合物，其包含如权利要求1所述的偶联物和医药学上可接受的载剂。

26.一种组合物，其包含如权利要求2所述的偶联物和医药学上可接受的载剂。

27.如权利要求25和24所述的组合物，其中所述组合物呈可吸入组合物、滴眼液制剂、用于局部或可注射使用的其它眼用组合物、灌肠剂、局部组合物、或包括用于持续释放的可注射植入物的可注射组合物的形式。

28.如权利要求1或2所述的偶联物，其中当在制剂中施用于受试者时，所述偶联物进入中枢神经系统。

29.如权利要求1或2所述的偶联物，其中当在制剂中施用于受试者时，所述偶联物与当不偶联至所述第一区时由所述第二区引出的不当免疫反应相比在受试者中引出减少的不当免疫反应。

30.一种治疗受试者的病状的方法，其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者，其中所述偶联物进入受试者的中枢神经系统。

31.一种治疗受试者的病状的方法，其包括将包含如权利要求21所述的偶联物的制剂施用于所述受试者，其中所述偶联物进入所述受试者的中枢神经系统。

32.一种治疗受试者的病状的方法，其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者，其中所述偶联物预防或改善所述受试者中对抗原肽的免疫反应以使

得以下至少一者存在:(a)肽暴露没有显著减少,以及(b)免疫介导的不良临床事件的发生率没有显著增加。

33.一种治疗受试者的干眼症的方法,其包括将包含如权利要求1或2所述的偶联物的制剂施用于所述受试者,其中所述第一区偶联至NEMO结合域。

34.如权利要求33所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

35.一种治疗受试者的自体免疫和发炎相关的脱发的方法,其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者,其中所述第二区是NEMO结合域。

36.一种治疗受试者的自体免疫和发炎相关的脱发的方法,其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者,其中所述第二区是NEMO结合域。

37.如权利要求35或36所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

38.一种治疗受试者的发炎性气道疾病的方法,其包括将包含如权利要求1所述的偶联物的可吸入制剂施用于所述受试者,其中所述第二区是NEMO结合域。

39.一种治疗受试者的发炎性气道疾病的方法,其包括将包含如权利要求2所述的偶联物的可吸入制剂施用于所述受试者,其中所述第二区是NEMO结合域。

40.如权利要求38或39所述的方法,其中所述发炎性气道疾病是慢性阻塞性肺病。

41.如权利要求38所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

42.一种治疗受试者的结肠直肠发炎性肠病的方法,其包括将包含如权利要求1所述的偶联物的灌肠制剂施用于所述受试者,其中所述第二区是NEMO结合域。

43.一种治疗受试者的结肠直肠发炎性肠病的方法,其包括将包含如权利要求2所述的偶联物的灌肠制剂施用于所述受试者,其中所述第二区是NEMO结合域。

44.如权利要求42或43所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

45.一种治疗受试者的病状的方法,其包括将包含如权利要求1所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

46.一种治疗受试者的病状的方法,其包括将包含如权利要求2所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

47.如权利要求45或46所述的方法,其中所述病状是不稳定性心绞痛、急性心肌梗塞以及急性中风中的至少一者。

48.如权利要求45或46所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多肽序列或其变体。

49.一种治疗受试者在短暂缺血后的缺血再灌注损伤的方法,其包括将包含如权利要求1所述的偶联物的全身制剂施用于所述受试者,其中所述第二区是NEMO结合域。

50.一种治疗受试者在短暂缺血后的缺血再灌注损伤的方法,其包括施用包含如权利要求2所述的偶联物的全身制剂,其中所述第二区是NEMO结合域。

51.如权利要求49或50所述的方法,其中所述缺血再灌注损伤是在脑、肾脏或心脏中。

52.如权利要求49或50所述的方法,其中所述NEMO结合域具有SEQ.ID No.20所列的多

肽序列或其变体。

53.一种治疗受试者的多囊性肾病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区是源自PC1CTT的融合蛋白质、源自PC1CTT的p200肽、或源自PC1CTT的p21肽。

54.一种治疗受试者的多囊性肾病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区是源自PC1CTT的融合蛋白质、源自PC1CTT的p200肽、或源自PC1CTT的p21肽。

55.如权利要求53或54所述的方法，其中所述第二区具有SEQ.ID No.23或26中的至少一者所列的多肽序列或其变体。

56.如权利要求53或54所述的方法，其中所述偶联物进一步偶联至非竞争性V2受体拮抗剂用于靶向集合管细胞的肾囊肿。

57.一种治疗受试者的溶酶体贮积病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述偶联物的所述第二区包含恢复具有遗传性酶缺乏的受试者中的酶功能的酶。

58.一种治疗受试者的溶酶体贮积病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述偶联物的所述第二区包含恢复具有遗传性酶缺乏的受试者中的酶功能的酶。

59.如权利要求57或58所述的方法，其中所述酶选自由葡糖脑苷脂酶、α-L-艾杜糖苷酸酶以及艾杜糖醛酸-2-硫酸酯酶组成的群组。

60.如权利要求57或58所述的方法，其中所述酶包括如SEQ.ID No.30、45或48中的至少一者所列的多肽序列或其变体。

61.一种治疗受试者的1型莱伯先天性黑朦的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包括RPE65的基因产物。

62.一种治疗受试者的1型莱伯先天性黑朦的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包括RPE65的基因产物。

63.如权利要求61或62所述的方法，其中所述RPE65的基因产物具有如SEQ.ID No.33所列的多肽序列或其变体。

64.一种治疗受试者的亨廷顿氏病的方法，其包括将如权利要求1所述的偶联物施用于所述受试者，其中所述第二区包括锌指肽。

65.一种治疗受试者的亨廷顿氏病的方法，其包括将如权利要求2所述的偶联物施用于所述受试者，其中所述第二区包括锌指肽。

66.如权利要求64或65所述的方法，其中所述锌指肽序列包括SEQ.ID No.36、29或42中的至少一者或其变体。

67.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 99-118表位肽。

68.一种治疗受试者的结核病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 99-118表位肽。

69.如权利要求67或68所述的方法，其中所述Ag85A 99-118表位肽序列包括SEQ.ID No.51、54、57或60中的至少一者或其变体。

70.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 70-78表位肽。

71.一种治疗受试者的结核病的方法，其包括将包含如权利要求2所述的偶联物的制剂施用于所述受试者，其中所述第二区包含Ag85A 70-78表位肽。

72.如权利要求70或71所述的方法，其中所述Ag85A 70-78表位肽序列包括SEQ.ID No.63、66或69中的至少一者或其变体。

73.一种偶联物，其通过包括以下步骤的方法制备：

将用包含编码如权利要求1所述的偶联物的核酸的表达载体转化的宿主细胞在提供所述偶联物在所述宿主细胞内的表达的条件下培养；以及

通过亲和纯化回收所述偶联物。

74.一种偶联物，其包含：

包含作为HOX D12同源域或HOX C12同源域的同源域结构的第一区；以及

与所述第一区并不天然缔合的包含功能或调控核酸或肽核酸的第二区。

75.一种偶联物，其包含：

包含具有SEQ ID No.1至19中的任一者所描述的多肽序列或其变体的同源域结构的第一区；以及

与所述第一区并不天然缔合的包含功能或调控核酸或肽核酸的第二区。

76.如权利要求74或75所述的偶联物，其中所述第二区包含具有细胞内生物活性的DNA、RNA、LNA、PNA、γPNA、或这些组分的组合。

77.如权利要求74或75所述的偶联物，其中所述第二区包括结合至miRNA21或DYRK1b的核酸。

78.如权利要求74至76中任一项所述的偶联物，其中所述第二区具有如SEQ ID No.76、78或79中的至少一者所列的核苷酸序列或其变体。

79.如权利要求74至77中任一项所述的偶联物，其中所述第二区偶联至所述第一区的C端。

80.如权利要求74至77中任一项所述的偶联物，其中所述第二区偶联至所述第一区的N端。

81.如权利要求74至77中任一项所述的偶联物，其中所述第一区用肽键连接至所述第二区。

82.如权利要求74至77中任一项所述的偶联物，其中所述第二区经由非肽键偶联至所述第一区。

83.如权利要求74至77中任一项所述的偶联物，其中所述第二区与细胞内标靶和/或核内标靶相互作用。

84.如权利要求83所述的偶联物，其中所述第二区经由与miRNA或结合miRNA的蛋白质复合物的相互作用而与细胞内标靶和/或核内标靶相互作用。

85.如权利要求84所述的偶联物，其中所述第二区是结合至miRNA132用于影响Ras路径的核酸或PNA。

86.如权利要求84所述的偶联物，其中所述第二区是稳定地杂交至如SEQ ID No.75所列的miRNA核苷酸序列或其变体的核酸序列或PNA。

87.如权利要求86所述的偶联物，其中所述第二区具有稳定地杂交至miRNA132的如SEQ ID No.78所列的核苷酸序列或所述核苷酸序列的一部分或其变体。

88.如权利要求86所述的偶联物，其中所述第二区基于与miRNA132的相互作用的特异性来选择。

89.如权利要求86所述的偶联物，其中所述第二区由用以增加稳定性或减少降解的修饰核苷酸组成。

90.如权利要求84所述的偶联物，其中所述第二区是结合至miRNA21用于影响肿瘤抑制路径或肿瘤生长的核酸序列或PNA。

91.如权利要求90所述的偶联物，其中所述第二区包含如SEQ ID No.76所列的核苷酸序列或其变体或可以稳定地杂交至如SEQ ID No.77所列的核苷酸序列或其变体。

92.如权利要求74和75至91中任一项所述的偶联物，其中所述第一区同源域结构具有如SEQ ID No.2所列的多肽序列或其变体。

93.如权利要求74至77中任一项所述的偶联物，其中所述第二区经由与mRNA相互作用用于影响拼接或蛋白质翻译而与细胞内标靶和/或核内标靶相互作用。

94.如权利要求93所述的偶联物，其中所述第二区结合至DYRK1b。

95.如权利要求94所述的偶联物，其中所述第二区具有如SEQ ID No.79所列的核苷酸序列或其变体。

96.如权利要求74所述的偶联物，其中所述第一区和所述第二区经由“点击化学”而连接。

97.如权利要求74所述的偶联物，其中所述第二区包含PNA，并且其中所述第一区和所述第二区经由肽键连接。

98.一种治疗受试者的癌症的方法，其包括将包含如权利要求74所述的偶联物的制剂施用于所述患者。

99.一种治疗受试者的癌症的方法，其包括将包含如权利要求93所述的偶联物的制剂施用于所述受试者。

100.一种治疗受试者的癌症的方法，其包括将包含如权利要求94所述的偶联物的制剂施用于所述受试者。

101.一种治疗受试者的癌症的方法，其包括将包含如权利要求95所述的偶联物的制剂施用于所述受试者。

102.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求74所述的偶联物的制剂施用于所述受试者。

103.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求93所述的偶联物的制剂施用于所述受试者。

104.一种治疗受试者的代谢综合症、脂质紊乱和/或肥胖的方法，其包括将包含如权利要求94所述的偶联物的制剂施用于所述受试者。

105.一种治疗受试者的代谢综合症、脂质紊乱或肥胖的方法，其包括将包含如权利要求95所述的偶联物的制剂施用于所述受试者。

106.如权利要求98所述的治疗癌症的方法，其包括施用作为静脉内溶液的所述制剂。

107.如权利要求102所述的治疗代谢综合症、脂质紊乱和/或肥胖的方法，其包括施用

呈持续释放形式的所述制剂。

108.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区包括Ag85A 99-118表位肽或Ag85A 70-78表位肽之一的至少一部分。

109.如权利要求108所述的治疗受试者的结核病的方法，其中：

经由吸入途径、鼻内途径和/或通过肌肉内、皮内或皮下肠道外注射将所述制剂至少一次施用于所述受试者；并且

所述制剂的施用引发防止所述受试者中结核分枝杆菌的活动性肺部感染发作的保护性免疫反应。

110.一种治疗受试者的结核病的方法，其包括将包含如权利要求1所述的偶联物的制剂施用于所述受试者，其中所述第二区具有SEQ ID No.51、54、57、60、63、66、69以及72中的至少一者的多肽序列或其变体。

111.一种治疗受试者的干眼症、自体免疫相关的脱发、发炎相关的脱发、慢性阻塞性肺病、发炎性气道疾病、不稳定性心绞痛、急性心肌梗塞、急性中风以及对器官的缺血再灌注损伤中的任何一者或多者的方法，其包括：

至少一次施用包含如权利要求1所述的偶联物的制剂，其中所述第二区包含NEMO结合域，并且

其中所述制剂的施用抑制NF-κB活性，降低TNF-α、IP-10、SAA、IL-6、Kc和/或GM-CSF的血液浓度，和/或增加血液中的白介素10水平。

112.如权利要求111所述的方法，其中所述第二区具有如SEQ ID No.20所列的多肽序列或其变体。

113.如权利要求111所述的方法，其中所述第一区包含如SEQ ID No.1或2中的任一者所列的多肽序列或其变体。

114.如权利要求1、2、74以及75中任一项所述的偶联物，其中所述第一区包含来源于人类基因的多肽序列。

115.如权利要求114所述的偶联物，其中所述第二区包括来源于人类基因的多肽序列或其变体。

116.如权利要求114所述的偶联物，其中所述第二区包括来源于人类基因的核酸序列或其变体。

117.如权利要求30至72和98至113中任一项所述的方法，其中所述受试者是人类。